Model 380B DNA Synthesizer

Version 1.1

User's Manual

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

APPLIED BIOSYSTEMS LIMITED WARRANTY

Applied Biosystems warrants to the customer that, for a period ending on the earlier of one year from completion of installation or 15 months from the date of shipment to the Customer ("the Warranty Period"), the Applied Biosystems Model 380B purchased by the customer (the "Instrument") will be free from defects in material and workmanship, and will perform in accordance with the performance specifications contained in the applicable Applied Biosystems product literature (the "Specifications").

This Warranty does not apply to the Instrument's valves, reagent lines, or performance, unless the Customer uses only reagents and solvents supplied by Applied Biosystems or expressly recommended by Applied Biosystems, or to any damages caused by reagents or solvents not supplied by Applied Biosystems, even though recommended by Applied Biosystems. This Warranty does not extend to any Instrument or part thereof that has been subjected to misuse, neglect or accident, that has been modified or repaired by anyone other than Applied Biosystems or that has not been used in accordance with the instructions contained in the Instrument Operator's Manual. Nor does this Warranty cover any Customer-installable consumable parts for the Instrument that are listed in the Instrument Operator's Manual.

Applied Biosystems obligation under this warranty is limited to repairs or replacements the Applied Biosystems deems necessary to correct covered defects or failures of which Applied Biosystems is notified prior to expiration of the Warranty Period. All repairs and replacements under this Warranty shall be performed by Applied Biosystems on-site at the Customer's location at Applied Biosystems expense.

No agent, employee, or representative of Applied Biosystems has any authority to bind Applied Biosystems to any affirmation, representation, or warranty concerning the Instrument that is not contained in the printer product literature or this Warranty Statement. Any such affirmation, representation or warranty made by any agent, employee, or representative of Applied Biosystems shall not be binding on Applied Biosystems.

Applied Biosystems shall not be liable for any incidental, special, or consequential loss, damage or expense directly or indirectly arising from the use of the Instrument. Applied Biosystems makes no warranty whatsoever in regard to products or parts furnished by third parties; such products or parts will be subject to the warranties, if any, of their respective manufacturers.

This Warranty is limited to the original Customer and is not transferable.

THIS WARRANTY IS THE SOLE AND EXCLUSIVE WARRANTY AS TO THE INSTRUMENT AND IS EXPRESSLY IN LIEU OF ANY OTHER EXPRESS OR IMPLIED WARRANTIES, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE AND OF ANY OTHER OBLIGATION ON THE PART OF APPLIED BIOSYSTEMS.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, chemical safety, and waste profiles.

About MSDSs

Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current material safety data sheet (MSDS) before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

⚠ WARNING CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.

Ordering MSDSs

You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

To order documents by telephone:

1	From the U.S. or Canada, dial **1.800.487.6809**, or from outside the U.S. and Canada, dial **1.858.712.0317**.
2	Follow the voice instructions to order documents (for delivery by fax). **Note** There is a limit of five documents per fax request.

To view, download, or order documents through the Applied Biosystems web site:

Step	Action
1	Go to **http://www.appliedbiosystems.com**
2	Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS.**
3	Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a pdf of the MSDS.

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

USER ATTENTIONS

Four "User Attention" words are utilized in the text of this manual. Categorically, each one implies a particular level of observation or action as follows:

NOTE: This word is used to call attention to information.

IMPORTANT: This information is indicated because it is necessary for proper instrument operation.

CAUTION: This word informs the user that damage to the instrument could result if the user does not comply with this information.

WARNING: Physical injury to the user or other persons could result if these required precaution(s) are not implemented.

MATERIAL SAFETY DATA SHEETS (MSDS)

WARNING: Some chemicals used with this instrument are considered hazardous. Hazards are prominently displayed on the labels of all hazardous chemicals. In addition there are MSDS which provide information about physical characteristics, hazards, precautions, first aid, spill cleanup and disposal procedures. The MSDS have been sent to you with the Preinstallation Manual. Please be sure to familiarize yourself with the information contained in these documents before attempting to operate the instrument or use the reagents. Additional copies of the MSDS are available from Applied Biosystems at no extra cost.

TABLE of CONTENTS

SECTION 4: SOFTWARE MENUS

GENERAL INTRODUCTION

The Applied Biosystems Model 380B DNA Synthesizer is a precision instrument which automatically performs all steps of DNA Synthesis to produce the highest quality oligonucleotides. In addition, the instrument provides automatic deprotection and cleavage of the DNA chain from the solid support. The system was designed for high reliability, ease of operation and efficient use of the operator's time.

The Model 380B uses the phosphoramidite method of oligonucleotide synthesis because of its inherently high coupling efficiency and the stability of the starting materials. The synthesis is performed with the growing nucleotide chain attached to a solid support which is contained within a column (the reaction chamber). Solid support synthesis allows excess reagents which are in the liquid phase to be removed by filtration and eliminates the need for purification between base additions. Typically, stepwise yields of 98 to 100% are obtained with the Model 380B enabling synthesis of oligonucleotides of 60 to more than 100 bases in length. With these high coupling efficiencies, it is often not necessary to purify oligonucletides with 15-25 bases which will be used as hybridization probes.

All Applied Biosystems chemicals are purified or analyzed to ensure high yield syntheses. Solvents and reagents are packaged in bottles that attach directly to the synthesizer. To prepare the chemicals, it is only necessary to dissolve the phosphoramidites in anhydrous acetonitrile.

Each phosphoramidite, column, reagent and solvent manufactured by Applied Biosystems is unconditionally guaranteed. If you are not completely satisfied by the product (and it is used prior to any applicable expiration date and under the correct operating conditions), it will be replaced at no charge.

Reagents and solvents are delivered to the reaction chamber by a pressure-driven chemical delivery system. The system uses patented zero-dead volume valves which increase reliability, eliminate cross-contamination, and reduce the reagent costs.

The Model 380B uses "menu-driven" software which is designed for simplicity and ease of operation. A touchscreen presents various options and necessary information about the synthesis or status of the instrument. In response, the user selects an option and gives instructions by pressing the appropriate area on the screen. To perform automated synthesis, stored cycles and procedures are used to complete all chemical reactions.

Applied Biosystems and its representatives maintain a large staff of fully trained service engineers strategically located throughout the world. If any questions arise while operating the DNA Synthesizer, call your service representative. In addition, technical experts are available to answer any questions about DNA synthesis, analysis and purification. Please feel free to extensively use these resources.

SECTION 1

SYSTEM DESCRIPTION

This section describes the components of the Model 380B DNA Synthesizer. Use this section to familiarize yourself with the synthesizer and its operations.

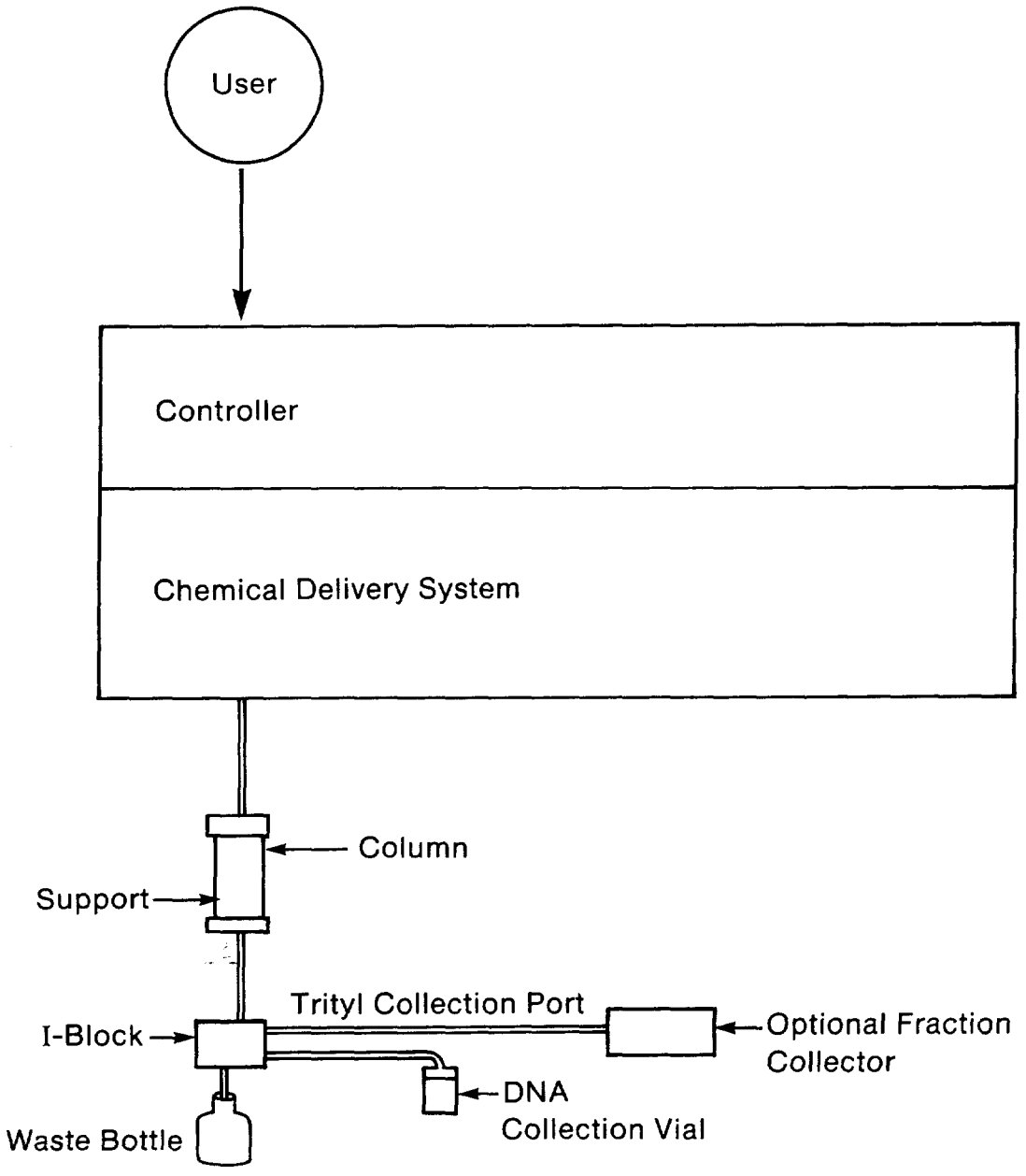
SYSTEM DESCRIPTION

INTRODUCTION

The major components of the Model 380B automated DNA Synthesizer are shown in Figure 1-1. All chemical steps required for DNA synthesis take place within a reaction chamber, called the COLUMN. The column contains the 3'-terminal nucleoside which is covalently attached to a SUPPORT. The DNA chain is built by adding one base at a time to the support-bound nucleoside.

All reagents and solvents necessary for synthesis are accessed by the CHEMICAL DELIVERY SYSTEM. In this system, a set of solenoid valves opens to create a pathway for chemical flow. Regulated argon pressure forces the chemicals to flow from their reservoirs through the pathway (consisting of one or more valve blocks and delivery lines) and then to the column. Following completion of a chemical step, the column effluent flows to either the WASTE BOTTLE, the TRITYL COLLECTION PORT (to collect the dimethoxytrityl cation for analysis) or the DNA COLLECTION VIAL (containing the DNA product).

The CONTROLLER directs and initiates all synthesizer activity and consists of a user interface, a microprcessor and associated electronics. Information is displayed on a touchscreen and the user gives instructions by touching the appropriate area on the screen.



Major Components of Model 380B

Figure 1-1

THE CHEMICAL DELIVERY SYSTEM

The flow of reagents, solvents and gases through the Model 380B is controlled by a positive-pressure chemical delivery system. The system components include a regulated pressure source, 18 reagent and solvent reservoirs, delivery valves, a column, a vented waste bottle and delivery lines which interconnect the components. All inner surfaces of the chemical delivery system are made of inert glass, Teflon, Kel-F or Kalrez.

PRESSURE

System pressure is provided by pre-purified (99.998%) argon. Argon's high density and low oxygen contamination make it preferable to nitrogen. Two argon cylinders (a primary tank and a back up tank) are connected to the inlet ports at the rear of the Model 380B using oxygen-impermeable tubing and gas-tight connectors. There are optimal pressure regulator settings specified for each synthesizer. The regulator from the primary tank is set at approximately 60 psi while the back up is set 4 psi lower. When the primary tank empties, the back up tank automatically delivers argon to the Synthesizer. A pair of check valves permits flow from one tank to another and facilitates changeover. This design permits replacement of an empty argon cylinder without interrupting an ongoing synthesis. Section 5 includes a procedure for changing an argon tank.

Argon entering the Synthesizer travels to a purge valve (which is used to eliminate air after changing cylinders), passes through check valves, a moisture trap, a moisture indicator, a 15-u particle filter and to three pressure regulators: A, B and D.

Regulator A delivers argon to a manifold which channels the gas to specific pressure valves used to pressurize the following reservoirs:

spare reservoir	bottle 8
acetonitrile	bottle 13
trichloroacetic acid	bottle 14
iodine	bottle 15
acetonitrile	bottle 16
spare reservoir	bottle 17
acetonitrile	bottle 18

When using Version 2.00 software, Regulator A is adjusted so that acetonitrile flows from Bottle 18 through the column and then to waste (exiting at the waste line in the waste bottle compartment) at a rate of 2.5 mL/min. plus or minus 0.2 mL/min. Versions 1.34 and 1.44 require a flow rate between 1.55 mL/min and 1.65 mL/min.

Regulator B delivers argon to pressurize the following reservoirs:

phosphoramidites	bottles 1 to 7
tetrazole	bottle 9
1-methylimidazole (NMI)	bottle 11
acetic anhydride	bottle 12

To pressurize the phosphoramidites, argon is delivered to one pressure valve which supplies the gas to a manifold pressurizing all seven bottles simultaneously. To do this, bottles must be attached to all seven phosphoramidite positions, even if some bottles are empty. The two capping reagents (NMI and acetic anhydride) share a pressure valve which channels the argon to a manifold to pressurize both bottles. The tetrazole is pressurized using a single valve.

Regulator B is adjusted so that acetonitrile flows from Bottles 1 to 7 and 9 to the lower column Luer fitting at a rate of 1.45 to 1.55 mL/min. Acetonitrile should flow from Bottles 11 and 12 to the lower column

Luer fitting at a rate of 1.60 to 2.00 mL/min. The regulator should read approximately 6 to 7 psi.

Regulator D pressurizes ammonium hydroxide (bottle 10) and supplies argon to valve blocks H, K and I. The regulator is adjusted so that aectonitrile flows from Bottle 10 to the DNA collection vial at a rate of 3.9 to 4.5 mL/min. Regulator D should read approximately 12 psi.

For accurate calibration, all the regulators must be set while reagents are flowing through the lines.

REAGENT AND SOLVENT RESERVOIRS

Each reservoir has a unique position on the instrument and is referred to by the number located above each receptacle. Position numbers are also printed on bottle labels.

Bottle reservoirs 1 through 12 are pushed upward around a Teflon insert and a Kalrez O-ring to form an airtight seal inside each bottle neck. Pressing the black button above the bottle position releases the grip on the bottle and allows for its removal. The button will return to its "out" position only when the reservoir is correctly seated.

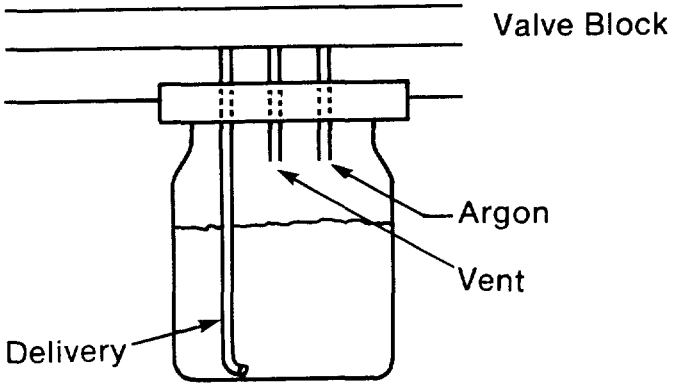
Bottles 13 through 18 screw snugly into a threaded cap assembly mounted on the Synthesizer. A Teflon insert and Kalrez gasket form an airtight seal between each cap and reservoir.

CAUTION: Do not overtighten the bottles or the gaskets will tear or distort and the bottle may break.

When changing a bottle, use a mirror to inspect the insert and check the condition of the gasket. A functional gasket appears as a flat black surface. If no black is seen or if the black gasket appears wrinkled or cracked, it must be replaced.

The pressure and delivery lines:

Each bottle has an argon pressure line and a delivery line entering through the cap insert. As shown in Figure 1-2, the argon line remains above the liquid level while the delivery line extends to the bottom of the bottle. Upon opening the correct set of valves, the reservoir headspace is pressurized by argon and the liquid is pushed into the delivery line and flows to its destination.



REAGENT RESERVOIR
Figure 1-2

Since all phosphoramidite reservoirs are pressurized simultaneously by a single valve, bottles must be attached to all seven positions (even if some are empty) or the argon will escape out any exposed pressure line. Also, bottles should be on all positions to keep the lines clean.

The vent line:

In addition to the pressure and delivery lines, the following bottles also have a vent line (See Figure 1-2):

phosphoramidites	bottles	1 to 7
spare reservoir	bottle	8
tetrazole	bottle	9
ammonium hydroxide	bottle	10
acetonitrile	bottle	18

Vent lines provide an escape route for pressurized gas and are used when changing bottles.

Ammonium hydroxide is extremely volatile. Therefore, before removal from the instrument, this reservoir should be depressurized by opening its vent line. This prevents a burst of vapor when changing the bottle. Venting occurs automatically during the bottle change procedure.

When fresh atmosphere-sensitive phosphoramidites and tetrazole are placed on the instrument, they are purged with argon to eliminate air. A purge delivers gas to the reservoir. As the gas is passed over the bottle's headspace, the air escapes out the open vent line. Purging is performed automatically during the bottle change procedure.

REAGENTS AND SOLVENTS

All chemicals have been thoroughly tested at Applied Biosystems to ensure repeatedly reliable syntheses. Each reagent and solvent has a unique position on the instrument and is referred to by the number (1-18) located above each receptacle. Position numbers are also printed on the bottle labels.

All reagents may be stored at room temperature, except ammonium hydroxide and the iodine solution which must be kept refrigerated. For waste disposal information, refer to Section 5, Changing the Waste Bottle.

WARNING: Consider each chemical in the synthesizer potentially harmful. Do not inhale. Work in a well ventilated area. Always use eye protection and wear acid-impermeable gloves. Do not leave any chemicals uncapped. If any chemical is ingested or comes in contact with the eyes, immediately consult a physician. If there is any physical contact with a chemical, wash immediately with water.

Bottles 1-7

Reservoirs 1 through 4 contain phosphoramidites dissolved in anhydrous acetonitrile. They are used to synthesize single species sequences and mixed sequence probes.

Contents	Reservoir
adenosine phosphoramidite (A)	1
guanosine phosphoramidite (G)	2
cytosine phosphoramidite (C)	3
thymidine phosphoramidite (T)	4

Positions 5 to 7 are spare phosphoramidite reservoirs and can be used for synthesizing with modified bases. Useful derivatives such as deoxyinosine and Aminolink 2 ™ (a linker for various substrates) are available from Applied Biosystems and can be placed in these positions.

IMPORTANT: Since all seven reservoirs are pressurized simultaneously with a single valve, all seven bottles must be attached to the instrument (even if some are empty) to perform synthesis.

Phosphoramidites are extremely sensitive to acid, oxygen and water. Once they are in solution and the protective cap is removed, quickly put them on the instrument to prevent their contamination.

IMPORTANT: Use anhydrous acetonitrile with less than 90 ppm water to dissolve the phosphoramidites. Do not use HPLC grade acetonitrile, its higher water content will decrease coupling efficiency.

Phosphoramidites are stable in powder form for one year and should be stored at room temperature in a desicator. Once they are dissolved, they should be used within approximately two weeks. After this time, coupling efficiencies may slowly begin to decrease. If they can not be used within this time, it is possible to freeze, store, thaw and then reuse the phosphoramidites. Although they may still show a slight loss of activity, they are usually adequate for synthesizing sequences of approximately 20 bases. For details on preparation and storage of phosphoramidites, see Section 5.

Bottle 8

This is a spare bottle position.

Bottle 9

Tetrazole (0.5 M) in anhydrous acetonitrile is used as the activator for the phosphoramidites. Once the protective seal is removed, quickly put the tetrazole on the instrument to prevent atmospheric water contamination.

IMPORTANT: Tetrazole will form a precipitate at approximately 12°C. Do not put precipitated tetrazole on the instrument.

Examine each bottle before using it. If the tetrazole has precipitated, warm it slightly until it is in solution. In cold climates, the tetrazole may precipitate in the delivery lines resulting in expensive repairs. Therefore, keep the room temperature above 18°C for operating the Model 380B and storing tetrazole.

Bottle 10

The recommended concentrated ammonium hydroxide is a 27% solution in water and is used to cleave the DNA from the support. It is extremely volatile and should be stored refrigerated. Any loss of ammonia concentration decreases the solution's effectiveness, therefore, only open it immediately before placement on the instrument. Use in a well ventilated area. This reservoir should be refilled with analytical grade ammonium hydroxide purchased from a local supplier.

Bottle 11

Acetic anhydride-lutidine-tetrahydrofuran (THF), (1:1:8) is one half of the capping reagent. Atmospheric water will reduce its efficiency. Upon opening the bottle, quickly place it on the instrument. Use in a well ventilated area, avoid inhalation. Store at room temperature.

Bottle 12

1-methylimidazole (NMI) is the second half of the capping reagent. Within 24 hours of combining NMI with the acetic anhydride reagent, the solution discolors and becomes viscous. To prevent this, the two reagents are stored in separate reservoirs and are simultaneously added to the column to perform capping. Upon opening the bottle, quickly place it on the instrument. Use in a well ventilated area and avoid inhalation. Store at room temperature.

Bottle 13

HPLC grade acetonitrile washes the support before and after the detritylation step. It is the same type of acetonitrile as used in bottle 18.

Bottle 14

Trichloroacetic acid (TCA) - dichloromethane (3% wt/vol) is the detritylating agent. Store at room temperature and use with caution.

Bottle 15

Iodine-water-pyridine-THF (0.1M:1:10:40) is the oxidizing reagent. It can be stored for up to 6 months from the date of manufacture. After this time, it may develop a tar (visible when the bottle is turned over) and should not be used.

Note the expiration date on the bottle. Any iodine which develops the tar before the expiration date will be replaced, without charge, by Applied Biosystems. Use in a well ventilated area and avoid inhalation.

Bottle 16

HPLC grade acetonitrile washes the support before and after the detritylation step. It is the same type of acetonitrile as used in bottle 18.

Bottle 17

This is a spare bottle position.

Bottle 18

HPLC grade acetonitrile is used to wash the support, valve blocks and delivery lines and to remove nucleophiles from the support prior to the coupling step.

IMPORTANT: Use HPLC or U.V. grade acetonitrile with a specification of less than 300 ppm of water.

Water contamination of even 1000 ppm will lead to a 1 to 2% decrease in coupling efficiency. If unsure of the water content, either check the acetonitrile using the Karl Fischer method of titration (requiring an automatic titration apparatus) or distill it over phosphorous pentoxide followed by redistillation over calcium hydride. Although Applied Biosystems supplies HPLC grade acetonitrile, it may be more economical to purchase it from local supplier such as Burdick and Jackson (Part Number 015).

To avoid contaminating the acetonitrile with atmospheric water, quickly refill bottle 18, replace the cap on the source acetonitrile and place bottle 18 on the instrument. As an additional precaution, keep the source acetonitrile dedicated only to this use.

REAGENTS AND SOLVENTS

Bottle Position	Reagent and Solvent Description		Part Number
1	Adenosine (A-bz) phosphoramidite	(0.25g)	400600
		(0.50g)	400330
		(1.0 g)	400326
2	Guanosine (G-ib) phosphoramidite	(0.25g)	400601
		(0.50g)	400331
		(1.0 g)	400327
3	Cytosine (C-bz) phosphoramidite	(0.25g)	400603
		(0.50g)	400332
		(1.0 g)	400328
4	Thymidine (T) phosphoramidite	(0.25g)	400602
		(0.50g)	400333
		(1.0 g)	400329
5-7	Positions 5, 6 and 7 are available for modified bases. For example:		
	Deoxyinosine phosphoramidite	(0.25g)	400402
9	Tetrazole/acetonitrile		400233
			400606*
10	Ammonium hydroxide**		
11	Acetic anhydride/lutidine/THF		400234
			400607*
12	1-Methylimidazole		400777
			400785*
14	Trichloroacetic acid		400236
15	Iodine/water/pyridine/THF		400753
16,18	Acetonitrile		400443
	Anhydrous Acetonitrile (for dissolving phosphoramidites)		400060

* For instruments with large bottle receptacles in positions 9, 11 and 12.

** Ammonium hydroxide should be purchased from a local supplier.

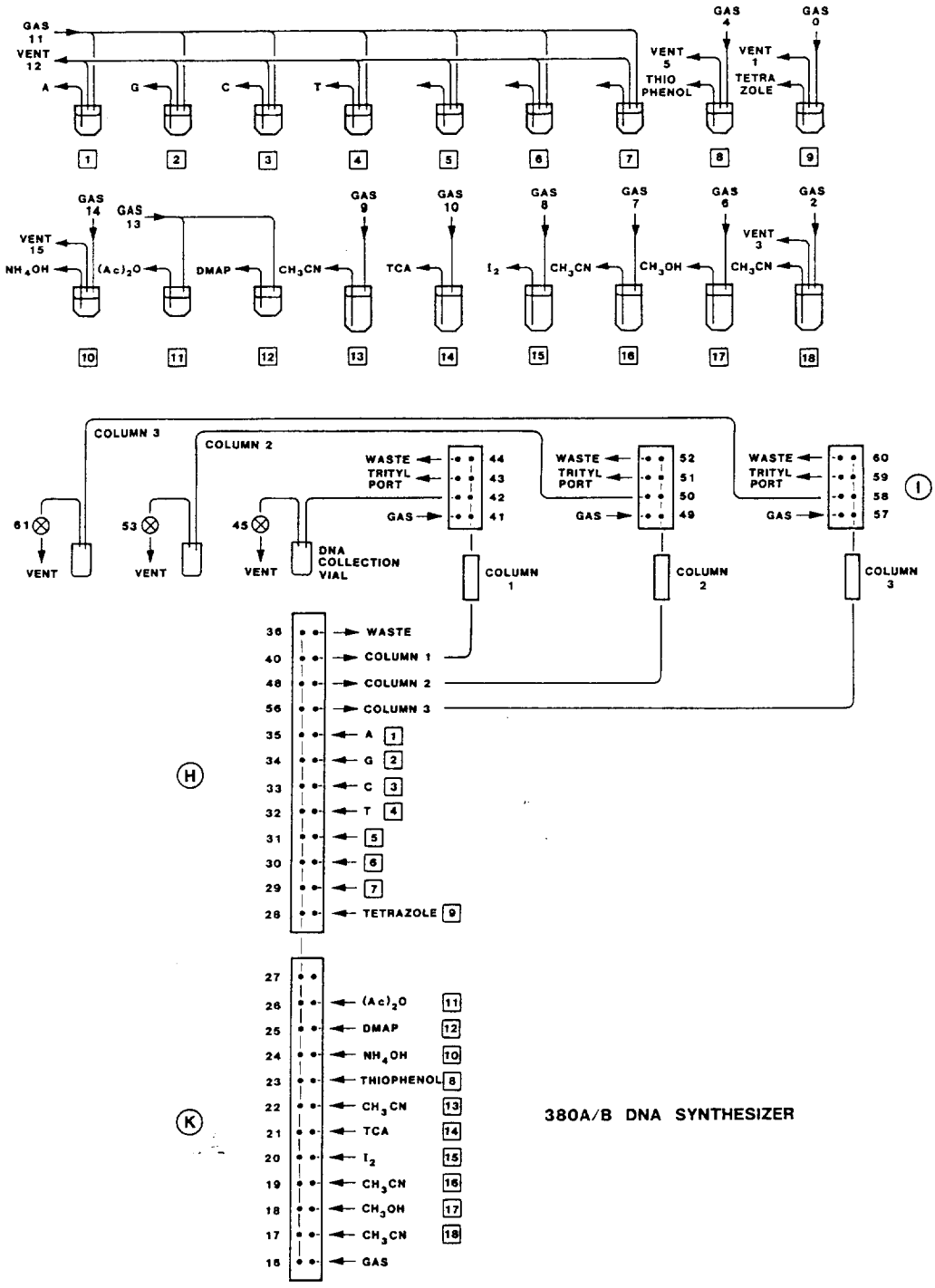
DELIVERY VALVES

The chemical delivery system includes three valve blocks: K, H and I. The valve blocks control gas and chemical flows to the column and exit ports. Figure 1-3 shows a schematic representation of all the valve blocks.

Valve block K controls the delivery of the following solvents and reagents:

spare bottle position	bottle 8
ammonium hydroxide	bottle 10
acetic anhydride	bottle 11
1-methylimidazole	bottle 12
acetonitrile	bottle 13
trichloroacetic acid	bottle 14
iodine	bottle 15
acetonitrile	bottle 16
spare bottle position	bottle 17
acetonitrile	bottle 18

Each of the above reagents flows from its reservoir through valve block K and then through valve block H which directs the flow to the column or waste. Valve block K also controls delivery of argon through valve blocks H and K.



380B DNA Synthesizer Schematic

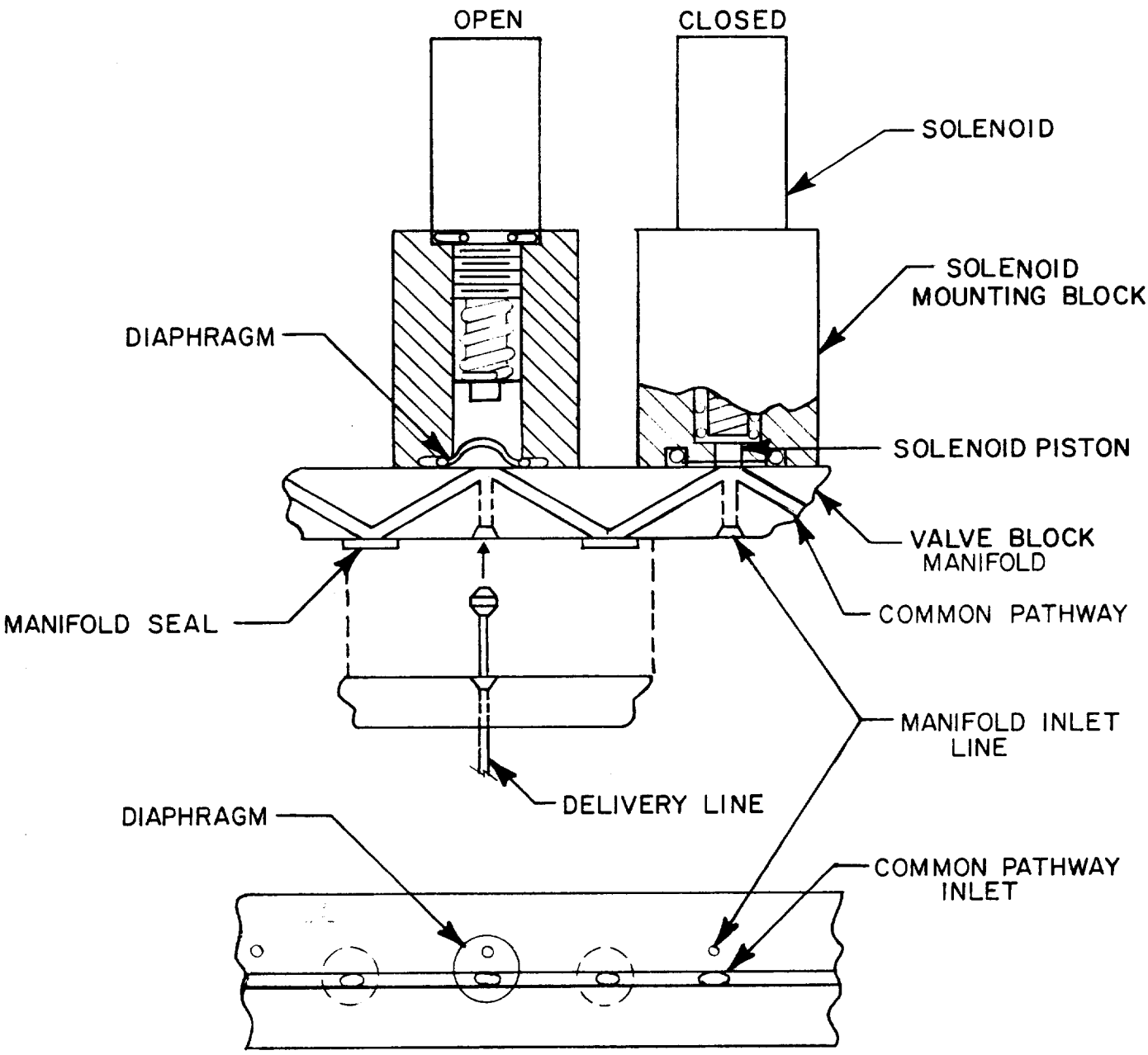
Figure 1-3

Valve block H controls the delivery of the phosphoramidites and the tetrazole to the column or waste and directs the flow from valve block K to the column or waste.

Valve block I directs the column effluent to either the waste, the trityl collection port or the DNA collection vial. It also controls the argon gas used to remove or flush the reagents from the column. There is one I block for each column.

The design of the valve blocks provides zero-dead volume. Delivery lines feed into each valve block and connect to the common pathway in the valve block manifold via a manifold inlet line and a solenoid-controlled diaphragm valve. See Figure 1-4.

The delivery lines enter the valve block through the manifold inlet lines. Passage between the manifold inlet line and the common pathway of the valve block is accomplished by an open solenoid valve. When a valve opens, the solenoid piston pulls away from a diaphragm located under the piston. With vacuum assist, an open solenoid will cause the diaphragm to form a 2-uL domed chamber. The domed chamber creates a passageway between the inlet line and the common pathway. The common pathway zig-zags through the valve block manifold and passes other closed valves which are unaffected by the flow. The direction of flow is determined by the pressures on either side of the valve block. During proper operation, the flow will be toward the column or one of the exit ports.



The Valve Block

Figure 1-4

VACUUM ASSIST

For proper valve block operation, it is crucial that each diaphragm forms a "domed" chamber. The vacuum assist creates a vacuum on the solenoid side of the diaphragm to form a domed chamber each time a solenoid valve opens. An aspirator pump provides vacuum assist to each valve block.

The aspirator pump requires that regulated argon enter the synthesizer at a gauge pressure of approximately 60 psi. The argon regulator should be connected to the synthesizer with the 1/4-inch tubing supplied with the instrument.

IMPORTANT: Do not use 1/8-inch tubing, it is too narrow to deliver the correct volume of gas.

The electrical vacuum pressure gauge should read between apprroximately 15 in. and 18 in. Hg. When the gauge drops below 15 (once every 8-12 hours), a valve opens to allow high pressure argon to flow to the aspirator pump. The gas is then diverted out of the pump and creates a Venturi vacuum when a second valve opens to the vacuum assist plumbing. If the gauge pressure drops below 15 in. Hg. more than once every 2 hours, there is a leak in the system. Although synthesis can continue with the leak, argon consumption will be greater than necessary. Call an Applied Biosystems service representative for repair instructions.

COLUMN FLOW

The normal flow into the column is from the bottom. By sending the liquid stream upward, the CPG particles are lifted and maintained in a fluidized state. The flow rates of the solvents and reagents have been set to achieve proper mixing of the particles.

THE DNA COLLECTION VIAL

Following DNA synthesis, the DNA is cleaved from the support with ammonium hydroxide and deposited into the DNA collection vial. This glass vial (4mL for 1-umol scale synthesis, and 10 mL for 10-umol scale) screws tightly into a permanent cap located behind the column. Inlet and vent lines are inserted through the cap to allow the ammonium hydroxide-crude DNA solution to flow into the vial. A gasket inside the cap provides an airtight seal with the vial.

WASTE AND VENTING

The ultimate destination of most chemical deliveries is the waste bottle. It is a one-liter glass container located on the left panel of the instrument. The bottle is held in place by a spring-loaded plug with an O-ring forming an inside diameter seal. By raising the horizontal black bar, the bottle may be removed for emptying.

WARNING: Synthesizer waste must be handled and disposed of properly and carefully. The Synthesizer generates 1 to 2 liters of hazardous, halogenated organic liquid waste per 100 cycles of operation. When handling the waste for disposal, wear gloves and eye protection, and avoid inhalation and skin contact. Place the liquid in a sealed container labeled "FLAMMABLE", "POISON B N.O.S." or absorb in vermiculite, dry sand or earth. Dispose of waste according to applicable government regulations.

Several lines enter the top of the spring-loaded plug of the waste bottle. They are the waste lines from the H and I blocks; and the vent lines from the phosphoramidite vent manifold, the DNA collection vial and bottles 8, 9, 10, and 18. The large line is the waste bottle vent which runs to a gas-tight fitting on the back panel of the instrument. Connect appropriate tubing to this fitting to vent vapors to a suitable exhaust such as a fume hood. Proper venting allows operation of the Model 380B on an open lab bench.

IMPORTANT: Prevent condensation from collecting in the vent line. This is done by continuously sloping the tube upward towards the fume hood. The tubing should not be horizontal or have troughs that will form collecting points. Condensation will eventually cause erratic deliveries and may even stop the flow.

IMPORTANT: The waste bottle is the low pressure side of the delivery system and must always be kept vented to atmosphere. If it is not properly vented, back pressure will be generated which will decrease the deliveries of reagents and solvents.

THE BATTERY

A rechargeable, nickel-cadmium battery is provided in the Model 380B. When the main power is turned off (either intentionally or during a power failure), the battery provides power so that all synthesis parameters are retained in memory for up to several months. These parameters include the DNA sequence, cycle modifications and bottle usage information. If a power failure occurs during an active synthesis, the synthesis will be interrupted and can only be resumed when the main power is restored.

The battery also maintains the synthesizer's internal clock. If a power outage does occur, the clock records the time the power failed and the time it is restored. A synthesis will automatically resume if the elapsed time of the power failure is less than the specified time set by the user. Refer to Section 4, The Power Fail Menu, for further instructions.

THE CONTROLLER

The controller directs and initiates all synthesizer activity. Its major components are the software, the microprocessor, the touchscreen and associated electronics.

The software defines all necessary operations for synthesis and is interpreted and executed by the microprocessor. The software is stored on a 3 1/2-inch hard cover microfloppy disk that is inserted into the disk drive.

The software is "menu-driven" where menus and pages of menus are shown on a touchscreen. These menus present various options and necessary information about the synthesis or status of the instrument. In response, the user selects options and gives instructions by touching the appropriate area on the screen. For a complete description of all menus, refer to Section 4.

To perform automated synthesis, the software uses a stored cycle consisting of a series of steps which complete all chemical reactions. These steps can be changed allowing the user to modify the existing cycle or create new ones.

Proper operation of the controller's electronic components is verified by a system self-test which should be performed routinely (approximately once a month) and whenever a malfunction occurs. See Section 4 for instructions.

IMPORTANT: A fan on the rear of the instrument distributes air through the electronics for cooling and should not be obstructed.

SECTION 2

CHEMISTRY FOR AUTOMATED DNA SYNTHESIS

Contents:

INTRODUCTION

DNA synthesis is quite simple in concept. A reactive 3' phosphorous group of one nucleoside is coupled to the 5' hydroxyl of another nucleoside. The former is a monomer, delivered in solution. The latter is immobilized on a solid support. An internucleotide linkage is thus formed. Three other chemical reactions are necessary to prepare the growing chain of DNA for the next coupling. In this way a synthesis cycle is conducted, adding one nucleoside monomer at a time. The desired sequence and length are defined by the operator on the synthesizer[1]. When the chain is complete, the crude DNA (*oligonucleotide*) must be *cleaved* from the support and *deprotected*. Further purification is usually advised. When the complete operation becomes routine, the synthesis of oligonucleotides becomes reliable and their biological activity is assured. Since most laboratories are not interested in this as a research project, the goals are for DNA synthesis to become cheaper, faster, better, easier, and more flexible. This chapter will help you understand the synthesis chemistry and how you can attain these goals.

The *phosphoramidite* method of oligonucleotide synthesis is the chemistry of choice for most laboratories because of efficient and rapid coupling and the stability of the starting materials[2]. The synthesis is performed with the growing DNA chain attached to a solid support so that excess reagents which are in the liquid phase can be removed by filtration[3]. Therefore, no purification steps are required between cycles. This support material is a form of silica, controlled-pore-glass (*CPG*) beads[4]. The particle size and the pore size have been

optimized for liquid transfer and mechanical strength. The synthesis cycle is depicted in Figure 2-1. The starting material is the solid support derivatized with the nucleoside which will become the 3'-hydroxyl end of the oligonucleotide. As shown in Figure 2-2, the nucleoside is bound to the solid support through a *linker* attached at the 3'-hydroxyl. The 5'-hydroxyl is blocked with a *dimethoxytrityl* (DMT) group.

The first step of the synthesis cycle is treatment of the derivatized solid support with acid to remove the DMT group (Figure 2-4). This frees the 5'-hydroxyl for the coupling reaction (Figure 2-5). An activated intermediate is created by simultaneously adding the phosphoramidite nucleoside monomer and tetrazole, a weak acid, to the reaction column. The tetrazole protonates the nitrogen of the phosphoramidite, making it susceptible to nucleophilic attack. This intermediate is so reactive that addition is complete within 30 seconds. As shown in Figure 2-5, the phosphoramidite is blocked at the 5'-OH with the dimethoxytrityl group.

The next step, capping, terminates any chains which did not undergo addition. Since the unreacted chains have a free 5'-OH, they can be terminated or capped by acetylation. These unreacted chains are also called "failure products". Capping is done with acetic anhydride and 1-methylimidazole[5]. Since the chains which reacted with the phosphoramidite in the previous step are still blocked with the dimethoxytrityl group, they are not affected by this step. Although capping is not required for DNA synthesis, it is highly recommended because it minimizes the length of the impurities and thus

facilitates product identification and purification (Figure 2-6).

The internucleotide linkage is then converted from the phosphite to the more stable phosphotriester. Iodine is used as the oxidizing agent and water as the oxygen donor. This reaction is complete in less than 30 seconds (Figure 2-7).

After oxidation, the dimethoxytrityl group is removed with a protic acid, either trichloroacetic or dichloroacetic acid. The cycle is repeated until chain elongation is complete. At this point, the oligonucleotide is still bound to the support with protecting groups on the phosphates and the exocyclic amines of the bases A, G, and C. The oligonucleotide is cleaved from the support by a one-hour treatment with concentrated ammonium hydroxide. Ammonia treatment also removes the cyanoethyl phosphate protecting groups. The crude DNA solution in ammonium hydroxide is then treated at 55°C for 8 to 15 hours to remove the protecting groups on the exocyclic amines of the bases (Figure 2-8).

Note that synthesis can be performed using methyl, or the newer cyanoethyl phosphoramidites[6]. These two versions of synthesis monomers differ only by the protecting group on the phosphorous oxygen. The synthesis chemistry and the resulting oligonucleotide are the same with both. Excellent results are obtained with either one[7]. The primary difference is that when using methyl phosphoramidites, thiophenol treatment is required to deprotect the internucleotide methyl phosphotriester groups at the end of synthesis. Thiophenol is foul smelling and toxic. In addition, this adds an extra 30-60 minutes

to deprotection time. For these reasons, use of cyanoethyl phosphoramidites is strongly recommended. Methyl phosphoramidites, since they offer no advantage or unique utility, are no longer available from Applied Biosystems.

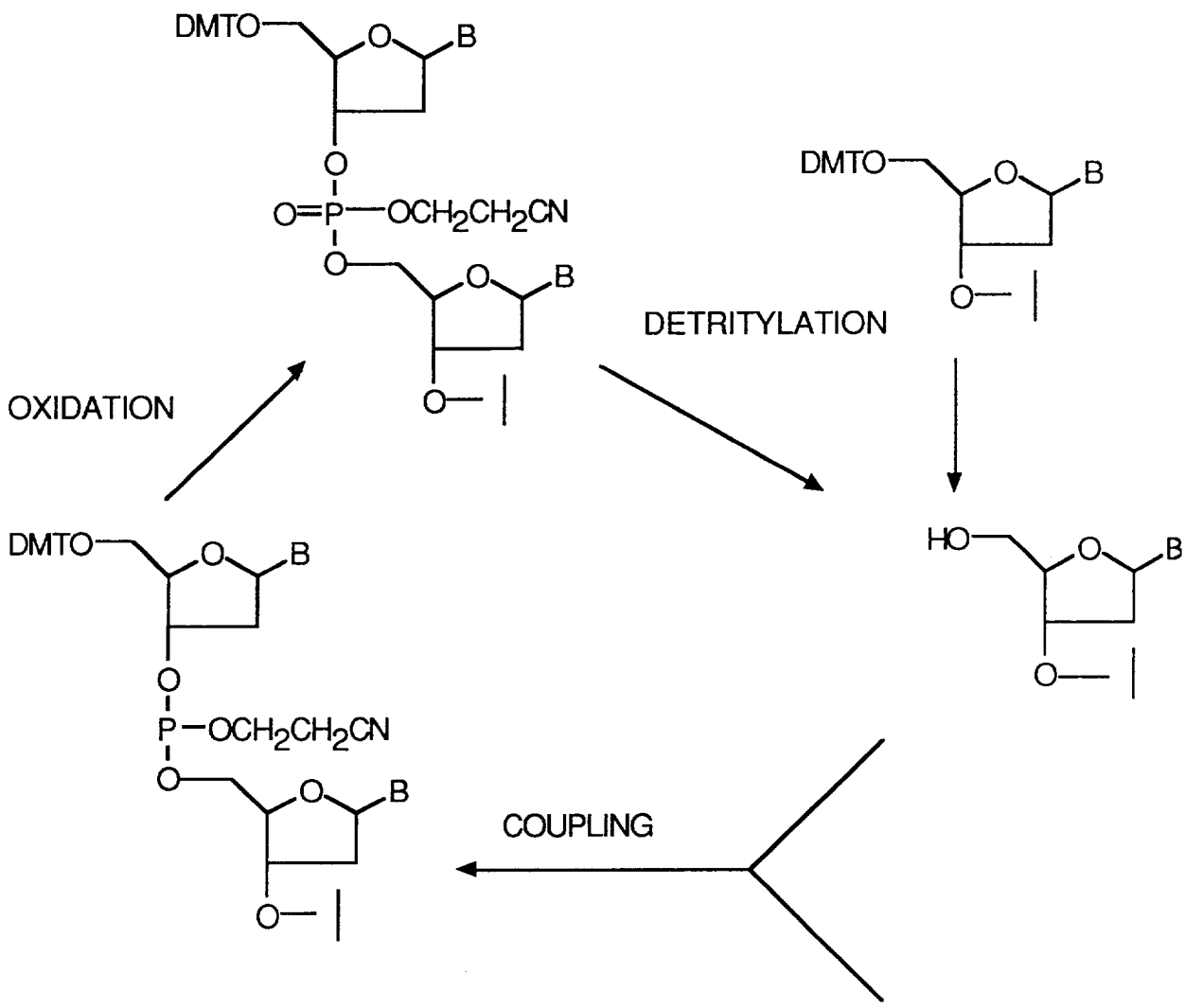


Figure 2-1
The Synthesis Cycle

THE SOLID SUPPORT - CPG

The Model 380B DNA Synthesizer uses a solid phase synthesis chemistry in which the growing DNA chain remains covalently attached to an insoluble support. All reagents and solvents flow through the support which is contained within a synthesis column.

The support used for DNA synthesis is Controlled-Pore-Glass (CPG)[4]. When used with the Model 380B and other Applied Biosystems reagents, CPG produces coupling efficiencies of 98 to 100%, as measured by the trityl cation assay. This enables the synthesis of oligomers up to 175 bases in length[8]. CPG is a porous, non-swelling particle which is about 150 microns in diameter and has 500Å pores. A wide-pore CPG support (1000Å) is also available and should be used when synthesizing oligonucleotides greater than 70 bases[8]. CPG is covalently derivatized with one of the four nucleosides (A, G, C or T), see Figure 2-2. The reactive groups on these nucleosides are blocked or protected to prevent unwanted side reactions. They are all blocked at the 5'-hydroxyl with a dimethoxytrityl group. As shown in Figure 2-3, the exocyclic amines on adenosine (A) and cytosine (C) are protected by a benzoyl group (A^{bz}, C^{bz}), and the exocyclic amine on guanosine (G) by an isobutyryl group (G^{ib}). Thymidine does not need a protecting group since the thymine ring has no exocyclic amines.

As shown in Figure 2-2, CPG has a linker attached to its surface via a siloxane bond. All free silanol groups are capped to prevent side reactions. The support is then derivatized by covalently attaching the 3'-hydroxyl of the nucleoside to the linker via a

succinate ester bond. The bond is base labile and allows for removal of the DNA from the support with ammonia. After synthesis is complete the oligonucleotide is quantitatively cleaved with a free 3'-hydroxyl.

The loading of nucleoside as measured by DMT release is 27 to 30 micromoles per gram of 500Å support. Prefilled columns contain 0.2 µmol, 1 µmol or 10 µmol of initial nucleoside. The 0.2 µmol scale provides sufficient amounts of purified oligomers for most applications. The 1 µmol scale is used when greater quantities of DNA are needed. The 10 µmol scale is useful for physical studies, such as X-ray crystallography or NMR. Wide-pore CPG supports are only available on the 0.2-micromole level. The loading of nucleoside is lower, about 15 µmol/gm of 1000Å support. For the synthesis of long oligonucleotides ("bigmers"), it has been shown that a lower loading of nucleoside and larger CPG pore size are critical requirements for success.

Typical quantities of oligonucleotide obtained from the different synthesis scales are given below:

synthesis cycle	crude yield*(O.D.) (20mer)
0.2μM	20-25
1 μM	100-120
10 μM	800-1000

* Yield figures based on a 20mer sequence. Absorbance measured at 260nm, assuming 33 micrograms/O.D. unit.

With automated synthesis, the DNA is built from 3' to 5'. Before beginning a synthesis, one of four support-bound nucleosides (A, C, G or T) contained within a column is placed on the instrument. This nucleoside is the 3'-terminus of the sequence.

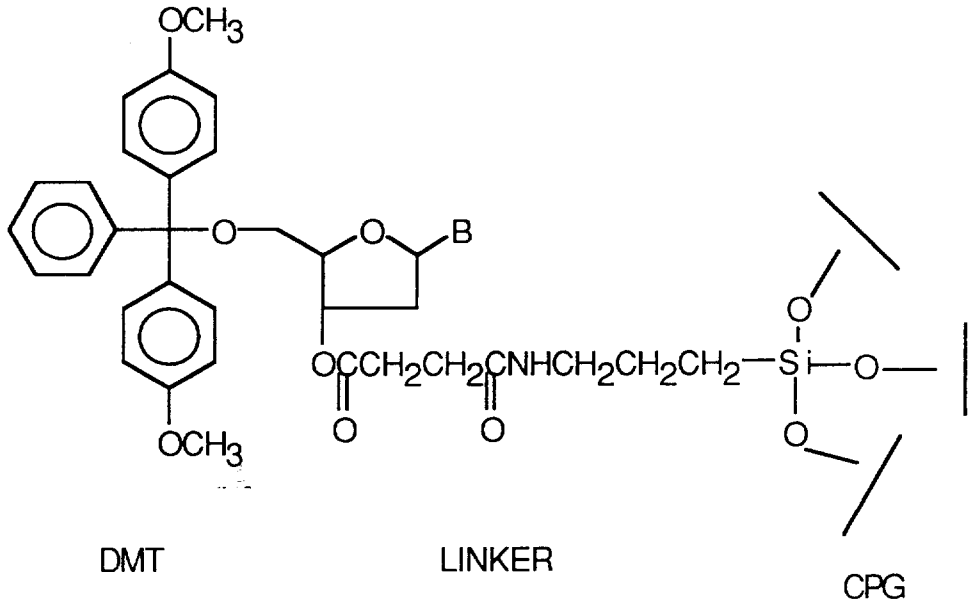
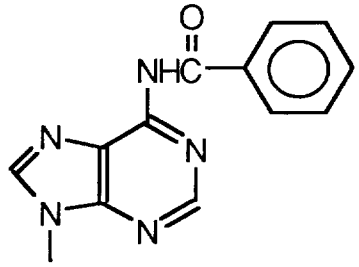
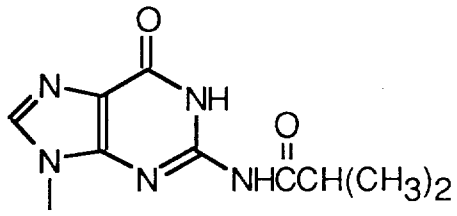


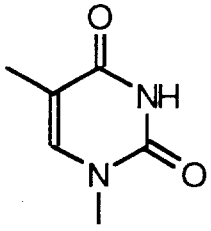
Figure 2-2
The DMT protected nucleoside is attached to the controlled pore glass (CPG) support
B = Base, A,G,C,T).



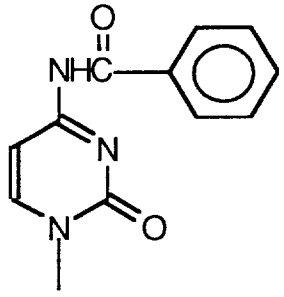
Adenosine-benzoyl protected



Guanosine-isobutyryl protected



Thymidine



Cytosine - benzoyl protected

Figure 2-3
Protected exocyclic base amines. Adenosine (A) and cytosine (C) are protected by a benzoyl group (Bz), and guanosine (G) by an isobutyryl group (Ib). Thymidine (T) does not need a protecting group.

DNA SYNTHESIS CHEMISTRY CYCLE

Each cycle of base addition consists of four steps:

1) Detritylation
2) Coupling
3) Capping
4) Oxidation.

These reaction steps are repeated in the above order until all bases are added. Following synthesis, the DNA chain is cleaved from the solid support and the cyanoethyl phosphate protecting groups are removed automatically. The Model 380B does not base deprotect the DNA. That must be completed manually. Each step of the synthesis cycle will be discussed in detail.

Detritylation

The first step in oligonucleotide synthesis is to remove the acid-labile, dimethoxytrityl (DMT) protecting group on the 5'-hydroxyl of the support-bound nucleoside. As shown in Figure 2-4, treatment with the protic acids, trichloroacetic acid (TCA) or dichloroacetic acid (DCA), will deprotect or detritylate the 5'end. This will yield a reactive 5' hydroxyl which can couple with a phosphoramidite during the following addition step. The two acids can be used interchangeably and are both available from Applied Biosystems. Dichloroacetic acid is a weaker acid and may yield lower levels of depurination in the synthesis of long oligonucleotides. However, this observation has not been consistently corroborated. For convenience, in this manual the detritylation acid will exclusively be referred to as TCA.

Detritylation in more detail:

Immediately before detritylation, the CPG support is washed with acetonitrile to eliminate traces of the preceding reagent. Next, TCA (bottle 14) is delivered to the column to cleave the trityl group. Detritylation under anhydrous conditions, is a reversible reaction. The DMT cation is highly reactive and can re-tritylate any reactive nucleophile. Detritylation is driven to completion by the removal of the DMT cation from the synthesis column. Therefore, detritylation is conducted by continuous delivery of TCA and elution of the DMT cation. Unlike the other reactions in the cycle, there is no wait period for detritylation. Any residual TCA must be removed by an acetonitrile wash to prevent detritylation of the incoming phosphoramidite. If the phosphoramidite monomer becomes detritylated, an unwanted dimer can form in solution and then couple to the support-bound nucleotide chain. Continued chain propagation would result in some sequences being longer than the product, making purification difficult.

Following both acetonitrile washes, the remaining solvent is forced out of the column by an *argon reverse flush* - argon passes through the column from top to bottom and pushes the liquid to waste. For a summary of these steps, see Table 2-1.

TABLE 2-1

DETRITYLATION STEPS

STEP DESCRIPTION	PURPOSE
acetonitrile (bottle 18) delivery to column	wash column and support, remove traces of preceding reagent
argon reverse flush	remove residual acetonitrile from column
TCA (bottle 14) delivery to column	detritylate support-bound nucleoside
acetonitrile (bottle 18) delivery to column	wash column, remove traces of TCA
argon reverse flush	remove residual acetonitrile from column

NOTE: The complete synthesis cycle contains steps to wash and flush the valve blocks and delivery lines.

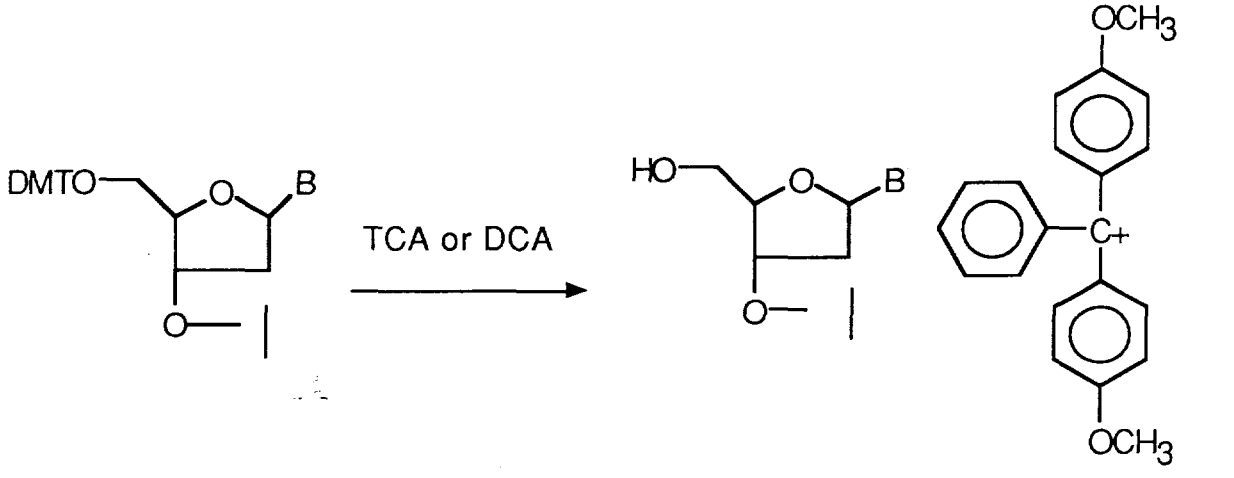


Figure 2-4

Detritylation. TCA or DCA is used to remove the DMT from the 5'end. This leaves a 5' hydroxyl to react with the incoming phosphoramidite in the coupling step. Quantitating the released trityl cation indicates the step-wise yield and can be used to monitor the instrument's performance.

Monitoring the Trityl Cation:

When the dimethoxytrityl, also referred to as DMT or trityl, protecting group is cleaved from the nucleotide, it exists as a cation (Figure 2-4). When in acid solution, this cation is relatively stable and produces a brilliant orange color. It has an absorbance maximum at about 498 nm and extinction coefficient of about 70,000 in most solvents, such as acetonitrile or dichloromethane. It is easily detected and quantitated spectrophotometrically.

To quantitate the trityl cation released at each detritylation step, the column effluent from the TCA delivery and the subsequent wash with acetonitrile is collected. Instead of the effluent flowing into the waste bottle, it is channelled through the *trityl collection port* into tubes in a fraction collector. The Model 380B cycles provide for the collection of trityl effluent fractions and include a signal to advance a fraction collector (user provided).

Next, the absorbance of the fractions is measured to quantify the trityl released in each addition cycle. Since the trityl solution is very concentrated, it must be diluted before quantitating or significant errors in the readings will occur. Typically, the trityl fraction is brought up to a volume of 10 mL with 0.1 M p-toluenesulfonic acid in acetonitrile. The trityl yield is then used to calculate the coupling efficiency of each addition step by dividing the next step absorbance value from the previous step value. From this data, an overall stepwise yield can be determined and the expected product yield can be estimated. The molar amount of DMT cation can be calculated using Beer's Law:

$A = eCl$ where: A - absorbance
e - extinction coefficient
C - concentration
l - path length

$$\text{overall yield} = (\text{coupling yield})^{\text{number of couplings}}$$

For more details, see the trityl assay procedure discussed in User Bulletin 13 (Revised) in the appendix.

Monitoring the trityl cation is very important, but the results must be interpreted with caution. The trityl assay is only an indirect measure of synthesis efficiency. Certainly, high trityl cation yields (>98%) must be present for good synthesis. However, high trityl yields can be present when a poor synthesis occurs. The reason for this discrepancy is that although this chemistry is highly refined, it is not perfect. Certain reactions contribute to the trityl cation released each cycle. In particular, a low level of extraneous chain growth other than the desired oligonucleotide can occur.

One other cause of imperfect results might be incomplete detritylation or capping. This could happen if the synthesizer is not operating correctly, the cycle is not optimized, or the reagents are wet or impure. These occasions generate species other than the desired product which release the trityl cation. Low trityl cation yields (<98%) always predict a less than optimal synthesis. In practical terms, trityl yields of less than 95% will not allow identification or purification of even short oligonucleotides. For the above reasons the trityl assay must be viewed only as a preliminary, yet convenient, monitor of the

synthesizer's performance. The assay is useful to aid in the early detection and diagnosis of instrument related problems. Many laboratories monitor their trityl fractions only by visual inspection. With experience, a failed synthesis is detected this way. Evaluation of the oligonucleotide by PAGE or HPLC is much more informative than the trityl assay. Synthesizer or reagent problems can be adequately diagnosed only by these methods, which are direct analyses of the product.

Depurination:

Trichloroacetic acid is a very effective protic acid detritylating agent. However, in the presence of protic acids, amine-protected purines (A^{bz} and G^{ib}) are susceptible to depurination (removal of the purine from its sugar). The chemical mechanism is initial protonation at N-7 of the purine ring, causing increased lability of the ribose 1' - purine N-9 bond. Cleavage of adenine and guanine bases yields a 1' hemiacetal ribose ring, the result of depurination. Oligonucleotides which contain apurinic sites are cleaved during the ammonium hydroxide treatment. Cleavage occurs at the internucleotide bonds on the 3' hydroxyl side of the apurinic deoxyribose (This is similar in effect to the chemistry of Maxam-Gilbert sequencing). Each purine in the oligonucleotide chain is exposed to acid at each detritylation step. Purines near the 3' end will have the longest cumulative exposure time and a greater chance for depurination. It has been reported that the 5' terminal purine is more susceptible to depurination than an internucleotide purine with a 3' and a 5' phosphorous[9].

The quantity of DNA fragments generated by apurinic ammoniolysis is usually insignificant. If the synthesis is conducted Trityl On, for purification by OPC or Trityl On HPLC, the 5' end fragment of apurinic ammoniolysis will bear a DMT group and may complicate purification. In practice however, using the ABI reagents and cycles optimized for DNA synthesis, depurination is not detectable or significant except for long oligonucleotides (>80 bases) which are purine rich near the 3' end[10]. To minimize depurination, each treatment with TCA should not be extended beyond the times specified in the cycles.

IMPORTANT: Do not stop a synthesis while the DNA is exposed to TCA. Do not increase TCA delivery times.

COUPLING

Phosphoramidites:

Phosphoramidites (shown in Figure 2-5a) are chemically modified nucleosides which are used as the building blocks for synthesizing DNA. They are added to the support-bound nucleotide chain one at a time until all bases in the sequence are coupled. The cyanoethyl phosphoramidite nucleosides have the following functional groups:

1. A diisopropylamino on a 3' trivalent phosphorous moiety[11]. The phosphoramidite is very stable and is made highly reactive by the activator, tetrazole.

2. A ß-cyanoethyl protecting group on the 3' phosphorous moiety. This group prevents side reactions and aids in solubility of phosphoramidites. It is removed upon completion of the synthesis by using ammonia. In deprotection, ammonia acts as a base to remove a proton on the methylene group bearing the nitrile group. This anion is formed only in low concentration, but rapidly fragments by a ß-elimination reaction to form acrylonitrile and the deprotected internucleotide phosphodiester group. Acrylonitrile then reacts irreversibly with ammonia to form 3-aminopropionitrile, an inert compound.

3. A dimethoxytrityl (DMT) protecting group on the 5' hydroxyl. The DMT is removed during each detritylation step leaving a reactive 5' hydroxyl available for coupling an incoming phosphoramidite.

4. A benzoyl protecting group on the exocyclic amines of A and C (A^{bz}, C^{bz}), and an isobutyryl protecting group on the exocyclic amine of G (G^{ib}). These amide groups prevent side reactions and are removed upon completion of the synthesis with ammonia. Since thymidine is unreactive and does not contain an exocyclic amine moiety, it is not protected.

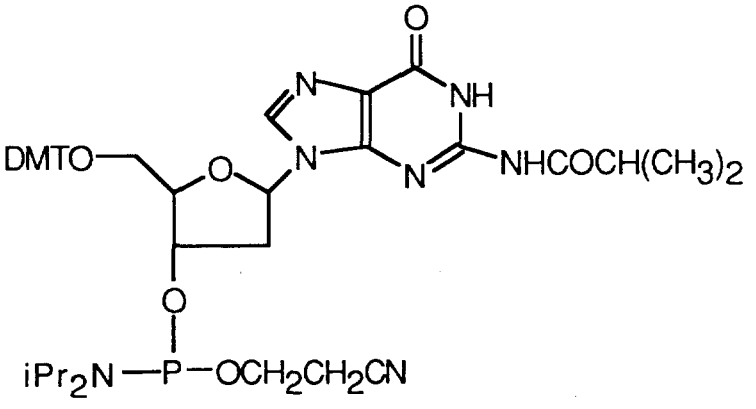


Figure 2-5a
Structure of guanosine cyanoethyl phosphoramidite

Coupling:

Before beginning the coupling step, the support is made anhydrous and free of nucleophiles (e.g. water) by an extensive wash with acetonitrile. Any extraneous nucleophiles will compete with the support-bound 5' hydroxyls for the activated phosphoramidite and will decrease coupling efficiency. The column is then dried by an argon reverse flush to remove residual acetonitrile. Tetrazole, the phosphoramidite activator, is next delivered to the column. According to the oligonucleotide sequence, one or more of the phosphoramidites (bottles 1 to 7) and tetrazole

(bottle 9) are then simultaneously delivered to the column. Depending on the synthesis cycle, alternate deliveries of tetrazole and then base-plus-tetrazole are repeated up to 3 times. When these reagents mix, the mild acid, tetrazole, (pKa = 4.8, Ref.8) transfers a proton to the nitrogen of the diisopropyl group on the 3' phosphorous (See Figure 2-5b). This protonated amine makes a very good leaving group upon nucleophilic attack by the tetrazole to form a tetrazolyl phosphoramidite[12] . This is the reactive intermediate which forms the internucleotide phosphite with the support bound 5' hydroxyl. A molar excess of tetrazole ensures that the phosphoramidite will be activated. The excess of phosphoramidite relative to free 5' hydroxyl ensures that the reaction is nearly quantitative (over 98% coupling). The coupling steps are summarized in Table 2-2.

Mixed sequence probes are synthesized by simultaneous delivery of up to 4 bases and tetrazole with near equivalent coupling. The four nucleoside phosphoramidites have slightly different reactivities, as all different molecules must[13]. The cyanoethyl phosphoramidites follow the reactivity order of T > G > C > A. When all four are delivered simultaneously, their representation will be (normalized to 100%):

T - 30%
G - 26%
C - 24%
A - 20%

These values are slightly dependent on cycle, location of the site in the oligonucleotide, age of the phosphoramidite solutions, etc. They have a range of about 3% because of these variables.

TABLE 2-2

COUPLING STEPS

STEP DESCRIPTION	PURPOSE
acetonitrile (bottle 18) delivery to column	remove nucleophiles render support
argon reverse flush	remove residual
tetrazole (bottle 9) delivery to column	deliver activator
tetrazole + phosphor-amidite delivery to column	activate phosphor-amidite, begin coupling reaction
tetrazole delivery to column	continue coupling
tetrazole + phosphor-amidite delivery to column	continue coupling
wait	complete coupling
argon reverse flush	remove the tetrazole

IMPORTANT: These deliveries are critical. Under-delivery causes low coupling efficiency. Over-delivery wastes reagents.

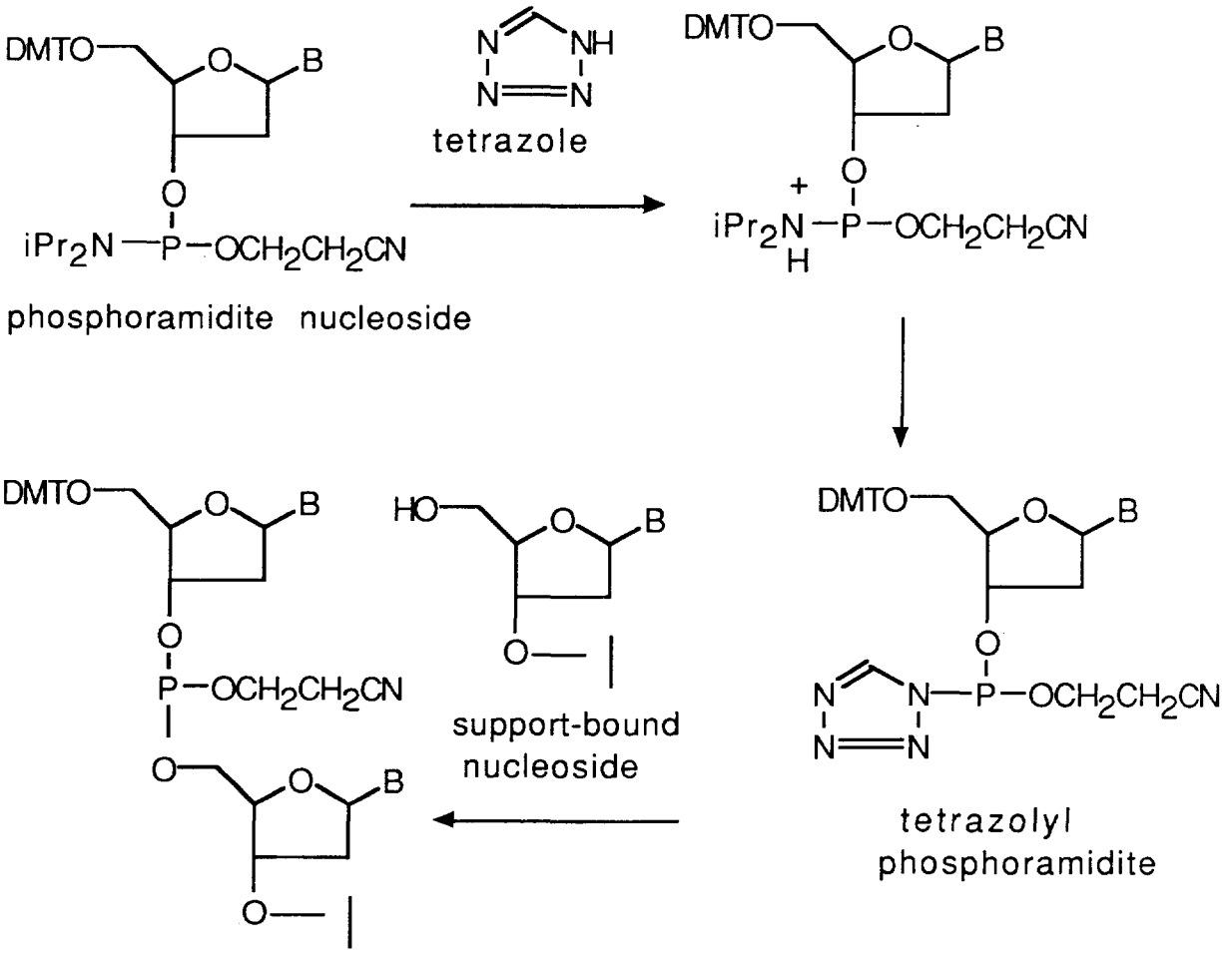


Figure 2-5b
The coupling step. Phosphoramidites and tetrazole are delivered to the column which contains the support-bound nucleotide. The diisopropylamine is protonated and displaced by tetrazole. When the 5' OH couples to the phosphorous, a 5' to 3' internucleotide linkage is created.

CAPPING

Because coupling is not always quantitative, a small percentage (up to 2%) of support-bound nucleotides can fail to undergo addition. These truncated, or *failure sequences*, will remain attached to the support. If they remain in the hydroxyl form, they can propagate in subsequent coupling steps. Failure sequences with one less base than the product would then be generated making isolation of the product more difficult. Capping the remaining free hydroxyls by acetylation eliminates this problem. The capped failure sequences are then prevented from participating in the rest of the synthesis reactions.

To cap, equal volumes and equimolar amounts of two binary reagents, acetic anhydride (bottle 11) and 1-methylimidazole[5], NMI, (bottle 12), are simultaneously delivered to the column. As shown in Figure 2-6, the reagents mix and create a powerful acetylating agent. The two reagents need to be segregated since the active acetylating agent is unstable. This agent reacts at the 5' hydroxyls rendering these moieties unreactive for the remainder of the synthesis. The excess reagents are then removed by an argon reverse flush. The capping time required to acetylate the 1 or 2% unreacted 5' hydroxyls is very brief, only a few seconds. It is important to minimize this time to prevent loss of cyanoethyl groups from the internucleotide linkages and to prevent base modification by-products. The efficiency of shorter capping time and the capping/oxidation order have been extensively demonstrated in studies at Applied Biosystems[14].

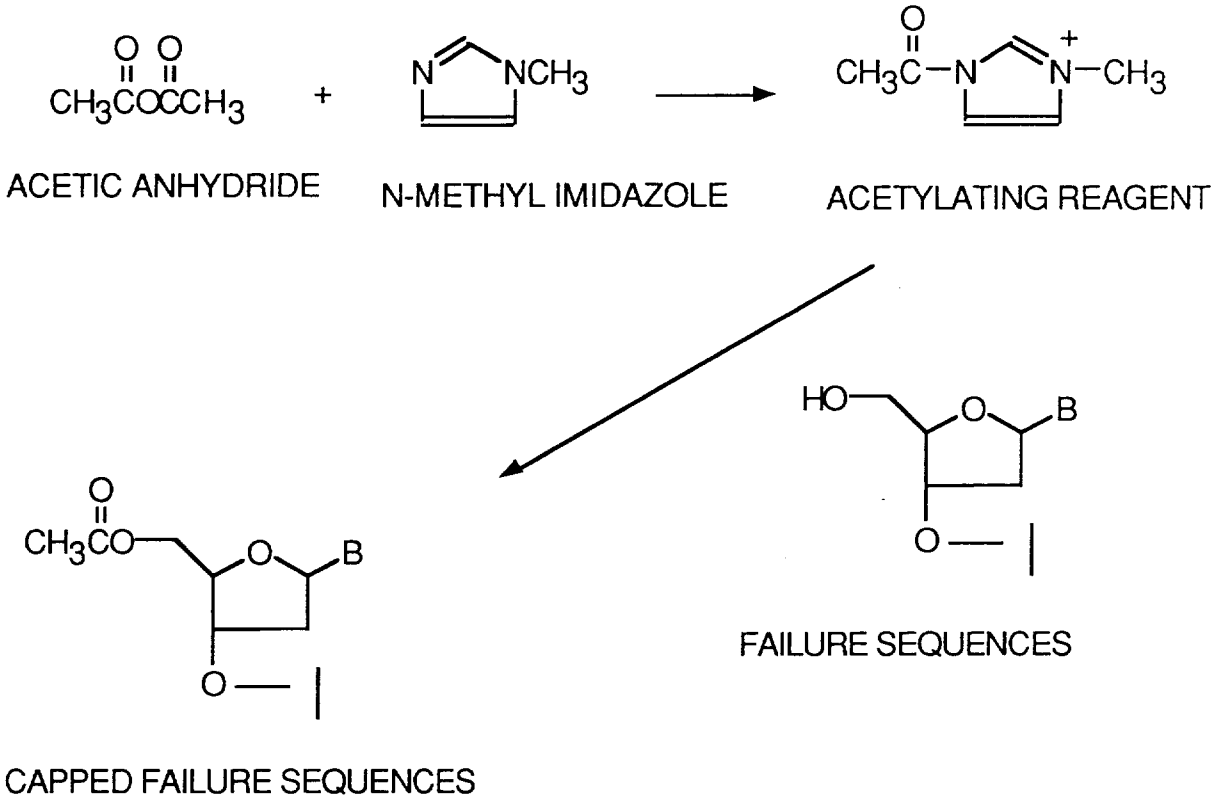


Figure 2-6
Capping of unreacted chains. The capping reagents, acetic anhydride and 1-methylimidazole (NMI), are used to terminate unreacted chains by acetylating the 5' hydroxyl groups.

OXIDATION

The newly formed internucleotide linkage is a phosphite (trivalent phosphorous) triester. The phosphite linkage is unstable and is susceptible to acid and base cleavage. Therefore, immediately after capping, the trivalent phosphite triester is oxidized to a stable pentavalent phosphate triester. This is shown in Figure 2-7.

Oxidation follows capping to eliminate the possibility of traces of water from the oxidizing solution causing acetic anhydride to form acetic acid during capping. This would expose the oligonucleotides to acid as well as make capping less effective. Also, scientists at Applied Biosystems have elucidated the complex chemical pathway whereby a small side-reaction, the phosphitylation of the O-6 position of guanosine can be minimized when capping immediately follows coupling[5,15]. The enzymatic digestion/base composition assay on oligonucleotides made with different cycle orders, capping then oxidation and oxidation then capping, shows markedly different results. The Applied Biosystems standard, capping then oxidation, virtually eliminates base-modified nucleosides.

Iodine is used as a mild oxidant in a basic tetrahydrofuran (THF) solution with water as the oxygen donor. When the iodine-water-pyridine-THF mixture (bottle 15) is delivered to the column, an iodine-pyridine complex forms an adduct with the trivalent phosphorous. This adduct is decomposed by water with production of a pentavalent phosphotriester internucleotide group. This is an extremely fast reaction, being quantitative in 30 seconds. The

iodine solution is removed by an argon reverse flush and several acetonitrile washes, each followed by an argon reverse flush.

Other oxidizing agents such as sulfur[16] can be used in place of oxygen to create nucleotide phosphate analogs or to introduce radioactive atoms.

IMPORTANT: Do not stop the synthesis while the phosphorous is unoxidized.

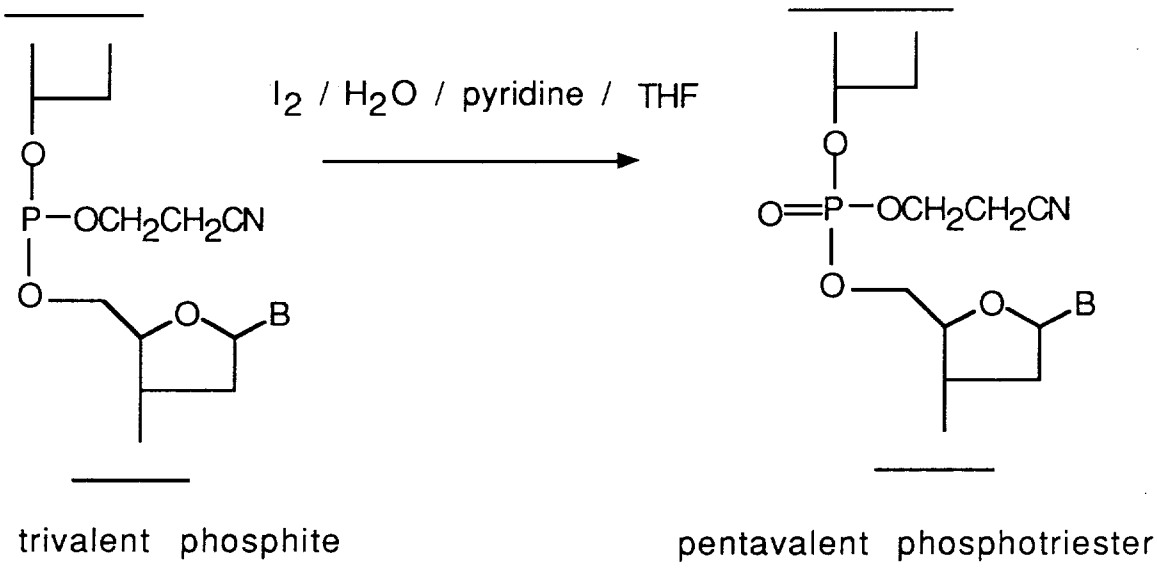


Figure 2-7
Oxidation of the trivalent phosphorous. The unstable trivalent phosphorous of the newly formed internucleotide linkage is oxidized to a stable pentavalent phosphorous using an iodine solution.

COMPLETION OF THE SYNTHESIS CYCLE

Following oxidation, a cycle of nucleotide addition is complete. The 5' terminus of the oligomer is protected by the dimethoxytrityl group. DNA synthesis continues by removing the 5' trityl and repeating another cycle of base addition. This is done until DNA of specified length has been fully synthesized.

Immediately after completing the synthesis, a trityl group may still be attached to the 5' terminus, according to the user's option. The ending method instructions may be programmed for the trityl group to remain attached (trityl on) or be cleaved (trityl off).

The oligonucleotides are usually detritylated when purifying by gel electrophoresis or ion exchange HPLC. The trityl groups are usually left intact when purifying by OPC or trityl-specific reverse phase HPLC. The ending method (trityl on or trityl off) is generally chosen before beginning a synthesis.

DEPROTECTION AND CLEAVAGE

When the synthesis is finished, the product and capped failure sequences (still attached to the support) exist as phosphate-protected, base-protected phosphotriesters. Complete deprotection is necessary to produce biologically active DNA. In addition, the oligonucleotides must be cleaved from the support. Cleavage and phosphate deprotection are performed automatically by the 380B. Base deprotection must be completed manually.

Cleavage:

Following synthesis, the DNA remains covalently attached to the support. The diester oligonucleotides are cleaved from the support by four consecutive 15-minute treatments with fresh, concentrated ammonium hydroxide. Alternatively, cleavage can be accomplished manually using the double syringe method[17]. As seen in Figure 2-8, the cleavage occurs at the base-labile ester linkage between the linker of the support and the 3' hydroxyl of the initial nucleoside. The cleaved DNA has a free 3' hydroxyl.

The column effluent from each treatment is forced into the DNA collection vial by an argon flush. The vial contains the crude mix (product and failure sequences) of base-protected oligonucleotides in ammonium hydroxide. Following cleavage, the column and the internal passages of the instrument are rinsed with acetonitrile and dried with argon. The used column and the DNA collection vial are removed. This leaves the synthesizer ready for another synthesis. The DNA is then base deprotected, desalted and purified for use.

Phosphate Deprotection:

The cyanoethyl protecting groups are also removed by treatment with ammonium hydroxide. This occurs at the same time as cleavage making phosphate deprotection very quick and simple (see Figure 2-8).

Base Deprotection:

The benzoyl and isobutyryl base protecting groups are removed by placing the vial of DNA at 55°C for 8 to 15 hours. This also cleaves the acetyl caps from the failure sequences. Base deprotection is an ammoniolysis reaction, where ammonia is a nucleophile, attacking the carbonyl of the amide protecting groups. For effective treatment, use fresh, concentrated ammonium hydroxide during cleavage. To ensure no decrease in ammonia concentration, store the reagent in a refrigerator, tightly capped. Discard thirty days after opening.

IMPORTANT: Use a tightly sealed DNA collection vial that can withstand positive pressure. The vial must also have a Teflon-lined cap. Rubber-lined caps have contaminants that leach out of the cap liner during deprotection. Teflon-lined caps can be ordered from Wheaton; Part Number 240408, size 13-425.

After completing deprotection, cool the ammonium hydroxide-DNA solution on ice to prevent losses from bubbling. Then remove the ammonia by vacuum. Ammonia is much easier to transfer at lower temperature than at room temperature. A note of caution: When drying down trityl-on syntheses, it is important to keep the oligonucleotide solution basic. Vacuum removal of the ammonia can lead to slightly acidic solution

conditions which may promote trityl removal. To maintain basic conditions during ammonia removal, add one drop of distilled triethylamine every 10 minutes. Also, avoid heating the sample.

If the 5' DMT group has been left on, it can be removed manually by treatment with 80% acetic acid/water for 20 minutes at room temperature. The acid is then diluted with ethanol and removed by vacuum, followed by several rinses with ethanol. This procedure is usually done after reverse phase HPLC or before radioactively labeling the 5' end prior to analysis by gel electrophoresis.

The deprotected, detritylated DNA has a free 5' and 3' hydroxyl and is biologically active. Desalting and purification may be necessary before use in experiments. An overview of these procedures is described later in this section. Details can be found in User Bulletin 13 (Revised).

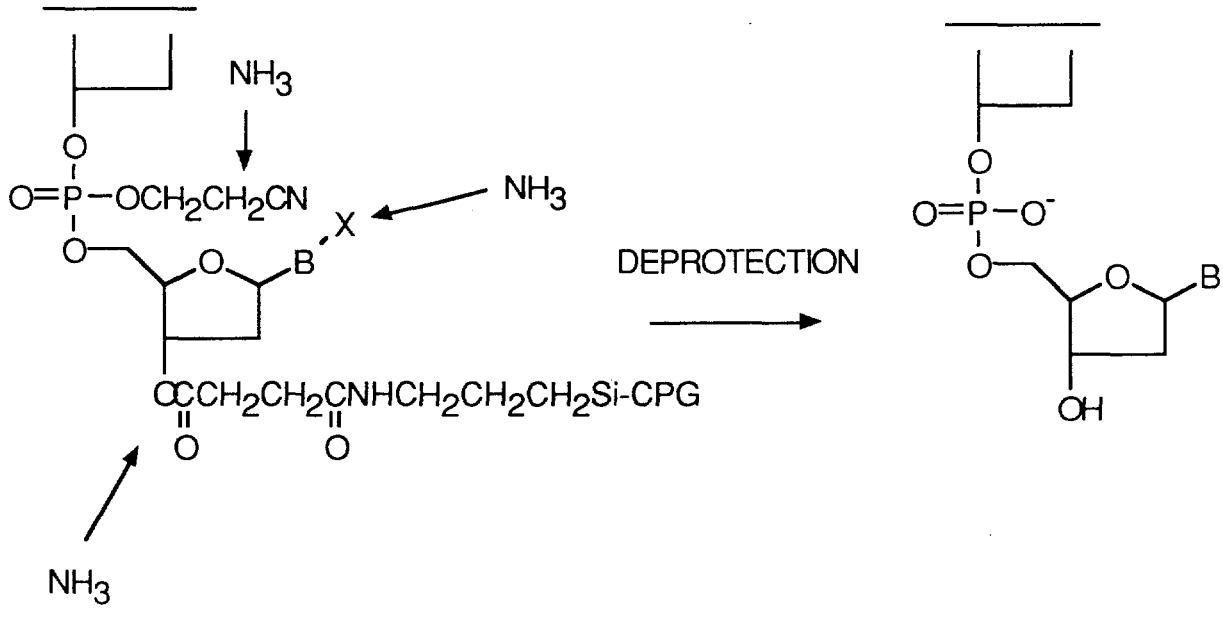


Figure 2-8
Deprotection and cleavage of ß-cyanoethyl protected oligonucleotides. Treatment with concentrated ammonium hydroxide removes the ß-cyanoethyl protecting groups and cleaves the oligonucleotides from the support. The benzoyl and isobutyryl base protecting groups (X) are removed by heating at 55°C in ammonia for 8 to 15 hours.

QUANTITATION OF THE OLIGONUCLEOTIDE

Nucleic acids of any variety are most easily quantitated by UV spectroscopy, measuring at or near their UV absorbance maxima, about 260 nm. A dilute aqueous solution of 1 ml or less, depending on the cuvette size, is measured by either scanning a region of about 200-350 nm or a single wavelength measurement. A scan of an oligonucleotide will show broad absorbance with a maxima near 260 nm. Using Beer's law, the concentration of the solution and absolute quantity can be calculated. As a useful approximation, 1 optical density unit (odu) of single-stranded oligonucleotide consists of about 33 micrograms, by mass. An approximation to relate absorbance to molar quantities is that a micromole of oligonucleotide has a number of odu equal to 10 times the number of bases. For example, a micromole of a 20mer would be 200 odu.

STORAGE OF THE OLIGONUCLEOTIDE

Most applications for synthetic oligonucleotides require less DNA than a synthesis provides. Fortunately, oligonucleotides can be stored easily, with little or no degradation for long periods of time. It is probably most convenient to store them refrigerated as a solution, in either a crude or purified state. The media may be concentrated ammonia, used to cleave and deprotect the crude oligonucleotide, water, or dilute buffer or salt. Typical aqueous media may contain ethanol, acetonitrile, triethylammonium acetate (TEAA), EDTA, etc. It is important to keep the oligonucleotides cold to minimize degradation and bacterial growth. Alternatively, oligonucleotides may be stored dried as a pellet in a clean, dry vessel, such as a microcentrifuge tube. The solution used to elute purified oligonucleotides from OPC, 20% acetonitrile, is a convenient and stable storage media. When stored by these means, oligonucleotides are stable for over a year. Avoid solutions that are mutagenic, oxidizing or outside the pH range of 3-12.

ANALYSIS AND PURIFICATION

This section reviews the most common methods for purification of synthetic oligonucleotides. HPLC (high performance liquid chromatography) and PAGE (polyacrylamide gel electrophoresis) can provide a high level of purity, but require initial capital investments and are labor intensive and time consuming. A short oligonucleotide (<30 bases) made with typical synthesis efficiency (>98% average DMT yield/cycle) may require a less stringent purification, with efficient desalting and removal of non-nucleoside synthesis byproducts. These methods are elaborated in detail in User Bulletin No. 13 Revised.

OPC

The Oligonucleotide Purification Cartridge (OPC, ABI part # 400771) was designed specifically for rapid, easy purification of synthetic oligonucleotides[18,19]. The method is based on a small, syringe-mounted cartridge containing an adsorbent material with an affinity for DMT oligonucleotides. The ammonia solution of the crude DMT oligonucleotide (10-20 crude odu) is applied directly to the cartridge. The DMT oligonucleotide product is retained. Byproducts, failure sequences not bearing a DMT group, and other impurities are not retained and are eluted. The DMT group of the OPC-bound oligonucleotide is removed with a mild acid solution, then the purified oligonucleotide is eluted (typically 1-5 odu) with about 1 ml of a 20% acetonitrile solution. The entire operation requires

15-20 minutes, and several OPC purifications can be conducted in parallel. Efficient, reliable purifications are achieved with oligonucleotides up to about 70 bases.

Several features of the OPC purification are noteworthy. No sample preparation is required. The OPC material is stable to concentrated ammonia. The ammonia solution provides a *denaturing* media, eliminating the purification complications of secondary structure and hydrogen-bonding. The DMT group is detached and retained in the cartridge. Thus, the purified, fully deprotected oligonucleotide is eluted in a small volume of 20% acetonitrile in water, completely desalted and ready for use.

PAGE

Polyacrylamide gel electrophoresis (PAGE) is a widely used method for the analysis and purification of oligonucleotides[21]. When a mixture of charged molecules are exposed to an electric field, they will migrate with velocities determined primarily by their mass to charge ratios. This ratio changes linearly with the log of the molecular weight of DNA of differing chain lengths. This allows a very ordered progression of oligonucleotides with mobility decreasing as the length increases. Proper PAGE technique can provide resolution, and preparative isolation, of single base length differences.

Oligonucleotide samples for PAGE are typically prepared by drying a known odu amount (by measuring absorbance) and redissolving in media such as formamide/1X TBE : 9/1 or 7M urea, which are denser

than the 1X TBE running buffer. These media and the gel matrix (containing 7M urea) should be denaturing to ensure disruption of secondary structure and hydrogen-bonding.

The most desirable format for gel electrophoresis of oligonucleotides is the slab gel. There are many commercial devices which essentially consist of a sandwich of two glass plates held apart by two side spacers. The thickness of the spacers determine the gel thickness. For UV shadowing, .75 - 1.5 mm is convenient. The acrylamide solution is poured in between and allowed to polymerize. A comb is also inserted, which forms wells where the olignuclotide is loaded. Each well forms a vertical lane during the electrophoresis. The plate length should be about 16-40 cm, depending on oligonucleotide length. The width depends only on the number of samples to be run. Dyes, such as bromophenol blue and xylene cyanol are run either in the samples or by themselves as indicators of the migration distance of the oligonucleotides. The acrylamide concentration of the gel matrix determines the velocity of the oligonucleotides. A range of 8-20% is typical. Longer oligonucleotides require lower acrylamide concentration.

When the dyes indicate the appropriate migration distance of the oligonucleotide product, the power is turned off and the gel sandwich is disassembled. There are several methods of analysis: UV shadowing, staining, and radiolabelling/autoradiography.

UV shadowing:

This is the simplest, easiest, and introduces the fewest artifacts into the analysis. Depending on oligonucleotide length, from 0.5 to 10 odu are loaded in a well. After electrophoresis, the gel is transferred from the plates to a clear plastic wrapped, fluorescent TLC plate, most conveniently plastic-backed, 20x20cm. The gel is visualized with a UV lamp at short wavelength (254 nm). The bands appear dark against a fluorescent green background. A permanent record can be made by photography through a green filter.

Staining:

Dyes such as methylene blue, ethidium bromide, Stains-All, and others, will visualize oligonucleotides in a polyacrylamide matrix. After electrophoresis, the gel is transferred to a pan containing the staining agent and let soak for some time period. This technique is more sensitive than UV shadowing, but is more time-consuming and uncertain than UV shadowing.

Radiolabelling:

This is the most sensitive, but most laborious method of analysis of oligonucleotides. Typically about 0.01 odu is phosphorylated enzymatically with T4 polynucleotide kinase and gamma-^{32}P ATP to give 5' ^{32}P phosphorylated oligonucleotides. Alternatively, ^{35}S ATP can be used, and the oligonucleotides can be labelled at either the 5' or 3' terminus. The radiolabelled samples are electrophoresed on the gel. The gel is wrapped in plastic and exposed to X-ray film in a dark room. An autoradiogram is generated in a time period ranging from minutes to days, depending on the specific activity of the ATP and other

radiolabelling parameters. The film has a finite capacity for development and exposure time must be carefully monitored, usually by taking several exposures. The gel pattern on the film may be quantitated by densitometry. Product identification is obvious when the appropriate tracking dye or oligonucleotide standard is also present on the gel.

By any of these techniques, the gel pattern of electrophoresis of an oligonucleotide can be very diagnostic about the course of synthesis. Many synthesizer and reagent problems can be diagnosed by the appearance and relative amounts of the "failure bands". Also, PAGE has a distinct advantage in that many samples can be analyzed and purified concurrently. The equipment is relatively inexpensive and easy to maintain. For bigmers, PAGE is usually the most efficient purification method and the only analytical method. The primary disadvantages of PAGE are that it is labor intensive and dependent on good technique.

Oligonucleotides can be purified by locating the product band by UV shadowing and excising the gel material therein with a clean razor blade. The gel material which is removed should be free of failure bands, most typically the lower N-1 band. In a preparative electrophoresis run, the product is run further, on a thicker gel, than in an analytical run, to maximize the separation of the product band from the N-1 band. The excised gel fragment(s) is soaked in an elution buffer. The gel debris is then removed by a desalting method, such as with an OPC cartridge.

HPLC

High Performance Liquid Chromatography (HPLC) is another efficient method which combines quantitative analysis and purification of oligonucleotides. One of the advantages of HPLC is a high level of automation. Systems are available which allow for repetitive programmed injection, analysis, and data manipulation and storage. Two different types of column adsorbents are popular for oligonucleotides; reverse-phase and ion-exchange.

Reverse-phase adsorbents discriminate by the hydrophobic differences between oligonucleotides of varying lengths and sequences. When the 5' DMT is on the oligonucleotide, this group is dominant in its interaction with the support adsorbent. Reverse-phase columns also can adequately resolve DMT off oligonucleotides with sufficient capacity. The mobile phase is typically a volatile buffer such as 0.1M triethylammonium acetate. The oligonucleotides are eluted with a gradient of increasing organic solvent, such as acetonitrile. These conditions are non-denaturing. Occasionally, certain sequences can exhibit unpredictable HPLC elution patterns, caused by inter- or intramolecular secondary structure and hydrogen-bonding effects.

Ion-exchange adsorbents elute oligonucleotides based on increasing charge, i.e. chain length. An increasing salt gradient in the mobile phase is used to displace the oligonucleotide phosphate anions. The salt anions, such as ammonium sulfate or sodium phosphate, and the DNA pair with the adsorbent-bound cation, usually alkylated ammonium species. Since ion-exchange analysis separates only on the basis of

increasing charge, the desired product oligonucleotide will always elute after the lesser charged failure sequences. The high salt mobile phase also provides a denaturing media. Both of these factors allow easy product identification. Preparative ion-exchange HPLC requires a final desalting operation, most efficiently conducted with OPC.

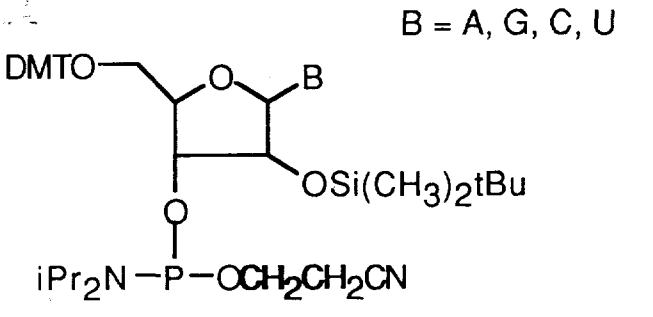
For more information, consult the chapter on HPLC in User Bulletin 13, Revised.

ALTERNATE CHEMISTRIES

In addition to the phosphoramidite chemistry method to prepare "normal" phosphodiester oligonucleotides, many alternative chemistries have been demonstrated on ABI synthesizers. These other products include; synthetic RNA, phosphate-analog oligonucleotides, and chemically derivatized oligonucleotides which can be covalently attached to other molecules.

RNA Synthesis

RNA oligoribonucleotides can be synthesized on the Model 380B[22,23]. Using 2' silyl 5' DMT cyanoethyl phosphoramidite RNA monomers, the only cycle change is to increase the coupling wait time of the corresponding DNA synthesis cycle to 600 seconds. RNA monomers bear a large 2' silyl protecting group and therefore require longer time to couple. Details can be found in Applied Biosystems User Bulletins No. 47 and 53.



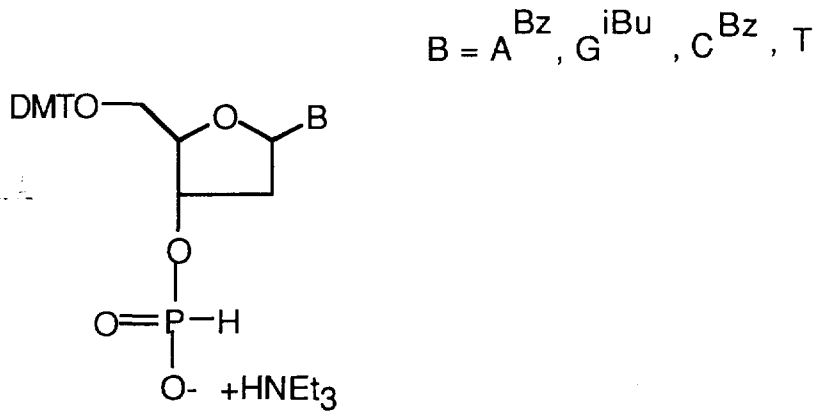
RNA phosphoramidites

Figure 2-9

Hydrogen-Phosphonate Chemistry

Hydrogen-phosphonate chemistry is useful and effective for preparing either "normal" phosphodiester oligonucleotides or phosphate-analog oligonucleotides[24]. Although the cycle is similar in that it contains coupling, capping[25], and detritylation steps, the reagents[26] are different from those used for the phosphoramidite method. User Bulletin No. 44 provides details regarding appropriate synthesis reagents and post-synthesis protocols.

The synthesis efficiency of hydrogen-phosphonate chemistry is routinely less than that of the phosphoramidite method (typically 95-96% trityl yields). The primary advantage of hydrogen-phosphonate chemistry is the potential for one-step, post-synthesis conversion of the internucleoside hydrogen-phosphonate groups to a variety of phosphate species[27] such as phosphodiesters, phosphoramidates, phosphotriesters and phosphorothioates.

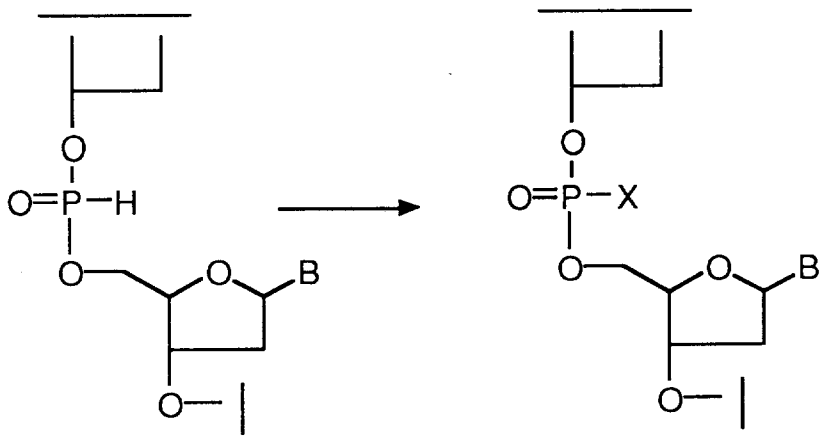


hydrogen-phosphonate monomers

Figure 2-10

Phosphorothioate DNA

Once hydrogen-phosphonate synthesis of an oligonucleotide is completed, the column may be removed and a sulfurizing reagent introduced by the double-syringe method[17,28]. All the internucleoside hydrogen-phosphonates are rapidly converted to phosphorothioates. After regular ammonia cleavage/deprotection, oligonucleotides bearing phosphorothioate linkages behave chemically similarly to "normal" phosphodiester DNA. They show the same electrophoresis and HPLC behavior. This class of phosphate-analog DNA has shown activity in anti-sense translation arrest experiments. In particular, both a sequence, and non-sequence specific effect has been observed in inhibiting in vitro viral replication of HIV[29]



CONVERSION OF HYDROGEN-PHOSPHONATE
TO PHOSPHATE ANALOGS

X	OXIDANT
O	I_2/pyr/Et_3N/H_2O/THF
S	sulfur/CS_2/pyr/Et_3N
NR_2	HNR_2/CCl_4
OR	ROH/base/CCl_4

Figure 2-11

5' Attachments

In the last several years many applications have been identified and developed for the covalent attachment of small molecules to oligonucleotides. These molecules include fluorescent dyes[30], biotin[31], proteins[32], and other species that allow the identification of oligonucleotides in biological systems. In addition, oligonucleotides can be derivatized to allow attachment to solid supports[33]. In this way, for example, an affinity matrix can be constructed to purify a sequence complementary to the support-bound sequence.

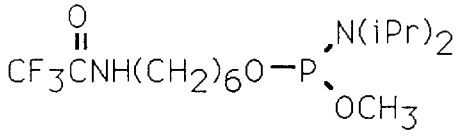
The attachment can be made at several sites on the oligonucleotide. However, this site must not impair hybridization or confer chemical instability. For these reasons and for synthesis ease and efficiency, the 5' terminus is usually the preferred location for derivatization of an oligonucleotide.

Fluorescent-dye Linked Sequencing Primers

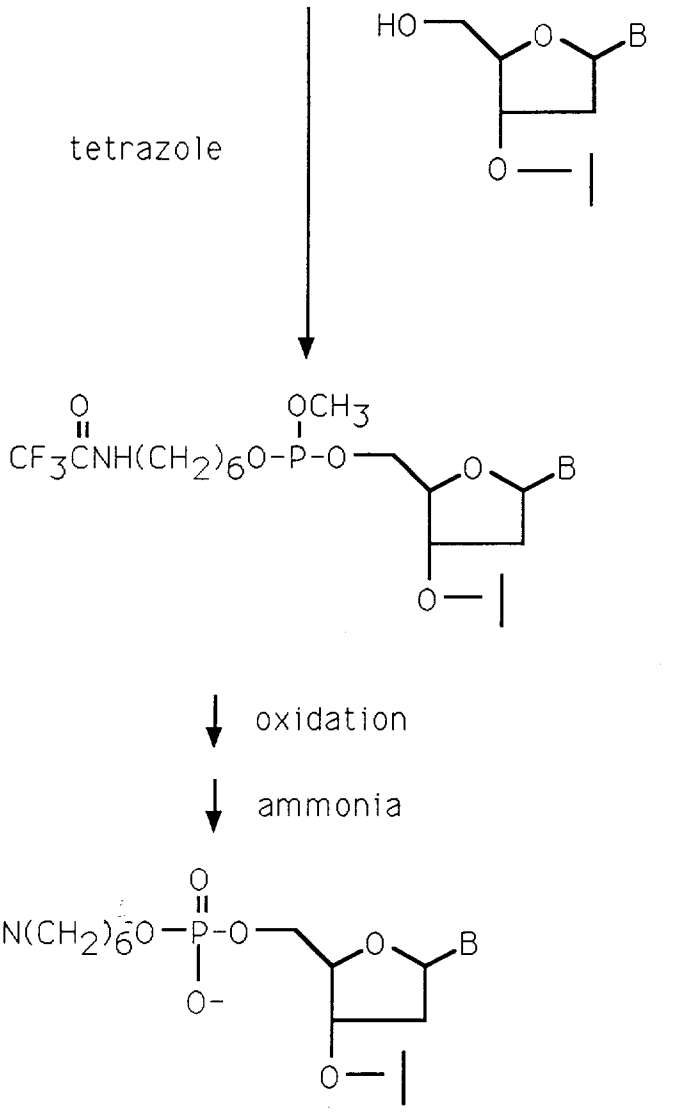
Aminolink 2:

The 5' fluorescent dye labelled sequencing primers used for the Applied Biosystems 370A DNA Sequencer consist of three parts; the oligonucleotide primer, a linker, and a fluorescent dye. The linker bears a highly nucleophilic primary amine group which reacts with the electrophilic N-hydroxy succinimide group of the fluorescent dye. This linker group is created with AMINOLINK 2 (ABI Part No. 400808)[34]. Aminolink 2 is a phosphoramidite molecule with a six carbon chain and a protected amine group (Figure

2-12). Although it comes as a viscous liquid rather than as a powder, this reagent is handled and used like a phosphoramidite nucleoside. Activation with tetrazole forms an active intermediate that couples to the 5' hydroxyl terminus of the support-bound oligonucleotide, in the final coupling cycle. Oxidation and ammonia cleavage/deprotection yields the aminolink-oligonucleotide, in solution. Coupling to the fluorescent dye-NHS ester, or other electrophilic species, is conducted in a homogeneous solution.



Aminolink 2



aminolinked oligonucleotide (in solution)

Figure 2-12

REFERENCES

1. Efcavitch, J.W., "Automated System for the Optimized Chemical Synthesis of Oligodeoxyribonucleotides", Macromolecular Sequencing and Synthesis, Selected Methods and Applications, pages 221-234, Alan R. Liss, Inc. (1988).

2. Beaucage, S.L. and Caruthers, M.H., Tetrahedron Letters 22, 1859 -1862 (1981). Beaucage, S.L. and Caruthers, M.H., United States Patent #4,668,777

3. Matteucci, M.D. and Caruthers, M.H., J. Am. Chem. Soc. 103, 3185 (1981)
Matteucci, M.D. and Caruthers, M.H., United States Patent #. 4,458,066

4. Adams, S.P., Kavka, K.S., Wykes, E.J., Holder, S.B., and Galluppi, G.R., J. Am. Chem. Soc. 105, 661 (1983).

5. Eadie, J.S. and Davidson, D.S., Nucleic Acids Research, 15, 8333-8349, (1987).
Farrance, I.K., Eadie, J.S., and Ivarie, R., Nucleic Acids Research, 17, 1231-1245, (1989).

6. Sinha, N.D., Biernat, J., and Koster, H., Tetrahedron Letters, 24, 5843-5846 (1983).

7. McBride, L.J., Eadie, J.S., Efcavitch, J.W., and Andrus, W.A., Nucleosides and Nucleotides , 6, 297-300 (1987).
Yeung, A.T., Dinehart, W.J. and Jones, B.K., Nucleic Acids Research, 16, 4539-4554 (1988).

8. Efcavitch, J.W., McBride, L.J. and Eadie, J.S., Biophosphates and Their Analogues - Synthesis, Structure, Metabolism and Activity, pages 65-70, Bruzik, K.S. and Stec, W.J. (Eds), Proceedings of the 2nd International Symposium on Phosphorus Chemistry Directed Towards Biology, Lodz, Poland, 1986.

9. Tanaka, T. and Letsinger, R.L., Nucleic Acids Research, 10, 3249-3260, (1982).

10. Efcavitch, J.W. and Heiner, C., Nucleosides and Nucleotides, 4, 267 (1985).

11. McBride, L.J. and Caruthers, M.H., Tetrahedron Letters, 24, 245-248 (1983).

12. Dahl, O., Phosphorus and Sulfur, 18, 201-204 (1983).
Dahl, B.H., Nielsen, J. and Dahl, O., Nucleic Acids Research, 15, 1729-1743, (1987).

13. Zon, G., Gallo, K.A., Samson, C.J., Shao, K., Summers, M.F. and Byrd, R.A., Nucleic Acids Research, 13, 8181-8196, (1985).

14. Applied Biosystems Nucleic Acid Research News, Issue No. 7, October 1988 "The Effect of the Synthesis Cycle on the Chemical Authenticity of Synthetic DNA".

15. Bauer, B.F. and Holmes, W.M., Nucleic Acids Research, 17, 812 (1989).

16. Stec, W.J., Zon, G., Egan, W. and Stec, B., J. Am. Chem. Soc. 106, 6077-6079 (1984).

17. The DOUBLE-SYRINGE METHOD for manual ammonia cleavage:

1. Attach an empty luer tip syringe, with plunger fully inserted, into one end of the synthesis column.
2. Load 2 ml of conc. ammonia in another luer tip syringe and attach to the other end of the column
3. Holding a syringe in each hand, carefully inject the reagent through the column to the empty syringe and return the reagent through the column several times.
4. Allow it to stand for at least one hour at room temperature.
5. Drain all of the reagent into one syringe, detach and eject contents carefully into an appropriate vial for heating to achieve complete deprotection.

18. L.J. McBride, McCollum, C., Davidson, S., Efcavitch, J.W., Andrus, A., and Lombardi, S.J., Biotechniques, 6, No. 4, 362-367 (1988) "A New, Reliable Cartridge for the Rapid Purification of Synthetic DNA". Applied Biosystems User Bulletin No. 51 "OPC Purification of Long Oligonucleotides"

19. "Rapid Purification, Desalting, and Detritylation of Synthetic Oligonucleotides", McCollum, C., Davidson, S., McBride, L.J., Efcavitch, J.W., and Andrus, A., poster, FASEB, Las Vegas, 1988

20. Horn, T. and Urdea, M.S., Nucleic Acids Research, 16, 11559-11571, (1988).

21. Efcavitch, J.W., "The Electrophoresis of Synthetic Oligonucleotides", Gel Electrophoresis of Synthetic Oligonucleotides, 2nd Ed., IRL Press, Practical Approach Series, *in press*.

22. Applied Biosystems User Bulletin No. 47 "RNA Synthesis" April 1988.

23. Usman, N., Ogilvie, K.K., Jiang, M.-Y., and Cedergren, R.J., J. Am. Chem. Soc., 109, 7845-7854 (1987). Ogilvie, K.K., Damha, M.J., Usman, N., and Pon, R.T., Pure & Applied Chem., 59, 325-330 (1987). Ogilvie, K.K., Usman,

N., Jiang, M.-Y., and Cedergren, R.J., Proc. Natl. Acad. Sci, 5764 (1988).

24. Froehler, B.C. and Matteucci, M.D. Tetrahedron Letters, 27, 469-472 (1986). Froehler, B.C., Ng, P.G., and Matteucci, M.D. Nucleic Acids Research, 14, 5399-5407 (1986). Garegg, P.J., Lindh, I., Regberg, T., Stawinski, J., and Stromberg, R., Tetrahedron Letters, 27, 4051-4057 (1986).

25. Andrus, W.A., Efcavitch, J.W., and McBride, L.J., United States Patent #4,816,571, "Chemical Capping By Phosphitylation During Oligonucleotide Synthesis".

26. Andrus, A., Efcavitch, J.W., McBride, L.J., and Giusti, B, Tetrahedron Letters, 29, 861-864 (1988).

27. Froehler, B.C., Tetrahedron Letters, 27, 5575-5578 (1986).

28. Andrus, A. and Zon, G., Nucleic Acids Research Symposium Series No. 20, 121-122 (1988). Applied Biosystems User Bulletin No. 44 "Oligonucleotide Synthesis with Hydrogen-Phosphonate Monomers"

29. Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, H., Reitz, M., Cohen, J.S., and Broder, S., Proc. Natl. Acad. Sci. USA, 84, 7706-7710 (1987). Stein, C.A., Subsinghe, C., Shinozuka, K. and Cohen, J.S., Nucleic Acids Research, 16, 3209-3221 (1988).

30. Smith, L.M. *etal*, Nature, 321, 674-679 (1986).

31. Chu, B.C.F. and Orgel, L.E., DNA, 4, 327-331 (1985). Chollet, A. and Kawashima, E.H., Nucleic Acids Research, 13, 1529-1541 (1985). Agrawal, S., Christodoulou, C. and Gait, M.J., Nucleic Acids Research, 14, 6227-6245 (1986).

32. Li, P., Medon, P.P., Skingle, D.C., Lanser, J.A. and Symons, R.H., Nucleic Acids Research, 15, 5275-5287 (1987). Chu, B.C.F. and Orgel, L.E., Nucleic Acids Research, 16, 3671-3691 (1988). Zuckermann, R.N., Corey, D.R. and Schultz, P.G., J. Am. Chem. Soc., 110, 1614-1615 (1988). Zuckermann, R.N. and Schultz, P.G., J. Am. Chem. Soc., 110, 6592-6594 (1988).

33. Ghosh, S.S. and Musso, G.F., Nucleic Acids Research, 15, 5353-5372 (1987).

34. Applied Biosystems User Bulletin No. 49. "Aminolink 2".

SECTION 3

PRINCIPLES OF OPERATION

This section describes the basic principles for operating the Model 380B DNA Synthesizer and includes information pertaining to Valves, Functions, Cycles and Procedures.

CONTENTS

INTRODUCTION

Automated DNA synthesis requires chemical deliveries to specified destinations, such as the column. These deliveries are controlled by valves that open and close, creating various pathways through the Model 380B.

Each solenoid valve is assigned a number which may be used to open or close the valve. A valve or set of valves opened simultaneously to perform a specific delivery or task is a function. Functions that are programmed to occur for a specified amount of time are steps.

A cycle is a series of steps that perform and repeat a chemical process. The Synthesis Cycle performs the steps that are necessary for one base addition. This cycle is repeated for the sequential addition of each base.

Procedures, like cycles, are a series of steps that perform a chemical process. However, procedures are performed once and are not repeated. The 380B Procedures includes: Begin Procedure; End Procedure; Bottle-Change Procedure; and the Shut-Down Procedure.

VALVES

The Model 380B has solenoid valves (numbered 0 through 61) which are listed in Table 3-1 with a description of what they control. Refer to Section 1 for a mechanical description of valves, and for the 380B DNA Synthesizer schematic to view valve placement.

Valves are opened (i.e., activated) electrically and are controlled through the microprocessor. During synthesis, valves open automatically to create a pathway for chemical and gas deliveries.

For user control and troubleshooting purposes, valves can also be manually activated and deactivated using the Manual Control Menu. Manual Control can be used either prior to the start of a synthesis or when synthesis is interrupted. Refer to Section 4 for use of the Manual Control Menu.

Valve Number	Valves Control
0, 2, 4, 6-11, 13-14	Argon Pressure to Reservoirs
1, 3, 5, 12, 15	Reservoirs Vent from Reservoirs
16-36, 40, 48, 56	Delivery Valves on Valve Blocks H and K
41-44, 49-52, 57-60	Deliveries to Column 1, 2 and 3 from Valve Block I
45, 53, 61	Collection Vial Vent Valves.

Table 3-1: Valve Numbers

Table 3-2 lists each reservoir with the pressure, vent and delivery valve numbers. The pressure and vent valves deliver gas to pressurize and vent the reservoir. The valve on the valve block opens to deliver that chemical.

RESERVOIR NUMBER	BOTTLE CONTENTS	PRESSURE VALVE	VENT VALVE	DELIVERY VALVE ON VALVE BLOCK
1	A phos	11	12	35
2	G phos	11	12	34
3	C phos	11	12	33
4	T phos	11	12	32
5	Reservoir 5	11	12	31
6	Reservoir 6	11	12	30
7	Reservoir 7	11	12	29
8	Spare Res.	4	5	23
9	Tetrazole	0	1	28
10	Ammonium hydroxide	14	15	24
11	Acetic anhydride	13	-	26
12	NMI	13	-	25
13	Acetonitrile	9	-	22
14	TCA	10	-	21
15	Iodine	8	-	20
16	Acetonitrile	7	-	19
17	Spare Res.	6	-	18
18	Acetonitrile	2	3	17
-	Argon gas	-	-	16

Table 3-2: Pressure, Vent and Delivery Valve Numbers Associated with Each Reservoir

FUNCTIONS

Some functions activate a valve or set of valves simultaneously so that various reagents can be delivered to a specified destination. Other functions are "Cycle Directives" and signal the controller. A few functions are "Relay Functions" and activate relays.

Functions are numbered and given an abbreviated name to describe the action they perform. For example, Function Number 9 is named "#18 to Column". When this function is activated, the acetonitrile in reservoir 18 is delivered to the column.

A standard set of functions, supplied on the disk, encompasses all processes necessary for synthesis. Refer to Appendix I for a list of 380B functions.

During synthesis, functions are activated automatically. After a specified time, the function is deactivated. For operator control and troubleshooting purposes, functions can also be manually activated and deactivated using the Manual Control Menu. Manual Control can be used either prior to the start of a synthesis or when synthesis is interrupted. Refer to Section 4 for use of the Manual Control Menu.

1. Valve Activating Functions:

These functions produce gas and chemical deliveries. From the reservoir, chemicals are delivered to the column, the waste bottle or the waste port. From the column, chemicals and gas are delivered to the waste bottle or waste port. To achieve flow of a reagent, functions are activated (i.e. a valve or set of valves open) to pressurize the reservoir with gas and then create a pathway from the reservoir to its destination.

Pathways can be traced using the flow schematic in Section 1. For example, Function Number 13, "#15 TO COLUMN", accomplishes the delivery of Iodine from the reservoir bottle to the column by opening valves 8, 20, 40, and 44. The pathway created is shown in Figure 3-1.

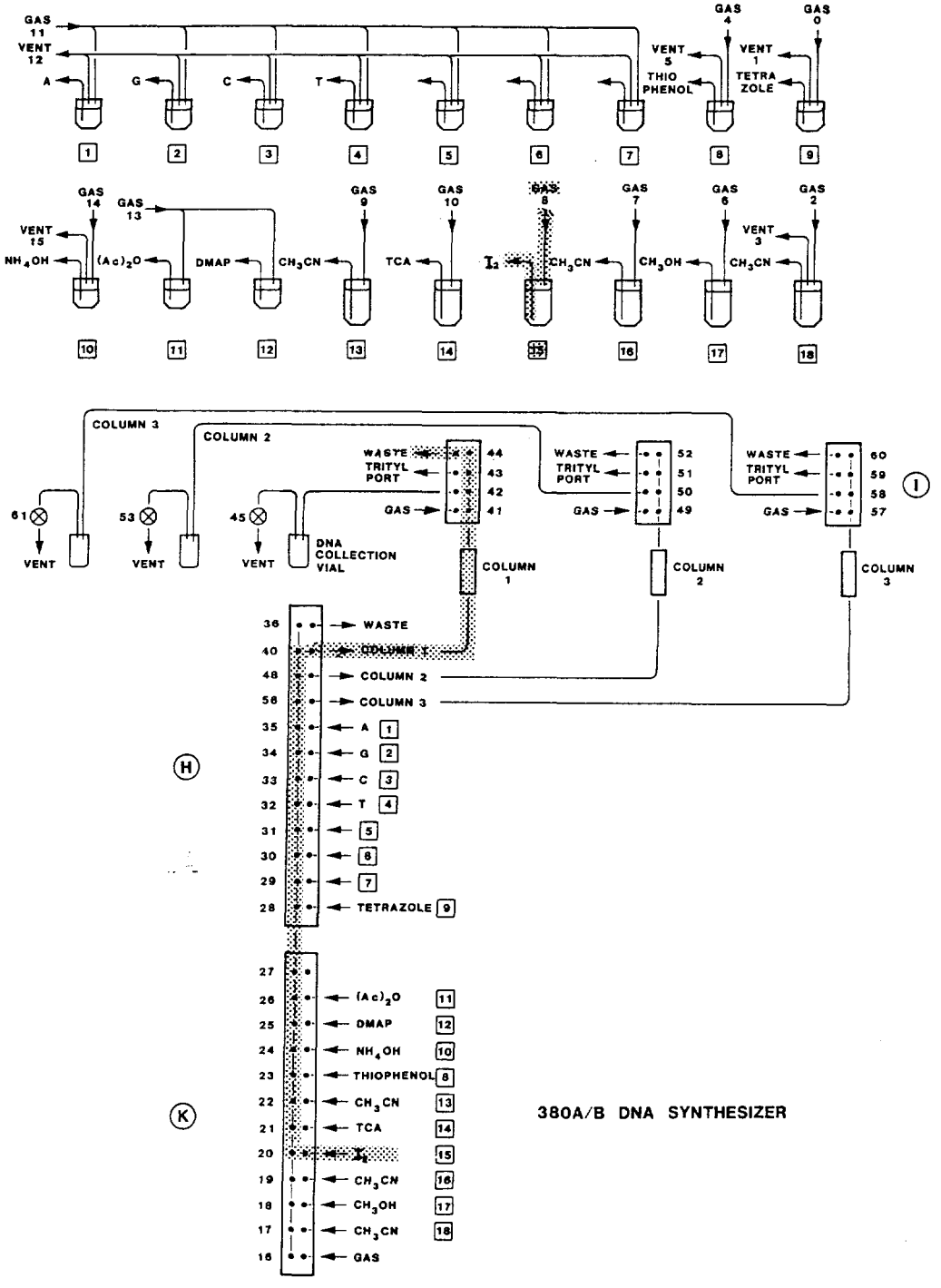


Figure 3-1: Function 13 - "#15 TO COLUMN"

Gas enters and pressurizes the iodine reservoir through valve number 8. With valves 20 and 40 open, a pathway is created from the reservoir, through the valve blocks, and to the column. Valve 44 is open to waste and flow proceeds from the pressurized bottle to the waste bottle which is at atmospheric pressure.

User-Defined Functions

User-defined functions can be created and used if chemical modification is desired. Function numbers 92 through 99 can be defined by the user. This is accomplished by selecting a set of valves to open, that creates a new pathway. Refer to Section 4, the Function Editor Menu for creating user-defined functions.

2. Cycle Directive Functions:

Cycle Directives are functions that signal the controller but do not open valves. Cycle Directive Functions are:

Function Number	Function Name
6	Waste-Port
7	Waste-Bottle
17	Interrupt
33	Cycle Entry
45	Group 1 On
46	Group 1 Off
47	Group 2 On
48	Group 2 Off
49	Group 3 On
50	Group 3 Off

Table 3-3: Cycle Directive Functions

Functions 6 and 7: "Waste-Port" and "Waste-Bottle"

Gas and reagents can be sent from the column to the waste bottle or to the trityl collection port. This is accomplished using Function 6 - Waste-Port, or Function 7 - Waste-Bottle.

By using Function 6, flow from the column will be directed to the trityl collection port. Activating "Waste to Port" signals the controller to activate the port valves (43, 51 or 59) instead of the waste-bottle valves (44, 52 or 60).

In the standard synthesis cycles, Function 6 is activated just prior to TCA delivery so that the trityl cation released during detritylation can be sent to a fraction collector for subsequent analysis.

Comparatively, by using Function 7, flow from the column will be directed to the waste bottle. Activating "Waste to Bottle", signals the controller to activate the waste-bottle valve (44, 52 or 60), instead of the port valve (43, 51 or 59).

Function 17: Interrupt

The Interrupt Function signals the controller to stop until a "continue" command is given. This function is used in the Bottle-Change Procedure when the bottle is replaced, and in the Shut-Down Procedure.

Function 33: Cycle Entry

DNA synthesis begins with detritylation, which is located in the middle of synthesis cycles. Function "Cycle Entry" is placed in a cycle just prior to the detritylation steps and signals to the controller where synthesis starts. (Cycle Entry is not step number one in synthesis cycles.) When creating new cycles, this function is required to be placed appropriately in the cycle.

NOTE: The DNA is chemically safe at Cycle Entry and at Cycle End and therefore is a preferred step for synthesis interruption. To interrupt synthesis, refer to the Interrupt Status Menu in Section 4.

Cycle End

This Cycle Directive has been incorporated into the software and is not a function. Cycle End occurs after the last step of a cycle and before step number 1 of the next cycle. The base number increments immediately after Cycle End.

Group Functions

Function Number	Function Name
45	Group 1 On
46	Group 1 Off
47	Group 2 On
48	Group 2 Off
49	Group 3 On
50	Group 3 Off

Table 3-4: List of Group Functions

Group On functions signal the controller to make deliveries to the specific column. For example: The functions that follow "Group 1 On" will deliver all chemicals to Column 1 by opening the appropriate valves. When a "Group 2 On" or "Group 3 On" function is activated in a one-column instrument, "Group 1 ON" is automatically activated.

Group Off functions signal the controller that subsequent functions are no longer column specific.

3. Relay Functions:

Relay functions are used to control external devices such as a strip chart recorder or a fraction collector. There are four numbered contact closures located at the rear of the Synthesizer on the terminal strip labeled SIGNAL.

Signal 1 can be used to control a strip chart recorder. Function Number 31 activates the contact closure to turn the recorder on while Function Number 32 turns the recorder off.

Signal 2 can be used to control a fraction collector. When a fraction collector is connected to Signal 2, Function Number 5 advances the fraction collector to the next tube by closing the electrical contact. The Pulse Width (i.e. the duration of the closure) can be programmed in the Configure Pulse Menu. Refer to Section 4 for use of this Menu. The Pulse Width programmed is dependant on the requirements of the device used.

Signals 3 and 4 can be connected to devices for user applications.

Function Number	Function Name
35	Relay 3 On
36	Relay 3 Off
37	Relay 3 Pulse
38	Relay 4 On
39	Relay 4 Off
40	Relay 4 Pulse

Table 3-5: Functions Controlling Relays 3 and 4

Functions 35 and 38 (Relay ON), activate a device by closing an electrical contact. Functions 37 and 40 (Relay Pulse), activates a device by "pulsing" the electrical contact. The Pulse width (i.e. the duration of the contact closure) can be programmed in the Configure Pulse Menu. Refer to Section 4 for use of this menu. The Pulse width programmed is dependant on the requirements of the device used. For assistance, contact your Applied Biosystems Service Representative.

CYCLES

Synthesis Cycle:

A cycle is a series of steps that perform and repeat a chemical process. The Synthesis Cycle performs the steps that are necessary for one base addition. This cycle is repeated for each base addition.

Applied Biosystems supplies seven Synthesis Cycles for use with 1-column instruments and seven Synthesis Cycles for use with 3-column instruments. Software Version 2.00 synthesis cycles are listed in Table 3-6 (page 3-19). The cycle print-outs can be found in Appendix II.

NOTE: Refer to User Bulletin 40 for further information on synthesis cycles in Version 1.34 software.

Selection of the desired Synthesis Cycle is made just prior to starting a synthesis. Refer to the Run Editor Menu, described in Section 4.

DNA synthesis begins at "Cycle Entry", which is located in the middle of the Synthesis Cycle. In three-column instruments, when synthesis on one column is interrupted and then the interrupt is cancelled, synthesis will start again when the cycle reaches Cycle Entry.

Upon completion of all base additions, the Synthesis Cycle will conclude at "Cycle Entry" when the trityl protecting group remains on the DNA. If the Ending Method is trityl off, detritylation will be performed and the cycle will conclude at "Cycle End". Refer to page 3-13 for an End Method definition.

Cycle Modifications

Cycles can be created or revised between syntheses from the Cycle and Procedure Menu. Cycle times can be modified during synthesis from the Cycle Monitor Menu. New cycles can be created through the Cycle and Procedure Editor. Refer to Section 4 for use of all menus.

Coordination of Cycles in 3-Column Synthesis

The Model 380B can simultaneously perform up to three independent syntheses. A synthesis for any column can be set up and started at any time regardless of the status of the other columns. When two or three columns are synthesizing, the cycle steps being performed are automatically synchronized. Gas and most reagents are simultaneously delivered to all active columns.

When a synthesis is started and one or two columns are already active, the synthesis will begin when the others reach the step, Cycle Entry. Similarly, if an interrupt is set and released, the synthesis will resume when the active cycle reaches Cycle Entry.

Note that during simultaneous deliveries for multiple column syntheses, the chemical flow rates are slightly slower and the delivery times must be slightly longer than during a one-column synthesis. For example, a 100-second delivery in a one-column synthesis will be 109 seconds in a 2-column synthesis and 118 seconds during a 3-column synthesis.

PROCEDURES

Procedures, like cycles are series of steps that perform a chemical process. However, Procedures are performed once and are not repeated. 380B Procedures include: the Begin Procedure; End Procedure; Bottle-Change Procedure; and the Shut-Down Procedure.

Begin Procedure:

At the beginning of a synthesis, the Begin Procedure fills all phosphoramidite and tetrazole delivery lines (from the reservoir to valve block H) with fresh reagent.

Oxygen and atmospheric water can slowly penetrate the delivery lines. Therefore, when an instrument is idle for more than 12 hours (or 6 hours in humid environments), or if one of the phosphoramidite reservoirs has not been accessed within 12 hours, the Begin Procedure should be performed.

IMPORTANT: First coupling failures may be seen if the Begin Procedure is not performed after the Synthesizer has been idle. To safeguard against this type of synthesis failure, perform

the Begin Procedure whenever the Synthesizer has been idle for more than 12 hours.

The standard Begin Procedure, "phos003" is supplied on the disk and a printout can be found in Appendix II. "phos003" can be modified or new Begin Procedures can be created. If other Begin procedures are created, they can be selected just before the start of synthesis from the Cycle Monitor Menu. Refer to Section 4 for use of this menu.

NOTE: Reagents used in the Begin Procedure are not counted as a "Cycle" in the Reservoir Status Menu. Therefore, the Begin Procedure is not included in tabulation of the reservoir alarm. Refer to the Reservoir Status Menu in Section 4.

<u>End Method and End Procedure</u>:

End Method:

At the conclusion of synthesis, the DNA chain can be:

- -Protected (with the trityl ON);
- -Unprotected (with the trityl OFF);
- -Automatically cleaved from the solid support; or
- -Left on the solid support for manual cleavage.

This is accomplished by selecting the desired End Method. End Method Choices are as follows:

TR ON MANUAL

The 5' terminus of the synthesized DNA chain will remain protected and cleavage from the solid support is not automatically performed.

TR ON AUTO	The 5' terminus of the synthesized DNA chain will remain protected and the 380B performs cleavage from the solid support.
TR OFF MANUAL	The 5' terminus will be deprotected, and cleavage from the solid support is not automatically performed.
TR OFF AUTO	The 5' terminus will be deprotected, with the 380B performing the cleavage.

End Procedure:

The End Procedure is performed at the conclusion of synthesis only when it is desired to have the DNA automatically cleaved from the solid support. Therefore, when the End Method selected is AUTO, the End Procedure is performed after the last Synthesis Cycle and accomplishes deprotection and cleavage.

Six standard End Procedures can be found in Appendix II. During the End Procedure, the DNA strand is cleaved from the solid support by four consecutive 15-minute treatments with concentrated ammonium hydroxide. Following each treatment, the cleaved DNA is deposited into the DNA collection vial.

"System Default" End Method and End Procedure:

The End Method and End Procedure used most often can be programmed as the System Default End Method and End Procedure through the Configure Standard End Menu in Section 4.

Bottle-Change Procedure:

Reservoirs can be changed between syntheses, or during an interrupted synthesis. Reservoirs are changed using the Reservoir Status Menu. Refer to Section 4, the Reservoir Status Menu, to change reservoirs.

When the empty reservoir is removed and replaced with a full one using the Reservoir Status Menu, the Bottle-Change Procedure is automatically performed. During this Procedure, the delivery line is flushed; the bottle is changed and repressurized; the delivery line is refilled with fresh reagent; and the valve block is rinsed and dried.

A standard Bottle-Change Procedure is supplied on the disk for each reservoir. The standard Procedures are named "bc1" through "bc18", and can be found in Appendix II.

Bottle-Change Procedure for Phosphoramidite and Tetrazole Reservoirs:

Below is a summary of the Bottle-Change Procedure for the phosphoramidite (Bottles 1-7) and tetrazole (Bottle 9) reservoirs. Performing this Procedure is especially important to prevent atmospheric oxygen and water from contaminating these sensitive reagents.

* The valve blocks are cleared by F1, "Block Flush", and rinsed by F10, "#18 to Waste".

* Old reagent in the delivery line is removed and forced back into the reservoir by an acetonitrile wash by delivering #18 to the designated bottle.

IMPORTANT: If the phosphoramidites are to be removed and saved for later use, do not complete these steps because HPLC grade acetonitrile will contaminate the bottles. Refer to Section 5 for instructions on how to store dissolved phosphoramidites.

* Argon clears the delivery lines using Function "Argon Flush" to the designated bottle.

* The bottles are removed and replaced.

* Argon purges the reservoir headspace to eliminate air using F44, "Phosphoramidite Purge".

* The delivery line is filled with fresh reagent by delivering reagent from the reservoir to waste.

* The reagent is rinsed from the valve blocks by F10 "#18 to Waste", and then cleared using F1, "Block Flush".

Shut-Down Procedure:

The Shut-Down Procedure should be performed when a Synthesizer is not going to be used for more than a week or two. It is selected to flush reagents from the delivery lines, and to wash and dry all chemical pathways. Refer to Section 4 for use of the Shut-Down Procedure. The standard Shut-Down Procedure is "clean003" and can be found in Appendix II. A summary is as follows:
*Reagents in all delivery lines are removed and forced back into the reservoirs by an acetonitrile wash. (via delivery of bottle #18 to the designated reservoir).

* The lines are cleared by an argon flush to each bottle.

* The user then removes all bottles and wipes the delivery lines with a lint-free tissue.

* Clean bottles are placed on the instrument by the user to protect the delivery lines during storage.

COMPLETE FILE DIRECTORY

VERSION 2.00

CYCLE TYPE		CYCLE NAME	
1-Micromole Cycles		Standard Cycle	Large Bottle Cycle
1-Column	ß-cyanoethyl	cef1	ceaf1
3-Column	ß-cyanoethyl	cef3	ceaf3
Small Scale Cycles		Standard Cycle	Large Bottle Cycle
1-Column	ß-cyanoethyl	sscef1	ssceaf1
3-Column	ß-cyanoethyl	sscef3	ssceaf3
Combined 1-Micromole & Small Scale Cycles		Standard Cycle	Large Bottle Cycle
1-Column	RNA	rnaf1	rnaaf1
1-Column	H-Phosphonate	(hpaf1)	hpaf1
3-Column	RNA	rnaf3	rnaaf3
3-Column	H-Phosphonate	(hpaf3)	hpaf3
10-Micromole Cycles		Standard Cycle	Large Bottle Cycle
1-Column	ß-cyanoethyl	10cef1	10ceaf1
1-Column	RNA	10rnaf1	10rnaaf1
1-Column	H-Phosphonate	10hpf1	10hpaf1
3-Column	ß-cyanoethyl	10cef3	10ceaf3
3-Column	RNA	10rnaf3	10rnaaf3
3-Column	H-Phosphonate	10hpf3	10hpaf3

STANDARD BEGIN PROCEDURE

phos003

STANDARD END PROCEDURES

deprce, deprce10, deprhp, deprhp10, deprna, deprna10

STANDARD BOTTLE-CHANGE PROCEDURES

bc1, bc2, bc3, bc4, bc5, bc6, bc7, bc8, bc9, bc10,
bc11, bc12, bc13, bc14, bc15, bc16, bc17, bc18.

STANDARD SHUT-DOWN PROCEDURE

clean003

SECTION 4

SOFTWARE MENUS

Each menu is described in detail. Use this section while viewing your 380B DNA Synthesizer.

CONTENTS:

CONTENTS (Continued)

INTRODUCTION

Interaction between the DNA Synthesizer and the user is accomplished through the touchscreen. The Model 380B software is menu-driven. The touchscreen displays a series of menus which provide options that can be selected. Options are displayed in the rectangular orange areas. To make a selection from any menu, touch the center of the square orange area with your finger. An asterisk will appear in the upper left hand corner of the selection. When your finger retracts from the screen, the selection is activated. After 60 minutes with no activity, the touchscreen will turn dark to prolong the tube life. To activate the darkened touchscreen, simply touch any part of the screen.

The Model 380B software is stored on a 3.5-inch hard cover microfloppy disk that is inserted into the disk drive. When the red light next to the disk drive is lighted, the disk is being read by the microprocessor.

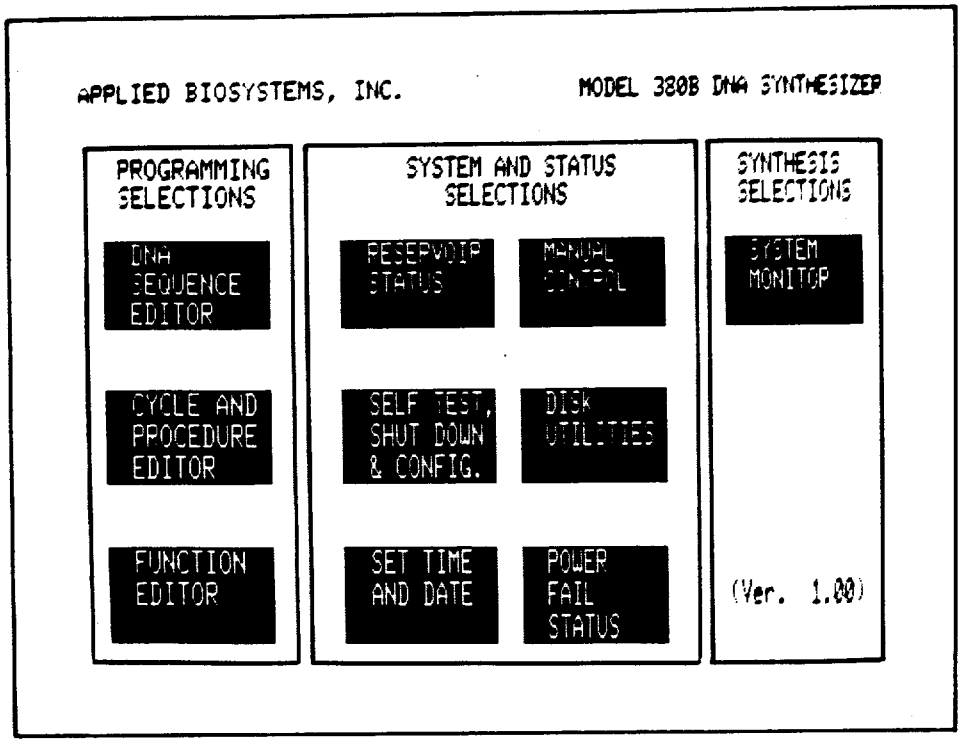
CAUTION: DO NOT take the disk out while the red light is on. Otherwise, the disk files could be lost.

The disk can be removed by simply pressing the bar under the disk drive. During synthesis, the disk should remain in the disk drive since it is sometimes read by the microprocessor.

When an option has been selected by touching the screen, the Synthesizer responds with a tone. When the selection is successful, another higher sounding tone can be heard. If a selection has been made that cannot be executed, a lower tone sounds indicating an error. The tone that can be heard when a message or prompt appears on the screen is between the success and error tones. In cases where immediate attention is necessary, an oscillating tone will sound for 15 seconds or until the screen in touched.

MAIN MENU

When the DNA Synthesizer power is turned on, software from the disk is loaded into the memory and the Main Menu appears on the screen. The Main Menu allows the user to choose Synthesis Selections, Programming Selections or System and Status Selections.



Synthesis Selections:

SYSTEM MONITOR

From this menu, the parameters for synthesis are set, and synthesis is begun. The System Monitor Menu displays on-going synthesis information. From this menu, synthesis interrupts can be programmed, and the touchscreen can be locked.

Programming Selections:

DNA SEQUENCE EDITOR	The DNA Sequence Editor is used to define and store DNA sequences on the disk for use during synthesis.
CYCLE AND PROCEDURE EDITOR	From this menu, synthesis protocols can be created or revised. These protocols are named and stored on the disk for use during synthesis.
FUNCTION EDITOR	Information about each function including the function name, and activated valves, can be viewed from this menu. The user can also define functions through this menu.

System and Status Selections:

RESERVOIR STATUS	RESERVOIR STATUS/BOTTLE CHANGE is used when changing a reservoir bottle and to set alarms so that synthesis is automatically interrupted when the reagent level is low.
SELF TEST SHUT DOWN & CONFIG.	SELF TEST is selected to perform an electrical check on various components in the Synthesizer. The CRT screen can be checked for alignment and a Shut-Down Procedure can be performed when the instrument will not be used for a long period of time.
SET TIME and DATE	In the SET TIME AND DATE Menu, the operator can set the hour, minute, month, day and year.

MANUAL CONTROL	From the MANUAL CONTROL Menu, the user can activate or deactivate functions and individual valves and the column(s).
DISK UTILITIES	DISK UTILITIES is selected to name a disk and to copy, rename, purge or list files stored on the disk.
POWER FAIL	POWER FAIL STATUS is a report which provides information on power failures. Also the user can modify the acceptable power-down time.

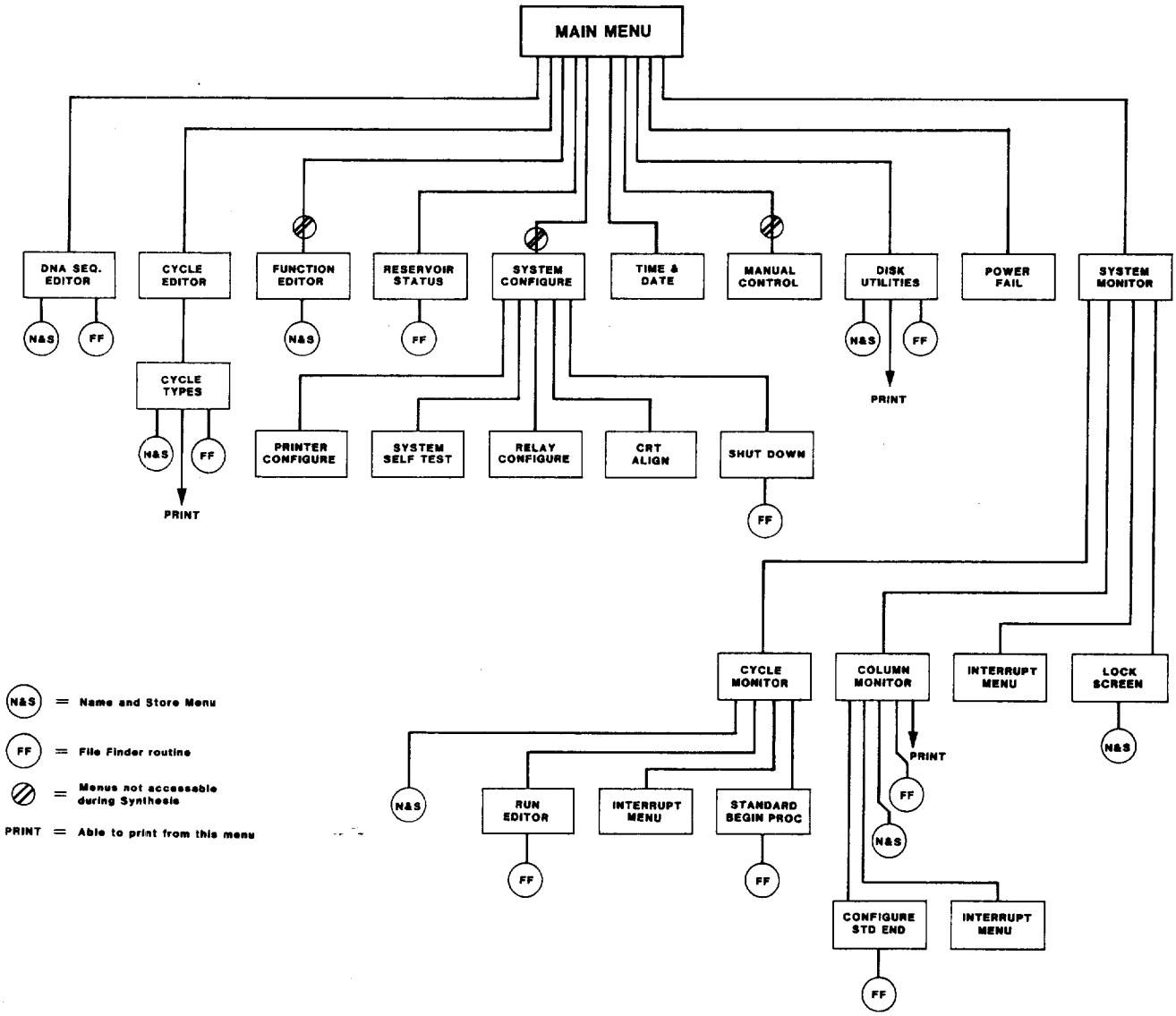


Figure 4-1: Software Menu Schematic

COMMON MENU OPERATIONS

There are some keys and menu pages common to many menus. These are the File Finder Routine, the Previous Menu key, the Name and Store Menu Page and the Numeric Keyboard.

FILE FINDER ROUTINE

All files are stored on the disk. The types of files are DNA Sequences, Cycles and Procedures. Files are chosen for use in synthesis or for file modification. To make the files available for selection, the 380B goes through a "File Finder Routine".

First, the disk directory searches for files stored on the disk and then displays them on the screen. The type of files are noted on the top of the page, such as "DNA Sequences found on disk" or "Synthesis Cycles found on disk". The file listed on the page, is then selected by touching it. Twenty selections can be shown on one page. The most recently stored files are displayed first. If there are more than twenty stored files, the option MORE FILES is displayed at the bottom of the page.

MORE FILES	When this option is selected the disk directory searches for more files and then displays them on the screen as options.

PREVIOUS MENU KEY

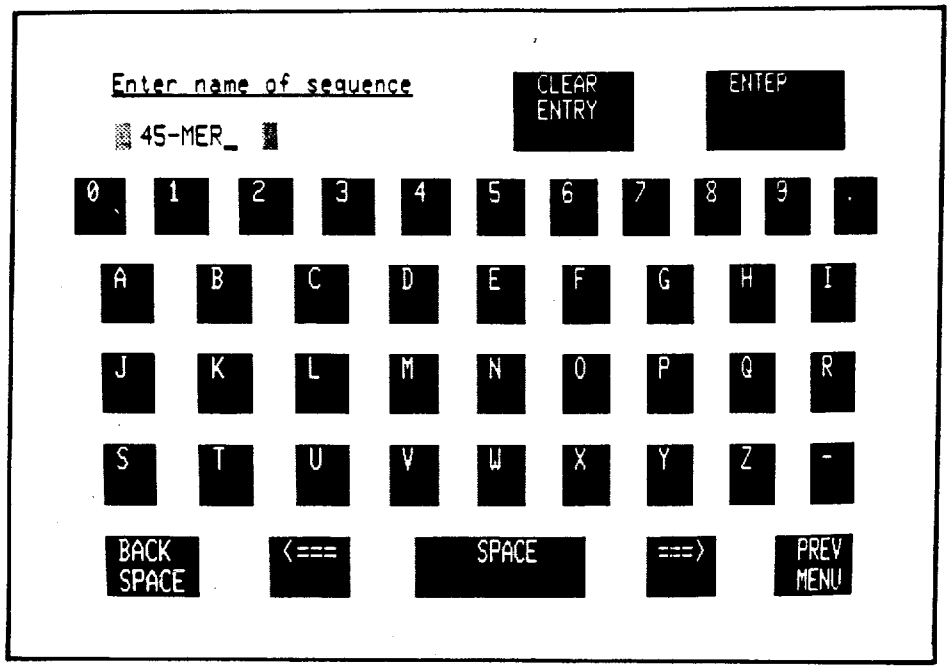
When the Previous Menu Key is selected, the preceding menu page is displayed as shown in Figure 4-1. The page that displays files from the File Finder Routine is NOT a menu and is not displayed when Previous Menu is selected. Also, when Previous Menu is selected from the NAME AND STORE menu, storage is cancelled.

THE NAME AND STORE MENU

The Name and Store Menu is used in many menus including the Cycle and Procedure Editor, the DNA Sequence Editor, Disk Utilities, Column Monitor and Function Editor. This menu allows:

* Files to be named and then stored on the disk;
* A comment to be created to describe to a DNA Sequence;
* User-defined functions to be named and stored; and,
* A code to be entered so that the touchscreen locks.

The Name And Store Menu:



From this menu page, naming a file is accomplished through use of the alphabet, numbers 0 to 9, a hyphen, space and a period. The characters are entered and displayed between two boxes in the upper left corner of the menu. Up to:

16 characters can be used to name a disk;
8 characters to name a Cycle or a DNA Sequence;
20 characters for a DNA Sequence comment;
3 characters to lock the touchscreen; and
14 characters to name a function.

SPACE	... enters one blank character.
CLEAR ENTRY	... erases all input made before ENTER is selected.
ENTER	... stores the DNA Sequence or Cycle on the disk.
--> <--	The arrows move the cursor one space in the direction of the arrow without any erasure.
BACKSPACE	... moves the cursor back one space and erases that character.

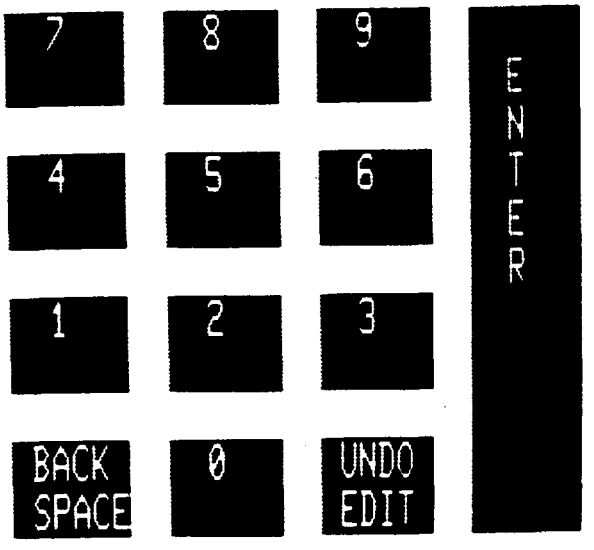
After the file is named, it is stored on the disk by pressing ENTER.

NOTE: The file is not stored when PREVIOUS MENU is selected. The menu returns to the previous menu with a message indicating that the storage was not done.

If a file had been previously stored, the name will be present in the Name area. If the file is to be given another name, either use Clear Entry or just type the new name over the old name and erase any extra characters using the space bar.

THE NUMERIC KEYBOARD

The numeric keyboard is included in several menus. The selection in the keyboard consists of numbers 0 to 9, Backspace, Undo Edit and Enter.



BACKSPACE	moves the cursor back one space and deletes that character.
UNDO EDIT	erases all changes made before Enter is selected.
ENTER	is used to store information when an entry is complete.

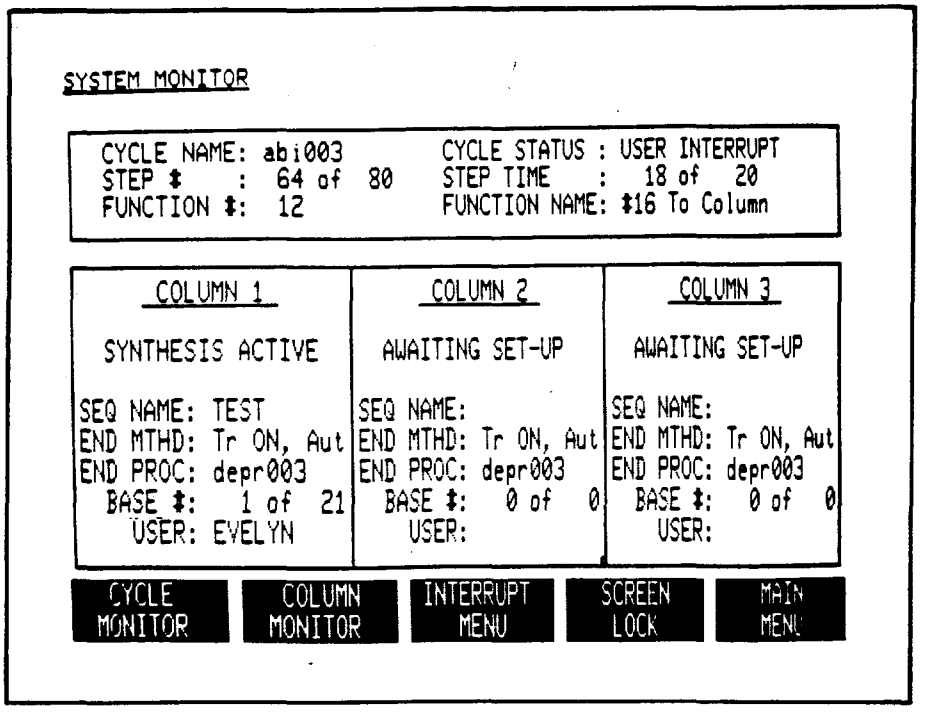
SYSTEM MONITOR

Introduction:

Most interaction between the user and the DNA Synthesizer will be made through the System Monitor Menu. From this menu the following can be performed:

* Select Synthesis Parameters;
* Start Synthesis;
* Program Synthesis Interruptions;
* Display Synthesis Information; and,
* Lock the Touchscreen.

The System Monitor Menu:



Selecting Parameters for Synthesis

From the System Monitor Menu, three menus are accessed to select the desired parameters for synthesis and are shown schematically in Figure 4-2. Synthesis parameters are selected from the menus in the following order:

I. COLUMN MONITOR MENU

Synthesis column(s) are "activated" by selecting a DNA sequence and choosing an End Method and End Procedure.

II. RUN EDITOR MENU

Cycles for synthesis are next selected

III. CYCLE MONITOR MENU

The Begin Procedure is selected and synthesis is then started.

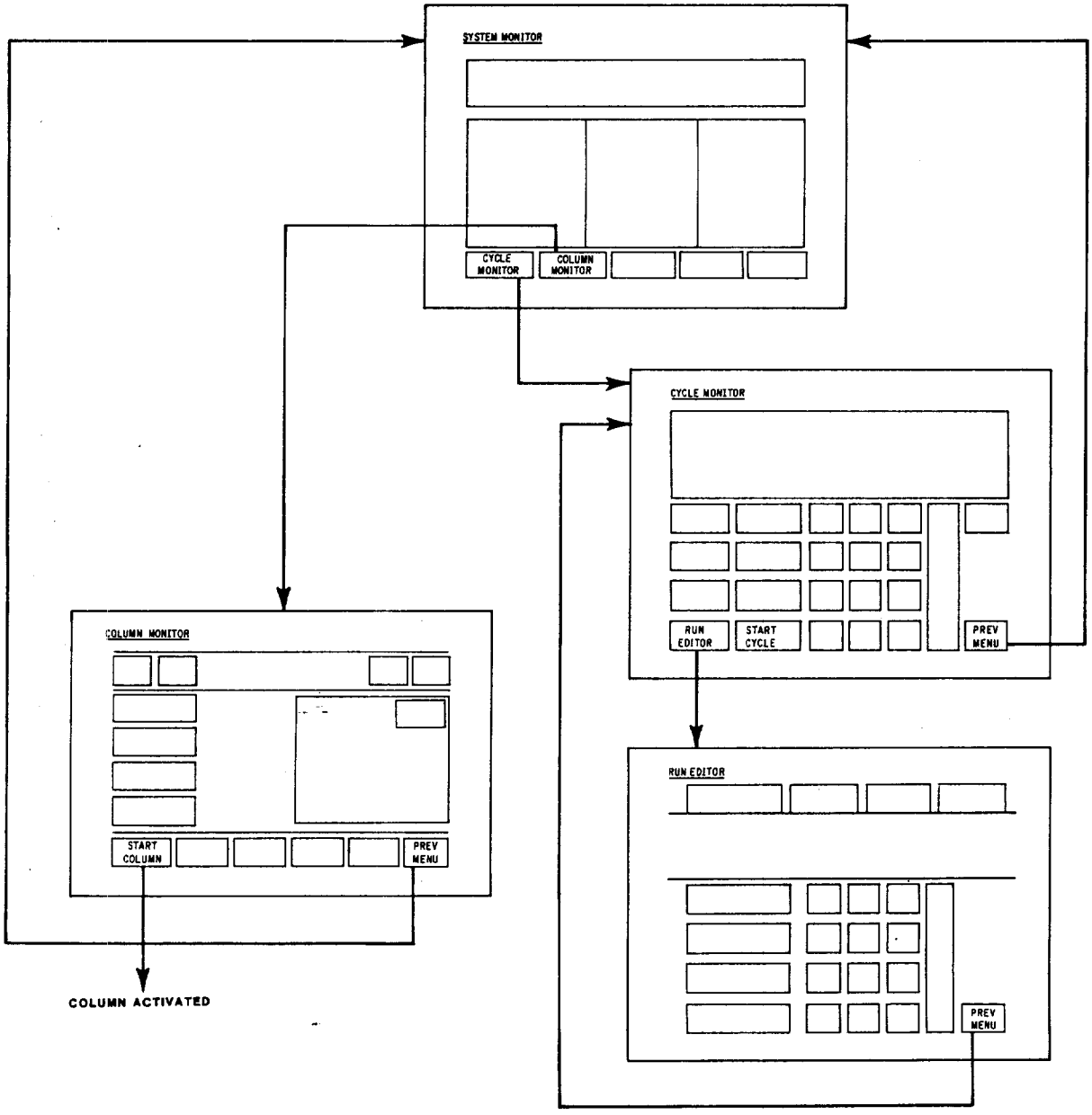
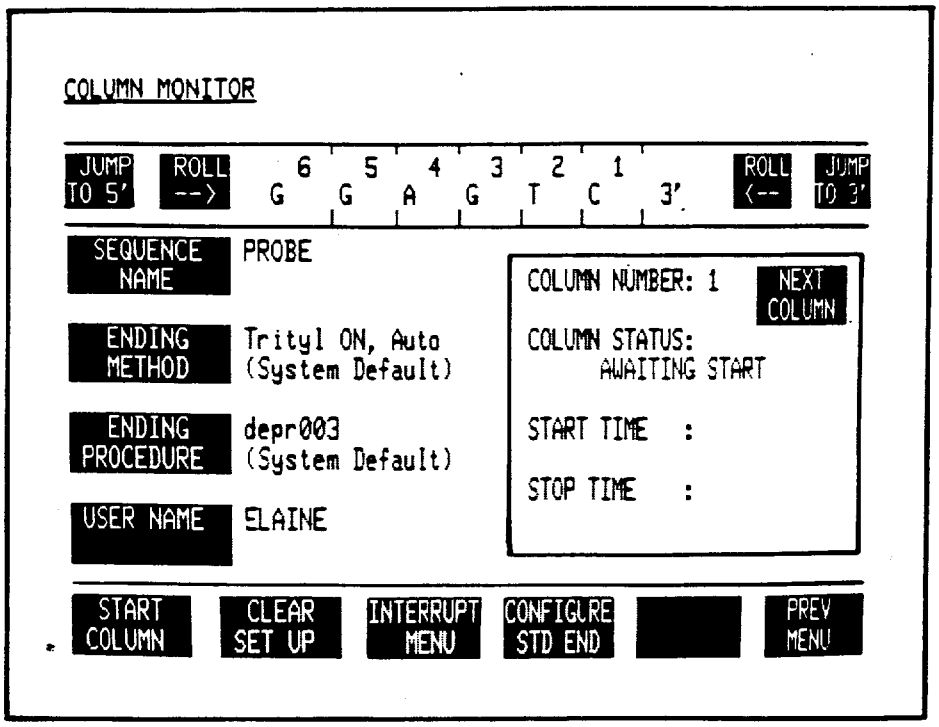


Figure 4-2: Menus Accessed from the System Monitor Menu

I. THE COLUMN MONITOR



ACTIVATING THE COLUMN(S):

1. If three columns are present, first choose the column to be set-up for synthesis by touching NEXT COLUMN.

NEXT COLUMN	Each time this key is selected, the "Column Number" being set-up changes from 1 to 2 to 3 to 1, etc.

2. Select a stored sequence by touching SEQUENCE NAME. The disk goes through the File Finder Routine and displays the "DNA Sequences found on the disk". Choose the desired DNA sequence for synthesis. The selected sequence is numbered and displayed between the ROLL --> and <-- ROLL keys. Initially viewed are the first 6 bases at the 3' end of the sequence:

JUMP TO 5'	ROLL --->	6	5	4	3	2	1	3'	ROLL <---	JUMP TO 3'
		C	G	A	G	C	T			

ROLL --> / ROLL <--	The arrow keys move other bases of the chain into view. The arrow key repeats this action when a finger is held against the key.
JUMP TO 3' / JUMP TO 5'	To view either the 3'or 5' terminal of the sequence, just touch these labeled areas.
CLEAR SET-UP	For the column that is indicated, this key will erase the displayed sequence, the sequence name, and the start and stop times. It will also set the Ending Method and Ending Procedure to "System Default" and the column status will be "Awaiting Set-up".

3. Next, an End Method and End Procedure are chosen for each column. Refer to Section 3 for descriptions.

End Method

At the end of synthesis, the DNA can remain protected (with the trityl ON), or unprotected (with the trityl OFF). The DNA can also be cleaved from the solid support automatically or this procedure can be performed manually. Choosing the appropriate End Method depends on the purification method used. Refer to the purification procedures in User Bulletin 13. The Ending Method choices are as follows.

TR ON MANUAL	The 5' terminus of the synthesized DNA chain will remain protected and cleavage from the solid support is not automatically performed.
TR ON AUTO	The synthesized DNA chain will remain protected and the 380B performs cleavage from the solid support.

TR OFF MANUAL	The 5' terminus will be deprotected, and cleavage from the solid support is not automatically performed.
TR OFF AUTO	The 5' terminus will be deprotected, with the 380B performing the cleavage.

End Procedure

The End Procedure is a series of steps, stored on the disk, that are performed at the end of the synthesis when an "AUTO" Ending Method is chosen. The standard End Procedure removes the methyl groups from the phosphates with thiophenol and cleaves the completed chain from the solid support with ammonia.

Selecting the desired protocols can be made either by, A) Touching the END METHOD and END PROCEDURE keys or by, B) Programming the "System Default" End Method and End Procedure from the Configure Std End Menu.

A) Selecting the End Method and End Procedure keys:

1. Selecting the End Method:

ENDING METHOD	By selecting this key, options TR On, Auto; TR On, Manual; TR Off, Auto; and TR Off Manual, become available.
UNDO EDIT	If this key is selected, the Ending Method displayed changes back to the previous selection and the previous option keys again become available.
SYSTEM DEFAULT	By selecting SYSTEM DEFAULT, the ending method will be as chosen in the Configure Standard End Menu.

2. Selecting the Ending Procedure:

ENDING PROCEDURE

When this key is selected, a message is viewed at the top of the menu: "Select System Default or View End Procs".

Options available to answer the prompt are SYSTEM DEFAULT, VIEW END PROCS, and UNDO EDIT.

SYSTEM DEFAULT

By selecting SYSTEM DEFAULT, the ending procedure will be as chosen in the Configure Standard End Menu.

VIEW END PROCS

When VIEW END PROCS is chosen the disk goes through the File Finder Routine and displays: "Ending Procedures Found on Disk". Selection of an End Procedure enters it into the microprocessor.

UNDO EDIT

If this key is selected, the Ending Procedure displayed changes back to the previous selection and the previous option keys become available.

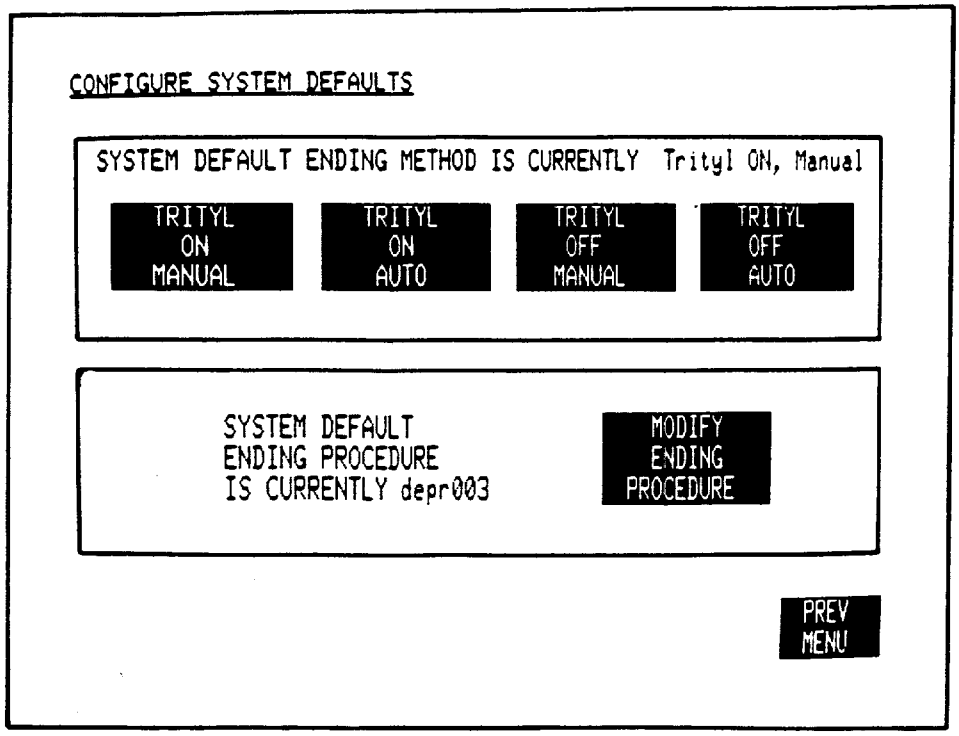
B) Programming "System Default" End Method and End Procedure

"System Default" End Method and End Procedure can be programmed from the CONFIGURE STD END Menu. The End Method and End Procedure that are selected from the Configure Std End Menu, will be automatically displayed in the Column Monitor Menu and will be used for synthesis unless a change is made.

CONFIGURE STD END

When this key is selected the menu page changes to the Configure Standard End Menu:

The Configure Std End Menu:



System Default protocols are programmed by touching the appropriate End Method and End Procedure options available.

4. Next, a name can be entered, if desired.

USER NAME

The user can enter his/her name or initials by selecting USER NAME.

The menu changes to the Name and Store page. Refer to page 4-8 for use of this menu. Up to 10 characters can be used.

5. After the DNA Sequence and the End Protocols have been selected, the column can be activated.

NOTE: CANCEL START can be used at any time to bring the user back to this point in setting up a column.

Activating the column begins by selecting START COLUMN.

START COLUMN

The START COLUMN key changes to read CONTINUE START and the key CANCEL START is available. The microprocessor performs a bottle check and a message is displayed.

For the bottle check, the microprocessor determines if a reservoir will empty before synthesis is complete, thereby causing synthesis to be interrupted. The microprocessor checks the reservoir alarm status; the length of the DNA sequence; and how often each reservoir is accessed for the cycles used in synthesis, and calculates when the interrupt will occur.

NOTE: The reservoir alarm is set by the user through the Reservoir Status Menu. For this bottle check to be useful, the alarm must be set accurately.

If the microprocessor determines that the reservoir bottles have enough reagent to complete synthesis, then the following message will appear:

"All reservoirs pass bottle check"

If the microprocessor determines that a reservoir will empty before synthesis is complete, a message will be displayed with the bottle number:

"Bottle Number 9 Will Empty First, 41 Cycles Ahead"

NOTE: If the microprocessor determines that two reservoirs will empty at the same time, the above message is displayed using the lowest numbered reservoir.

b) When CONTINUE START is selected next, another message is displayed:

"Put On x-Column and Collection Vial.
(x=3'end)

Place the desired column on the synthesizer and place a collection vial appropriately. Refer to Section 5 for operational procedures.

c) Touching CONTINUE START again displays the next message:

"Check Waste Bottle--Touch CONTINUE To Start".

d) Pressing CONTINUE START this last time activates the column. The Column Status changes from "Awaiting Status" to "Awaiting Cycle Entry" and the Start Time is displayed.

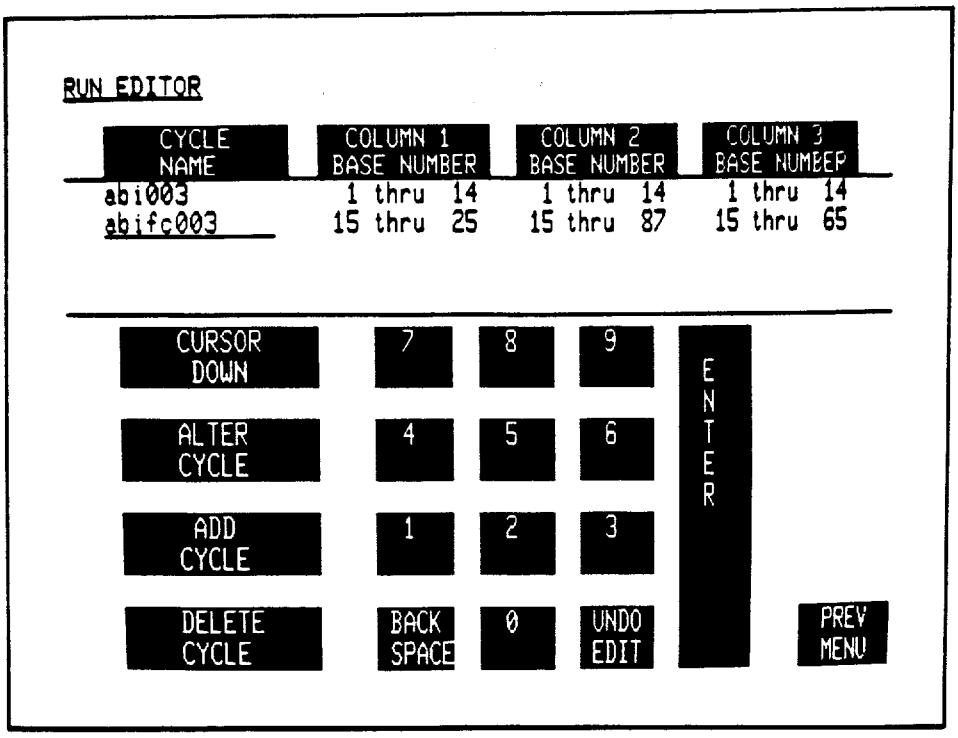
6. Selecting PREV MENU displays the System Monitor Menu for next selection.

II. THE RUN EDITOR

Introduction:

Up to 4 cycles can be used for DNA synthesis. Cycles to be run for synthesis are selected through the Run Editor. To display the Run Editor, first select the CYCLE MONITOR key from the System Monitor Menu, and then select the RUN EDITOR key.

The Run File Menu:



Menu Descriptions:

There are three keys that are only present in certain situations. DELETE CYCLE is only available when there is more than one cycle listed in the run. Option ALTER CYCLE changes to option ALTER BASE NUMBER when the cursor is in a Base Number column. ADD CYCLE is no longer available when four cycles are listed in the Run Editor.

CYCLE NAME	COLUMN 1 BASE NUMBER	COLUMN 2 BASE NUMBER	COLUMN 3 BASE NUMBER
abifc002	<Inactive>	1 thru 7	1 thru 24
SPECIAL 1			25 thru 53
SPECIAL 2			54 thru 240

Up to four different cycles can be selected for a synthesis. The Cycle listed under Cycle Name, will be performed for the addition of those bases indicated.

To Add Cycles to a Run:

ADD CYCLE

By selecting ADD CYCLE, the disk goes through the File Finder Routine and displays a list of cycles that can be chosen. The selected cycle is placed in the run at the end of the list.

To Alter Base Numbers:

If more than one cycle is to be used for synthesis, it is necessary to indicate the starting base number for the different cycles.

1. For three-column instruments, first place the cursor on the line and in the column where the change is to take place. The cursor can be moved several ways. By touching CYCLE NAME, COLUMN 1 BASE NUMBER, COLUMN 2 BASE NUMBER, or COLUMN 3 BASE NUMBER, the cursor will move to the indicated column.

CURSOR DOWN	Each time the CURSOR DOWN key is selected, the cursor moves down one line. To move the cursor to the first line, place the cursor on the last line and select this key again.

2. Next, enter the starting base number by selecting:

ALTER BASE NUMBER	When this key is selected, a box is displayed at the right of the menu to prompt the user to select the desired base number.

After the base number is selected, press ENTER. The Run Editor automatically adjusts the base numbers accordingly.

To change the listed Cycles:

A listed cycle can be replaced with another one, or a cycle can be deleted from the list.

To replace a listed cycle, first place the cursor in the Cycle column, under the cycle name to be changed. Then select ALTER CYCLE.

ALTER CYCLE	When this key is selected, the disk goes through the File Finder Routine and displays "Synthesis Cycles Found On Disk". The selected cycle replaces the previous cycle listed.

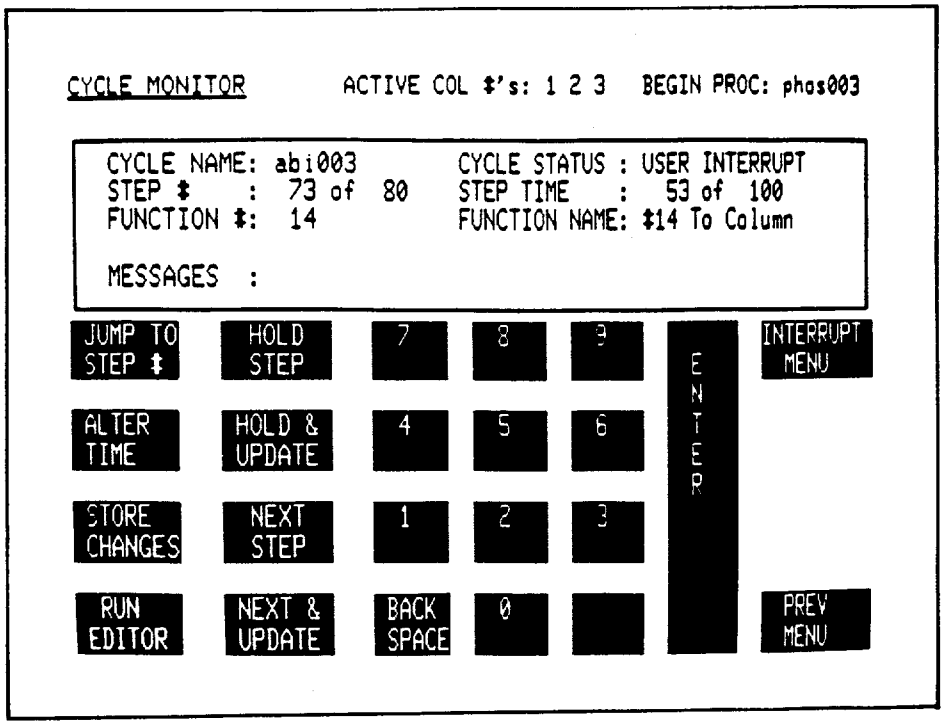
To delete a cycle from the list, first place the cursor under the cycle to be omitted.

DELETE CYCLE	By selecting DELETE CYCLE, the cycle at the cursor is deleted and the bases are renumbered.

III. THE CYCLE MONITOR

With the column(s) activated and the cycles chosen, synthesis can be started.

The Cycle Monitor Menu



To Start Synthesis

If a Begin Procedure is to be run, it can be selected at this point. The Begin Procedure is listed at the upper right corner of the menu and can be changed by selecting the ALTER BGN PROC key. The disk will then go through the File Finder Routine and display the Begin Procedures on the screen for selection. To start synthesis, select the START CYCLE key.

NOTE: The START CYCLE key is available only after a column has been set up; the column status is "Awaiting Cycle Entry"; and the run cycles have been selected.

START CYCLE	When START CYCLE is selected the prompt, "Do You Wish To Run The BEGIN PROCEDURE First?", is displayed.

Options to answer the prompt are YES, NO or CANCEL START.

If you do not wish to start synthesis at this time, select CANCEL START.

CANCEL START	When this key is selected, the previous option keys become available with the START key again available.

Synthesis will begin after selecting either YES or NO. By selecting YES, the Begin Procedure listed will be run and synthesis will start. By selecting NO the Begin Procedure will not be run and synthesis will start.

Synthesis information can be viewed either from the System Monitor or from the display area of the Cycle Monitor.

Editing Cycles during Synthesis

During synthesis, cycles can be altered by changing the time of any step. Step times can be changed in two ways. 1) A new step time can be directly entered into a Synthesis Cycle with the use of the ALTER TIME key and, 2) Step times can be changed while observing that step, with the use of some of the keys on the menu.

1) Editing Cycles Directly:

After synthesis has started, the new time for a particular step can be directly entered into a cycle by selecting ALTER TIME.

ALTER TIME

When selected, the prompt "Enter # of Step To Be Altered" is displayed at the top of the page. Also, at the right of the menu is a box to display the step number selected.

Using the keyboard, enter the step number and press ENTER. The "MESSAGE:" area displays the step as it appears in the cycle. At the top of the menu, a prompt is shown:

"Enter new time for step shown in MESSAGE".

Using the keyboard, enter the new time for the step and press ENTER. When this is complete, the "MESSAGE:" area reads:

"Active Synthesis Cycle Has Been Altered"

The step is now updated with the new time and will be run in each subsequent cycle of the current synthesis.

2. Changing Step Times While Observing A Cycle:

During synthesis, the duration of a step can be changed while the step is being observed. With the use of the keys that become available after synthesis has started, the user has the ability to increase or decrease the step time as it occurs. The altered step time can be stored in the cycle under a new name, if desired. In this manner, cycles can be easily optimized.

1. To jump over many steps to get to the area of the cycle to be observed, choose the JUMP TO STEP # key.

CAUTION: When jumping from one step to another, care must be taken not to mix reagents together in the valve block, or delivery lines. Otherwise, salts could form and blockage could occur requiring replacement of expensive parts.

JUMP TO STEP #	When this key is selected, all other option keys become blank, to prompt the user to make a numerical entry from the keyboard.

2. Step times can be controlled using either "Non-Update keys" or "Update keys".

Non-Update Keys:

By using the Non-Update key HOLD STEP or NEXT STEP, the time of a step can be increased or decreased. The change in time performed for the step is Not stored in memory.

HOLD STEP	When HOLD STEP is selected, the step being performed continues until Next Step is selected. When the STEP TIME: has counted down to 1, the total time will start to increment showing the additional amount of time that this step has been performed.

NEXT STEP	NEXT STEP ends the current step and starts the next step.

Update Keys:

By using the Update key HOLD & UPDATE or NEXT & UPDATE, the time of a step can be increased or decreased respectively. The "Updated" time is recorded in memory which can later be stored on the disk.

HOLD & UPDATE	When HOLD & UPDATE is selected, the step continues until the NEXT STEP key is selected. The step has been lengthened and the increased amount of time is held in memory.

NOTE: No update will be made if the HOLD & UPDATE step is stopped (by selecting NEXT STEP) before the time reaches "0".

UNDO EDIT	If HOLD & UPDATE has been selected and it is not desired to update this step then select UNDO EDIT.

NEXT & UPDATE	When NEXT & UPDATE is selected, the step being performed ends and the next step begins. The decreased amount of time is held in memory.

To store the time changes from using the Update keys, select the key STORE CHANGES.

STORE CHANGES	Using this key displays the Name and Store Menu. The new step times can be stored by giving the cycle a new name. The new name given to this cycle cannot exist for any file already stored on the disk. For use of Name and Store Menu, refer to page 4-8.

The new cycle will be performed, displayed next to CYCLE NAME in the System Monitor Menu, and will be found in the Run Editor in place of the cycle which was altered.

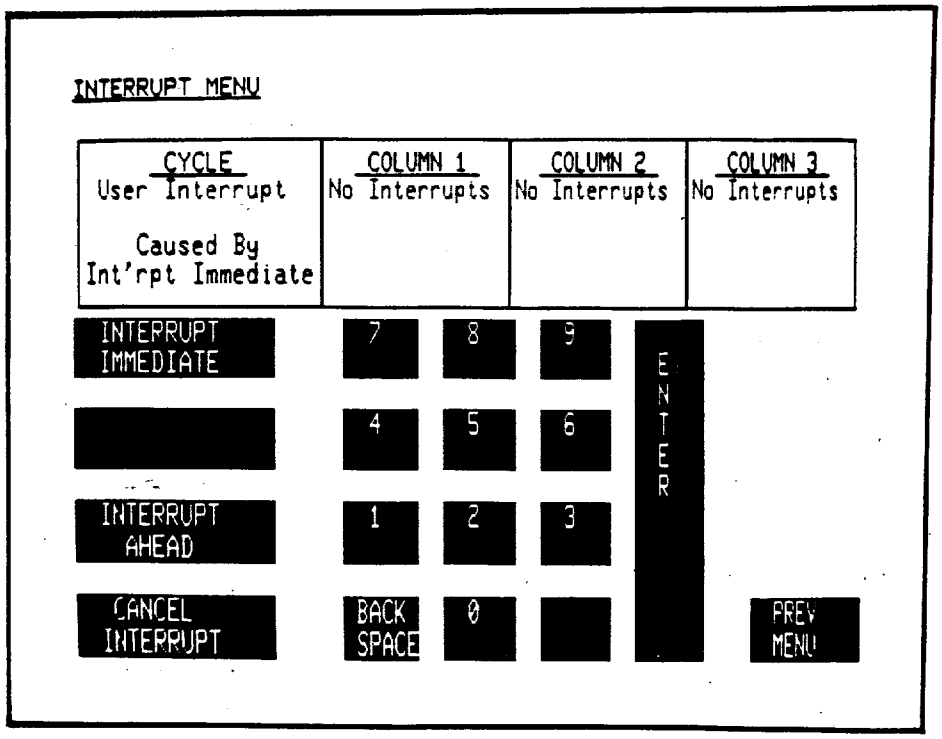
INTERRUPT STATUS

An "interrupt" is anything that causes synthesis to halt. It may be desire to interrupt synthesis for such reasons as to replace chemicals or to correct a problem. When synthesis is interrupted, it can be restarted or aborted.

INTERRUPT MENU

When this key is selected, the Interrupt Menu is displayed. This menu displays synthesis interruptions and allows the user to set synthesis interrupts.

The INTERRUPT MENU key is accessed from three menus: System Monitor, Column Monitor and Cycle Monitor.



This menu provides many choices on how synthesis is to be interrupted. Synthesis can be interrupted immediately or it can be programmed to interrupt ahead. In both instances, the Cycle can be interrupted in which all synthesis will pause, and/or the Column(s) can be interrupted in which synthesis for that particular column will stop.

INTERRUPT IMMEDIATE:

Synthesis pauses immediately by using the INTERRUPT IMMEDIATE key. Both the Cycle (all synthesis), and a particular Column synthesis can be interrupted immediately.

Cycle Interrupt Immediate:

To interrupt a Cycle immediately, first select the INTERRUPT IMMEDIATE key and then the CYCLE key.

INTERRUPT IMMEDIATE	When this key is selected, the CYCLE key becomes available.
CYCLE	When this key is selected, all synthesis pauses.

To resume synthesis, first select the CANCEL INTERRUPT key and then the CYCLE key.

CANCEL INTERRUPT	When this key is selected and only a Cycle interrupt is in progress, the CYCLE key becomes available.
CYCLE	By selecting this key, the Cycle interrupt is cancelled.

Column Interrupt Immediate:

To interrupt a particular column synthesis immediately, the Cycle is interrupted first (as described above) and then the Column can be interrupted.

INTERRUPT IMMEDIATE	When this key is chosen and the Cycle is interrupted, keys become available to select the COLUMN to be interrupted.

If all three columns are activated, the keys will be COLUMN 1, COLUMN 2, and COLUMN 3. Select the desired column(s).

To resume synthesis, the column interrupt is cancelled first and then the cycle interrupt is cancelled.

CANCEL INTERRUPT	When this key is selected with both the Cycle and Column interrupt in progress, the interrupted COLUMN keys become available.

Selecting the appropriate Column key, cancels the Column interruption. The Cycle can then be cancelled by touching the CANCEL INTERRUPT key again, and then the CYCLE key. Synthesis will then continue.

If CANCEL INTERRUPT is selected and there are no interrupts to cancel, the keys in the first column will be blank. In this instance, select UNDO EDIT from the keyboard.

UNDO EDIT	Can be selected when there are no other selections available in the first column.

NOTE: With an interrupt immediate in progress, no columns can continue synthesis until all interrupts are cancelled so that synthesis remains in synchrony. There is an exception, however. If the cycle is interrupted at Cycle End or Cycle Entry a column can be interrupted immediately with on-going synthesis of the other columns.

Situations that warrant a column interrupt immediate may be when it is imperative to abort the synthesis for that column. To pause synthesis of only one or two columns and continue synthesis with the third, it will be necessary to Interrupt Ahead.

IMPORTANT: Do not interrupt a synthesis at step 1 of the synthesis cycle. If this is done, the instrument will skip the addition of one base upon resuming the synthesis.

II. Interrupt Ahead

Cycle and Column synthesis can be interrupted at a designated point by selecting the INTERRUPT AHEAD key. By programming a synthesis interrupt, the Cycle interrupt can then be cancelled so that synthesis can continue for the uninterrupted column(s).

INTERRUPT AHEAD	When this key is selected, the CYCLE key and active COLUMN keys become available. When all three columns are activated, COLUMN 1, COLUMN 2 and COLUMN 3 keys will be available.

To Program a CYCLE Interrupt Ahead:

After selecting INTERRUPT AHEAD, choose the CYCLE key. A prompt is displayed at the right of the menu:

CYCLES AHEAD 0	Using the keyboard, enter the cycle number that the interruption is to occur and press ENTER. The next prompt is displayed:

STEP NUMBER 0	Using the keyboard, enter the cycle number for the interruption and press ENTER.

The Interrupt will be indicated in the CYCLE column as shown below. In this example, the interrupt will occur just before step 23, 7 cycles from now. The "CYCLES Ahead:" number will count down each time a "Cycle Entry" is reached.

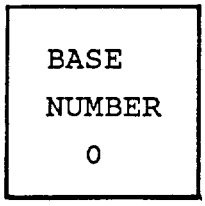
CYCLE	COLUMN 1	COLUMN 2	COLUMN 3
User Interrupt	No Interrupts	No Interrupts	No Interrupts

CYCLES Ahead:7
STEP Number :23

To Program an Interrupt Ahead for COLUMN 1; COLUMN 2; or COLUMN 3:

After selecting INTERRUPT AHEAD, choose the Column Number to be interrupted. Options will only include the activated column numbers. If all three columns are activated, the column options will be COLUMN 1, COLUMN 2, and COLUMN 3.

To program a Column Interrupt Ahead, select COLUMN 1, COLUMN 2, or COLUMN 3. A prompt is displayed at the right of the menu:



Using the keyboard, enter the base number for the interruption to occur. (Bases are counted from the 3' end.)

Synthesized DNA is most stabile at Cycle Entry and at Cycle End. Refer to Section 3 for a Cycle Entry and Cycle End description. To program a Column 'interrupt ahead', select one of the two options:

AT CYCLE ENTRY (Tr+)	If this key is selected, then the column will be interrupted at a Cycle Entry step. The DNA will be protected with the trityl on.
AT CYCLE END (Tr-)	If this key is selected, the column will be interrupted after the end of a Cycle and the DNA will be unprotected with the trityl off.

The column will stop synthesizing at Cycle Entry or Cycle End of the base number entered. The interrupt will be displayed in the appropriate column as shown below.

CYCLE	COLUMN 1	COLUMN 2	COLUMN 3
No Interrupts	No Interrupts	Int. Pending	No Interrupts
		Base # 9	
		At Cycle Entry	

CANCEL INTERRUPT

After this key is selected, the CYCLE key will be available.

After the Cycle interruption is cancelled by selecting CYCLE and then CANCEL INTERRUPT is selected again, the interrupted COLUMN keys become available. Select the desired Column number key to cancel the column interruption. Synthesis continues for the column selected.

In three column instruments:

If a synthesis is in progress when the column interrupt is cancelled, synthesis will continue at the next Cycle Entry step.

If all syntheses are interrupted, the first column interrupt that is cancelled will continue synthesis immediately. The other column(s) will continue synthesis at the next Cycle Entry step after the column interrupt is cancelled. In this manner, synthesis remains in synchrony for all columns.

TO ABORT SYNTHESIS:

To abort synthesis, 1) a Column Interrupt must be in progress, then 2) an abort key will then be accessible from the Column Monitor Menu.

1. If the column synthesis is in progress, first interrupt column synthesis from the Interrupt Menu. Refer to the preceding pages for a discussion of 'Interrupt Immediate' of the Interrupt Menu. A short version is given below.

CAUTION: When synthesis is interrupted rinse all chemicals from delivery lines and valve blocks. Salts could form if chemicals remain in these lines causing blockage. Replacement of expensive parts may be necessary.

From the Interrupt Menu, first select INTERRUPT IMMEDIATE; then select, CYCLE. By selecting INTERRUPT IMMEDIATE again, the selected COLUMN can be interrupted.

2. From the System Monitor Menu, select Column Monitor for this menu to be displayed. Then indicate the column to be aborted by selecting NEXT COLUMN. The ABORT COLUMN key is located in the lower left of the menu. When selected, a message will be displayed:

"Are you sure you wish to abort the column?"

Options are YES or NO. By selecting YES, synthesis for the column indicated, is aborted. Other columns will continue synthesis uninterrupted.

LOCK SCREEN

The touchscreen can be locked so that it does not respond to touch when unattended.

To lock the screen first select LOCK SCREEN from the System Monitor Menu.

LOCK SCREEN	When this key is selected, the menu page changes to the Name And Store page with the message "Input lock screen password".

Select any 3 characters as a code, from the alpha-numeric board and press ENTER.

NOTE: Record the code entered to lock the screen. If forgotten, the instrument will have to be turned off to unlock the screen.

The System Monitor Menu will be displayed and the only available option is to unlock the screen.

To unlock the screen, select UNLOCK SCREEN.

UNLOCK SCREEN	When this key is selected, the menu page changes to the Name And Store page with the message "Password to unlock screen.

Enter the same 3-character code used to lock the screen. The System Monitor Menu will be displayed with all options available.

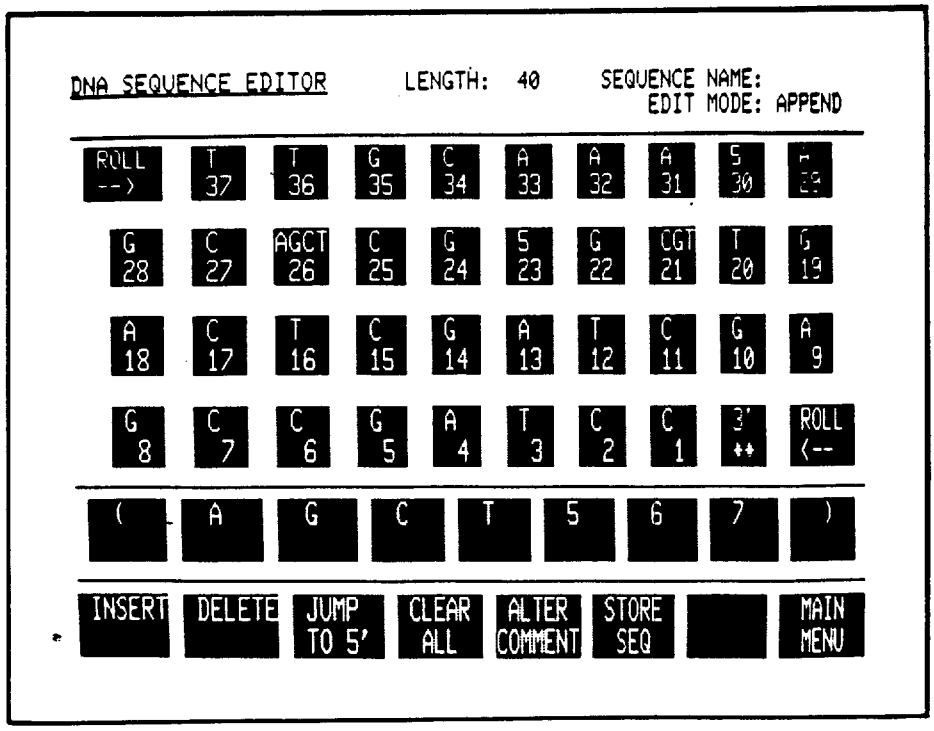
DNA SEQUENCE EDITOR

Introduction

From the DNA Sequence Editor:

* The base sequence can be created, given a name and stored on the disk;
* A stored DNA Sequence can be modified;
* The modified sequence can be be stored on the disk under the same name or it can be re-named;
* A comment identifying the sequence, can be given; and,
* The entire DNA Sequence can be viewed.

The DNA Sequence Editor:



Sequence Editor Description:

Types of Keys

The Sequence Editor can be divided into three sets of keys. Sequence keys will be located between the 3' and 5' terminals. Base keys are A,G,C,T,5,6,7 and the parentheses. Action keys are located at the last row of the menu.

The Cursor

The blinking box is the cursor position and dictates placement of the base. When the DNA Sequence Editor is first chosen, the cursor is at the 3' terminal. Base selections are placed at or next to the cursor, depending on the Edit Mode.

Edit Modes:

The Edit Mode indicates the type of editing in progress. Edit Modes are Modify, Append, Insert or Delete and differ by the location of the cursor. In the Append mode, the cursor is at the 3' terminal and a base selection is placed in front of cursor. With Edit Mode: Modify, the cursor is along the Sequence keys and not at the 3' terminal. In the Modify mode, base selections are placed at the cursor. When the Edit Mode: reads Insert, the cursor is a blank Sequence key where base selections will be placed. In the Delete mode, the cursor is moved to the position that is deleted.

To create a new sequence:

The DNA sequence entry can begin immediately by selecting the Base keys in the desired order beginning at the 5' end. When an Base key is chosen, it is viewed as a Sequence key.

Up to 36 Sequence keys can be viewed on screen at one time. To view other bases in the chain, the following selections can be made.

JUMP TO 5'	JUMP TO 5' is available only when more than 36 bases are displayed. When selected, this key moves the bases at the 5' terminal into view, and the JUMP TO 3' key becomes available.

JUMP TO 3'	This key moves the bases at the 3' terminal into view, and the JUMP TO 5' becomes available.

ROLL <---	ROLL --->	Each time an arrow is selected, the Sequence keys roll one position to view a higher or lower number. When a finger is held continuously against this key, the action is repeated.

Recovering From Entry Mistakes:

1. To recover from mistakes while entering the sequence, follow the discussion under "Insert" and "Delete" on the following page.

2. To clear the sequence from view:

CLEAR ALL	CLEAR-ALL is used to recover from numerous mistakes and erases the entire DNA sequence.

"Respond YES If You Wish To Clear", prompts the user to respond by selecting YES or NO. Responding "YES" erases all bases.

To Store the DNA Sequence:

When the base selections are complete the sequence is ready to be named and stored.

STORE SEQ	After the user selects STORE SEQ., the display changes so the DNA Sequence can be named and stored on the disk. Refer to page 4-8 for use of the Name and Store Menu.

To Modify a Sequence:

A DNA sequence can be modified by 1)changing, 2)adding or 3)deleting bases. First select the DNA Sequence to be modified, by choosing the Action key LOAD.

LOAD SEQ

When LOAD SEQ is selected, the disk goes through the File Finder Routine and displays DNA Sequences stored on the disk. The selected sequence is displayed between the 3' and 5' terminals.

LOAD is available only when the Sequence Editor is first selected and when the menu is clear of Sequence keys. If Sequence keys are present, LOAD SEQ changes to STORE SEQ. To change from option STORE SEQ to option LOAD SEQ, select CLEAR ALL and YES.

1) To change a base, bring the cursor to the desired position by touching the appropriate Sequence key. The Sequence key will be blinking and the "Edit Mode: Modify" will be displayed. The base is replaced with the selected base key.

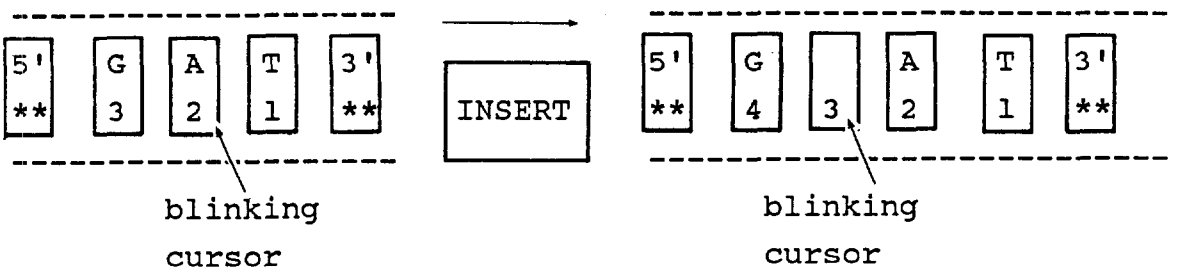
2) To add a base between two bases, the INSERT key is used. The menu page changes slightly in that the previous Action keys are no longer available, EXIT INSERT becomes an option, and "Edit Mode: Insert" is displayed.

INSERT

INSERT places an empty Sequence key at the left of the cursor. The empty key is now the cursor position.

When INSERT is selected and the cursor is at a Sequence key, the empty key will appear to the left. The base that occupied that position moves to the left. Also, an empty Sequence key can be moved simply by touching any Sequence key.

An example is shown below:



When the empty key is in position, select the desired base key to be inserted into the sequence. The Sequence key to the left will be empty, numbered and blinking.

More bases can be inserted if desired by repeating this procedure. The empty Sequence key is removed when EXIT INSERT is touched.

EXIT INSERT

EXIT INSERT makes the previous keys keys available. The Edit function changes to the Append or Modify mode, depending on the cursor position.

3) To delete a base from the DNA chain, first select DELETE. The menu page changes slightly and the Edit Mode: reads Delete. Action key EXIT DELETE becomes available. .

DELETE

DELETE changes the menu page and activates the delete mode.

To delete a base from the chain, simply touch the Sequence key to be deleted. This will rid the sequence of the base and renumber the chain. This procedure can be repeated if desired. After a deletion has been performed, the key UNDO DELETE becomes an option.

UNDO DELETE

UNDO DELETE brings only the last base deleted back into view.

The delete mode is active until EXIT DELETE is touched.

EXIT DELETE	EXIT DELETE makes the previous Action keys available and ends the option to undo any deletes made.

Recovering from Entry Mistakes:

CLEAR ALL	CLEAR-ALL is used to recover from numerous mistakes and erases the entire base sequence.

The message "Choose YES if you wish to clear" appears. Selecting YES erases the entire sequence. By selecting NO, the sequence remains and the previous option keys are available.

To Store the DNA Sequence

When the base selections are complete the sequence is ready to be named and stored.

STORE SEQ	After answering a prompt, STORE SEQ. changes the menu page so the DNA Sequence can be named and stored on the disk. Refer to page 4-8 for use of the Name and Store Menu.

DNA Sequence Comment

A comment can be given to the sequence for further identification or information by selecting ALTER COMMENT.

ALTER COMMENT	When this key is selected, the Name and Store Menu is displayed. A 20 character comment can be given.

CYCLE AND PROCEDURE EDITOR

Introduction:

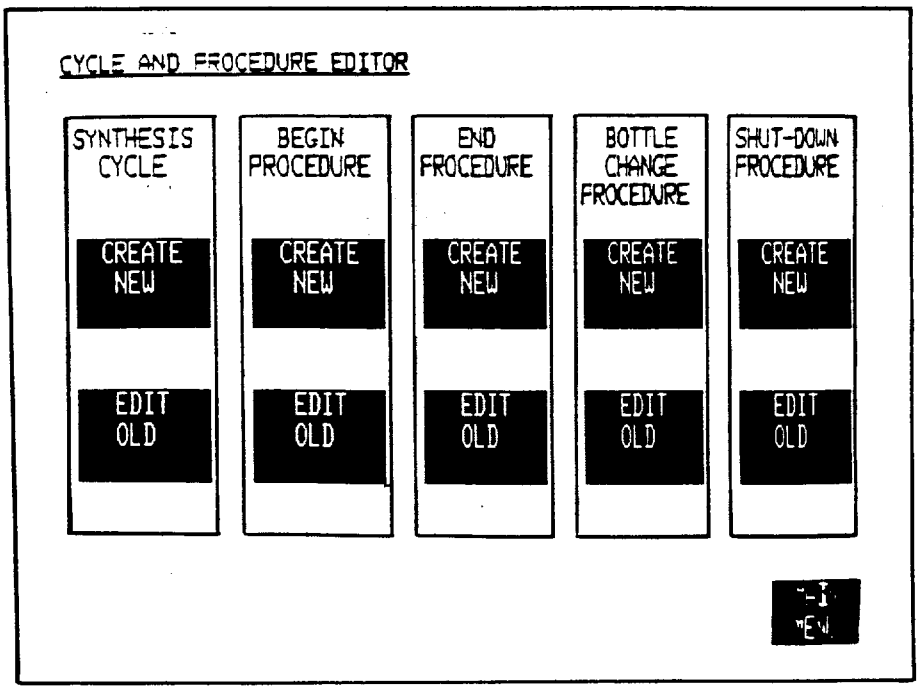
From the Cycle and Procedure Editor, the user can create or revise Cycles and/or Procedures that are named and stored on the disk. Refer to Section 3 for a description of each Cycle and Procedure.

Creating a new Cycle or Procedure consists of entering steps. A step consists of a function number and the amount of time the function is to occur. The functions are entered in an order to produce an end chemical result. The new Cycle or Procedure is then named and stored on the disk.

NOTE: Function "Cycle Entry" is required to be in all Cycles. Refer to Section 3 for a discussion. Refer to User Bulletin #34 for other required functions when creating new cycles.

To revise an existing Cycle or Procedure, a function or amount of time for a particular step can be changed. Steps can also be added or deleted from the cycle. The revised Cycle or Procedure is named and stored on the disk.

The Cycle And Procedure Editor:



CREATE NEW

CREATE NEW is selected to make a new cycle.

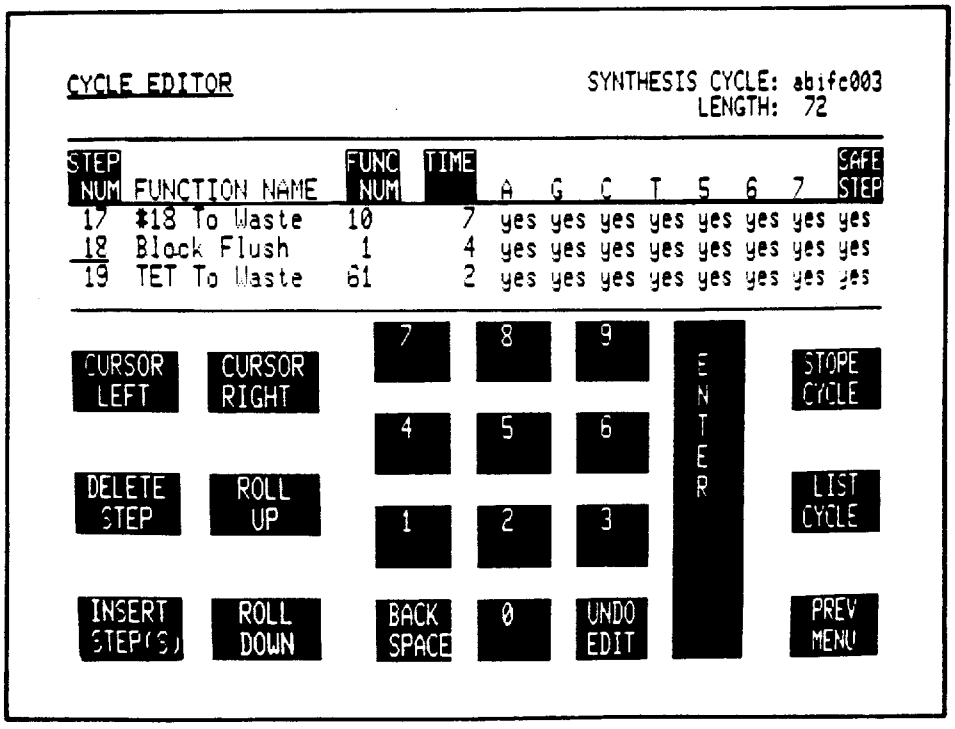
To create a cycle, first select CREATE NEW in the desired column. The menu page changes to view the Cycle Editor.

EDIT OLD

EDIT OLD is selected to revise an existing Cycle or Procedure.

To revise a cycle, first select the "Edit Old" option listed in the desired column. The disk then goes through the File Finder Routine and searches for all files of that type and displays them as choices on the screen. After a Cycle or Procedure is selected, the Cycle Editor Menu is displayed.

The Cycle Editor:



Cycle Editor Description:

Three steps or lines of a Cycle or Procedure are viewed as shown below:

STEP NUM	FUNCTION NAME	FUNC NUM	TIME	A	G	C	T	5	6	7	SAFE STEP
10	Block Flush	1	10	YES	YES	YES	YES	YES	YES	YES	YES
11	#18 To Column 9		25	YES	YES	YES	YES	YES	YES	YES	YES
12	#18 To Column 9		10	YES	YES	YES		YES	YES	YES	YES

The Cursor

The cursor moves only along the "edit line" which is the middle step line. All entries are made here. The cursor can be moved across the edit line in a number of different ways:

The cursor moves to the appropriate column by touching the STEP NUM, FUNC NUM, TIME, or SAFE STEP keys.

CURSOR LEFT	CURSOR RIGHT

Using CURSOR LEFT and CURSOR RIGHT moves the cursor over one column; and,

ENTER

The ENTER key automatically moves the cursor to the next column.

YES or NO

For each base (A, C, G and T), user-defined phosphoramidites (5, 6, and 7) and for SAFE STEP, a "YES" or "NO" can be programmed. A YES in the base columns (A C G T 5 6 7), indicates that the step will be performed for the addition of that base. A blank (or "NO") in a base column, indicates that the step will not be performed for that base addition. In the above example, Step 12 will not be performed for the addition of T.

Safe Step:

Steps are indicated as SAFE or UNSAFE in the event of a power failure. A "YES" in the SAFE STEP column, indicates that if synthesis is interrupted by a power failure, then synthesis will continue when the power returns.

Unsafe Step:

A blank in the Safe Step column indicates that the step is UNSAFE. If a power failure occurs at an UNSAFE step and exceeds the maximum power fail time*, then synthesis will not restart when the power comes back on. Also "User Interrupt" will be listed under "Cycle" in the Interrupt Menu.

Synthesis can then be continued or aborted. When the interrupt is cleared, synthesis will resume at the step where the power failure occurred. In the above example, synthesis will continue if a power failure occurs at any of the steps displayed.

* Refer to the Power Fail Menu to set the Maximum Power Fail time.

To Create Cycles:

When a cycle is being created, steps can be entered immediately. The prompt reads:

"Enter FUNCTION NUMBER and TIME for step:".

With the cursor in the FUNC NUM column, enter the function number using the keyboard, and press ENTER. The cursor automatically moves to the TIME column. Next, enter the amount of time for that step to occur, and then press ENTER.

YES, automatically appears in the base and Safe Step columns. If it is desired not to perform this step for a particular base addition, place the cursor in the appropriate base column and select NO. A blank will appear, indicating that this step will not be performed for that base addition.

Next, if the step is to be designated as UNSAFE, place the cursor in the SAFE STEP column and select NO. A blank indicates an Unsafe step.

When all the steps are entered, the cycle can be viewed and checked for errors by selecting LIST CYCLE. If input errors have occurred, follow the discussion below on revising cycles.

When complete, the cycle can then be given a name and stored on the disk. Refer to page 4-8 to Name and Store the Cycle.

To Revise Cycles:

For any kind of modification, the STEP must first be brought to the edit line.

ROLL UP	ROLL DOWN	When selected, these keys move a step up or down one line respectively.

STEP NUM	When this key is selected, enter the number of the step to be placed on the edit line.

Then, follow the procedures below on how to modify an existing step; add a step in a cycle; or delete a step in a cycle.

1. To Modify an Existing Step

With the step on the edit line, place the cursor in the column where the change is to take place (FUNC NUM, TIME, A, G, C, T, 5, 6, 7 or SAFE STEP). Enter the number using the keyboard, or YES or NO and then press ENTER.

UNDO EDIT	If UNDO EDIT is selected, the edit line will show the step as it appeared before editing.

2. To Add a Step

Adding a step in a cycle is accomplished by using the INSERT key. First, bring the step that will appear before the added step, to the edit line.

STEP NUM	FUNCTION NAME	FUNC NUM	TIME	A	G	C	T	5	6	7	SAFE STEP
24	#18 To Column	9	15	YES	YES	YES	YES	YES	YES	YES	YES
25	Reverse Flush	2	5	YES	YES	YES	YES	YES	YES	YES	YES
26	Block Flush	1	4	YES	YES	YES		YES	YES	YES	YES

INSERT	INSERT adds a blank step at the edit line. The option to EXIT INSERT becomes available.

STEP NUM	FUNCTION NAME	FUNC NUM	TIME	A	G	C	T	5	6	7	SAFE STEP
25	Reverse Flush	2	5	YES	YES	YES	YES	YES	YES	YES	YES
25			___	YES	YES	YES	YES	YES	YES	YES	YES
26	Block Flush	1	4	YES	YES	YES	YES	YES	YES	YES	YES

The step that occupied the edit line moves to the line above the edit line. The edit line is blank and given the next step number. All subsequent steps are renumbered. In this manner, the new step is placed before the step that previously occupied the edit line.

With the cursor in the Function Number column, enter the desired Function number and select ENTER. Next, with the cursor in the Time column, enter the amount of Time for that function to occur and select ENTER.

YES automatically appears in the base and Safe Step columns. If it is desired <u>not</u> to performed this step for a particular base addition, place the cursor in the appropriate base column and select NO. A blank indicates that this step will not be performed for the base addition.

Next, if the step is to be programmed as as UNSAFE, place the cursor in the SAFE STEP column and select NO. A blank indicates an unsafe step. Refer to the previous page for an explanation.

EXIT INSERT

EXIT INSERT removes the blank line from the edit line and brings the previous menu options into view.

3. To Delete a Step

Erasing a step in a cycle is accomplished by using the DELETE key. First, bring the step to be deleted to the edit line.

DELETE	DELETE erases the step in the edit line and moves the next step into that position. All subsequent steps are renumbered.
UNDO EDIT	UNDO EDIT brings back to view only the last step deleted.

To view steps in a cycle:

LIST CYCLE can be used to view more steps of the cycle on the screen. If a printer is connected and indicated present from the Self Test Menu, the cycle can also be printed.

LIST CYCLE	When a printer is connected and LIST CYCLE is selected, options SCREEN and PRINT become available.

If a printer is not connected, PRINT is not an option. To display 12 steps of the cycle on the touchscreen, select SCREEN. To print the steps, select PRINT.

ROLL UP / ROLL DOWN	moves the steps up or down one line.
NEXT PAGE / PREV PAGE	brings the next or previous 12 steps into view.

Name and Store the Cycle:

When cycle entries are complete, the cycle can be named and then stored on the disk. The option STORE CYCLE, changes the menu to the Name and Store Menu page. The prompt reads "Enter desired file name". A cycle can be named using up to 8 characters. Refer to page 4-8 for use of this menu.

NOTE: Standard files, named with lower case letters, cannot be overwritten or purged.

If the cycle is not stored and the Prev Menu key is selected, the following message is displayed:

WARNING: Cycle not stored! Exit to Prev Menu?

This message is to prevent loss of a cycle that has inadvertently not been stored.

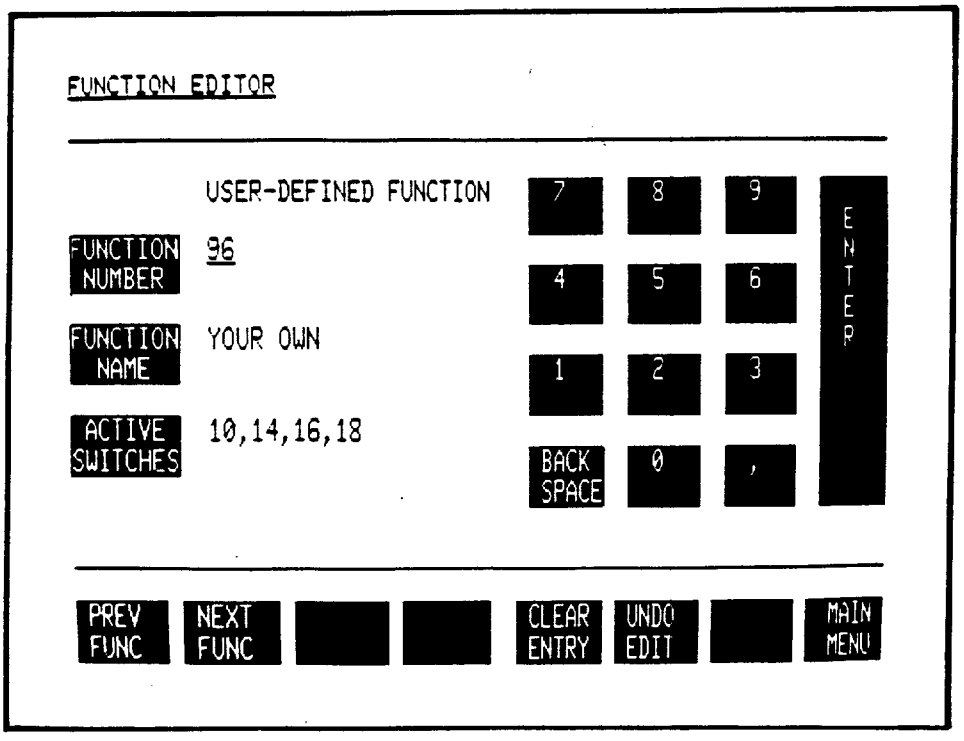
FUNCTION EDITOR

Introduction:

Functions for all processes necessary for synthesis are included with each 380B instrument. Information on each function including the function name and valves can be viewed in the Function Editor. Refer to Appendix I for a list of all standard functions.

The user may define new functions for use in cycles, procedures or manual control and test. Functions are defined by listing the valve(s) which will be activated and by giving the function a name.

The Function Editor:



1. To view function information:

When this menu is first selected, Function Number 1 with the function name and a list of valves that are activated is displayed. To view the next or previous function number, select one of the following keys.

NEXT FUNCTION

NEXT FUNCTION and PREVIOUS FUNCTION displays information on the next or the previous numbered function.

To view a specific function number, place the cursor next to the Function Number key and enter the function number using the keyboard. Then, press ENTER. The function name and valve(s) activated are displayed.

2. To program user-defined functions:

Function Numbers 92 through 99 are undefined functions that the user can define by programming which valve(s) are to be activated, and by giving the function a name.

To define a function, select the FUNCTION NUMBER key by touching it. Then, using the numeric keyboard, touch-in the number (valid entries are 92 through 99), and press ENTER. If the function has not been defined previously, the function name reads "Undefined" and no active valves will be listed.

Next, a function is named. When the FUNCTION NAME key is selected, the Name And Store Menu is displayed where a 14- character name can be given. Refer to page 4-8 for use of the Name And Store Menu.

Then the valve number(s) that will be activated by this function are entered. With the cursor at the ACTIVE VALVES key, enter the valve numbers(s) using a comma between each number.

CURSOR LEFT

CURSOR LEFT and CURSOR RIGHT move the cursor across a line.

CLEAR ENTRY	CLEAR ENTRY erases all the characters on the line at the cursor.
UNDO EDIT	UNDO EDIT displays the function as it was before editing.

If a printer is connected to the 380B, user-defined functions can be printed using the PRINT key. The function is stored when PREV MENU is selected.

NOTE: Standard Functions cannot be altered or printed.

IMPORTANT: User-defined functions are stored on the disk that is in the Synthesizer at the time they are defined. Therefore, when a disk is changed the user-defined functions must be redefined and stored on the new disk.

A few valve numbers cannot be included in user-defined functions and include numbers 37, 38, 39, and 46 or greater. The message Invalid switch number will appear at the top of the menu if any of these have been used. For use of valve number 27, refer to Section 3.

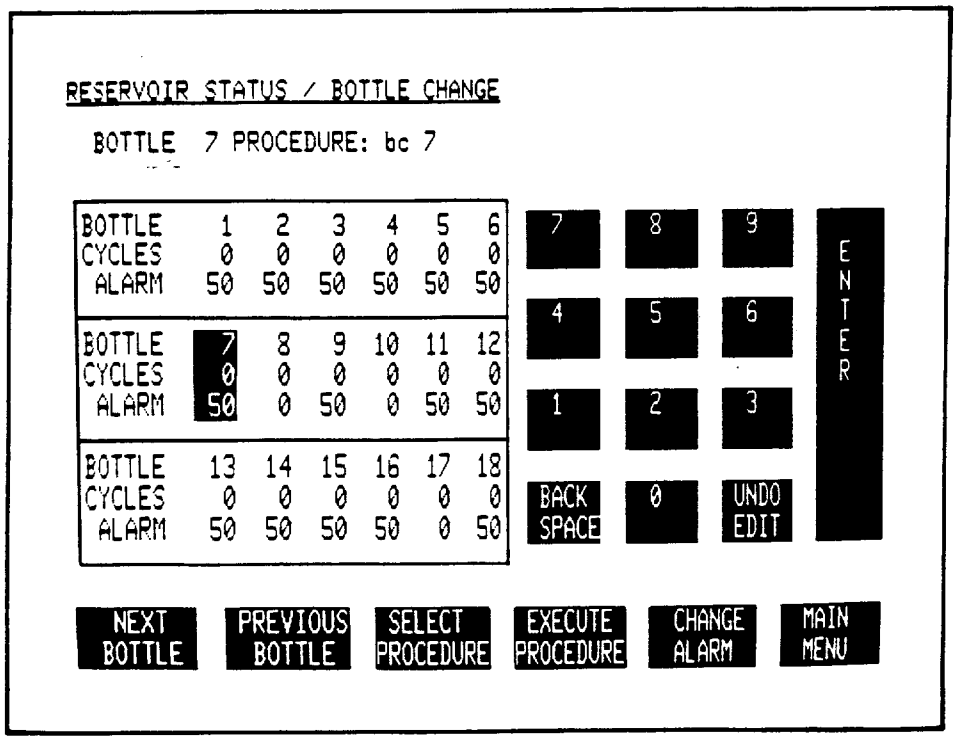
RESERVOIR STATUS/BOTTLE CHANGE

The Reservoir Status/Bottle Change Menu is used to change reservoir bottles. When a bottle is changed, a "Bottle-Change Procedure" is performed. During this Procedure the delivery line is flushed; the bottle is changed and re-pressurized; the delivery line is purged with fresh reagent; and the valve block is rinsed and dried.

A bottle can be changed during synthesis by interrupting synthesis at a safe step, preferably at "Cycle Entry" or at "Cycle End". To interrupt synthesis, refer to the Interrupt Status Menu.

An alarm can be set through the Reservoir Status Menu. The alarm is set by entering the number of cycles that a bottle can be accessed before the reagent is completey used. When the bottle has been accessed for the "set" number of cycles, synthesis will be interrupted at Cycle Entry and the alarm will sound.

The Reservoir Status Menu



The cursor is the highlighted area which contains a number for the BOTTLE, CYCLE and ALARM. The BOTTLE is the reservoir number. The CYCLE number is a count of the cycles since the bottle was last changed. Each time the cycle reaches "Cycle End" the CYCLE number increments. The ALARM is the number of cycles the bottle can be accessed before the reagent empties. When the ALARM equals the CYCLE number, synthesis will be interrupted at "Cycle Entry" and the alarm will sound.

WARNING: Consider each chemical in the Synthesizer potentially harmful. Do not inhale. Work in a well ventillated area. Do not leave any chemicals uncapped. Immediately consult a physician if any chemical in ingested. If there is any physical contact, wash immediately with ample water. Wear eye protection. In case of contact with eyes consult a physician immediately.

To change a reservoir bottle:

1. Indicate the reservoir to be changed by placing the highlighted cursor over the bottle number.

NEXT BOTTLE	When selected, the highlighted cursor moves to the next higher numbered bottle.
PREVIOUS BOTTLE	When selected, the highlighted cursor moves to the next lower numbered bottle.

2. The Bottle-Change Procedure is noted at the top of the menu. If desired, another Procedure can be selected by choosing SELECT PROCEDURE.

SELECT PROCEDURE	When this key is selected, the disk goes through the File Finder Routine and displays the Bottle-Change Procedures stored on the disk. The selected Procedure will be noted on the menu page and will be run.

3. Next Select:

START PROCEDURE	When this key is selected, the menu changes so that the Procedure activities can be viewed at the right of the menu. The EXECUTE PROCEDURE and CANCEL PROCEDURE keys become available.

NOTE: The Bottle-Change Procedure does not start until EXECUTE PROCEDURE is selected. This was incorporated to prevent inadvertent selection of the start key.

EXECUTE PROCEDURE	When this key is selected the Procedure listed at the top of the menu is performed.

NOTE: Once this key is selected, the Bottle-Change Routine cannot be aborted, however, it can be paused by selecting PAUSE PROC.

When the Bottle-Change Procedure, reaches the interrupt function, a message is displayed at the top of the menu page notifying the user that the bottle can be changed.

After the bottle is changed select CONTINUE PROCEDURE. The Procedure continues and re-pressurizes the bottle, purges the delivery line and flushes the valve block.

To set the alarm:

To set the alarm, first place the highlighted cursor over the desired bottle number. Then select:

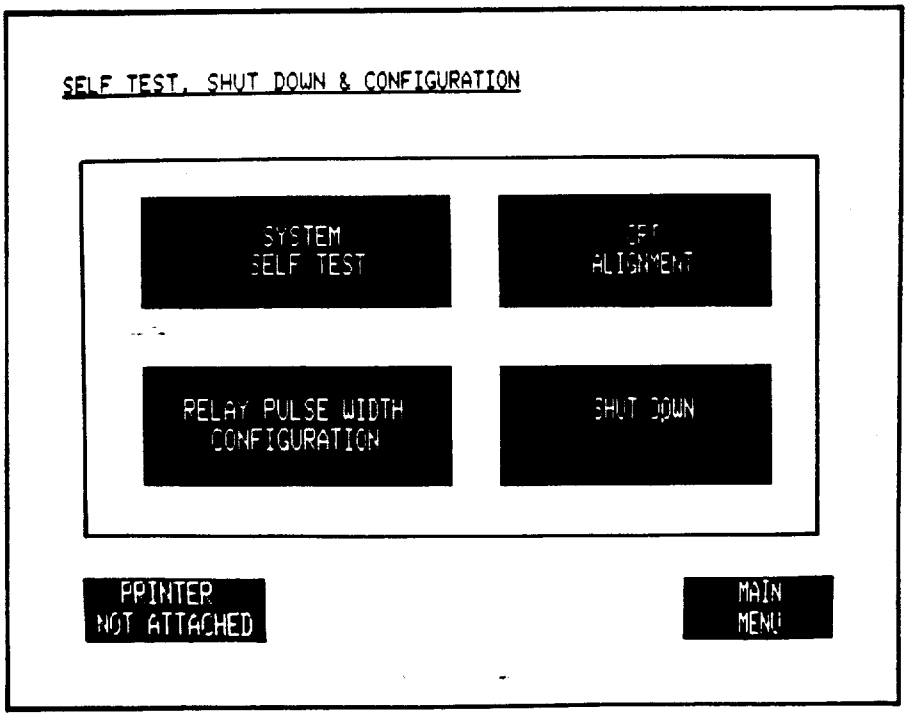
CHANGE ALARM	When this key is selected, a prompt is displayed to enter the number to set the alarm. Enter the number of cycles that the reservoir can be accessed before it empties.

SYSTEM SELF TEST, CRT ALIGNMENT, SHUT DOWN PROCEDURE and RELAY CONFIGURATION

Introduction:

From this menu, several tasks can be performed:

1) It is indicated that a printer is connected or not connected to the Synthesizer.
2) An electrical test of component boards can be performed through Self Test.
3) The CRT can be checked for alignment.
4) A Shut-Down Procedure can be performed when the instrument will not be in use for long periods of time.
5) The pulse width to the relay port can be set.



1) PRINTER ATTACHED/NOT ATTACHED

When a printer is connected to the Synthesizer, it must be indicated as present. This is accomplished with use of the Printer Attached/Not Attached key. This key rolls to view ATTACHED and NOT ATTACHED, each time it is touched.

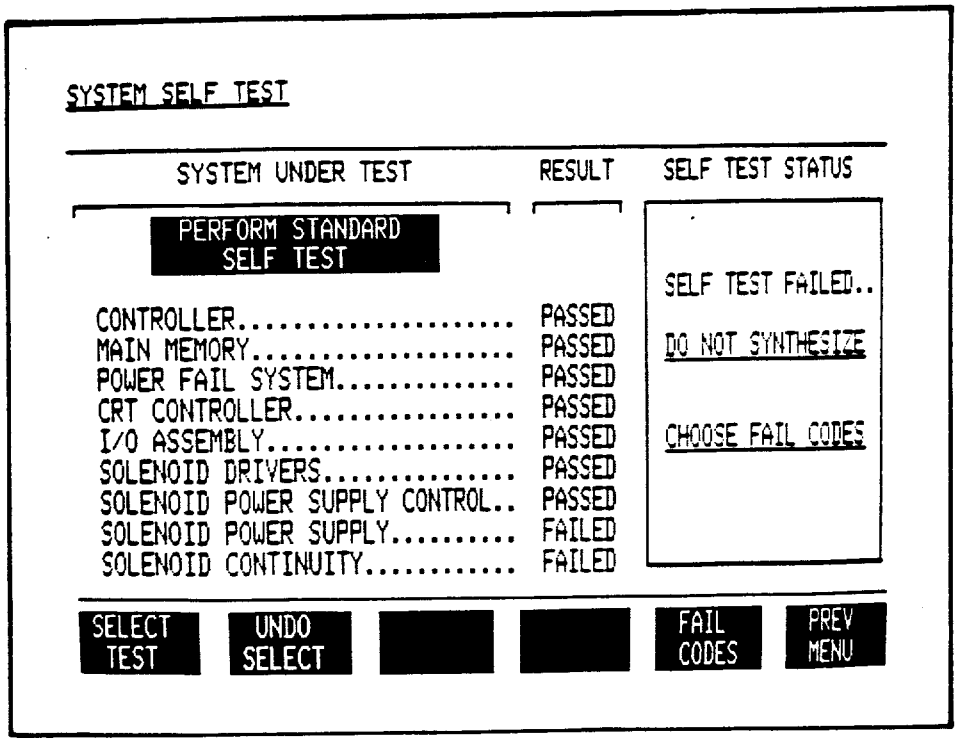
PRINTER ATTACHED	When a printer is connected to the Synthesizer and the PRINTER ATTACHED key is viewed, the PRINT key will be available in various menus.
PRINTER NOT ATTACHED	The PRINTER NOT ATTACHED key should be viewed when the printer is not connected to the Synthesizer.

2) SYSTEM SELF TEST

Introduction:

System Self Test performs an electrical check of component boards. System Self Test should be performed periodically to ensure the 380B is electrically functioning properly. The boards that are checked, are listed in the "System Under Test" column of the Self Test Menu. Results, can be either Passed or Failed. When an component board fails, a code can be viewed which provides information of the specific test failed.

The Self Test Menu:



Either the Standard Self Test, or a Selected Self Test can be performed. The Standard Self Test checks all the component boards, and the Selected Self Test checks only the component boards that have been designated.

Standard Self Test

PERFORM STANDARD SELF TEST	When this key is selected, all the tests listed on the menu are performed once. This key is replaced with the CANCEL TEST IN PROGRESS key.

As each component board completes the check, either "Passed" or "Failed" is displayed in the Result column. When all tests Passed, the message "Electronics ready for operation" is displayed at the right of the menu. If the component board failed, the message prompts the user to select FAIL CODE. (The Fail Code description, follows the discussion on Select Self Test.)

CANCEL TEST IN PROGRESS	When this key is selected, all testing stops and the option PERFORM STD SELF TEST is available.

Select Self Test

It may be desired to test only one or more of the component boards listed. This can be done using the SELECT TEST key.

SELECT TEST	When the SELECT TEST key is touched, an arrow is displayed next to one of the component boards. The arrow moves down to the next line each time this key is touched. Place the arrow next to the desired system to be tested.

When the arrow is pointing at the desired component board, then touch ENTER SELECT.

ENTER SELECT	When ENTER SELECT is chosen, the selected board will be tested when Self Test is started. The selected component board is designated by a dash.

Other boards can be chosen to be tested by repeating this procedure. To omit a selected board from the Selected Self Test, choose UNDO SELECT.

UNDO SELECT	When this key is selected, the component board next to the arrow, will not be tested.

To start the Select Self Test, choose either START ONCE or START REPEAT.

START ONCE	When this key is touched, Self Test is run once for each of the selected boards.
START REPEAT	When this key is touched, Self Test is run and repeated continuously for each of the selected boards. Testing stops when CANCEL TEST IN PROGRESS is selected or until a failure has occurred. A count of the number of times the test has repeated is displayed at the right of the menu.

As each component board completes the check, either "Passed" or "Failed" is displayed in the Result column. When all the selected tests Pass, a message is displayed at the right of the menu:

"Selected Tests Passed, Be Sure Standard Self Test Has Been Performed!"

If the component board Failed, the message prompts the user to select FAIL CODE.

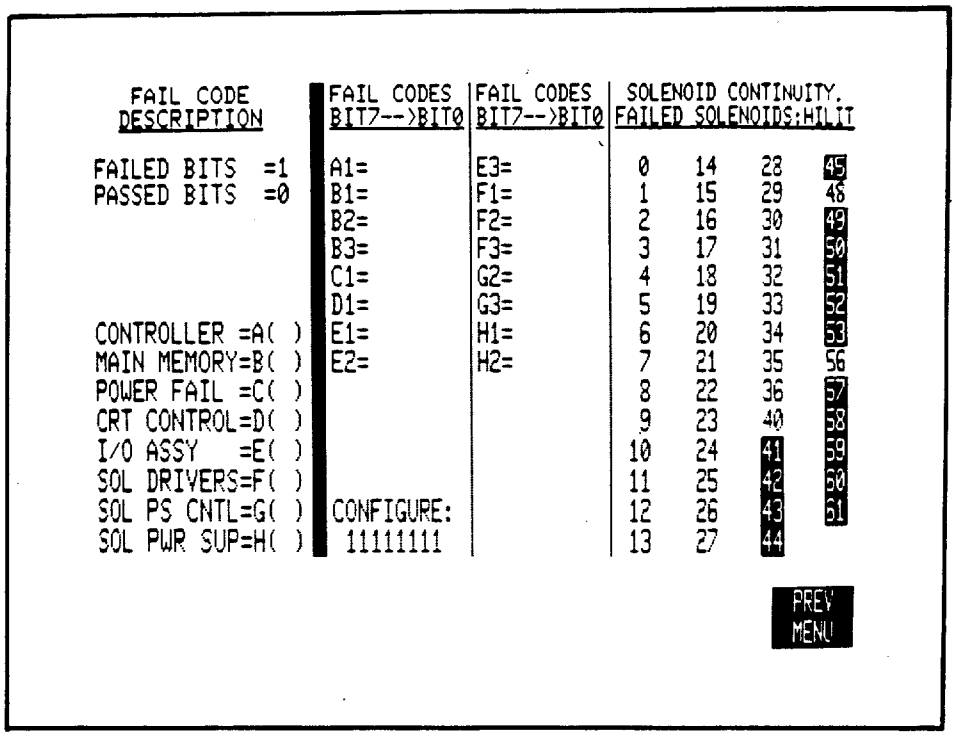
Fail Codes

The Fail Code Menu displays a code or a failed valve number that can be helpful in determining the cause of the failure.

NOTE: This menu can only be displayed after a component board has failed the Self Test.

FAIL CODE	FAIL CODE, changes the menu page to view the specific failure that occurred in the form of a code.

The Fail Code Menu:



Failed valves

The right columns will display any valves that have failed the Solenoid Continuity Test by highlighting the valve numbers. Note any highlighted switch numbers and call your Applied Biosystems Service Representative.

Fail Codes

The fail codes describe the specific test that failed and are listed in the two "Fail Code" columns.

An example of a fail code is: H1 = 00001010

Letters range from A to H and represent each component board tested. These are listed below and in the left column of the menu.

Controller =A	I/O Assy =E
Main Memory =B	Sol Drivers =F
Power Fail =C	Sol PS Cntl =G
CRT Control =D	Sol Pwr Sup =H

A component board may go through as many as 3 different tests. The number beside the letter can be 1, 2, or 3 and indicates the specific test that failed.

The last 7 digits of the fail code are noted in BITs and are displayed as 0's and 1's. A display of "1" indicates a failure while "0" indicates the test passed. BITs are read from the right-most column or column 0 to the left-most column or column 7.

In the example fail code, (H1 = 00001010), the failure occurred in test 1 of the Solenoid Power Supply at BITs 1 and 3. Should a fail code appear, note it down and contact your Applied Biosystems Representative.

Configuration Switch

The Configure Switch is read by the Synthesizer during Self Test, and indicates that the instrument is run by 50 Hz or 60 Hz and that the instrument has 1 or 3 columns. Depending on the configuration of the instrument, the switch will read as follows:

	50Hz	60Hz
1 Column	00000000	00000001
3 Column	00000010	00000011

Configuration Switch Readings

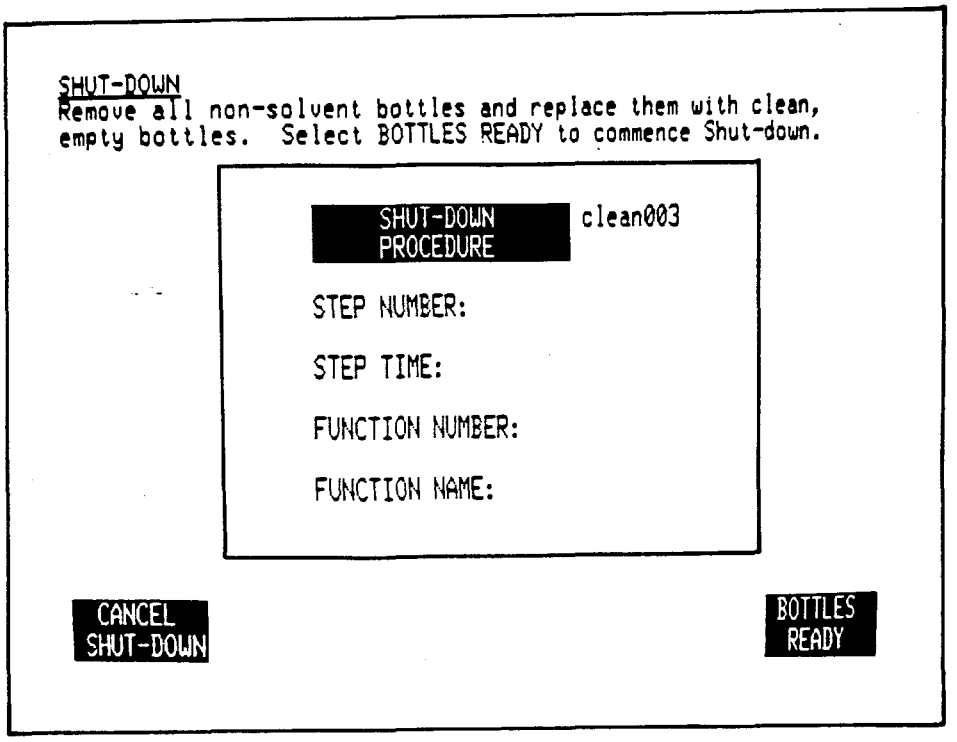
3) CRT DISPLAY ALIGNMENT

The CRT can be periodically checked for proper alignment. First place your finger in the middle of the line labeled VERTICAL. An asterisk will appear at the top of this line. Keeping your finger against the screen move your finger to the middle of the line labeled SIZE. An asterisk should appear over this line. This procedure can be repeated with the HORIZONTAL line.

If the asterisk appears while your finger is elsewhere, the touchscreen may be out of alignment and needs adjustment. Call your Applied Biosystems Service Representative.

4) SHUT-DOWN PROCEDURE

The Shut-Down Procedure should be performed when the instrument is not going to be used for 2 weeks or longer. This Procedure rinses and cleans the lines and valve blocks.



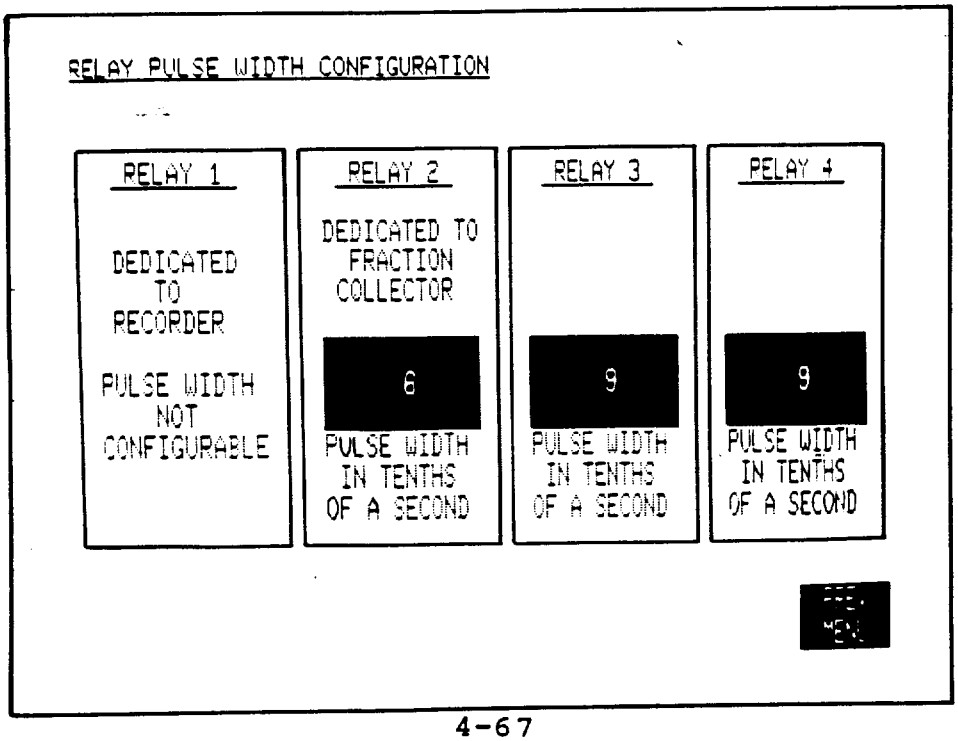
First, if the desired Shut-Down Procedure is not displayed, touch the SHUT-DOWN PROCEDURE key. The disk goes through the File Finder Routine and the desired Procedure can be selected by touching it. Next, select START SHUT-DOWN. A prompt appears at the top of the menu:

> Remove all non-solvent bottles and replace them with clean, empty bottles. Select BOTTLES READY to commence Shut-down.

After following these instructions and BOTTLES READY is selected, the Procedure starts with the activities being displayed on the screen.

5) RELAY CONFIGURATION

The RELAY PULSE WIDTH CONFIGURATION key is used to set the pulse width. The pulse width is the duration of the electrical pulse sent to the relay port on the back of the 380B. This pulse creates a contact closure for the operation of an external event such as a recorder or a Fraction Collector. The pulse is set according to the individual requirements of the external event.



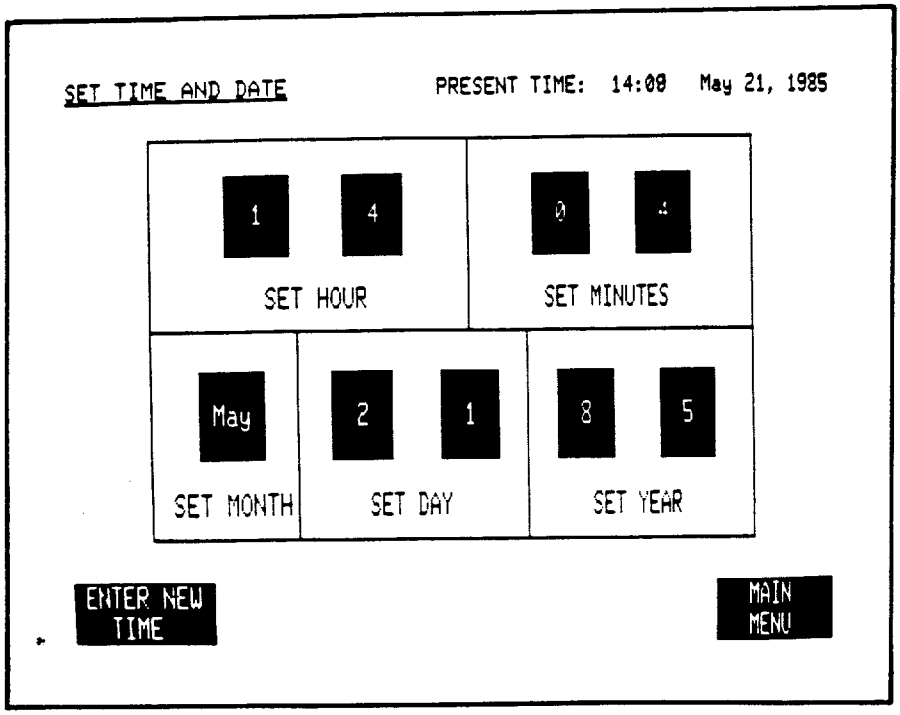
There are four relays in the Model 380B, numbered 1 through 4. Relay 1 is dedicated for use with Strip Chart Recorder and the pulse width cannot be changed. Relay 2 is dedicated to connect to a Fraction Collector and should be set to the appropriate pulse width of the Fraction Collector used. Relay 3 and Relay 4 can be connected to external events in accordance with the users needs and the pulse width can be set to the external event requirements.

The pulse duration for Relays 2, 3 and 4, can be individually set for 0.1 to 1.0 seconds in 0.1 second increments through this menu. To set the pulse width, touch the number key in the desired relay column. Each time the key is touched, the numbers increment. The numbers in the key are in tenths of a second.

SET TIME AND DATE

From the Set Time And Date Menu, the user can set or change the hour, minute, month, day and year. Time and date are used in menus, such as the System Monitor to display the start time and stop time, in creating or modifying files and in printouts.

The Set Time And Date Menu:

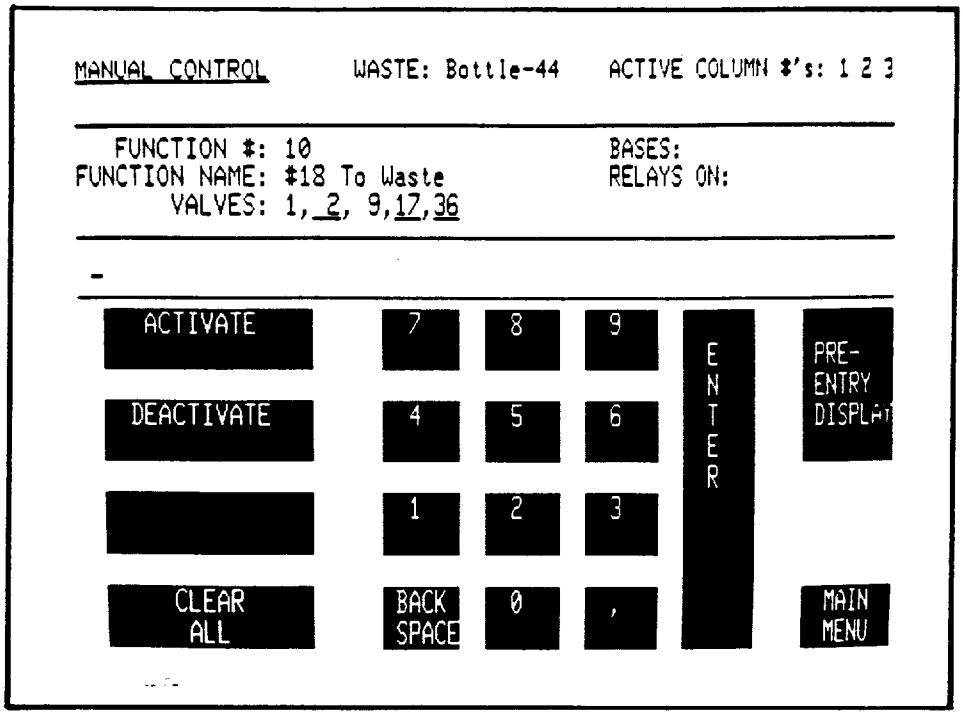


Touching the keys that indicate the time and date, increment the numbers or month. When the desired numbers are in place, select ENTER NEW TIME.

Introduction:

Activation and deactivation of functions and individual valves and the column(s) can be performed from the Manual Control Menu.

The Manual Control Menu:



Menu Description:

Waste can be directed either to the waste bottle through valve number 44 or to the waste port through valve 43, and is indicated on the first line of the menu. Activated columns are also noted on the first line of the menu.

When the Manual Control Menu is first selected, options displayed in the first column are ACTIVATE, DEACTIVATE, and CLEAR ALL. When the Activate or Deactivate key is selected, this column of keys will change to display FUNCTION NUMBER, VALVE NUMBER(S), COLUMN NUMBER(S) and CLEAR ENTRY. The COLUMN NUMBER(S) key is only present in three-column instruments.

Turning Valves, Functions and Columns On and Off:

To turn a valve, function or column on or off, first choose option ACTIVATE or DEACTIVATE.

ACTIVATE

Valves, functions or columns are turned on by using the ACTIVATE key.

NOTE 1: In three-column instruments each column must be activated before chemical deliveries can be made. In one column instruments the column is always activated.

NOTE 2: Chemicals accessed through the Manual Control Menu, are not counted as CYCLES used in the Reservoir Status Menu. Refer to the Reservoir Status Menu for details.

DEACTIVATE

Valves, functions or columns are turned off by using the DEACTIVATE key.

This column of keys change for further selections that define what can be activated or deactivated. The choices are:

FUNCTION NUMBER	VALVE NUMBER	COLUMN NUMBER(S)

Using the keyboard, enter the number of the valve, function or column to be activated or deactivated. Selections are displayed in the display area.

Activating Valves or Columns:

More than one valve or column can be activated or deactivated at a time by using a comma between each valve number.

Activating Functions:

Only one function can be activated at a time. When a second function is activated, the first function will automatically be deactivated.

1. Activating Column-Specific Functions:

Column-Specific Functions include function numbers 19 through 24 and are shown in the Table below.

Function Number	Function Name	Valves Activated
19	B+TET To Col 1	0,11,28,40,44
20	B+TET To Col 2	0,11,28,48,52
21	B+TET To Col 3	0,11,28,56,60
22	Cap To Col 1	13,25,26,40,44
23	Cap To Col 2	13,25,26,48,52
24	Cap To Col 3	13,25,26,56,60

Table 4-1: Functions 19 through 24

Activating Functions 19 through 21:

To deliver Base + TET to the column(s), first the column(s) must be activated. If the column is not activated, no delivery will be made.
Next, enter the Base + TET function number. A message will be displayed prompting selection of the bases to be delivered. The keyboard changes and makes A, G, C, T, 5, 6, and 7 available. Select up to four bases to be delivered to the column with this function. If no base has been indicated, then only TET will be delivered to the column. The function will activate when the ENTER key is touched and an asterisk will appear next to the function number, indicating that it is a Column-Specific Function.

Activating Functions 22 through 24:

To activate Column-Specific Functions 22, 23, and 24, the column(s) must first be activated. If the column is not activated, no delivery will be made. The function will activate when the ENTER key is touched and an asterisk will appear next to the function number, indicating that it is a Column-Specific Function.

2. Activating "Block to Column" (Function Number 3):

In instruments with three columns, this function can be used when checking flows through the columns. First, the appropriate column(s) can be activated to allow chemicals to be delivered. Next, a set of valves that produce a chemical delivery to the valve block can be activated. Chemicals will then be delivered to the column when function number 3 "Block to Column" is activated.

3. Activating Cycle Directive Functions:

The following table is a list of the Cycle-Directive Functions.

Function Number	Function Name
6	Waste-Port
7	Waste-Bottle
17	Interrupt
33	Cycle Entry
45	Group 1 On
46	Group 1 Off
47	Group 2 On
48	Group 2 Off
49	Group 3 On
50	Group 3 Off

Table 4-1: Cycle Directive Functions

Cycle Directive Functions do not open valves. However, each Cycle Directive performs a specific task by signalling the controller. For example, when function number 6 is performed, all chemicals directed to waste will be directed to the waste trityl port. Comparatively, when function 7 is performed, all chemicals that are directed to waste will be directed to the waste bottle.

Function number 17, "Interrupt" signals the controller to stop until a continue command is given. Function number 33, "Cycle Entry" is a signal that indicates where the cycle will first start. In three column instruments, function numbers 45 to 50 are used to control deliveries to specific columns. If these functions are activated through the Manual Control menu, all valves will remain closed.

To View the Function Name:

Before a function is activated by pressing ENTER, the function name and switch(es) can be viewed.

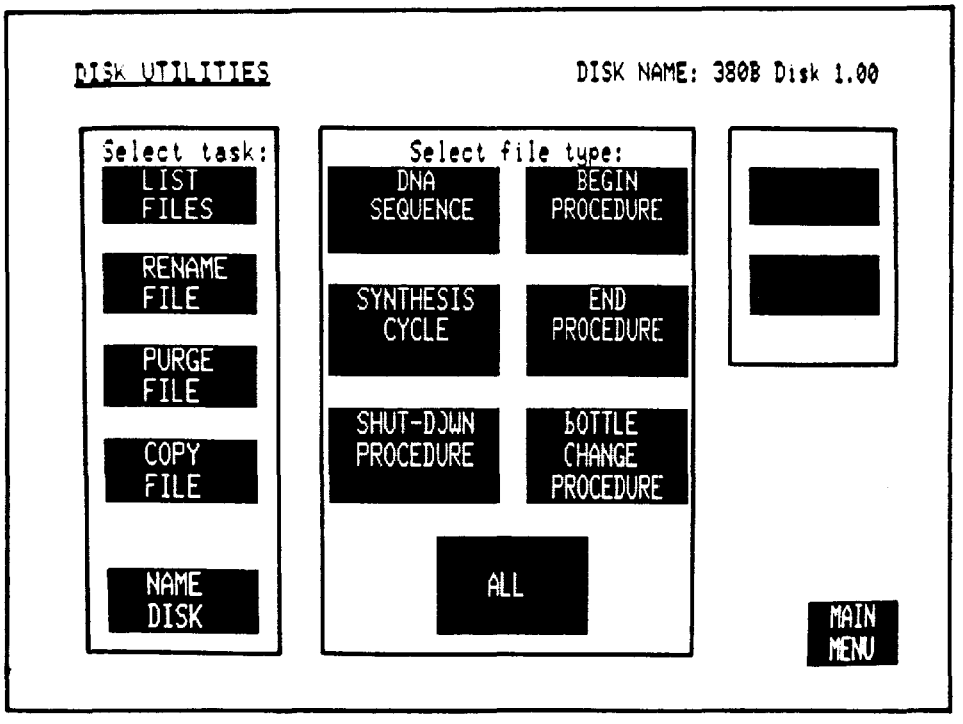
PRE-ENTRY DISPLAY	PRE-ENTRY DISPLAY displays the function name and valve numbers activated by the function number just entered.
CLEAR ALL	CLEAR ALL turns all valves off and erases the pre-entry display area.

DISK UTILITIES

Introduction:

The Disk Utilities Menu provides a means of organizing files on the disk. Files consist of DNA Sequences, Synthesis Cycles, Shut-down Procedures, End Procedures, and Bottle-Change Procedures. These files can be listed, given new names, purged, and/or copied. Also, disks can be named.

The Disk Utilities Menu:



To List Files

LIST FILES

A list of all files or of a file type is displayed by LIST FILES.

If a printer is connected, options SCREEN and PRINT are available to give the user the choice to view the files on the screen or have them printed.

Next, select the file type. All the file types can be listed by selecting ALL or just one file type can be listed.

When a file type is chosen to be viewed on the screen, the menu page changes and displays a list of the file names, with the dates each was created and last accessed.

Twenty files can be listed on the screen at a time. If there are more than 20 files, the option MORE FILES will be displayed at the bottom of the screen. Selecting this option displays additional files. To return to the Disk Utilities Menu, select PREV MENU.

To Rename a File:

RENAME A FILE

Files created by the user, are given a new name by selecting RENAME A FILE.

After RENAME A FILE is selected, select the file type. The disk goes through the File Finder Routine and searches for the files of the selected file type and displays them on the screen as options.

NOTE: Files that are presently listed for use for synthesis cannot be renamed. These files include: the Begin Procedure that is listed at the top of the Cycle Monitor menu; the Cycles listed in the Run Editor; and, the DNA Sequence and the End Procedure listed in the Column Monitor menu. To rename these files, clear them from the appropriate menus.

Next, select the name of the file to be renamed. To ensure the desired file was chosen, the screen displays a prompt with the file type and the file name. Answer the prompt using options YES or NO.

When NO is selected, the disk goes through the File Finder Routine again and displays the file names found on the disk. Choose the correct file to be renamed.

Selecting the option YES changes the menu to the Name and Store page. The file can be copied under a new name or with the same name. If the file name remains the same select ENTER. If a change in name is desired refer to the use of the Name and Store page, discussed on page 4-8.

NOTE: Standard files named with lower case letters cannot be renamed to prevent loss of the files. These are easily differentiated from user-files that use upper case letters.

To Copy A File:

COPY A FILE

Files are duplicated using option COPY A FILE.

After choosing COPY A FILE, select the file type. The disk goes through the File Finder Routine and searches for these files which are then displayed on the screen. After the file is selected, it is displayed on the screen with a prompt that ensures the desired file was selected. For example, when copying a synthesis cycle the prompt will be:

"Copy synthesis cycle xxx ?"

Answer the prompt using options YES or NO.

To Name A Disk:

NAME DISK

Using the option NAME DISK, a disk is named for identification.

When the option NAME DISK is selected, the Name and Store page is displayed. Refer to page 4-8 for use of the Name And Store Menu.

To Purge a File:

PURGE A FILE

This option deletes files from disk storage.

After PURGE FILE is chosen, select the file type. The disk goes through the File Finder Routine, searching for the selected file type which are then displayed on the screen as options.

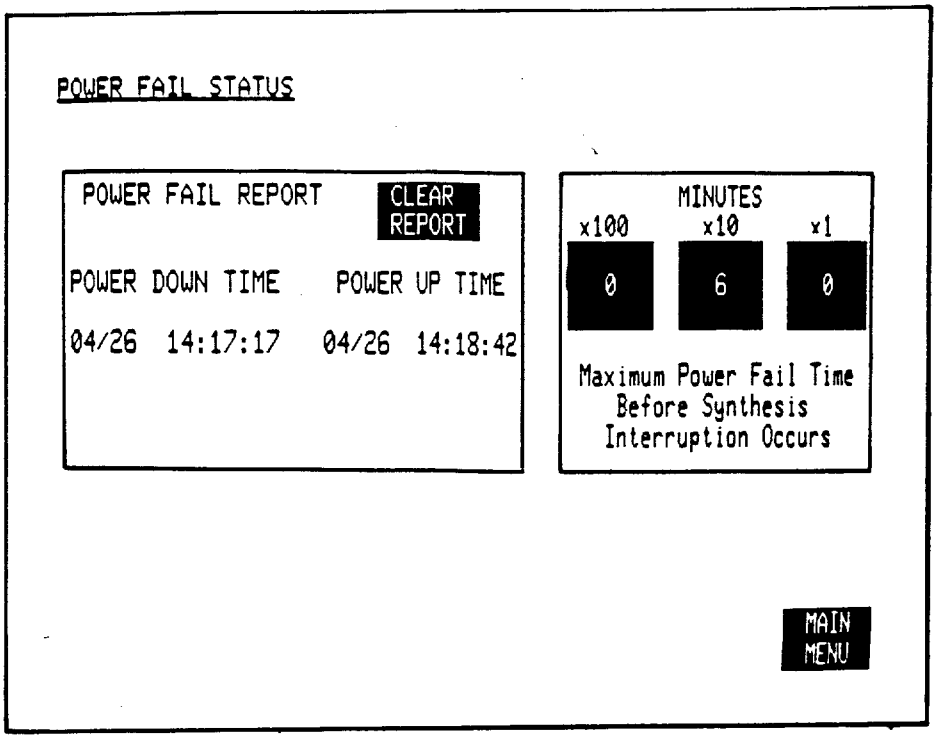
NOTE: Files that are presently listed for use for synthesis cannot be purged. These files include: the Begin Procedure that is listed at the top of the Cycle Monitor menu; the Cycles listed in the Run Editor; and, the DNA Sequence and the End Procedure listed in the Column Monitor menu. To rename these files, clear them from the appropriate menus.

Then select the name of the file to be purged. To ensure the desired file was chosen, the screen displays a prompt with the file type and the file name. Answer the prompt using options YES or NO.

Selecting YES purges the file. When NO is selected, the File Finder Routine starts again and displays the file names found on the disk. Choose the correct file or select PREV MENU.

NOTE: Standard files named with lower case letters cannot be purged to prevent loss of the files. These are easily differentiated from user-files that use upper case letters.

POWER FAIL



The Power Fail Menu displays a record of power failures to the 380B by listing the time that the power failure occurred and the time that the power was restored. The time a power failure started is listed under "Power Down Time" and the time the power failure ended is listed under "Power Up Time". Up to four power failures are viewed at one time. If more than four power failures have occurred, the most recent ones are displayed.

CLEAR REPORT	CLEAR REPORT erases the list of power failures.

Through the Power Fail Menu, the user can set a maximum time that a power failure can occur before synthesis will be interrupted and will not continue. When the power failure is less than the maximum power fail time, synthesis will automatically proceed as normal when the power is restored.

If a power failure exceeds the maximum power fail time and the failure occurred at an UNSAFE* step, then synthesis will not restart when the power is restored. In this instance, "User Interrupt" will be listed in the Cycle in the Interrupt Menu. Synthesis can either be continued or aborted. When the interrupt is cleared, synthesis will proceed at the step the power fail occurred. Refer to the Interrupt Menu in Section 4, to continue or abort synthesis.

The number displayed in each of the three "Minutes" keys is the maximum power fail time. To reset the number, touch the "Minutes" keys. The numbers increment each time these keys are touched.

* Steps are programmed as SAFE or UNSAFE in the Cycle Editor Menu. Refer to the Cycle Editor Menu for programming a step as Safe and Unsafe.

SECTION 5

OPERATION

Use this section as a step-by-step procedure for 380B operation. These procedures refer to the use of many menus. If questions arise regarding use of any menu, please refer to Section 4 where all menus are described in detail.

CONTENTS:

PART I: Preparations Prior to Synthesis

1. Check the chemical levels of the reagent bottles and replace those that are low. To change bottles 9-18 access the Reservoir Status menu and select the corresponding bottle change procedure. A prompt will tell you when to remove the old bottle and install the new one. Refer to Part V of this section for additional information.

   Before changing bottles 1-7 the phosphoramidites must first be reconstituted with acetonitrile. Detailed instructions are included in the phosphoramidite preparation procedure found in Part V of this section.

NOTE: Since all phosphoramidite reservoirs (1-7) are pressurized simultaneously during synthesis, it is necessary to have a bottle (even if empty), at each phosphoramidite position.

2. Access the Reservoir Status menu and check the bottle alarm settings. Refer to Section 4 for details on how to use the Reservoir Status menu.

3. Check the argon tank regulator for ample gas and pressure. Argon should be delivered to the synthesizer at a pressure of 60 psi. Change the tank if its total pressure drops below 400 psi. With average synthesizer use, an argon tank should last approximately 3 months. Refer to Part V in this section for a procedure on changing an argon tank.

4. Check the waste level.
   The synthesizer generates 1 to 2 liters of hazardous, halogenated, organic liquid waste per 100 base additions. When the waste bottle is full, it must be emptied and the waste disposed of properly. Waste can be emptied during synthesis when synthesis is interrupted. To empty the waste bottle, refer to the instructions in Part V of this section.

5. Fill the fraction collector with 10- to 15-mL volumetric tubes to collect the trityl cation released at each detritylation step. Use one tube for each base in the sequence. Be sure to label the tubes and align the fraction collector so the first tube collects the first trityl.

PART II: Starting a Synthesis:

I. First, define the DNA sequence from the DNA Sequence Editor menu and store it on a disk.

II. From the System Monitor menu, activate the column(s) to be used for synthesis by selecting the COLUMN MONITOR menu.

1. In three-column instruments, first select the column for synthesis set-up.

2. Select CLEAR COLUMN if a synthesis was previously performed and remove the old column.

3. Select the SEQUENCE NAME key and select one of the DNA Sequences displayed on the screen.

4. Check that the Ending Method and Ending Procedure, listed next to these keys are the ones that are desired for this synthesis.

   If you want to change the Ending Method, first touch the ENDING METHOD key. Then select one of the four Ending Methods options, now displayed at the last row of keys.

   The Ending Procedure will only be performed if the Ending Method is AUTO. If you want to change the Ending Procedure, first touch the ENDING PROCEDURE key. By touching the VIEW END PROCS key, the screen will display the Ending Procedures stored on the disk. Select the desired Ending Procedure.

5. If desired, USER NAME can be selected and the operators name or initials can be entered.

6. The START COLUMN key can then be selected.

   When this key is selected, the microprocessor checks the reservoir status alarm against the length of the DNA chain, the cycles listed in the Run Editor, and the reservoirs used for those cycles and determines if the reservoirs have sufficient reagent. A message will be displayed indicating that the Bottle Check Routine passed or displays the first reservoir that will empty.

7. Select the CONTINUE START key.

   A message is displayed to place the appropriate column and the collection vial on the instrument by following the procedure below.

   <u>To install the column:</u>

   a) Columns are packaged individually and have a year shelf-life. For synthesis of oligonucleotides of from 0 to 70 bases standard pore (500A) CPG is recommended. For synthesis of oligonucleotides of greater than 70 bases, wide pore (1000A) CPG is recommended.

   b) Check that the type of column matches the 3' base displayed on the screen. Record the column's serial number to identify the synthesis if deprotection and cleavage are performed later. Otherwise, label the

collection vial when the DNA is cleaved from the support in the column.

c) Next, firmly push either end straight up onto the upper male Luer fitting on the instrument. The column is symmetrical and has no "top" or "bottom". Therefore, it can be aligned in any way.

d) Finally, firmly push the lower male Luer fitting straight up into the bottom of the column. The column should fit securely. Do not twist the fittings or the filters may crimp or tear.

<u>To install the collection vial</u>:
Simply screw the collection vial onto the Synthesizer.

8. With the column and collection vial in place, select CONTINUE START again.

   A message is displayed to check the level of waste in the waste bottle. A procedure for changing waste is in Part V of this section.

9. With the waste level satisfactory, select CONTINUE START. The column is now activated. The Column Status changed from "Awaiting Status" to "Awaiting Cycle Entry" and the Start Time is displayed.

10. If a printer is connected, the column set-up can be printed by selecting the PRINT key.

NOTE: Additional columns to be used for synthesis can be configured and activated now following the same procedure described above.

11. When all the desired columns have been configured and activated, select PREV MENU to display the System Monitor menu.

III. Check that the cycles to be run for this synthesis are listed properly in the Run File Editor.

1. From the System Monitor menu, select the CYCLE MONITOR key.

2. From the Cycle Monitor menu, select the RUN EDITOR key.

   Cycles can be added, deleted or changed from those listed in the Run Editor. Refer to the Run Editor in Section 4 if changes are desired.

3. When the Run Editor lists the desired cycles, return to the Cycle Monitor by selecting PREV MENU.

IV. Check that the Begin Procedure, listed at the top right of the menu, is the desired procedure for this synthesis.

If you want to change the Begin Procedure, then select ALTER BEGIN PROCS. Select the desired Begin Procedure by touching it.

V. When steps I, II, III and IV are complete, synthesis can begin by selecting START CYCLE.

PART III: During Synthesis

Checks Following Synthesis Start:

1. Check that the columns do not leak when fluid is being delivered to them.

2. Note if and when Reservoir Bottles need to be changed during synthesis.

Tasks That Can/Cannot Be Performed During Synthesis:

* The Self Test, Manual Control and Function Editor menus cannot be accessed during synthesis. However, all other menus are accessible during synthesis.

* Synthesis can be interrupted. Refer to the Interrupt menu in Section 4 on how to interrupt synthesis.

* Cycles can be altered during synthesis by changing the time of a step. Refer to Section 4, the Cycle Monitor menu, for a description.

* The touch screen can be locked. Refer to the Lock Screen portion of Section 4.

To Abort Synthesis:

If desired, the synthesis can be aborted. When synthesis is aborted, all valves close and the option to continue synthesis will NOT be available. To abort synthesis, 1) first, Column Interrupt must be set from

the Interrupt menu. 2) Then abort key will be accessible from the Column Monitor menu.

1. Interrupt the column synthesis from the Interrupt Menu. Refer to section 4 for a detailed discussion of 'Interrupt Immediate' of the Interrupt Menu. A short version is given below.

CAUTION: Synthesis should be interrupted at a safe step so that the DNA is left chemically safe and so that chemicals are not left in valve blocks and lines. Salts could form and block delivery if chemicals are left in the valve blocks and lines. If this occurs, replacement of expensive parts may be necessary. Cycle Entry and Cycle End are the suggested steps for synthesis interruptions.

From the Interrupt menu, first select INTERRUPT IMMEDIATE; then select, CYCLE. By selecting INTERRUPT IMMEDIATE again, the selected COLUMN can be interrupted.

2. In three-column instruments, synthesis will continue when the Cycle Interrupt is cancelled. To do this, select CANCEL INTERRUPT and then select CYCLE.

3. From the System Monitor menu, select Column Monitor for this menu to be displayed. Then indicate the column to be aborted by selecting NEXT COLUMN. The ABORT COLUMN key is located in the lower left of the menu. When selected, a message will be displayed:

"Are you sure you wish to abort the column?"

Options are YES or NO. By selecting YES, synthesis for the column indicated, is aborted.

4. When synthesis is aborted at a step other than Cycle Entry or Cycle End, the lines and valve blocks will need to be rinsed and dried. To accomplish this, enter the Manual Control menu and select ACTIVATE followed by FUNCTION. Enter the function number to individually activate the following functions for the specified times. Delivery for longer than the specified times will not cause a problem.

FUNCTION		TIME (sec)
10	(#18 To Waste)	10
9	(#18 To Column)	10
1	(Block Flush)	5
2	(Reverse Flush)	10

PART IV: At Synthesis Completion

1. Remove the column. The column can be discarded if automatic deprotection and cleavage have been performed.

2. Remove the DNA collection vial and place at 55°C for 8-15 hours to remove the isobutyryl and benzoyl protecting groups.

3. Remove the trityl tubes. Perform the trityl cation assay to determine the coupling efficiency of the synthesis. Refer to User Bulletin 13 for instructions.

   Once the column, collection vial and the trityl tubes have been removed, the instrument is ready to begin another synthesis.

4. Analyze the crude oligoneucleotide mixture by one of the methods (such as HPLC or gel electrophoresis) described in User Bulletin 13.

5. Prepare the DNA for use in experiments by removing the ammonia and then desalting. Purify the product, if necessary. Refer to User Bulletin 13 (Revised) for instructions.

PART V: Procedures

A. CHANGING RESERVOIR BOTTLES

Introduction:

The Reservoir Status/Bottle Change menu is used when changing reservoir bottles. When a bottle is changed, a "Bottle-Change Procedure" is performed. During this Procedure, the delivery line is flushed; the bottle is changed and re-pressurized; the delivery line is primed with fresh reagent; and the valve block is rinsed and dried.

A reservoir bottle can be changed during synthesis by interrupting synthesis at a safe step, preferably at "Cycle Entry" or at "Cycle End". Refer to Section 4 for details on using the Interupt menu. Synthesis will be interrupted automatically when the reservoir alarm is reached. Refer to the Reservoir Status menu for details.

WARNING: Consider each chemical in the synthesizer potentially harmful. Do not inhale fumes. Work in a well ventilated area. Do not leave any chemicals uncapped. Immediately consult a physician if any chemical is ingested. If there is any physical contact, wash immediately with ample water. Wear eye protection. In case of contact with eyes consult a physician immediately.

The phosphoramidites, tetrazole and acetonitrile are atmosphere sensitive. Upon opening one of these bottles, quickly place it on the instrument to prevent contamination. Refer to Section 1 about handling synthesizer reagents and solvents.

Procedure for Changing Reservoirs 1 through 12:

1. Access the Reservoir Status menu and execute the appropriate bottle change procedure. Wait until a prompt instructs you to remove the old bottle.

2. To remove a bottle, firmly pull it straight down while pressing the black button above its receptacle. If the bottle seems to stick, move it carefully from side to side while pulling it off. Cover or recap the bottle to minimize the release of vapors. Wipe the delivery line with a lint-free tissue.

3. Next, tear off the aluminum cap in the direction of the arrow and remove the rubber septum from the fresh reagent bottle. Wipe any crystals or drops of reagent from the bottle neck.

4. To install the bottle, firmly push it up around its receptacle while pressing the black button. As necessary, maneuver the bottle into place by carefully moving it side to side while pushing upwards.

5. When the bottle is correctly in place, release the black button. If the button remains pressed in, the bottle is not installed properly and must be repositioned.

Procedure for Changing Reservoirs 13 through 18:

Bottles 13, 16 and 18 may be refilled with fresh UV or HPLC grade acetonitrile.

IMPORTANT: Acetonitrile that is contaminated with 1000 ppm water will lead to a 1 to 2% decrease in coupling efficiency. Therefore, follow

the precautionary measures in a) and b) below.

a) Use HPLC or U.V. grade acetonitrile with a specification of less than 300 ppm of water.

b) Avoid contaminating the acetonitrile with atmospheric water by quickly refilling the bottle; replace the cap on the stock acetonitrile; and place the bottle on the instrument. As an additional precaution, keep the stock acetonitrile dedicated only to this use.

1. To remove a bottle, unscrew it by turning clockwise. Recap it to minimize the release of vapors.

2. Open the full bottle and screw it snugly into its threaded cap on the instrument. There is a Teflon insert and Kalrez gasket which form an airtight seal between each cap and reservoir.

IMPORTANT: Do not overtighten a bottle or the gasket will tear or distort.

3. When changing a bottle, inspect the insert to check the condition of the gasket, using a mirror. A functional gasket appears as a flat black surface. If no black is seen or if the black gasket appears

Procedure for Changing Reservoirs 13 through 18:

Bottles 13, 16 and 18 may be refilled with fresh UV or HPLC grade acetonitrile.

IMPORTANT: Acetonitrile that is contaminated with 1000 ppm water will lead to a 1 to 2% decrease in coupling efficiency. Therefore, follow

B. CHANGING THE ARGON TANK

The primary tank is typically set at 60 psi with the secondary tank set at 4 psi lower (typically 56 psi), although optimal tank pressures are determined at installation. When the primary tank becomes depleted, the back-up tank (set 4 psi lower), will automatically start providing the synthesizer with argon. A pair of check valves permit flow from one tank to another and facilitates automatic change over. Replacement can be made during a run without interrupting the run in any manner. The empty primary tank should be replaced as soon as possible to avoid exhausting the entire supply.

WARNING: Damage to the synthesizer could occur if the argon supply is permitted to deplete. Reagents and solvents can mix with each other in the reservoirs and the plumbing causing plugging and damage to delivery valves. Chemical vapors and/or liquids can backflow to the argon manifold, necessitating a lengthy disassembly, cleaning and reassembly process. Most important, since several chemicals are corrosive, caustic and toxic, chemical leaks that can occur due to component damage represent a potential safety hazard to the operator.

To Replace an Empty Tank:

1. Close both the main supply valve on top of the tank and the needle valve on its regulator.

2. Remove the regulator from the empty cylinder and install it on a full replacement cylinder.

3. Open the main cylinder supply valve and check for leaks at the regulator connection using a soap solution.

4. Open the appropriate "tank purge" valve, located on the rear panel of the Synthesizer, and then open the needle valve on the argon regulator at the cylinder. Permit the purge (of the line between the tank and the synthesizer) to continue for approximately 30 seconds.

5. Close the "purge valve" and verify that:

   a. The cylinder regulator setting returns to its original position. (Pressure from primary tank is optimally set by by an Applied Biosystems Service Representative at installation and is recorded on the installation report.)

   b. The regulator on the secondary tank is set at 4 psi lower than the primary tank.

   c. The moisture trap in the front of the instrument is dry by noting that the indicator is blue in color. If the moisture trap is white (indicating that water is present) contact an Applied Biosystems service representative.

C. CHANGING THE WASTE BOTTLE

The synthesizer generates 1 to 2 liters of hazardous, halogenated, organic liquid waste per 100 base additions. The waste is collected in a one-liter glass container. The bottle is sealed by a spring-loaded cap and O-ring. When full, the waste bottle must be emptied.

Emptying the Waste Bottle:

WARNING: Synthesizer waste must be disposed of properly and carefully. When handling the waste for disposal, wear gloves and eye protection, and avoid inhalation and skin contact.

1. Raise the black bar above the waste and carefully pull the bottle out. Immediately cover the bottle to prevent the release of vapors.

2. Discard the waste as follows:

   Place the liquid in a sealed container labeled "FLAMMABLE", "POISON B N.O.S." or absorb in vermiculite, dry sand or earth. Dispose of waste according to applicable government regulations.

3. After emptying the waste, raise the black bar and replace the bottle securely.

IMPORTANT: The waste bottle creates the low pressure necessary for chemical deliveries. Therefore the waste bottle must always be kept vented. With the vent closed, back pressure will result and deliveries of reagents and solvents may be decreased. Therefore, check that the vent line is properly routed to a fume hood.

D. PHOSPHORAMIDITE PREPARATION

The prepackaged phosphoramidites are bottled as powders and sealed under argon pressure. In this state, they are stable for at least 1 year. To prepare them for use, they are dissolved in anhydrous acetonitrile.

Since the phosphoramidites are extremely sensitive to acid oxygen and water, special care must be taken when dissolving them. The following instructions will help avoid contamination, prevent degradation and ensure high coupling yields.

1. Use anhydrous acetonitrile with less than 90 ppm water to dissolve the phosphoramidites. Use of Applied Biosystems anhydrous acetonitrile, Part Number 400060, is strongly recommended. Do not use HPLC grade acetonitrile, its higher water content will decrease coupling efficiency.

   After opening the acetonitrile, keep it blanketed with argon to avoid contamination with air. Do not contaminate the acetonitrile bottle with traces of phosphoramidites.

2. When transferring the acetonitrile to a phosphoramidite bottle, use a clean, dry, glass syringe with a needle. Store the syringe in a 100-120 ^{o}C oven to prevent atmospheric moisture contamination. Keep the syringe dedicated to this use. Use acetonitrile to rinse it, do not use water.

3. Add the correct amount of acetonitrile to each phosphoramidite as follows:

ß-cyanoethyl Phosphoramidite	Volume of Acetonitrile	Weight of Phosphoramidite	Molarity
A	2.8 mL	.25 grams	0.1 M
	5.6	.50	0.1
	11.2	1.0	0.1
G	2.9	.25	0.1
	5.8	.50	0.1
	11.6	1.0	0.1
C	2.9	.25	0.1
	5.9	.50	0.1
	11.8	1.0	0.1
T	3.3	.25	0.1
	6.6	.50	0.1
	13.2	1.0	0.1
Deoxyinosine	3.4	.25	0.1

The above concentrations result in a 10-fold excess of soluble phosphoramidite over support-bound nucleoside.

Dissolving Phosphoramidites:

1. To prepare the phosphoramidite bottle, pull back the aluminum tab in the direction of the arrow. Do not yet remove it, simply expose the septum. Place a needle (any gauge, without a syringe) into the rubber septum. This vents the bottle when acetonitrile is added and prevents accidental splashing of reagent when the bottle is opened.

2. Unscrew the cap from the anhydrous acetonitrile bottle and quickly replace it with a clean rubber septum. The acetonitrile is packaged under argon. Since argon is heavier than air, argon should continue to blanket the acetonitrile after this transfer.

3. Remove the syringe/needle from the oven and allow it to cool to room temperature. Pierce the septum of the acetonitrile bottle with the needle and remove the correct amount of acetonitrile.

4. Pierce the septum a few millimeters with the needle/syringe and slowly add the acetonitrile. Make sure the needle can not touch the phosphoramidite powder. When finished, remove both the needle and the needle/syringe and gently swirl the bottle to dissolve the phosphoramidites. Once dissolved, they are ready to load on the instrument.

5. The acetonitrile bottle must be purged with dry argon. To do so, place a needle on the upper male Luer fitting on the front of the synthesizer. Remove the column if necessary. Select MANUAL CONTROL from the main menu and activate function 2, a reverse flush, for the appropriate column. Argon will then flow out of the needle.

6. Next, push the acetonitrile bottle onto the needle so that the needle pierces the bottle's septum. Hold it for 5 seconds and then remove the bottle with the needle. The pressure will immediately be released but the bottle will remain blanketed with argon. Remove the needle and continue dissolving additional phosphoramidites if necessary.

Once in solution, the phosphoramidites are stable for 2 weeks. After this time, coupling efficiencies will slowly begin to decrease. If they can not be used within this time, it is possible to freeze, store, thaw and reuse them.

Phosphoramidites that are frozen and thawed may show some loss of activity. However, the activity is adequate for synthesizing short sequences of approximately 20 bases or less.

Storing Dissolved Phosphoramidites:

1. Purge the phosphoramidite bottles with argon by activating function 44 for about 10 seconds from the Manual Control menu.

2. Deactivate function 44 and remove the phosphoramidite bottles and quickly cap with previously unused rubber septa (Aldrich catalog number Z10,074-9). Flush the line with acetonitrile and then with argon, using the appropriate function numbers. Place a clean bottle on the position.

NOTE: Do not use the Bottle Change Procedure for this bottle removal.

3. Seal the bottle(s) with Parafilm, place in a dessicator containing Drierite and keep dessicator in a freezer at -20°C.

4. To use, thaw the phosphoramidite(s) and remove them from the dessicator. Then, place them on the instrument using the Bottle Change Procedure from the Reservoir Status menu.

PART VI: USING DEOXYINOSINE, AMINOLINK 2™, AND OPC

A. DEOXYINOSINE

Deoxyinosine base pairs with A, T and C, which makes it a suitable choice for synthesis where the degree of degeneracy needs to be reduced. A 23-mer with five inosine substitutions used as a probe was found to bind to a complementary strand, and have a similar dissociation temperature as a 17-mer. It is not well understood how deoxyinosine affects the stabilization of the DNA duplex, however.

Martin, et al determined the stability of oligodeoxyribonucleotide duplexes with each of the four normal bases using optical techniques for measuring melting temperatures.[1] They observed large neighboring-base effects upon the stability of the base pairs between inosine and the normal bases. The results obtained by this group indicate that deoxyinosine reduces the specificity of hybridization probes.

Ohtsuka, from the faculty of Pharmaceutical Sciences and Institute for Molecular and Cellular Biology at Osaka, Japan, has found that synthetic oligonucleotides with deoxyinosine residues at ambiguous points are useful as hybridization probes.[2]

Takahashi, also from Osaka University, used a synthetic probe containing deoxyinosine to isolate the cholecytokinin gene directly from a human genomic library.[3]

References

1. Martin, F.M., Castro, N.M., Aboula-ela, F., Tinoco, I., Base Pairing Involving Deoxyinosine: Implications for Probe Design, Nuc. Acids Res. 13, 8927-8938, (1985)

2. Ohtsuka, E., Matsuki, S., Ikehara, M., Takahashi, Y., and Matsubara, K., An Alternate approach to Deoxyoligonucleotides Hybridization Probes by Insertion of Deoxyinosine at Ambiguous Codon Positions, J. Biol. Chem. 260, (5), 2605-2608, (1985)

3. Takahashi, Y., Kato, K., Hayashizaki, Y., Wakabayashi, T., Ohtsuka, E., Matsuka, S., Ikehara, M., and Matsubura, K., Molecular Cloning of Human Cholecytokinin Gene by Use of a Synthetic Probe Containing Deoxyinosine, PNAS 82, 1931-1935, (1985)

B. AMINOLINK 2™

Introduction

Aminolink 2 is a DNA synthesis reagent which affords a convenient method to introduce an aliphatic primary amine at the 5' end of oligonucleotides. This amine can react to form oligonucleotide conjugates with a variety of substrates such as biotin [1,2], fluorescent dyes[3], EDTA[4], or alkaline phosphatase[5]. Applications include:

- Non radio-labeled hybridization probes[1,2,5,6]
- Sequence specific cleavage of single stranded DNA[4]
- Automated DNA sequencing
- Affinity chromatography

Aminolink 2 offers many advantages. Because it is a phosphoramidite, it reacts like conventional nucleoside phosphoramidites. The compound is dissolved in anhydrous acetonitrile and placed on an extra phosphoramidite port. It is activated with tetrazole and couples with the same high efficiency found in standard oligonucleotide synthesis. When using Aminolink 2 you do not need to modify the synthesis cycle. Other featues include:

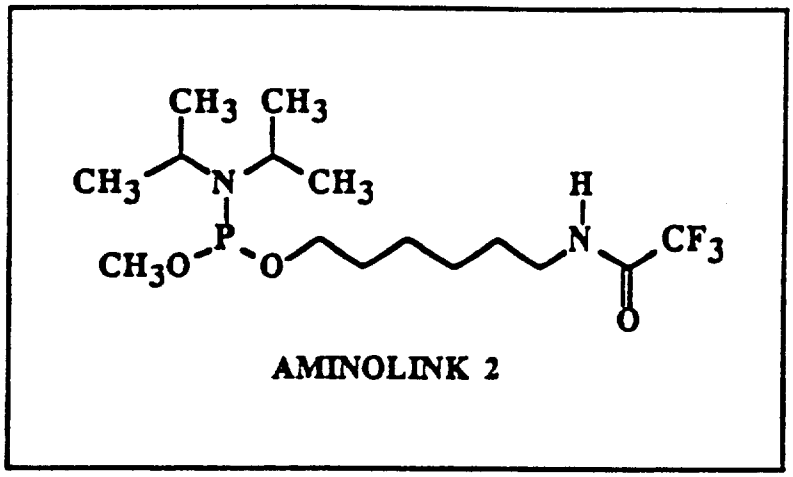
- Solution stability is comparable to nucleoside phosphoramidites (at least 2 weeks).
- No special cycles or protocols are required.

- Labeling at the 5'-terminal amine does not interfere with hybridization.

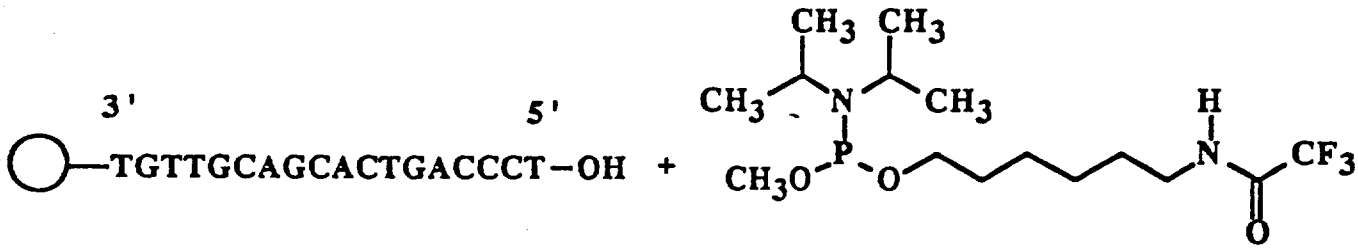
- Aminolinked oligonucleotides can be easily coupled with a variety of commercially available reagents such as fluorescein isothiocyanate and biotin NHS ester.

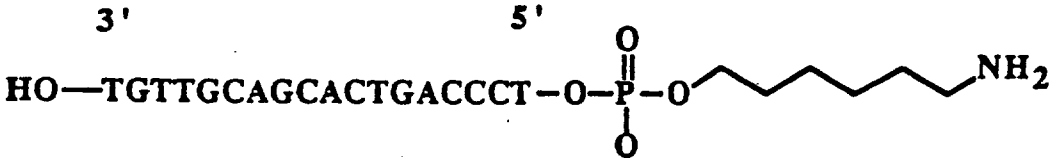
Chemical Description

Aminolink 2 (structure shown in Figure 5-1) generates an amino hexyl phosphate linker with a methoxy phosphate protecting group. Prior to deprotection, the phosphite triester is oxidized to the phosphate triester using standard oxidizing reagents. The amine group is protected by a trifluoroacetyl group, which is removed during standard ammonia deprotection procedures.



AMINOLINK 2





AMINOLINK 2 —— OLIGONUCLEOTIDE

Figure 5-1
Addition of the Aminolink 2 reagent to an oligonucleotide primer.

How to Use Aminolink 2

Aminolink 2 (Part Number 400808) is supplied in 250 mg quantities and comes as a viscous liquid, not a powder. Store the unopened linker desiccated and away from heat and moisture.

1. Dilute Aminolink 2 with 3.4 mL of anhydrous acetonitrile following the instructions in How to Prepare Phosphoramidites. (Once diluted and placed on the instrument, the compound is stable for at least 2 weeks.)

2. Place Aminolink 2 on one of the extra bottle positions.

3. Using the DNA Editor, enter the Aminolink bottle position as the first base, 5' end of the sequence.

4. Configure the synthesis "Trityl ON" since detritylation is not necessary after Aminolink 2 addition.

5. Deprotect and cleave the Aminolink-oligonucleotide. Although Aminolink has a methyl phosphate protecting group, thiophenol deprotection is not necessary. Ammonia treatment will demethylate the single methyl phosphotriester group of the Aminolink-oligonucleotide without adversely affecting product purity.

Analysis and Purification of Aminolink™-Oligonucleotides

Aminolink-oligonucleotides can be analyzed and purified by the same methods used for conventional oligonucleotides. Since the 5'-hydroxyl, usually available for phosphorylation by kinase and ATP, is blocked by the linker, an alternate 3'-end labeling procedure must be used to radiolabel Aminolink-oligonucleotides. To effect a 3'-end label, the enzyme Terminal Deoxynucleotidyl Transferase (TDT), is used. TDT adds a single nucleotide to the 3'-hydroxyl. Because the enzyme would continue propagation beyond the first nucleotide addition, a dideoxynucleotide analogue of [alpha-^{32}P]-dATP is used. The analogue, [alpha-^{32}P]-Cordycepin-5'-triphosphate, does not have a 3'-hydroxyl and therefore further addition is prevented. Below is a protocol for the labeling procedure.

3'-End-Labeling:

1. Prepare a stock solution of 10X 3'-end-labeling buffer:

   1.4 M Sodium Cacodylate*
   10 mM Cobaltous Chloride
   1.0 mM Dithiothreitol
   pH 7.5 with HCl

   *This chemical is carcinogenic, handle with care.

2. Aliquot 2 pmol of oligonucleotide into an Eppendorf-type tube (1.5 mL), dry in a Speed-Vac and ethanol rinse with 10 µL of 95% Ethanol, dry.

3. Add the following to the tube, while on ice:

   1 µL of 10X 3'end-labeling buffer
   1 µL of [alpha-^{32}P]-Cordycepin-5'Triphosphate (New England Nuclear #NEN-114, specific activity greater than 5000Ci/mmol)
   7 µL of deionized water
   1 µL of TDT (ENZO Biochemicals, 5 units per microliter)

4. Spin the tube very briefly in a microcentrifuge to bring contents of tube to the bottom. Incubate sample at 37°C for one hour.

5. Add 25 µL of 1.0 mM EDTA in deionized formamide loading buffer to the sample. Bromophenol Blue and Xylene Cyanol can be added as marker-dyes to the formamide prior to addition to the sample. The sample can be loaded onto an analytical gel in the same manner as described in Applied Biosystems DNA Synthesis User Bulletin No. 13 (Revised), Analysis and Purification of Synthetic Oligonucleotides. The linker retards the migration of the oligonucleotide by approximately one-half of a base in the gel.

   There is occasionally a radioactive contaminant which is a byproduct of the labeling procedure. This contaminant is most likely a type of enzyme-Cordycepin complex which co-electrophoreses with the sample and may appear on the autoradiogram as a fuzzy band in the region of a 16-mer. It is recommended that a blank sample containing the constituents of the reaction but *without DNA* be loaded on the gel so that the contaminant can be compared with the DNA sample. Note that since

phosphorylation of the 5'-hydroxyl by T4 kinase should be blocked by the presence of the linker, this procedure can be used as a qualitative analysis of the efficiency of Aminolink 2 addition.

Anion-exchange HPLC, described in User Bulletin No. 13 (Revised) can be used for Aminolink-oligonucleotide analysis or purification. The inherent problem with using reverse phase HPLC for purification is that the oligonucleotide and the Aminolink-oligonucleotide are not well resolved. Gel purification seems to give better results. Once a dye is attached, reverse phase HPLC may be used to purify the derivatized oligonucleotides.

Applications

Aminolink-oligonucleotides can be analyzed and purified by the same methods used for conventional oligoGeneral Protocol for Tag/Labeling Substrate. Described below is a generic protocol from which to extrapolate individual application protocols. Note that most commercially available biotinylation reagents come with usage protocols.

1. Prepare a solution of Aminolink-oligonucleotide at a concentration of 1mM or greater in a 50mM-200mM $NaHCO_3/Na_2CO_3$ Buffer, pH 9.

2. Add a 50-molar excess of the solid tag/labeling substrate (NHS-Biotin, Dye, etc.) and vortex to dissolve. If the tag or DMSO is insoluble in aqueous solutions, it can be dissolved in dimethylformamide before addition to the Aminolink-

oligonucleotide. The final concentration of organic solvent in the reaction mixture should not exceed 30%.

3. After several hours reaction time at room temperature, the mixture can be purified by gel-exclusion on a G-50 or G-25 Sephadex™ column to remove the excess tag. Use 100mM TEAA as the eluant.

NOTE: Reactions with fluorescent dyes should be done in the dark.

References

1. B.C.F. Chu and L.E. Orgel, DNA 4, 327-331 (1985).

2. A. Chollet and E.H. Kawashima, Nucleic Acids Research 13, 1529-1541 (1985).

3. L.M. Smith et al., Nature 321, 647-679 (1986).

4. G.B. Dreyer and P.B. Dervan. Proc. Natl. Acad. Sci. USA 82, 968-972 (1985).

5. E. Jablonski, et al., Nucleic Acids Research 14, 6115-6128 (1986).

6. B.A. Connolly, Nucleic Acids Research 15, 3131-3137 (1987).

C. OLIGONUCLEOTIDE PURIFICATION CARTRIDGE (OPC)

OPC (Part No. 400771) is a rapid purification cartridge used specifically for synthetic DNA. It provides the level of purity required for the common applications of synthetic DNA. Using OPC eliminates the need to purify by time-consuming and labor-intensive polyacrylamidge gel electrophoresis (PAGE) or HPLC. If you currently only desalt your synthetic oligonucleotides, you can now use OPC to desalt *and* purify in less time than it takes to desalt alone.

OPC is fast, easy to use and delivers consistent results. The time required to go from the deprotected, crude oligonucleotide to its use in an experiment is greatly reduced. Complete purification requires 15-20 minutes.

After completing deprotection and cleavage, an aliquot of the crude oligonucleotide, still in ammonia, is diluted with water and then loaded directly onto the OPC cartridge. This eliminates the need to evaporate the sample prior to purifying. The remaining portion of the crude synthesis can then be stored in ammonia either refrigerated or at -20°C without harming the DNA. Storage in ammonia is preferable to storage as an evaporated sample or dissolved in water. The ammonia protects the sample from acidic byproducts present in the crude mixture.

Detritylation is also done on the cartridge, eliminating the need for post-purification work-up. The purified oligonucleotide is then eluted as a trityl-off species in a volatile buffer. Quantification can be easily accomplished and only the volume containing the amount required for the

experiment need be evaporated. The remainder can be stored at -20°C for later use. Once dry, the pure oligonucleotide can be redissolved in an appropriate buffer and used in your experiment.

The OPC purification protocol appears at the end of this section. It successfully purifies sequences up to 209 bases. Note that for sequences >40 bases long, you must perform an additional step, number 9a. This step adds several washes with dilute ammonia after detritylation with 2% trifluoroacetic acid. The extra basic wash preferentially removes any shorter sequences that had trityl protecting groups attached and thus had co-purified with the desired product.

The purification of longer oligomers (>70-mers) using OPC can be made more difficult because of the potential for depurination. To address this, a protocol has been developed by Thomas Horn and Mickey Urdea of Chiron Co.[1] in which you pre-treat the oligonucleotides with lysine and then perform the OPC purification procedure. This lysine treatment is described in detail in User Bulletin #52.

Although depurination is a valid concern during DNA synthesis of long oligonucleotides, the degree of depurination encountered during synthesis is highly sequence and reagent dependent. Since the 3' bases of an oligo (the initial couplings) have the greatest reagent exposure, varied purine content in this area will generate varied potential for depurination. Another factor in depurination is the purity of the acid used for detritylation. Contaminants, such as water or HCl, in the trichloroacetic or dichloroacetic acid will greatly promote depurination. Applied Biosystems supplies high purity trichloroacetic and

dichloroacetic acid reagents. In a study involving the synthesis of a 72-mer, the use of Applied Biosystems reagents did not promote detectable depurination. Analysis of the 72-mer synthesis, OPC purified with and without prior lysine treatment, showed no detectable purity difference.

[1] Horn, T. and Urdea, M. S., *Nuc. Acids Res. 16* (24), 11559-11574 (1988).

SECTION 6
ACCESSORIES

THE PRINTER

A Hewlett-Packard Think Jet 2225 C Printer (Centronics Interface), may be connected to the DNA Synthesizer for hard copy reports of synthesis protocols. Some other printers can be used with the Model 380B. These printers require a Centronics Interface and a receptacle (for the cable that connects the printer with the Synthesizer) that is Epson compatible.

1. Connecting the Printer:

A cable (Applied Biosystems Part Number 400222), connects the printer to the Model 380B Synthesizer. One end of the cable is connected to the rear of the printer and the other end is connected to the rear of the 380B.

2. Plugging the Printer:

As supplied, the printer is configured to operate when the power cord is plugged into a 120 VAC receptacle. A 120 VAC receptacle is located at the rear of the 380B for this purpose.

However, especially for users outside the U.S, the printer can be re-configured to operate at a variety of input voltages. Instructions to re-configure the printer to a voltage other than 120 VAC can be found in the HP printer manual.

WARNING: DO NOT plug the printer into 240 VAC unless it has been re-configured according to HP instructions. Damage to the printer and physical harm could result if it is plugged into an improper receptacle.

When operated outside the U.S. the appropriate plug needs to be supplied. The cord uses the international color code as follows: brown-line; blue-neutral; green/yellow-ground.

3. Set the Operation Code:

For the printer to operate with the DNA Synthesizer, it is necessary to set an operation code at the back of the printer. The code uses 8 switches that can be placed in an up (1) or down (0) position. Set the operation code to "00001100". All switches should be down except for the switches 5 and 6 which are in the up position.

4. Printer Start-Up Procedure:

Refer to the printer manual for the start-up procedure.

5. Select PRINTER ATTACHED:

Indicate that the printer is connected to the Synthesizer. From the 'Self Test, Shut Down & Config. Menu', touch the key PRINTER NOT ATTACHED so that the key reads PRINTER ATTACHED. With the PRINTER ATTACHED key viewed, the PRINT keys that appear in the software menus will be operational.

6. Printer Paper:

Though the printer will print on any standard 8.5 x 11 (European size A4) single or fanfold paper, best print quality can be assured by using HP specified ink jet paper. Additional paper stock is available from Hewlett-Packard under the following part numbers:

92261M	Ink Jet Paper	500 Sheets	Single Sheets
92261N	Ink Jet Paper	2500 Sheets	Fanfold

If an error message occurs: when attempting to use the printer, go through the following list.

* Check that the printer has the proper operation code and is set as discussed above.

* Check that the printer is plugged in, turned on and properly loaded with paper.

* Check that the cable attaching the printer to the Synthesizer is securely plugged in at both ends.

* Turn the printer off and then on again to establish a fresh communication link between the Synthesizer and the printer.

* Check the printer fuse. Refer to the HP printer manual for details.

Printouts:

The following can be printed:

DNA Sequences;
A column set-up for synthesis;
Synthesis Cycles;
The Begin Procedure;
The End Procedure;
Bottle-Change Procedures;
The Shut-Down Procedure; and
User-defined functions.

To print, select the PRINT key from the appropriate menus.

1. DNA Sequences can be printed using the DNA Sequence Editor. The printout lists the base sequence between the 5' and 3' termini.

2. Column Set-Up is printed from the Column Monitor menu. This printout lists:

COLUMN #
VERSION #

USER NAME:
CYCLES USED:
ENDING METHOD:
ENDING PROCEDURE:
SEQUENCE NAME:
SEQUENCE LENGTH:
DATE:
TIME:
COMMENT:

DNA SEQUENCE (5' ...to... 3')

3. Using the Disk Utilities Menu, a Partial or a Complete Directory Listing can be printed.

4. User-defined functions can be printed from the Function Editor.

5. Synthesis Cycles and Procedures are printed using the Cycle and Procedure Editor. Cycle printouts are shown in Appendix II.

THE FRACTION COLLECTOR

A Fraction Collector receives and holds the trityls produced from the detritylation step. The trityls are later analyzed to monitor the efficiency of a synthesis.

The Fraction Collector must be placed adjacent to the Synthesizer, near the trityl collection port. Any commercially available Fraction Collector with an external advance switch will suffice. The Fraction Collector should be able to contain 10-15 mL tubes.

The Fraction Collector is connected by two wires to the rear of the Synthesizer at the number 2 position of the terminal strip labeled SIGNAL. When connected in this manner and when Function 5 is activated, an electrical signal is sent to the Signal 2 position and produces a contact closure to advance the Fraction Collector. The duration of the electrical pulse (i.e. the Pulse Width) can be set from the Pulse Width Configuration Menu. The pulse is set according to the individual requirements of your Fraction Collector. Refer to Section 4, the Relay Configure Menu to set the Pulse Width.

Fraction Collectors With Three-Column Instruments:

There are two methods for collecting the trityl fractions when using the Applied Biosystems Model 380B DNA Synthesizer with 3-column capability.

The first method is to use a single fraction collector with the ability to hold three 1/16" trityl tubes in parallel. These fraction collectors typically use moveable cartridges for holding the tubes.

The second method is to use three fraction collectors, each collecting a trityl fraction from one column. When using multiple units, ensure that each fraction collector is identical to the others and is connected with the same polarity. This can most easily be accomplished by connecting the fraction collectors with twisted wire of two colors. All wires of one color are connected to the same terminal on the instrument and to identical terminals on the fraction collectors. This is illustrated below in Figure 6-1. Without these precautions, damage to the fraction collectors or to the DNA Synthesizer may occur.

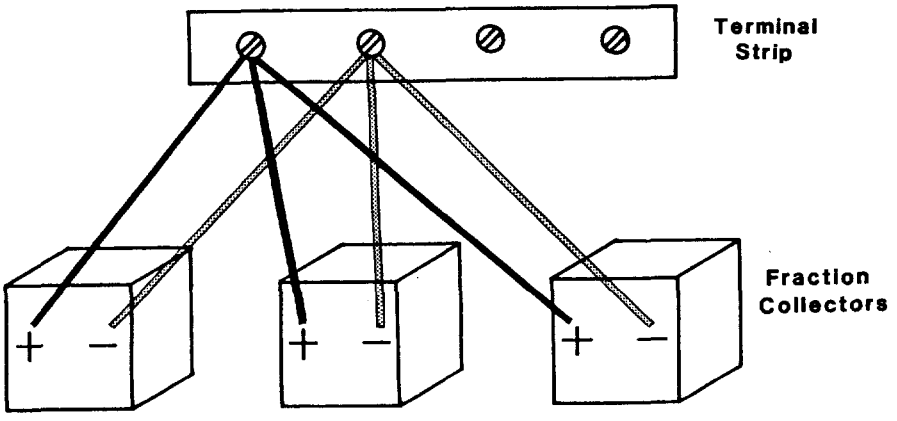


Figure 6-1: Connecting Multiple Fraction Collectors

APPENDIX I

APPENDIX I

FUNCTIONS FOR THE MODEL 380B DNA SYNTHESIZER
(Ver. 2.00)

* Functions can be of three basic types. A function can activate Valves or Relays (R) or a function can be a Cycle Directive (CD). Refer to Section 2 for a description of each.

FUNCTION NUMBER	FUNCTION NAME	ACTIVATED SWITCHES	FUNCTION TYPE*
1	Block Flush	16,36,41,44,49,52,57,60	
2	Reverse Flush	36,40,41,48,49,56,57	
3	Block To Column	40,44	CD
4	Wait	-	
5	Advance FC RELAY	-	
6	Waste-Port	-	CD
7	Waste-Bottle	-	CD
8	Flush To CLCT	16,40,42,45	
9	#18 To Column	2,17,40,44	
10	#18 To Waste	2,17,36	
11	#17 To Column	6,18,40,44	
12	#16 To Column	7,19,40,44	
13	#15 To Column	8,20,40,44	
14	#14 To Column	10,21,40,44	
15	#13 To Column	9,22,40,44	
16	Cap Prep	13	
17	Interrupt	-	CD
18	#16 To Waste	7,19,36	

19	B+TET To Col 1	0,11,28,40,44	
20	B+TET To Col 2	0,11,28,48,52	
21	B+TET To Col 3	0,11,28,56,60	
22	Cap To Col 1	13,25,26,40,44	
23	Cap To Col 2	13,25,26,48,52	
24	Cap To Col 3	13,25,26,56,60	
25	#17 To #8	5,6,18,23	
26	#8 To Column	4,23,40,44	
27	#10 To Collect	14,24,40,42,45	
28	Phos Prep	0,11	
29	Flush To # 8	5,16,23	
30	#17 To Waste	6,18,36	
31	Recorder On	-	R
32	Recorder Off	-	R
33	Cycle Entry		CD
34	Flush to Waste	16,40,44	
35	Relay 3 On		R
36	Relay 3 Off		R
37	Relay 3 Pulse		R
38	Relay 4 On		R
39	Relay 4 Off		R
40	Relay 4 Pulse		R
41	#8 Vent	5	
42	#10 Vent	1,15	
43	#18 Purge	2,3	
44	Phos Purge	11,12	
45	Group 1 On		CD
46	Group 1 Off		CD
47	Group 2 On		CD
48	Group 2 Off		CD

49	Group 3 On		CD
50	Group 3 Off		CD
51	Tet Purge	0,1	
52	A To Waste	11,35,36	
53	G To Waste	11,34,36	
54	C To Waste	11,33,36	
55	T To Waste	11,32,36	
56	#5 To Waste	11,31,36	
57	#6 To Waste	11,30,36	
58	#7 To Waste	11,29,36	
59	Cap A To Waste	13,26,36	
60	Cap B To Waste	13,25,36	
61	TET To Waste	0,28,36	
62	Flush To A	12,16,35	
63	Flush To G	12,16,34	
64	Flush To C	12,16,33	
65	Flush To T	12,16,32	
66	Flush To #5	12,16,31	
67	Flush To #6	12,16,30	
68	Flush To #7	12,16,29	
69	Flush To TET	1,16,28	
70	Flush To #18	3,16,17	
71	#18 To A	2,12,17,35	
72	#18 To G	2,12,17,34	
73	#18 To C	2,12,17,33	
74	#18 To T	2,12,17,32	
75	#18 To #5	2,12,17,31	
76	#18 To #6	2,12,17,30	
77	#18 To #7	2,12,17,29	
78	#18 To TET	1,2,17,28	

79	#8 To Waste	4,23,36
80	#10 To Waste	14,24,36
81	#15 To Waste	8,20,36
82	#14 To Waste	10,21,36
83	#13 To Waste	9,22,36
---	---	---
84	#18 To #14+#15	2,17,20,21
85	Flush - #14+#15	16,20,21
86	#18 To #11+#12	2,17,25,26
87	Flush - #11+#12	16,25,26
88	#18 To #10	2,17,24
89	Flush To #10	16,24
---	---	---
90	Tet To Column	0,28,40,44
91	Cap To Column	13,25,26,40,44
---	---	---
92	Undefined	User-Functions
93	Undefined	
94	Undefined	
95	Undefined	
96	Undefined	
97	Undefined	
98	Undefined	
99	Undefined	

380B OPERATING SYSTEM ERRORS:

Error Number	Error Message	Error Description
100 (64H)	Hardware Failure	Floppy Disk Controller was busy or the DNA transfer failed. No recovery is available and file integrity is lost. Call a service representative.
101 (65H)	Invalid Filename	The filename sent to the operating system was all blanks. This indicates a problem in the application software. File integrity is maintained.
102 (66H)	Invalid Access Type	An attempt was made to read to a file open in write access or write to a file open in read access. This indicates a problem in the application software. File integrity is lost.
103 (67H)	Invalid Record Length	The record length sent into the open write function was equal to zero. This indicates a problem in the application software. File integrity is maintained.
104 (68H)	Disk Directory Full	The disk directory is full on a disk on which an open write was attempted. This can be resolved by purging a file from the disk or using another disk which has directory space available. File integrity is maintained.
105 (69H)	FCB Directory Full	An open, either read or write, was attempted and the maximum number of open files has already been reached. Indicates a problem in application software such that a file or group of files have not been closed, thus causing a shortage of FCB's. File integrity is maintained.
106 (6AH)	File Not Found	The filename passed into the operating system could not be found on the disk. Indicates an application software problem. File integrity is maintained.

Error Number	Error Message	Error Description
107 (6BH)	File Already Exists	The filename specified on an open write already exists on the disk. Must purge the file from the disk before the new open write can be successful. File integrity is maintained.
108 (6CH)	No Available AUs	A new file was attempted to be created on a disk and there were no allocation units available for the file's data. If the error is returned on an open write function call, then file security is maintained. If the error is returned on a write record function call, then file integrity is lost. Can be resolved by using another disk with sufficient disk space or by purging a file from the current disk.
109 (6DH)	Cannot Purge Open File	An attempt was made to purge a file which is currently open. The file must be closed before the purge can be attempted. File integrity is maintained.
110 (6EH)	Invalid File Token	An invalid file token was passed to a read or write record function call. Indicates an application software problem. File integrity is lost.
111 (6FH)	Attempted Read Past EOF	An attempt was made to read from a file after the EOF REACHED warning was returned. Indicates an application software problem. File integrity is maintained.
112 (7OH)	Cannot Rename Open File	An attempt was made to rename a file which is currently open. The file must be closed before the rename can be attempted. File integrity is maintained.
113 (71H)	Invalid Drive Specifier	During a rename, the drive specified in the OLD parameter and the drive specified in the NEW parameter did not match up. This indicates a problem in application software. File integrity maintained.

Error Number	Error Message	Error Description
114 (72H)	Security Violation	A file was attempted to be opened in read access before it was closed from its creation. Indicates an application software problem. The file opened in write access must be closed before the read access open can be attempted. File integrity is maintained.
115 (73H)	Disk ID Invalid	The wrong disk is in the drive to do a read or write record to an opened file. Placing the correct disk in the drive and attempting the read write again is all that is necessary to do the record I/O. File integrity is maintained.
116 (74H)	OS Internal Error	Either a drive, track, or sector number was invalid when passed to a disk I/O routine. This indicates a problem with the operating system software. File security is lost.
117 (75H)	Disk Write Protected	The disk in the drive is software write protected so no purge, open write, or rename function call can be successfully attempted on the disk. Turning off the software write protect on the disk allows these commands to be attempted once again. File integrity is maintained.
118 (76H)	No Disk In Drive	An attempt was made to do something with the drive and there is no disk presently found there. Placing the proper disk into the drive and retrying the action will resolve the problem. File integrity is maintained.
119 (77H)	Hardware Write Protect	The disk in the drive is hardware write protected. This is not allowed in this operating system because file access dates cannot be updated with hardware write protection on. The operating system will not allow anything to happen with the disk in its protected state. File integrity is maintained.

Error Number	Error Message	Error Description
120 (78H)	Disk Not Formatted	The disk in the drive is not formatted to the proper specification. Placing a disk in with the proper format will allow the operating system to perform with this drive again. File integrity is maintained.
121 (79H)	EOF Reached	Only a warning. Indicates that the last record in a file open for read has been read and that no record was returned on the call just made. File security is maintained.
122 (7AH)	Wrong Software	The disk in the drive does not contain the same version of software that the system was booted with so the subprogram load cannot be done. File integrity is maintained.
123 (7BH)	Cannot Read Side 1	The system has a single-sided drive configuration in it, but a read has been attempted on the second side of a double-sided disk in the drive. File integrity is maintained.

APPENDIX II

Appendix II contains all the current (December, 1989) synthesis cycles and procedures found on the Version 2.00 software disk. The text in the User Manual has not yet been fully updated to reflect software Version 2.00 and may refer to obsolete cycles. Refer to User Bulletin 54 for further information about Version 2.00 synthesis cycles.

VERSION 2.00

CURRENT SYNTHESIS CYCLES
MODEL 380B DNA SYNTHESIZER

CYCLE TYPE	CYCLE NAME	
1-Micromole Cycles	Standard Cycles	Large Bottle Cycles
1-Column ß-cyanoethyl	cef1	ceaf1
3-Column ß-cyanoethyl	cef3	ceaf3
Small Scale Cycles	Standard Cycles	Large Bottle Cycles
1-Column ß-cyanoethyl	sscef1	ssceaf1
3-Column ß-cyanoethyl	sscef3	ssceaf3
Combined 1 Micromole & Small Scale Cycles	Standard Cycles	Large Bottle Cycles
1-Column RNA	rnaf1	rnaaf1
1-Column H-phosphonate	(hpaf1)	hpaf1
3-Column RNA	rnaf3	rnaaf3
3-Column H-phosphonate	(hpaf3)	hpaf3
10 Micromole Cycles	Standard Cycles	Large Bottle Cycles
1-Column ß-cyanoethyl	10cef1	10ceaf1
1-Column RNA	10rnaf1	10rnaaf1
1-Column H-phosphonate	10hpf1	10hpaf1
3-Column ß-cyanoethyl	10cef3	10ceaf3
3-Column RNA	10rnaf3	10rnaaf3
3-Column H-phosphonate	10hpf3	10hpaf3

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: ssceaf3
NUMBER OF STEPS: 81
DATE: Nov 2, 1989
TIME: 18:54

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	21	B+TET To Col 3	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	91	Cap To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	13	#15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: ssceaf3
NUMBER OF STEPS: 81

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
55	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: ceaf3
NUMBER OF STEPS: 87
DATE: Nov 2, 1989
TIME: 10:57

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	C	G	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	20	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	21	B+TET To Col 3	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: ceaf3
NUMBER OF STEPS: 87

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	13	#15 To Column	12 vs 23	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
61	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
80	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
81	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
82	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
83	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
84	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
85	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10ceaf3
NUMBER OF STEPS: 73
DATE: Nov 2, 1989
TIME: 19:00

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	21	B+TET To Col 3	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	13	#15 To Column	70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: 10ceaf3
NUMBER OF STEPS: 73

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
47	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
58	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
59	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
61	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
62	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
63	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: hpaf3
NUMBER OF STEPS: 59
DATE: Nov 2, 1989
TIME: 19:01

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	20	B+TET To Col 2	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	21	B+TET To Col 3	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	95	Undefined	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
31	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	14	#14 To Column	12			Yes	Yes	Yes	Yes	Yes	No
42	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
43	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: hpaf3
NUMBER OF STEPS: 59

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10hpaf3
NUMBER OF STEPS: 55
DATE: Nov 2, 1989
TIME: 19:03

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	20	B+TET To Col 2	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	21	B+TET To Col 3	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	95	Undefined	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	• 1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
29	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	5	Advance FC	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
39	14	#14 To Column	35			Yes	Yes	Yes	Yes	Yes	No
40	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
41	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
43	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10hpaf3
NUMBER OF STEPS: 55

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: rnaaf3
NUMBER OF STEPS: 87
DATE: Nov 2, 1989
TIME: 19:05

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	21	B+TET To Col 3	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: rnaaf3
NUMBER OF STEPS: 87

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
61	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
80	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
81	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
82	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
83	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
84	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
85	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10rnaaf3
NUMBER OF STEPS: 73
DATE: Nov 2, 1989
TIME: 19:06

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	21	B+TET To Col 3	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	13	#15 To Column	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: 10rnaaf3
NUMBER OF STEPS: 73

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
47	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
58	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
59	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
61	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
62	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
63	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: sscef3
NUMBER OF STEPS: 81
DATE: Nov 2, 1989
TIME: 19:08

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	21	B+TET To Col 3	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	91	Cap To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	13	#15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: sscef3
NUMBER OF STEPS: 81

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	15	#13 TTo Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye s
50	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
55	18	#16 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
80	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
81	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: cef3
NUMBER OF STEPS: 87
DATE: Nov 2, 1989
TIME: 19:10

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	21	B+TET To Col 3	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: cef3
NUMBER OF STEPS: 87

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
61	18	#16 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
80	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
81	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
82	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
83	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
84	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
85	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10cef3
NUMBER OF STEPS: 73
DATE: Nov 2, 1989
TIME: 19:12

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	21	B+TET To Col 3	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	13	#15 To Column	70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: 10cef3
NUMBER OF STEPS: 73

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
47	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
58	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
59	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
61	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
62	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
63	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10hpf3
NUMBER OF STEPS: 55
DATE: Nov 2, 1989
TIME: 19:13

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	20	B+TET To Col 2	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	21	B+TET To Col 3	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	95	Undefined	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
29	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
39	14	#14 To Column	35			Yes	Yes	Yes	Yes	Yes	No
40	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
41	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
43	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10hpf3
NUMBER OF STEPS: 55

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: rnaf3
NUMBER OF STEPS: 87
DATE: Nov 2, 1989
TIME: 19:14

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	20	B+TET To Col 2	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	20	B+TET To Col 2	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	21	B+TET To Col 3	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	21	B+TET To Col 3	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: rnaf3
NUMBER OF STEPS: 87

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
61	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
63	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
64	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
65	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
66	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
67	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
68	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
69	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
70	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
72	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
73	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
74	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
75	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
76	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
77	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
78	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
79	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
80	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
81	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
82	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
83	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
84	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
85	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
86	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
87	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10rnaf3
NUMBER OF STEPS: 73
DATE: Nov 2, 1989
TIME: 19:16

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	+47	Group 2 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	20	B+TET To Col 2	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	-48	Group 2 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	+49	Group 3 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	21	B+TET To Col 3	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	-50	Group 3 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	13	#15 To Column	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: 10rnaf3
NUMBER OF STEPS: 73

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
47	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
50	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
51	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
52	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
53	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
54	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
55	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
56	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
58	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
59	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
61	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
62	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
63	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
64	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
65	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
66	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
67	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
68	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
69	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
70	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
71	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
72	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
73	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: ssceaf1
NUMBER OF STEPS: 60
DATE: Nov 2, 1989
TIME: 19:18

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	91	Cap To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	13	#15 To Column	10 vs 15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
37	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: ssceaf1
NUMBER OF STEPS: 60

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
58	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

CYCLE NAME: ceaf1
NUMBER OF STEPS: 62
DATE: Nov 2, 1989
TIME: 19:19

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
39	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: ceaf1
NUMBER OF STEPS: 62

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
58	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
59	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10ceaf1
NUMBER OF STEPS: 58
DATE: Nov 2, 1989
TIME: 19:20

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	13	#15 To Column	70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
35	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
43	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10ceaf1
NUMBER OF STEPS: 58

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: hpaf1
NUMBER OF STEPS: 48
DATE: Nov 2, 1989
TIME: 19:22

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	4	Wait	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	95	Undefined	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
23	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	14	#14 To Column	12			Yes	Yes	Yes	Yes	Yes	No
31	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
32	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
33	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
34	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
35	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
36	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
37	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
38	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
39	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
40	14	#14 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
41	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
43	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: hpaf1
NUMBER OF STEPS: 48

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	9	#18 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
48	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10hpaf1
NUMBER OF STEPS: 44
DATE: Nov 2, 1989
TIME: 19:23

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	4	Wait	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	95	Undefined	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
21	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
28	14	#14 To Column	35			Yes	Yes	Yes	Yes	Yes	No
29	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
30	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
31	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
32	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
33	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
34	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
35	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
36	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
37	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
38	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
39	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
40	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
41	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: rnaaf1
NUMBER OF STEPS: 62
DATE: Nov 2, 1989
TIME: 19:24

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
39	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: rnaaf1
NUMBER OF STEPS: 62

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
58	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
59	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10rnaaf1
NUMBER OF STEPS: 58
DATE: Nov 2, 1989
TIME: 19:26

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
s											
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	13	#15 To Column	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
35	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
43	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10rnaaf1
NUMBER OF STEPS: 58

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: sscef1
NUMBER OF STEPS: 60
DATE: Nov 2, 1989
TIME: 19:27

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	91	Cap To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	13	#15 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
37	18	#16 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: sscef1
NUMBER OF STEPS: 60

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
58	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
59	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: cef1
NUMBER OF STEPS: 62
DATE: Nov 2, 1989
TIME: 19:29

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
39	18	#16 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: cef1
NUMBER OF STEPS: 62

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
F											
58	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
59	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10cef1
NUMBER OF STEPS: 58
DATE: Nov 2, 1989
TIME: 19:30

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	13	#15 To Column	70	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	4	Wait	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	**33**	**Cycle Entry**	1	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
35	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
43	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10cef1
NUMBER OF STEPS: 58

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10hpf1
NUMBER OF STEPS: 44
DATE: Nov 2, 1989
TIME: 19:32

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	13	#15 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	19	B+TET To Col 1	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	4	Wait	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	28	Phos Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	95	Undefined	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	13	#15 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
21	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
28	14	#14 To Column	35			Yes	Yes	Yes	Yes	Yes	No
29	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No
30	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
31	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
32	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
33	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
34	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
35	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
36	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
37	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
38	14	#14 To Column	35	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
39	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
40	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
41	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
44	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: rnaf1
NUMBER OF STEPS: 62
DATE: Nov 2, 1989
TIME: 19:33

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	90	TET To Column	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	19	B+TET To Col 1	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	9	#18 To Column	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	91	Cap To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	4	Wait	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	13	#15 To Column	12	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	2	Reverse Flush	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	34	Flush to Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
39	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	15	#13 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

CYCLE NAME: rnaf1
NUMBER OF STEPS: 62

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	10			Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	1			Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	34	Flush to Waste	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	14	#14 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
57	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
58	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
59	34	Flush to Waste	8	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
60	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
61	12	#16 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
62	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

SYNTHESIS CYCLE
VERSION 2.00

CYCLE NAME: 10rnaf1
NUMBER OF STEPS: 58
DATE: Nov 2, 1989
TIME: 19:34

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	28	Phos Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	+45	Group 1 On	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	90	TET To Column	2	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	19	B+TET To Col 1	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	-46	Group 1 Off	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	4	Wait	600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	16	Cap Prep	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	91	Cap To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	4	Wait	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	10	#18 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	81	#15 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	13	#15 To Column	80	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	4	Wait	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	**33**	**Cycle Entry**	**1**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
35	83	#13 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	15	#13 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	6	Waste-Port	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	5	Advance FC	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	82	#14 To Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
42	14	#14 To Column	30			Yes	Yes	Yes	Yes	Yes	No
43	34	Flush to Waste	3			Yes	Yes	Yes	Yes	Yes	No

(Continued next page.)

CYCLE NAME: 10rnaf1
NUMBER OF STEPS: 58

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
45	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
46	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
47	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
48	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
49	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
50	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
51	34	Flush to Waste	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
52	14	#14 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
53	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
54	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
55	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
56	7	Waste-Bottle	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
57	12	#16 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
58	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BEGIN PROCEDURE
VERSION 2.00

PROCEDURE NAME: phos003
NUMBER OF STEPS: 11
DATE: Nov 3, 1989
TIME: 17:26

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	28	Phos Prep	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	52	A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	53	G To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	54	C To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	55	T To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	56	#5 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	57	#6 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	58	#7 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	61	TET To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	10	#18 To Waste	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	1	Block Flush	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprce
NUMBER OF STEPS: 27
DATE: Nov 3, 1989
TIME: 17:10

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye
s											
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	27	#10 To Collect	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprce10
NUMBER OF STEPS: 26
DATE: Nov 3, 1989
TIME: 17:09

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	8	Flush To CLCT	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	8	Flush To CLCT	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	9	#18 To Column	90	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	120	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprhp
NUMBER OF STEPS: 45
DATE: Nov 3, 1989
TIME: 17:08

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	92	Undefined	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	94	Undefined	240	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	93	Undefined	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	94	Undefined	180	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	9	#18 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	9	#18 To Column	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	9	#18 To Column	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	2	Reverse Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	27	#10 To Collect	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	9	#18 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
44	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprhp10
NUMBER OF STEPS: 49
DATE: Nov 3, 1989
TIME: 17:05

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	92	Undefined	120	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	94	Undefined	240	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	93	Undefined	120	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	94	Undefined	180	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	9	#18 To Column	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	2	Reverse Flush	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	27	#10 To Collect	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
31	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
32	27	#10 To Collect	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
33	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
34	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
35	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
36	27	#10 To Collect	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
37	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
38	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
39	4	Wait	900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
40	8	Flush To CLCT	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
41	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
42	8	Flush To CLCT	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
43	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

(Continued next page.)

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprhp10
NUMBER OF STEPS: 49

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
44	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
45	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
46	9	#18 To Column	90	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
47	2	Revverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Ye s
48	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
49	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprna
NUMBER OF STEPS: 27
DATE: Nov 3, 1989
TIME: 16:55

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	27	#10 To Collect	18	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	27	#10 To Collect	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	8	Flush To CLCT	9	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	9	#18 To Column	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

END PROCEDURE
VERSION 2.00

PROCEDURE NAME: deprna10
NUMBER OF STEPS: 26
DATE: Nov 3, 1989
TIME: 16:56

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	2	Reverse Flush	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	27	#10 To Collect	30	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	10	#18 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	4	Wait	1800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	8	Flush To CLCT	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	27	#10 To Collect	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	8	Flush To CLCT	15	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	1	Block Flush	4	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	9	#18 To Column	90	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	2	Reverse Flush	120	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 1
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:10

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	71	#18 To A	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	62	Flush To A	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	52	A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yess	Yes	Ye s
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 2
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:09

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	72	#18 To G	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	63	Flush To G	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	53	G To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 3
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:20

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	73	#18 To C	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	64	Flush To C	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	54	C To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 4
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:19

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	74	#18 To T	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	65	Flush To T	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	55	T To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 5
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:22

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	75	#18 To #5	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	66	Flush To #5	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	56	#5 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 6
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:23

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	76	#18 To #6	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	67	Flush To #6	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	57	#6 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 7
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:23

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	77	#18 To #7	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	68	Flush To #7	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	44	Phos Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	58	#7 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 8a
NUMBER OF STEPS: 12
DATE: Apr 28, 1985
TIME: 16:24

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	79	#8 To Waste	120	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	25	#17 to #8	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	79	#8 To Waste	200	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	25	#17 to #8	40	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	79	#8 To Waste	200	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	79	#8 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	30	#17 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 9
NUMBER OF STEPS: 9
DATE: Apr 28, 1985
TIME: 16:24

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	78	#18 To TET	3	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	69	Flush To TET	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	51	Tet Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	61	TET To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 10
NUMBER OF STEPS: 4
DATE: Apr 28, 1985
TIME: 16:25

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	4	Wait	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	42	#10 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	4	Wait	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 11
NUMBER OF STEPS: 7
DATE: Apr 28, 1985
TIME: 16:26

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	16	Cap Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	59	Cap A To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 12
NUMBER OF STEPS: 7
DATE: Apr 28, 1985
TIME: 16:26

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	S	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	16	Cap Prep	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	60	Cap B To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 13
NUMBER OF STEPS: 6
DATE: Apr 28, 1985
TIME: 16:27

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	83	#13 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 14
NUMBER OF STEPS: 6
DATE: Apr 28, 1985
TIME: 16:29

STEP NUMBER	FUNCTION #	FUNCTION NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	82	#14 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 15
NUMBER OF STEPS: 6
DATE: Apr 28, 1985
TIME: 16:29

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	81	#15 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 16
NUMBER OF STEPS: 6
DATE: Apr 28, 1985
TIME: 16:30

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	18	#16 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 17
NUMBER OF STEPS: 6
DATE: Apr 28, 1985
TIME: 16:30

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	30	#17 To Waste	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

BOTTLE CHANGE PROCEDURE
VERSION 2.00

PROCEDURE NAME: bc 18
NUMBER OF STEPS: 7
DATE: Apr 28, 1985
TIME: 16:31

STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	10	#18 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	70	Flush To #18	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	17	Interrupt	1	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	43	#18 Purge	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

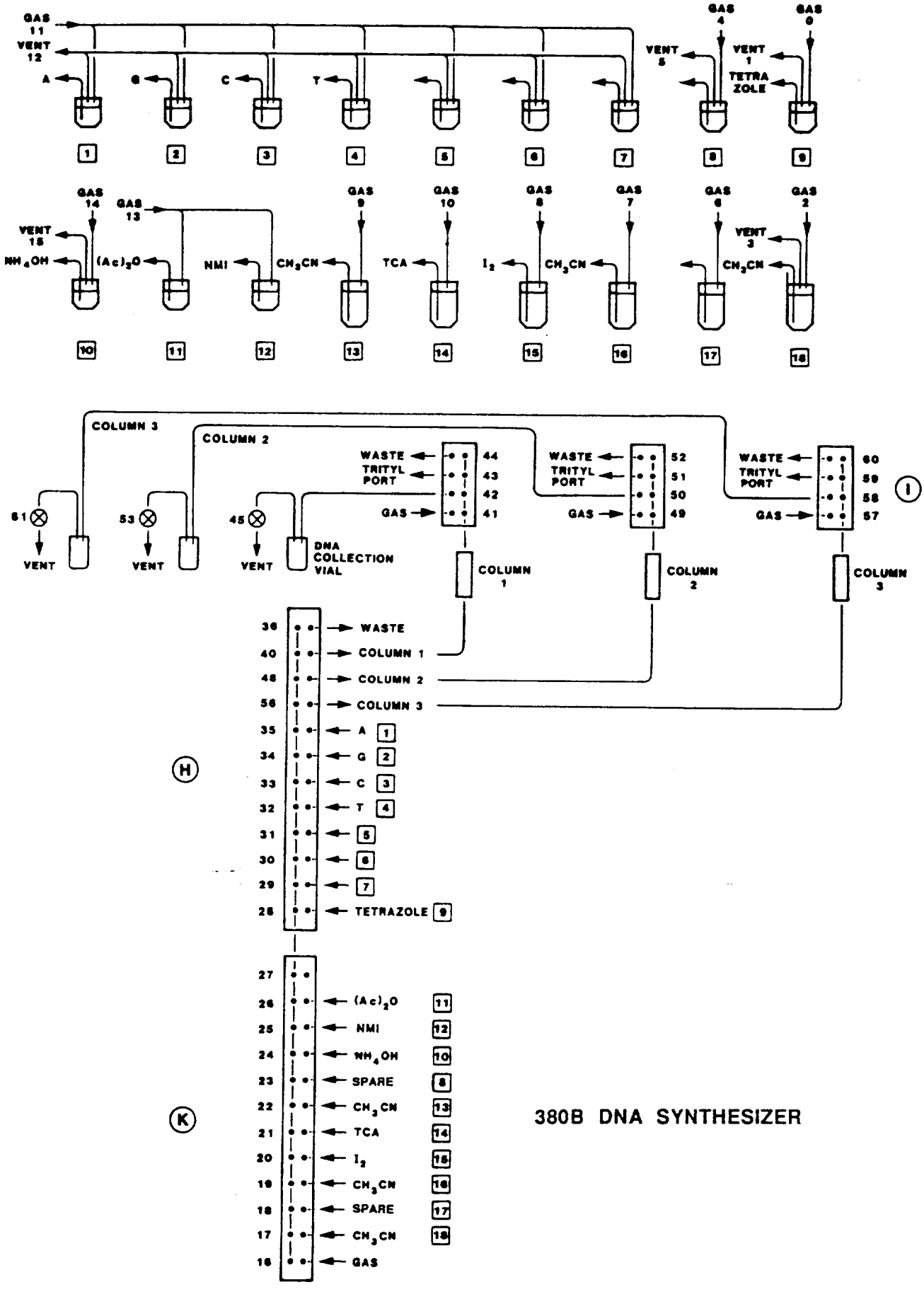
SHUT-DOWN PROCEDURE
VERSION 2.00

PROCEDURE NAME: clean003
NUMBER OF STEPS: 30
DATE: Apr 28, 1985
TIME: 16:32

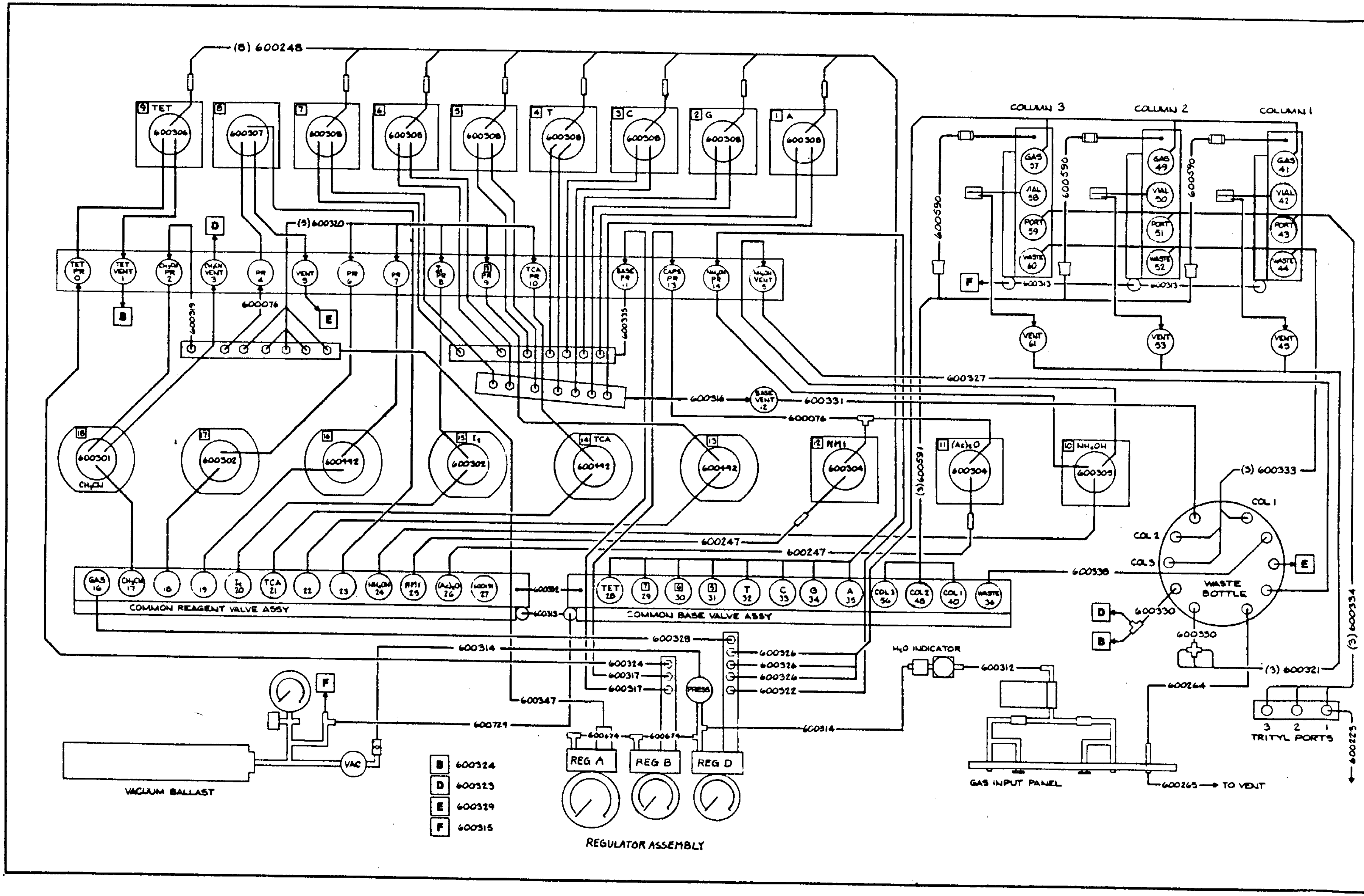
STEP NUMBER	FUNCTION #	NAME	STEP TIME	STEP ACTIVE FOR BASES A	G	C	T	5	6	7	SAFE STEP
1	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	10	#18 To Waste	10	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	71	#18 To A	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4	72	#18 To G	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	73	#18 To C	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	74	#18 To T	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
7	75	#18 To #5	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8	76	#18 To #6	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	77	#18 To #7	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	78	#18 To TET	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	88	#18 to #10	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	86	#18 to #11+#12	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
13	84	#18 to #14+#15	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
14	62	Flush To A	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
15	63	Flush To G	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
16	64	Flush To C	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
17	65	Flush To T	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
18	66	Flush To #5	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
19	67	Flush To #6	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20	68	Flush To #7	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
21	69	Flush To TET	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
22	89	Flush to #10	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
23	87	Flush- #11+#12	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
24	85	Flush- #14+#15	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
25	1	Block Flush	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
26	30	#17 To Waste	7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
27	25	#17 to #8	6	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
28	29	Flush to #8	60	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
29	41	#8 Vent	5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
30	1	Block Flush	20	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

APPENDIX III

Applied Biosystems



380B DNA SYNTHESIZER



(8) 600248
COLUMN 3
COLUMN 2
COLUMN 1
COMMON REAGENT VALVE ASSY
COMMON BASE VALVE ASSY
WASTE BOTTLE
COL 1
COL 2
COL 3
TRITYL PORTS
GAS INPUT PANEL
600265 → TO VENT
H_2O INDICATOR
VACUUM BALLAST
REG A
REG B
REG D
REGULATOR ASSEMBLY
B 600324
D 600323
E 600329
F 600315

APPENDIX IV

SYNTHESIS LOG SHEET

SEQUENCE NAME: _____

SEQUENCE: _____

DATE: _____ USER: _____

	Nucleoside	Absorbance of Trityl Solution	Micromoles
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			

380B REAGENT/SOLVENT LOG

MONTH: _____

	#1	#2	#3	#4	#5	#6	#7	#8	
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

NOTES

380B REAGENT/SOLVENT LOG

MONTH: _____

	#9	#10	#11	#12	#13	#14	#15	#16	
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

NOTES

380B REAGENT/SOLVENT LOG

MONTH: _____

	#17	#18	ARGON	WASTE					
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

NOTES

Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free: +1 800.345.5224
Fax: +1 650.638.5884

Worldwide Sales Offices
Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For international office locations, please call our local office or refer to our web site at www.appliedbiosystems.com.

www.appliedbiosystems.com

Applied Biosystems

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Printed in the USA, 06/2001
Part Number 900168 Rev. C

an Applera business