Applied Biosystems 3400 DNA Synthesizer

User Guide

Applied Biosystems

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Part Number 4334680 Rev. C
06/2010

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Preface

How to Use This Guide

Purpose of This Guide

The *Applied Biosystems 3400 DNA Synthesizer User Guide* provides operating information for the Applied Biosystems 3400 DNA Synthesizer. It describes the instrument components, standard operating procedures, instrument software, and synthesis chemistry.

Audience

This guide is intended for 3400 DNA Synthesizer users who use the instrument for performing low-throughput synthesis of oligonucleotides.

Assumptions

This guide assumes that your 3400 DNA Synthesizer has been installed by an Applied Biosystems technical representative.

Text Conventions

This guide uses the following conventions:

- **Bold** indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow bracket (>) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File > Open > Spot Set**.

  Right-click the sample row, then select **View Filter > View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

Examples of the user attention words appear below:

Note: The size of the column affects the run time.

Note: The Calibrate function is also available in the Control Console.

IMPORTANT! To verify your client connection to the database, you need a valid Oracle user ID and password.

IMPORTANT! You must create a separate Sample Entry Spreadsheet for each 96-well microtiter plate.

Safety Alert Words

Safety alert words also appear in user documentation. For more information, see "Safety Alert Words" on page xviii.

How to Obtain More Information

Related Documentation

If you need more information on...	See the ...	Part Number
site preparation	*Applied Biosystems 3400 DNA Synthesizer Site Preparation and Safety Guide*	4334679

Send Us Your Comments

Applied Biosystems welcomes your comments and suggestions for improving its user documents. You can e-mail your comments to:

techpubs@appliedbiosystems.com

How to Obtain Services and Support

To contact Applied Biosystems Technical Support from North America by telephone, call **1.800.831.6844**.

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Services and Support**.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety and EMC Compliance Information

This section includes the following topics:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where a user needs to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

– Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

– Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

– Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for IMPORTANTs, each safety alert word in an Applied Biosystems document always appears accompanied by an open triangle figure that contains one of a variety of hazard symbols. *These hazard icons–hazard symbols bounded by open triangles–are identical to and signify the same hazard types as the hazard icons that are affixed to Applied Biosystems instruments* (see “Safety Symbols” on page xix).

Examples

The following are some specific examples of the use of safety alert words:

IMPORTANT! You must create a separate a Sample Entry Spreadsheet for each 96-well microtiter plate.

The lamp is extremely hot. Do not touch the lamp until it has cooled to room temperature.

CHEMICAL HAZARD. **Formamide**. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

ELECTRICAL HAZARD. Failure to ground the instrument properly can lead to an electrical shock. Ground the instrument according to the provided instructions.

Symbols on Instruments

Electrical Symbols on Instruments

The following table describes the electrical symbols that may be displayed on Applied Biosystems instruments.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety Symbols

The following table describes the safety *s*ymbols that may be displayed on Applied Biosystems instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety Labels on Instruments" on page xx). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.

	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Applied Biosystems instruments in combination with the safety symbols described in the preceding section.

English	Francais
CAUTION Hazardous chemicals. Read the Material Safety Data Sheets (MSDSs) before handling.	**ATTENTION** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant la manipulation des produits.
CAUTION Hazardous waste. Read the waste profile (if any) in the site preparation guide for this instrument before handling or disposal.	**ATTENTION** Déchets dangereux. Lire les renseignements sur les déchets avant de les manipuler ou de les éliminer.
CAUTION Hazardous waste. Refer to MSDS(s) and local regulations for handling and disposal.	**ATTENTION** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
WARNING Hot lamp.	**AVERTISSEMENT** Lampe brûlante.
WARNING Hot. Replace lamp with an Applied Biosystems lamp.	**AVERTISSEMENT** Composants brûlants. Remplacer la lampe par une lampe Applied Biosystems.
CAUTION Hot surface.	**ATTENTION** Surface brûlante.
DANGER High voltage.	**DANGER** Haute tension.
WARNING To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Applied Biosystems qualified service personnel.	**AVERTISSEMENT** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié de Applied Biosystems.
DANGER Laser radiation present when open and interlock defeated. Avoid direct exposure to laser beam.	**DANGER** Rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de sécurité. Eviter toute exposition directe avec le faisceau.

English	Francais
DANGER Laser radiation when open. Avoid direct exposure to laser beam.	**DANGER** Rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 laser radiation present when open and interlock defeated. Do not stare directly into the beam	DANGER de Class 2 Rayonnement laser en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 laser radiation present when open. Do not stare directly into the beam.	DANGER de Class 2 Rayonnement laser en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 LED when open and interlock defeated. Do not stare directly into the beam.	DANGER de Class 2 LED en cas d'ouverture et d'une neutralisation des dispositifs de securite. Eviter toute exposition directe avec le faisceau.
DANGER Class 2 LED when open. Do not stare directly into the beam.	DANGER de Class 2 LED en cas d'ouverture. Eviter toute exposition directe avec le faisceau.
CAUTION Moving parts.	**ATTENTION** Parties mobiles.

General Instrument Safety

WARNING **PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Applied Biosystems may result in personal injury or damage to the instrument.

Moving and Lifting the Instrument

CAUTION **PHYSICAL INJURY HAZARD.** The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Operating the Instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Material Safety Data Sheets (MSDSs).

Chemical Safety

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

WARNING **CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING **CHEMICAL HAZARD.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING **CHEMICAL STORAGE HAZARD**. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page xxii.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical Waste Safety

Chemical Waste Hazard

CAUTION **HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

WARNING **CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING **CHEMICAL STORAGE HAZARD**. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Profiles

A waste profile for the Applied Biosystems 3400 DNA Synthesizer is provided in the *Applied Biosystems 3400 DNA Synthesizer Site Preparation Guide.*

Waste profiles show the percentage compositions of the reagents in the waste stream generated during installation and during a typical user application, even though the typical application may not be used in your laboratory.

The waste profiles help you plan for the handling and disposal of waste generated by operation of the instrument. Read the waste profiles and all applicable MSDSs before handling or disposing of chemical waste.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Electrical Safety

DANGER **ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the 3400 DNA Synthesizer without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

DANGER **ELECTRICAL SHOCK HAZARD.** Improper fuses or high-voltage supply can damage the instrument wiring system and cause a fire. Before turning on the instrument, verify that the fuses are properly installed and that the instrument voltage matches the power supply in your laboratory.

WARNING **FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER **ELECTRICAL HAZARD.** Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER **ELECTRICAL HAZARD.** Use properly configured and approved line cords for the voltage supply in your facility.

DANGER **ELECTRICAL HAZARD.** Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage Rating

The 3400 DNA Synthesizer has an installation (overvoltage) category of II, and is classified as stationary equipment

Physical Hazard Safety

Compressed Gases

WARNING **PHYSICAL HAZARD. Nonflammable compressed gas (argon).** Contents are under pressure. Receive proper training on the handling of compressed gases before use. Exposure to rapidly expanding gas may cause frostbite. High concentrations of vapors in the immediate area can displace oxygen and cause asphyxiation. Use only in areas with adequate ventilation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **EXPLOSION HAZARD.** Pressurized gas cylinders are potentially explosive and can cause severe injury if not handled properly. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Solvents and Pressurized Fluids

WARNING **PHYSICAL INJURY HAZARD.** Always wear eye protection when working with solvents or any pressurized fluids.

WARNING **PHYSICAL INJURY HAZARD.** To avoid hazards associated with high-pressure fluids in polymeric tubing:

- Be aware that PTFE tubing is a polymeric material. Use caution when working with any polymer tubing that is under pressure.
- Always wear eye protection when in proximity to pressurized polymer tubing.
- Extinguish all nearby flames if you use flammable solvents.
- Do not use PTFE tubing that has been severely stressed or kinked.
- Do not use PTFE tubing with tetrahydrofuran or concentrated nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause PTFE tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40 mL/min) may cause a static charge to build up on the surface of the tubing. Electrical sparks may result.

Safety and Electromagnetic Compatibility (EMC) Standards

This section provides information on:

- U.S. and Canadian Safety Standards
- Canadian EMC Standard
- European Safety and EMC Standards
- Australian EMC Standards

U.S. and Canadian Safety Standards

This instrument has been tested to and complies with standard UL 3101-1, "Safety Requirements for Electrical Equipment for Laboratory Use, Part 1: General Requirements."

This instrument has been tested to and complies with standard CSA 1010.1, "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part 1: General Requirements."

Canadian EMC Standard

This instrument has been tested to and complies with ICES-001, Issue 3: Industrial, Scientific, and Medical Radio Frequency Generators.

European Safety and EMC Standards

Safety

This instrument meets European requirements for safety (Low Voltage Directive 73/23/EEC). This instrument has been tested to and complies with standards EN 61010-1:2001, "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part 1: General Requirements" and EN 61010-2-010, "Particular Requirements for Laboratory Equipment for the Heating of Materials."

EMC

This instrument meets European requirements for emission and immunity (EMC Directive 89/336/EEC). This instrument has been tested to and complies with standard EN 61326 (Group 1, Class B), "Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements."

Australian EMC Standards

This instrument has been tested to and complies with standard AS/NZS 2064, "Limits and Methods Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radio-frequency Equipment."

About the 3400 DNA Synthesizer 1

This chapter covers:

Instrument Overview

Description

The Applied Biosystems 3400 DNA Synthesizer automates all steps of single-stranded oligonucleotide synthesis. The 3400 DNA Synthesizer produces the highest quality of synthetic DNA currently attainable, while minimizing synthesis time and cost.

The instrument has:

- Four column positions with eight monomer positions (10-mL bottles)
- Nine reagent and solvent positions:
  - Six 180-mL bottles for the ancillary reagents
  - Two 2-L bottles for TCA/DCM and DCM
  - One 4-L bottle for acetonitrile (ACN)

Besides automating the general solid-phase synthesis chemistries for oligonucleotides, phosphorothioates, and RNA, the instrument can also cleave the oligonucleotides from solid support with ammonium hydroxide and collect them for deprotection in glass vials.

Synthesis Scales

The 3400 DNA Synthesizer produces oligonucleotides in three synthesis scales:

- 40 nmol (ABI LV40® Column)
- 0.2 µmol (ABI LV200™ Column and 0.2 µmol)
- 1 µmol

Note: LV = low volume

For plain oligonucleotides, the average length is 25 bases. The throughput (including automated cleavage) and yields for four-column synthesis of plain 25 mers is as follows:

Synthesis Scale	Throughput (Hours)	Yield (ODU*/base)
40 nmol	~ 4	0.25
0.2 µmol	~ 4	1
1 µmol	~ 4	5

*ODU = Optical Density Units

Instrument Components

The major components of the 3400 DNA Synthesizer are listed in the table below.

Component	See Page …
Controller	1-4
Chemical Delivery System	1-5

Synthesis Process

1. Prepare the instrument by checking all reservoirs and installing all required chemicals.
2. Create sequences and (optional) custom cycle scripts using the controller (which consists of the 3400 DNA Synthesizer software, an LCD screen, and a keypad).
3. Set up the synthesis run by selecting the sequences to be synthesized in each column, the cycle script, ending options, and (optional) trityl options.
4. Start the run.
5. Pressure-regulated argon forces the chemicals to flow from their reservoirs through the cycle-specific pathway and then to a column. This is the chemical delivery system.
6. Synthesis occurs within the column(s).
7. During synthesis, effluent flows out of the columns to the following containers:
   – Waste containers (chlorinated and nonchlorinated)
   – DNA collection vial

Controller

The controller directs and initiates all activity on the 3400 DNA Synthesizer. You use the controller to set up your synthesis runs on the instrument.

Controller Components

The major components of the controller are:

- The 3400 DNA Synthesizer software
- A four-line liquid crystal display (LCD) screen
- Keypad

Software

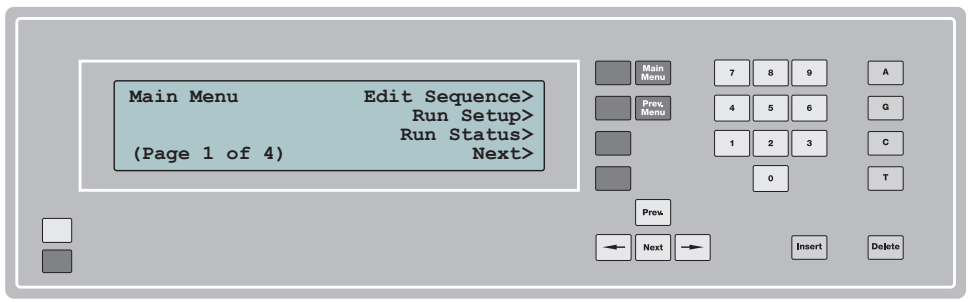
The 3400 DNA Synthesizer software defines operations for synthesis and is interpreted and executed by a microprocessor.

The software i*s menu driven,* which means that menus present various options and information about the synthesis or status of the instrument. In response, you select an option and give instructions by pressing the appropriate keys on the keypad. Detailed descriptions of all the menus are provided in Appendix B.

LCD Screen and Keypad

Synthesis information is shown on a four-line LCD screen. You interact with the information provided on the LCD screen by selecting keys on the keypad. The keypad consists of:

- Numeric keys (0 to 9)
- Base keys (A, G, C, T)
- Left- and right-arrow keys
- Soft keys, which are variable. The labels and actions of the soft keys change depending on the information displayed to the left of them.
- Six command keys (Main Menu, Prev. Menu, Prev., Next, Insert, Delete)



GR2269

Figure 1-1 LCD screen and keypad

Chemical Delivery System

The 3400 DNA Synthesizer uses a pressure-driven chemical delivery system to deliver reagents and solvents to the column. In this system, a set of solenoid valves opens to create a pathway for chemical flow. Pressure-regulated argon forces the chemicals to flow from their reservoirs through the pathway. The pathway consists of one or more valve blocks and delivery lines.

Reagent and solvent deliveries also rely on Applied Biosystems proprietary zero dead-volume valves, which increase reliability, eliminate cross-contamination, and reduce reagent costs.

System Components

The major components of the chemical delivery system are:

- Argon cylinder
- Pressure regulators
- Reagent bottles
- Pressure and delivery lines
- Pressure/vent lines
- Valve blocks
- Columns
- Waste containers

Figure 1-2 illustrates the components of the chemical delivery system. Descriptions of each component follow.

Note: All inner surfaces of the chemical delivery system are made of inert materials.

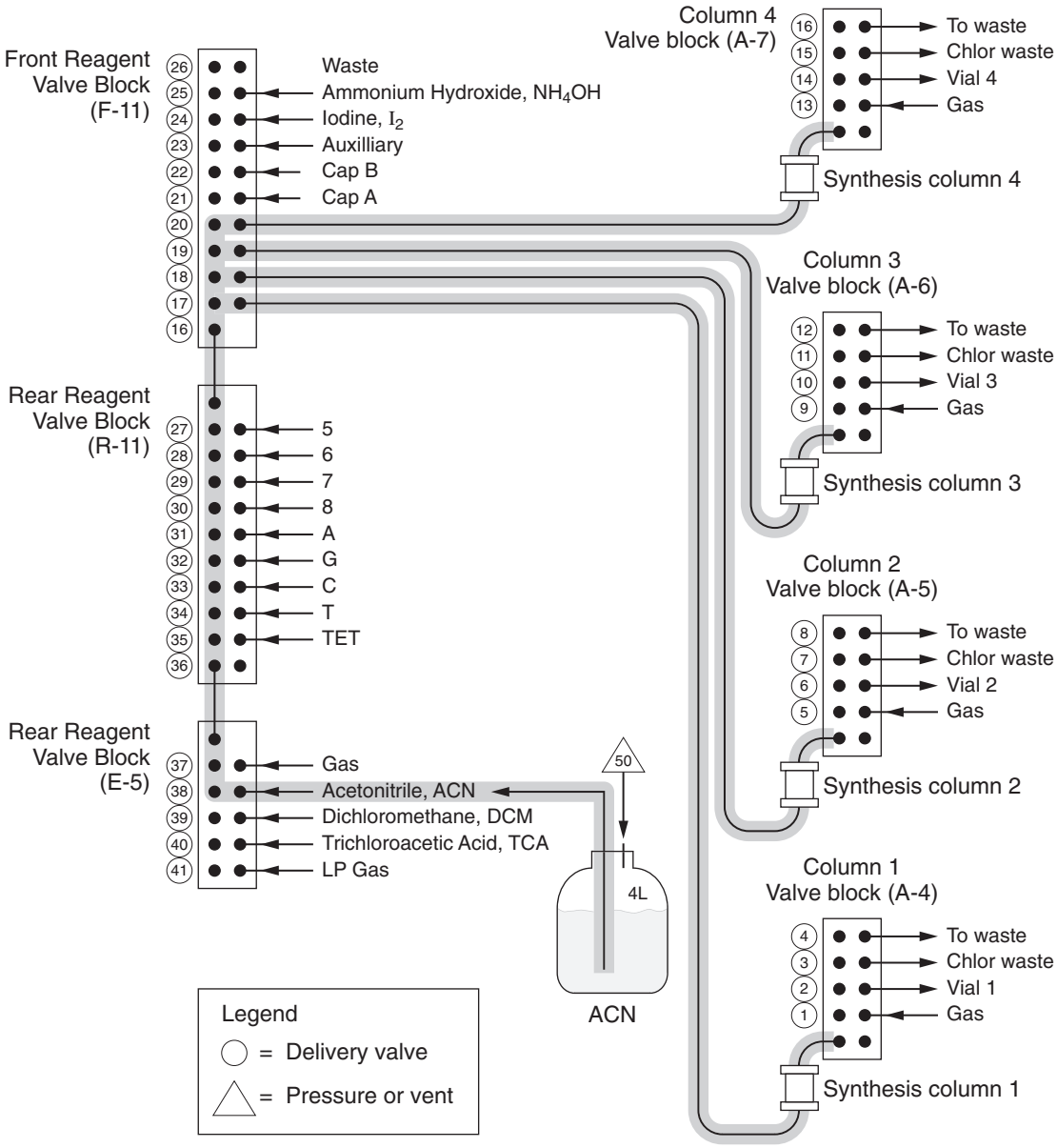


Figure 1-2 Chemical delivery system. Note that this diagram is simplified and does not show the valves used to pressurize and vent reagent reservoirs.

Argon Cylinder

System pressure is provided by pre-purified (99.998%) argon. Its high density and low oxygen contamination make it preferable to nitrogen.

An argon cylinder is connected to the inlet port at the right rear of the 3400 DNA Synthesizer. The pressure regulator on the tank is set to 60 psi. This high-pressure argon is used to supply argon to the regulators in the synthesizer.

Pressure Regulators

Argon entering the synthesizer travels through a 10-µm particle filter to three pressure regulators. The pressure regulators supply argon to the columns and reagent valve blocks. In addition, the regulators deliver argon to specific pressure valves used to pressurize the reagent and solvent reservoirs:

- The phosphoramidite bottles (positions A, G, C, T, 5, 7, 7, and 8) have one pressure valve, which supplies argon to a manifold pressurizing all eight phosphoramidite bottles simultaneously.
- The two capping reagent bottles (1-methylimidazole and acetic anhydride) share a pressure valve that channels the argon to a tee to pressurize both bottles.
- All other reagent bottles (tetrazole, ammonia, auxiliary, iodine, trichloroacetic acid, acetonitrile, and DCM) are pressurized by a single valve for each bottle.

IMPORTANT! For proper pressurization, bottles must be attached to *all positions,* even if some are empty.

Reagent Bottles

Bottle Type/Size	Label/Contents	Description
Phosphoramidites, 10-mL	A, G, C, T, 5, 6, 7, and 8	These bottles are pushed upward around a Teflon® insert containing an O-ring, which forms an airtight seal inside each bottle neck.
Auxiliary reagents, 180-mL	Tetrazole	These bottles screw snugly into a threaded cap mounted on the 3400 DNA Synthesizer. A disposable polyethylene insert forms an airtight seal between each cap and bottle.
	Ammonia	
	1-Methylimidazole	
	Acetic anhydride	
	Auxiliary	
	Iodine	
External	These bottles do not attach directly to the instrument.	
4-L	ACN	The external bottles are placed inside protective carriers on the left side of the instrument. A cap assembly, which includes the delivery and gas lines, screws onto each bottle and connects to the instrument. The acetonitrile cap has a Teflon insert and a rubber gasket. The DCM and TCA bottles have Teflon inserts and Kalrez® gaskets.
2-L	DCM	
	TCA	

Pressure and Delivery Lines

Each bottle has an argon pressure line and a delivery line entering through the cap insert. Most lines are color coded:

- Red lines are 0.5 mm ID
- Blue lines are 0.8 mm ID

As shown in Figure 1-3, the argon line remains above the liquid level while the delivery line extends to the bottom of the bottle. When the correct set of valves opens, the reservoir headspace is pressurized by argon, which pushes the liquid through the delivery line to its destination.

IMPORTANT! Bottles must be attached to *all positions,* even if some are empty. This helps to maintain argon pressure and keep the lines clean.

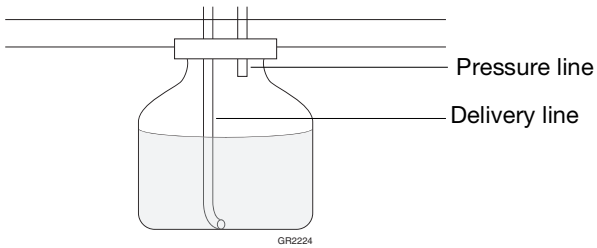


Figure 1-3 Reagent reservoir

Pressure/Vent Lines

For the phosphoramidite bottles, the pressure lines also function as vent lines. Fresh phosphoramidites are atmosphere sensitive; when they are placed on the instrument, they are purged with argon to eliminate air. A purge delivers gas through the delivery line. As the gas is passed through the bottle, the air escapes out the pressure/vent line.

IMPORTANT! Be sure the pressure/vent line is routed to a suitable exhaust (for example, a fume hood). If the pressure/vent line is blocked, back pressure will be generated and inhibit the delivery of reagents. To verify proper ventilation requirements, see the ventilation drawing in the *Applied Biosystems 3400 DNA Synthesizer Site Preparation and Safety Guide* (PN 4334679).

Valve Blocks

The valve blocks control gas and chemical flows to the columns and exit ports. The design of the valve blocks provides zero dead-volume when the solenoids are in the closed position.

Delivery lines feed into each valve block and connect to the common pathway in the valve block manifold through a manifold inlet line and a solenoid-controlled diaphragm valve. 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. An open solenoid causes the diaphragm to form a 2-µL 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.

The 3400 DNA Synthesizer has the following valve blocks:

- Two 11-port reagent valve blocks. These control the flow from the bottles to the bottom of the columns and to waste. Except for the phosphoramidites and tetrazole, reagent and solvent deliveries are made to all of the active columns simultaneously. Delivery times to each column are automatically adjusted for the slight flow rate change when more columns are active.
- Four 5-port column valve blocks. These direct the column effluent to the waste ports or the DNA collection vial. They also control the argon gas used to remove or flush the reagents from the column and the column valve block.
- One 6-port valve block. Two ports control the high-pressure and low-pressure gas (argon) flow. Three ports control ACN, DCM, and TCA. The last port is common to all.

Columns

Chemical steps for DNA synthesis take place within 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.

The initial support-bound nucleoside is contained in a disposable column that, besides the column body, has two retaining frits and two end fittings (Figure 1-4). The retaining frits are porous polyethylene held in place by the end caps. The inlet and outlet are female luer fittings designed for the male luer fittings on the instrument. The column is symmetrical (that is, no top or bottom, no front or back) and can be attached to the male luer fittings in any way.

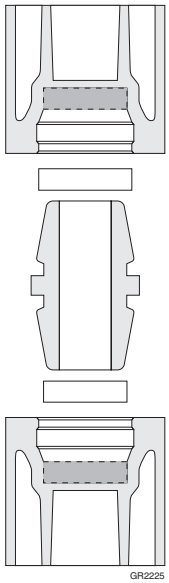


Figure 1-4 The column

The 3400 DNA Synthesizer supports four columns. Each column is color-coded to show the initial nucleoside.

Column	Color Code
A	Green
G	Yellow
C	Red
T	Blue

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

Waste Containers

The ultimate destination of most chemical deliveries is a waste container. The 3400 DNA Synthesizer has two waste containers:

- A 10-L (2.5-gal) polyethylene container for nonchlorinated waste
- A 6-L (1.5-gal) polyethylene container for chlorinated waste

The waste containers are free-standing. A waste line attaches to the caps of the waste containers and carries liquid waste from the 3400 DNA Synthesizer to the waste containers. Be sure that the waste containers are placed near the 3400 DNA Synthesizer on the floor or on a bench that is lower than the instrument.

About Valves and Cycle Scripts 2

This chapter covers:

Overview of 3400 Valves

The 3400 DNA Synthesizer has 53 solenoid valves that are opened and closed electrically and controlled through the microprocessor. The valves are numbered 1 to 53.

How the Valves Work

During a synthesis, valves are automatically opened to create the correct chemical pathways and allow reagent and gas deliveries. For flow to actually occur, several valves must be opened simultaneously. See "Overview of 3400 Valve Operations" on page 2-6.

You can also manually operate individual valves by using the Manual Control Menu. See "Using the Manual Control Menu" on page 6-20 for more information.

Valves 1 to 16

Valves 1 to 16 control input and output flows from the four column valve blocks, as described below.

Valve	Function
1	Controls the flow of argon to column 1
2	Controls the flow of DNA/RNA output to vial 1
3	Controls the flow of trityl output from column 1 to chlorinated waste
4	Controls the flow of waste from column 1
5	Controls the flow of argon to column 2
6	Controls the flow of DNA/RNA output to vial 2
7	Controls the flow of trityl output from column 2 to chlorinated waste
8	Controls the flow of waste from column 2
9	Controls the flow of argon to column 3
10	Controls the flow of DNA/RNA output to vial 3
11	Controls the flow of trityl output from column 3 to chlorinated waste
12	Controls the flow of waste from column 3
13	Controls the flow of argon to column 4
14	Controls the flow of DNA/RNA output to vial 4
15	Controls the flow of trityl output from column 4 to chlorinated waste
16	Controls the flow of waste from column 4

Valves 17 to 41 and 53

Valves 17 to 41 and 53 control the input of bases, reagents, and argon to the reagent valve blocks as well as output to the columns, as described below.

Valve	Function
17	Directs output from the reagent valve block to column 1
18	Directs output from the reagent valve block to column 2
19	Directs output from the reagent valve block to column 3
20	Directs output from the reagent valve block to column 4
21	Controls the input of acetic anhydride to the front reagent valve block
22	Controls the input of NMI to the front reagent valve block
23	Controls the input of Auxiliary to the front reagent valve block
24	Controls the input of Iodine or Auxiliary to the front reagent valve block
25	Controls the input of NH_4OH to the front reagent valve block
26	Output to waste, for both reagent valve blocks
27	Controls the input of base from position 5 to the reagent valve block
28	Controls the input of the position 6 base
29	Controls the input of the position 7 base
30	Controls the input of the position 8 base
31	Controls the input of base A to the rear reagent valve block
32	Controls the input of base G to the rear reagent valve block
33	Controls the input of base C to the rear reagent valve block
34	Controls the input of base T to the rear reagent valve block
35	Controls the input of tetrazole
36	Controls the connection between the third reagent block and the rear reagent block
37	Controls the input of argon (HP gas)
38	Controls the input of CH_3CN
39	Controls the input of DCM
40	Controls the input of TCA
41	Controls the pressure transducer
53	Controls the input of argon (LP gas)

Valves 42 to 52

Valves 42 to 52 are pressure valves that control the flow of argon to the manifolds (or bottles), as described below.

Valve	Argon (or vent) to...
42	Iodine (pressure)
43	manifold A (pressure manifold for all bases)
44	manifold B (vent manifold for all bases)
45	NH_4OH (pressure)
46	NH_4OH (vent)
47	Tetrazole (pressure)
48	Aux (pressure)
49	Acetic anhydride and NMI (pressure)
50	CH_3CN (pressure)
51	DCM (pressure)
52	TCA (pressure)

Valve Schematic

Figure 2-1 below is a simplified 3400 DNA Synthesizer schematic, illustrating the placement of the most important valves. For a more detailed schematic, see Appendix E.

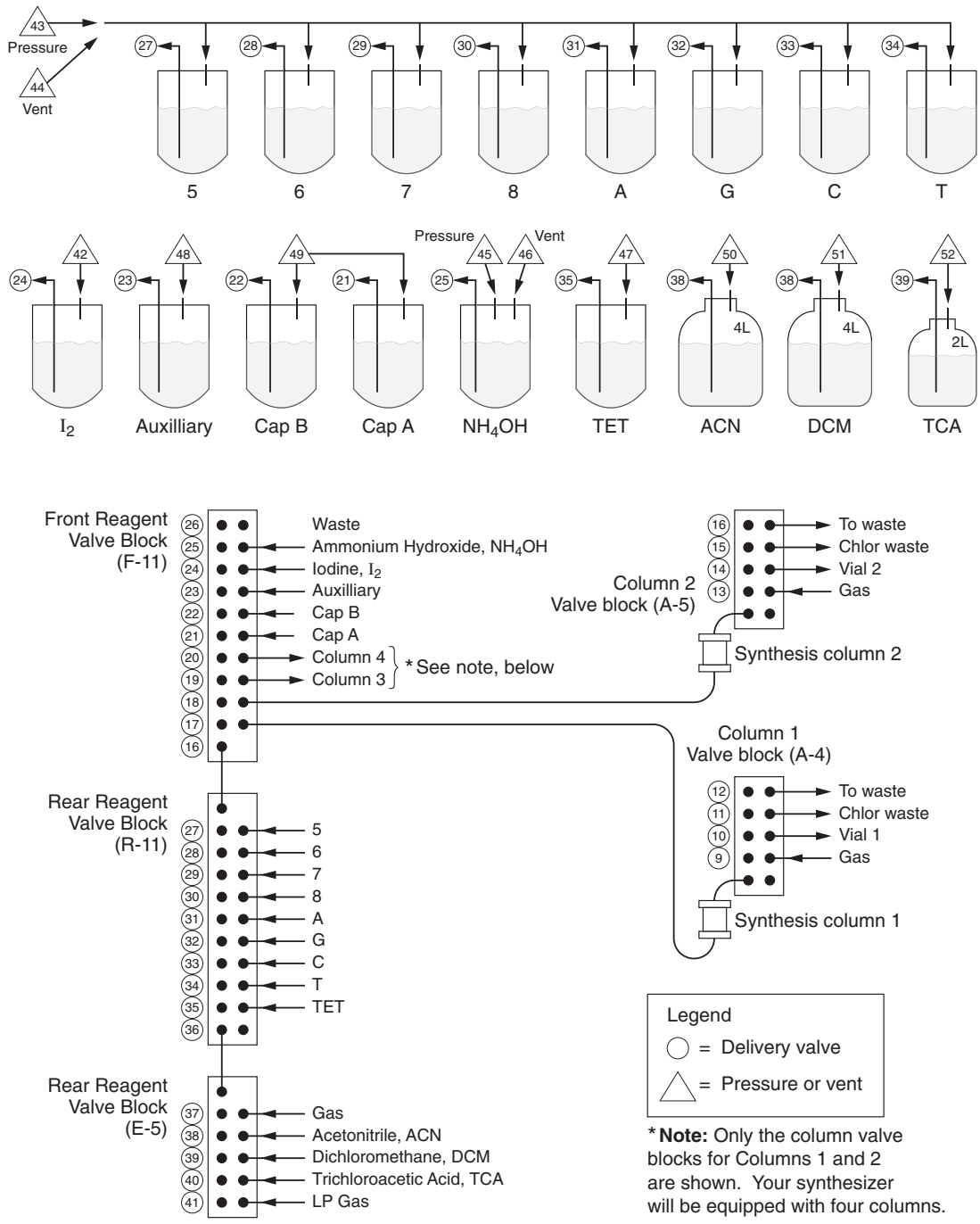


Figure 2-1 Simplified 3400 DNA Synthesizer schematic (valves)

Overview of 3400 Valve Operations

Valves, Valve Groups, and Valve Operations

Automated DNA synthesis requires chemical deliveries to specified destinations on the 3400 DNA Synthesizer, such as the column. These deliveries are controlled by electrically activating the solenoid valves to open and close, creating various pathways through the instrument.

Valve Groups

As described in the previous section (page 2-2), each solenoid valve is assigned a number that can be used to open or close it. A valve or set of valves opened to perform a specific delivery or task is a *valve group*. For example, the valve group, "ACNToWaste" represents those valves that need to be opened to deliver acetonitrile to waste (valves 50, 38, 36, and 26).

Valve Operations

During synthesis, the instrument often needs to open several valve groups simultaneously. For example, to deliver ACN to columns 2 and 3, both the "ACNToColumn2" and "ACNToColumn3" valve groups need to be opened. A shorthand notation for these two groups is "ACNToColumn(2,3)." The opening or closing of one or more valves or valve groups is called a *valve operation*.

Valve operations may involve a variable set of valve groups, indicated by the presence of one or more words that begin with dollar sign ($) in the list of groups. For instance, during the amidite delivery routine in a cycle script (see below), the notation "($Base,Tet)ToColumn($Col)" can be used to deliver the appropriate amidite into the currently active column. In this example, the variable $Col represents the currently active column, whereas $Base represents the amidite delivered into this column. During synthesis, such variables are substituted with actual synthesis data, for example, "(A,Tet)ToColumn(1)," "(T,Tet)ToColumn(2)," and so on.

Valve Codes

Using the instrument front panel, you can enter various valve operations into the built-in cycle script editor and the Manual Control menu using a set of *valve codes*. For example, the valve code 337 refers to the valve group "ACNToWaste." Similarly, the code 123 maps to "FlushTo(C,Tet)," which in turn is a shorthand for the set "FlushToC,FlushToTet".

For a complete list of all valve codes and associated valve groups provided with the 3400 DNA Synthesizer software, see Appendix A.

Valve Codes by Type of Task

To find the valve code for a task type, refer to the page indicated below.

Task Types	See Page
Delivering Reagents to the Columns	2-9
Rinsing or Flushing Chemical Pathways	2-10
Rinsing or Flushing Chemical Pathways	2-10
Priming the Delivery Lines	2-11

Task Types	See Page
Preparing Reagents for Delivery	2-12
Delivering Argon to Reservoirs	2-14
Testing the Instrument	2-15

Activating or Accessing Valves and Valve Groups

The valves in the 3400 DNA Synthesizer are activated automatically during synthesis runs and during certain operations (for example, bottle change, autodilution, and manufacturing tests).

When you create custom cycle scripts using the instrument keypad, you can control reagent delivery by entering either single valve numbers or valve codes that map to one or more sets of valve groups. For more information, see “Overview of the Cycle Scripts” on page 2-16, “Creating Custom Cycle Scripts” on page 2-18, and “Edit Cycle Menu” on page B-15.

Similarly, you can open and close valves directly through the Manual Control Menu. See “Manual Control Menu” on page B-34 for more information.

How Valve Operations are Performed

DNA synthesis requires various reagents to be delivered to a specified destination. A reagent can flow from its reservoir to the waste container, or through the column and then to either the two waste containers or the collection vials. To achieve flow of a reagent, specific valves must open simultaneously so that:

1. The reservoir is pressurized.
2. The pathway from the reservoir to the valve block is opened.
3. An exit is provided out of the valve block (that is, to waste, or to the column and then to either the waste ports or the collection vials).

Valve codes are provided with the 3400 DNA Synthesizer to perform each necessary delivery. A list of 3400 DNA Synthesizer valve codes by action type is provided on page 2-6.

Example: ACNToColumn1

The flow path created by activating a valve group can be traced for a single column using the valve group ACNToColumn1 as an example. Using the Manual Control Menu, this valve group can be accessed by entering valve code 237, followed by the number 1 key to select the corresponding column. (See Figure 2-2 on page 2-8.)

After you select this valve group, the instrument opens in sequence:

- Valve 50 to allow argon to flow into the Acetonitrile bottle to pressurize it
- Valve 38 to open the pathway between the bottle and the valve block
- Valve 36 to direct flow toward the selected column
- Valve 17 to open the pathway from the valve block to column 1
- Valve 4 to provide an exit (the waste container)

When all five valves are open, the argon pressure forces the acetonitrile out of the reservoir, through the reagent valve blocks, through the column, and out of the synthesizer to the waste container. When ACNToColumn1 is deactivated, all valves close and the flow stops.

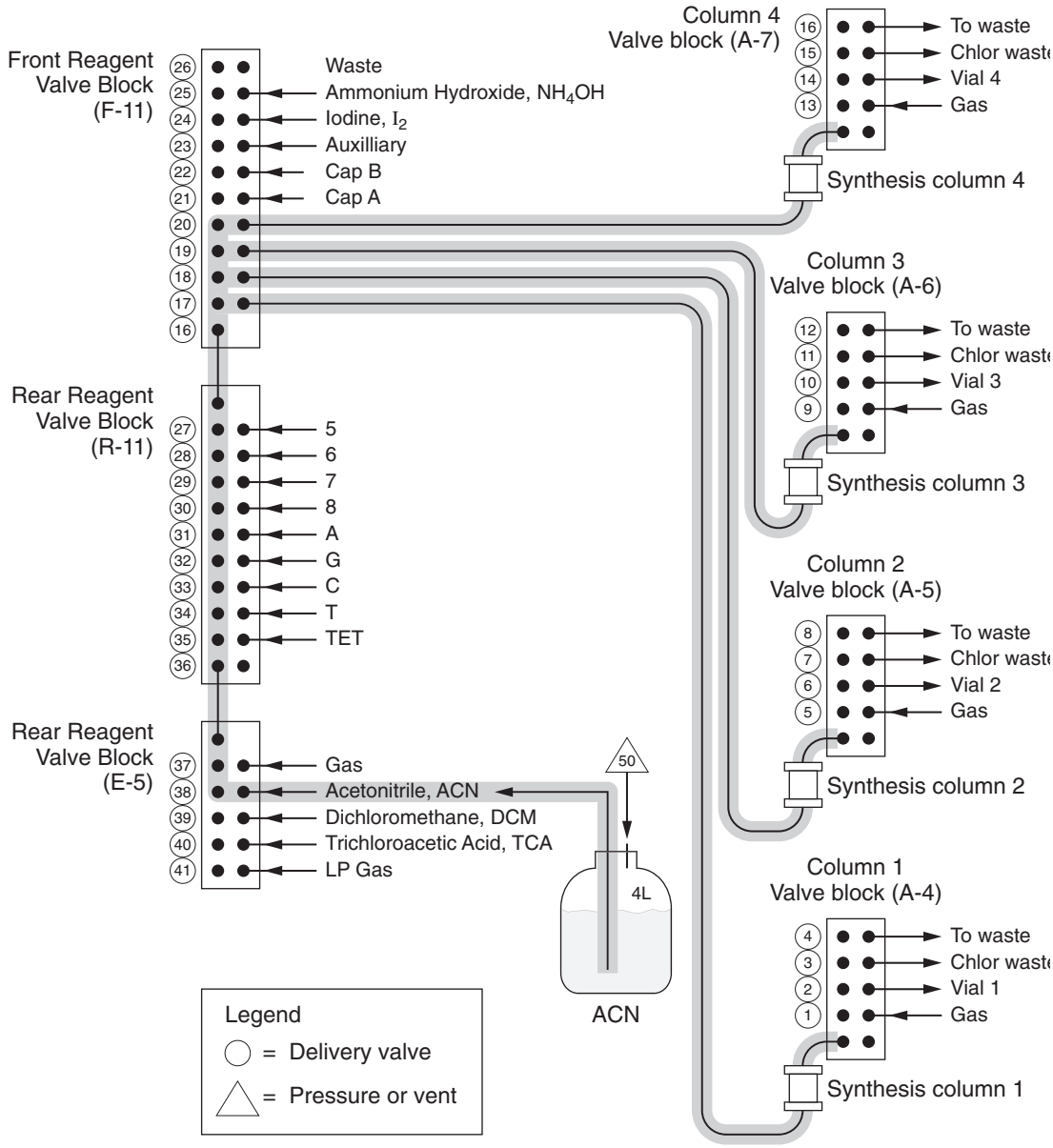


Figure 2-2 Flow path of ACNTtoColumn1 (code number 237)

Delivering Reagents to the Columns

Valve codes that deliver reagents to columns are *column specific*. They map to different valve groups, depending on which columns are active. For example, if only column 2 is active, then only the valve group that deliver reagents to column 2 is opened. After a specified time, the valve groups are closed.

Flow Path/Action

There are many valve codes provided to deliver reagents to the column. The flow path for the corresponding valve operations is: Reagent (driven by argon pressure) flowing from its reservoir, through the valve block, through the column, and ultimately to the waste container.

The table below describes the flow path and action of the most frequently used valve operations for delivering reagents to columns.

Valve Operation		Valves	Description
Code	Group		
220	($Base,Tet)ToColumn($Col)	X,43,47,35,Z	According to the sequence, the selected phosphoramidite(s) and tetrazole are simultaneously delivered to the column to perform the coupling reaction.
229	TetToColumn($Col)	47,35,Z	Tetrazole is delivered to the column; activates phosphoramidites for coupling reaction.
233	CapABToColumn($Col)	49,22,21,Z	Both capping reagents, acetic anhydride (cap A) and NMI (cap B) are simultaneously delivered to the column; used following coupling to terminate or cap unreacted nucleotide chains.
234	AuxToColumn($Col)	48,23,Z	Auxiliary contents is delivered to the column
235	IodineToColumn($Col)	42,24,Z	Iodine is delivered to the column; used to oxidize/sulfurize the DNA.
237	ACNToColumn($Col)	50,38,36,Z	Delivers acetonitrile to wash the column before or after reagent delivery.
247	ACNToCWaste($Col)	50,38,36,Y	Delivers acetonitrile through the column to chlorinated waste to clean up after TCA/DCM delivery.
248	DCMToColumn($Col)	51,39,36,Y	DCM is delivered to the column and chlorinated waste.
249	TCAToColumn($Col)	52,40,36,Y	TCA is delivered to the column and chlorinated waste; used to detritylate the support-bound oligonucleotides prior to coupling.

$Base = One or more of the following bases or corresponding IUB (degeneracy) codes: A, G, C, T, 5, 6, 7, 8
$Col = One or more of the columns: 1, 2, 3, 4
X = One to four of the following valves: 31, 32, 33, 34, 27, 28, 29, 30, depending on $Base
Y = One or more of the valve pairs: (17,3); (18,7); (19,11); (20,15); depending on $Col
Z = One or more of the valve pairs: (17,4); (18,8); (19,12); (20,16); depending on $Col

Rinsing or Flushing Chemical Pathways

Valve operations that rinse or flush chemical pathways are *column specific*. They map to different valve groups, depending on which columns are active. For example, if only column 2 is active, then only the valve group that deliver reagents to column 2 is opened. After a specified time, the valve groups are closed.

These valve operations are used throughout the synthesis to clear the valve blocks, the column, and the interconnecting delivery lines. They are also performed prior to a chemical delivery to remove residual reagent from a previous delivery.

Flow Path/Action

The table below describes the flow path and action for eight valve operations that rinse or flush chemical pathways.

Valve Operation		Valves	Description
Code	Group		
100	BlockFlush	37,36,26,1,4,7, 5,8,9,12,15,16	Removes any solvent or reagent from the reagent valve blocks and the column valve blocks. Argon enters the three valve blocks simultaneously and forces all liquid to the waste container.
101	ReverseFlush($Col)	26,W	Removes any reagent or solvent from the column and support; argon flows from the column valve block, through the column, into the reagent valve block and then to waste. This forces the re agent out of the column in the reverse direction of normal flow (that is, it drains the column from the top to the bottom).
102	FlushToCWaste($Col)	37,36,Y	Used during detritylation to flush TCA or CH_3CN through the column to the chlorinated waste container.
103	FlushToCollect($Col)	37,36,X	During the end procedure, ammonia is flushed from the column into the collection vial.
104	FlushToColumn($Col)	37,36,Z	Argon flows through the column from the bottom to the top and exits at the waste container.
105	FlushToWaste($Col)	37,36,26	Removes any reagent in the reagent valve blocks.
237	ACNToColumn($Col)	50,38,36,Z	Acetonitrile is delivered to rinse the column. Argon pressure forces the acetonitrile from its reservoir, then through the reagent valve block, the column, the column valve block, and finally to the waste container.
337	ACNToWaste	50,38,36,26	Acetonitrile is delivered to rinse the reagent valve blocks. Argon pressure forces acetonitrile from its reservoir, through the reagent valve blocks to rinse them thoroughly and then to the waste container.

$Col = One or more of the columns: 1, 2, 3, 4
W = One or more of the valve pairs: (17,1); (18,5); (19,9); (20,13); depending on $Col
X = One or more of the valve pairs: (17,2); (18,6); (19,10); (20,14); depending on $Col
Y = One or more of the valve pairs: (17,3); (18,7); (19,11); (20,15); depending on $Col
Z = One or more of the valve pairs: (17,4); (18,8); (19,12); (20,16); depending on $Col

Priming the Delivery Lines

Flow Path/Procedures

The table below describes the flow path and action for some of the valve operations that prime the delivery lines.

Valve Operations		Valves	Description
Code	Group		
311	AToWaste	43,31,26	Prime A line
312	GToWaste	43,32,26	Prime G line
313	CToWaste	43,33,26	Prime C line
314	TToWaste	43,34,26	Prime T line
315	5ToWaste	43,27,26	Prime 5 line
316	6ToWaste	43,28,26	Prime 6 line
317	7ToWaste	43,29,26	Prime 7 line
318	8ToWaste	43,30,26	Prime 8 line
319	TetToWaste	47,35,26	Prime Tet line
330	AmmoniaToWaste	45,25,26	Prime Ammonia line
331	CapAToWaste	49,21,26	Prime Cap A line
332	CapBToWaste	49,22,26	Prime Cap B line
335	IodineToWaste	42,24,26	Prime Iodine line
337	ACNToWaste	50,38,36,26	Prime Acetonitrile line
338	DCMToWaste	51,39,36,26	Prime DCM line
339	TCAToWaste	52,40,36,26	Prime TCA line

Preparing Reagents for Delivery

Flow Path/Procedures

The table below describes the flow path and action for eight valve operations that prepare reagents for delivery.

Valve Operation		Valves	Description
Code	Group		
820	Pressure (Amidite,Tet)	43,47	Prepare for amidite+tet delivery
830	PressureAmmonia	45	Prepare for ammonia delivery
833	PressureCapAB	49	Prepare for CapA+CapB delivery
834	PressureAux	48	Prepare for auxiliary delivery
835	Pressurelodine	42	Prepare for iodine delivery
837	PressureACN	50	Prepare for acetonitrile delivery
838	PressureDCM	51	Prepare for DCM delivery
839	PressureTCA	52	Prepare for TCA delivery

Delivering Acetonitrile to Reservoirs

Flow Path/Procedures

The table below describes the flow path and action for 17 valve operations that deliver acetonitrile to designated reservoirs.

Valve Operation		Valves	Description
Code	Group		
411	ACNToA	50,38,36,31,44	Push acetonitrile to A bottle
412	ACNToG	50,38,36,32,44	Push acetonitrile to G bottle
413	ACNToC	50,38,36,33,44	Push acetonitrile to C bottle
414	ACNToT	50,38,36,34,44	Push acetonitrile to T bottle
415	ACNTo5	50,38,36,27,44	Push acetonitrile to 5 bottle
416	ACNTo6	50,38,36,28,44	Push acetonitrile to 6 bottle
417	ACNTo7	50,38,36,29,44	Push acetonitrile to 7 bottle
418	ACNTo8	50,38,36,30,44	Push acetonitrile to 8 bottle
429	ACNToTet	50,38,36,35	Push acetonitrile to Tet bottle
430	ACNToAmmonia	50,38,36,25,46	Push acetonitrile to Ammonia bottle
431	ACNToCapA	50,38,36,21	Push acetonitrile to Cap A bottle
432	ACNToCapB	50,38,36,22	Push acetonitrile to Cap B bottle
433	ACNToCapAB	50,38,36,21,22	Push acetonitrile to Cap A and Cap B bottles
434	ACNToAux	50,38,36,23	Push acetonitrile to Auxiliary bottle
435	ACNToIodine	50,38,36,24	Push acetonitrile to Iodine bottle
438	ACNToDCM	50,38,39	Push acetonitrile to DCM bottle
439	ACNToTCA	50,38,40	Push acetonitrile to TCA bottle

Delivering Argon to Reservoirs

Flow Path/ Procedures

The table below describes the flow path and action for 21 valve operations.

Valve Operation		Valves	Description
Code	Group		
100	BlockFlush	37,36,26,1,4,5,8, 9,12,13,16	Flush the valve block
101	ReverseFlush($Col)	26,W	Reverse flush reagents from columns in reverse to waste
102	FlushToCWaste($Col)	37,36,Y	Flush reagents from columns to chlorinated waste
103	FlushToCollect($Col)	37,36,X	Flush reagents from columns to collection vials
104	FlushToColumn($Col)	37,36,Z	Flush reagents from columns to nonchlorinated waste
111	FlushToA	37,36,44,31	Flush to bottle A
112	FlushToG	37,36,44,32	Flush to bottle G
113	FlushToC	37,36,44,33	Flush to bottle C
114	FlushToT	37,36,44,34	Flush to bottle T
115	FlushTo5	37,36,44,27	Flush to bottle 5
116	FlushTo6	37,36,44,28	Flush to bottle 6
117	FlushTo7	37,36,44,29	Flush to bottle 7
118	FlushTo8	37,36,44,30	Flush to bottle 8
119	FlushToBases	37,36,44,31,32, 33,34,27,28,29,30	Flush to all amidite bottles at once (A, G, C, T, 5, 6, 7, 8)
129	FlushToTet	37,36,35	Flush to Tet bottle
130	FlushToAmmonia	37,36,25,46	Flush to Ammonia bottle
133	FlushToCapAB	37,36,21,22	Flush to Cap A and Cap B bottles
135	FlushToIodine	37,36,24	Flush to Iodine bottle
137	FlushToACN	37,38	Flush to acetonitrile bottle
138	FlushToDCM	37,39	Flush to DCM bottle
139	FlushToTCA	37,40	Flush to TCA bottle

$Col = One or more of the columns: 1, 2, 3, 4
W = One or more of the valve pairs: (17,1); (18,5); (19,9); (20,13); depending on $Col
X = One or more of the valve pairs: (17,2); (18,6); (19,10); (20,14); depending on $Col
Y = One or more of the valve pairs: (17,3); (18,7); (19,11); (20,15); depending on $Col
Z = One or more of the valve pairs: (17,4); (18,8); (19,12); (20,16); depending on $Col

Testing the Instrument

Flow Path/ Procedure

The table below describes the flow path and action of 16 valve operations that can be used to test valves and delivery lines.

Valve Group		Valves	Description
Code	Name		
211	AToColumn($Col)	43,31,Z	Deliver A to the specified column(s)
212	GToColumn($Col)	43,32,Z	Deliver G to the specified column(s)
213	CToColumn($Col)	43,33,Z	Deliver C to the specified column(s)
214	TToColumn($Col)	43,34,Z	Deliver T to the specified column(s)
215	5ToColumn($Col)	43,27,Z	Deliver 5 to the specified column(s)
216	6ToColumn($Col)	43,28,Z	Deliver 6 to the specified column(s)
217	7ToColumn($Col)	43,29,Z	Deliver 7 to the specified column(s)
218	8ToColumn($Col)	43,30,Z	Deliver 8 to the specified column(s)
229	TetToColumn($Col)	47,35,Z	Deliver Tet to the specified column(s)
231	CapAToColumn($Col)	49,21,Z	Deliver CapA to the specified column(s)
232	CapBToColumn($Col)	49,22,Z	Deliver CapB to the specified column(s)
234	AuxToColumn($Col)	48,23,Z	Deliver auxiliary reagent to the specified column(s)
235	IodineToColumn($Col)	42,24,Z	Deliver iodine to the specified column(s)
237	ACNToColumn($Col)	50,38,36,Y	Deliver acetonitrile to the specified column(s)
238	DCMToColumn($Col)	51,39,36,Y	Deliver DCM to the specified column(s)
239	TCAToColumn($Col)	52,40,36,Y	Deliver TCA to the specified column(s)

$Col = One or more of the columns: 1, 2, 3, 4
Y = One or more of the valve pairs: (17,3); (18,7); (19,11); (20,15); depending on $Col
Z = One or more of the valve pairs: (17,4); (18,8); (19,12); (20,16); depending on $Col

Overview of the Cycle Scripts

Definition

A *cycle script* controls the execution of a synthesis run. It consists of several *cycle procedures* (begin, detritylation, amidite delivery, and so forth). Each cycle procedure consists of a sequence of commands, such as valve operations, that are invoked throughout the synthesis run.

A command that is programmed to occur for a specified amount of time is a *step* (for example, the valve group ACNToColumn1 can be held open for 10 seconds).

Cycle Scripts Provided

The 3400 DNA Synthesizer software provides 12 cycle scripts, as listed in the table below.

Note: For information on each of the 3400 DNA Synthesizer cycle scripts, see Appendix A.

Cycle Script		Cycle Script		Cycle Script	
0.2 µm-PO*	For DNA synthesis	0.2 µm-PS†	For Phos-phorothioate DNA synthesis	0.2 µm-RNA	For RNA synthesis
1 µm-PO		1 µm-PS		1 µm-RNA	
LV40-PO		LV40-PS		LV40-RNA	
LV200-PO		LV200-PS		LV200-RNA	

*PO = Phosphorothioate oxidization
†PS = Phosphorothioate sulfurization

Cycle Procedures in Each Cycle Script

Each of the cycle scripts includes nine cycle procedures, as listed in the table below.

Cycle Procedure	Variable Arguments Provided	When the Cycle Procedure Is Invoked
BEGin	$Col – active columns	Once before the actual cycle
DETRitylate	$Col – active columns	Once per cycle Also, if DMT removal has been selected for any of the active columns, it is invoked immediately after all cycles have been completed (that is, all bases have been synthesized).
PREpare	$Col – active columns	Once per cycle, to prepare for amidite delivery/coupling
DELIVer	$Col – active columns $Base – active amidite(s): A, G, C, T, 5, 6, 7, 8 $TTime – amidite delivery time	Once for each active column in every cycle, to deliver amidites
COUPle	$Col – active columns $CTime – coupling time	Once per cycle, to couple

Cycle Procedure	Variable Arguments Provided	When the Cycle Procedure Is Invoked
SULFurize	$Col – active columns	Once per cycle,* only in phosphorothioate DNA synthesis cycle scripts
CAP	$Col – active columns	Once per cycle
OXIDate	$Col – active columns	Once per cycle,* only in standard DNA and RNA synthesis scripts
CLEave	$Col – active columns	At the very end of the cycle, if cleaving has been selected for any active columns
WASH	$Col – active columns	Invoked after a synthesis run is aborted using the "Abort and Clean Up" menu item

*Some cycle scripts contain the SULFurize procedure; others contain the OXIDate procedure. Every cycle script contains one or the other, but not both.

Cycle Procedure Instrument Command Conventions

The 3400 DNA Synthesizer cycle procedures use four commands:

TRANsfer <*valves*> <*time*> – Transfers reagents by holding open a set of valves and/or valve groups for a specified amount of time.

<*valves*> is a comma-separated list of one or more valves and/or valve groups (described in "Overview of 3400 Valve Operations" on page 2-6).

<*time*> is the step time; that is, how long to perform the reagent transfer. The time is in seconds.

MONitor <*valves*> <*time*> – This command is intended for use during the TCA delivery step inside the detritylation procedure. It works similarly to the TRANsfer command. However, during delivery, the conductivity sensor attached to each active column is monitored to get a trityl reading.

Two types of monitoring takes place:

- Delivery monitoring – After a trityl baseline (low reading) and trityl peak (high reading) are determined, delivery of reagent to a given column may terminate prior to the given step time if the trityl reading approach the baseline within a certain percentage of the peak delta (that is, the difference between the peak height and the baseline).

- Yield monitoring – All trityl readings that were in the upper half of the peak delta are added together to produce a peak area. Cycle after cycle, the peak areas are compared to come up with a trityl yield, or average step-wise yield. If, during the synthesis, the yield of a particular column falls below a set threshold, that column is terminated.

SLEep <*time*> – Wait for a given number of seconds.

SAFe {Yes | No} – Turn on or off safe mode. While safe mode is on (Yes), the Pause function on the front panel takes effect before the next step. While safe mode is off, the pause request is deferred.

Creating Custom Cycle Scripts

The cycle scripts provided with the 3400 DNA Synthesizer are write protected. Although you cannot modify or delete these cycle scripts, you can use them as a basis to create your own custom cycle scripts using the Edit Cycle Menu. See "Creating a Custom Cycle Script" on page 5-27 for more information.

How Cycles Work

A cycle completes operations for one base addition and then is repeated until the oligonucleotide length is fully synthesized. The four essential chemical reactions necessary for synthesis are, in order:

- Detritylation
- Coupling
- Capping
- Oxidization

Although each reaction requires different treatment, the following generalizations apply:

- Before the chemical reaction:
  - The valve blocks, the column, and the interconnecting delivery lines are rinsed with acetonitrile and flushed dry with argon.
  - The reagent reservoir(s) are prepared for delivery.
- Performing the chemical reaction requires reagent delivery to the column, often followed by a Sleep step to complete the reaction.

Synthesis Scales

The 3400 DNA Synthesizer produces oligonucleotides in three synthesis scales:

- 40 nmol (ABI LV40® Column)
- 0.2 µmol (ABI LV200™ Column and 0.2 µmol)
- 1 µmol

Note: LV = low volume

The 40-nmol or 0.2-µmol scale provides sufficient quantities of oligonucleotide for most applications. When larger quantities of DNA are needed, the 1-µmol scale cycle script can be used. The table below shows average yields for synthesis of a 20-mer oligo.

Synthesis Scale	Cycle Script Name	Yield of Crude Oligonucleotide	
		ODU*	Amount
40 nmol (LV40)	LV40-PO	5 to 10	165 to 330 µg
	LV40-PS		
	LV40-RNA		

0.2 µmol (LV200)	0.2 µm-PO	20	660 µg
	LV200-PO		
	0.2 µm-PS		
	LV200-PS		
	0.2 µm-RNA		
	LV200-RNA		
1 µmol	1 µm-PO	100	3.3 mg
	1 µm-PS		
	1 µm-RNA		

*ODU = Optical Density Units

Getting Started 3

This chapter covers:

Setting Up the 3400 DNA Synthesizer

Starting the Instrument

To start the instrument:

1.	Plug the 3400 DNA Synthesizer into a dedicated power source. **Note:** For more information about a dedicated power source, refer to the *Applied Biosystems 3400 DNA Synthesizer Site Preparation and Safety Guide* (PN 4334679).
2.	Power on the instrument. The controller board in the instrument beeps three times within 6 sec and there is an additional beep 7 sec later.

3.	Wait 2 to 3 min while the instrument starts up. The following screen is displayed briefly:
	
	`Applied Biosystems`
	`3400 DNA Synthesizer`

4.	If necessary, adjust the intensity of the display by pushing the intensity buttons. The intensity buttons are on the instrument, just below and to the left of the display. One is for darker intensity; the other is for lighter intensity.

Understanding the LCD Screen/Keypad

The 3400 DNA Synthesizer software is *menu driven*. Menus and pages of menus are shown on a four-line by 40-character liquid crystal display (LCD) screen.

The menus present various options and necessary information about the synthesis run or status of the instrument. In response, you select an option and provide instructions by pressing the appropriate keys on the keypad.

Physical Features

Figures 3-1 shows the 3400 DNA Synthesizer LCD screen/keypad with Page 1 of the Main Menu displayed. See Table 3-1 below for label descriptions.

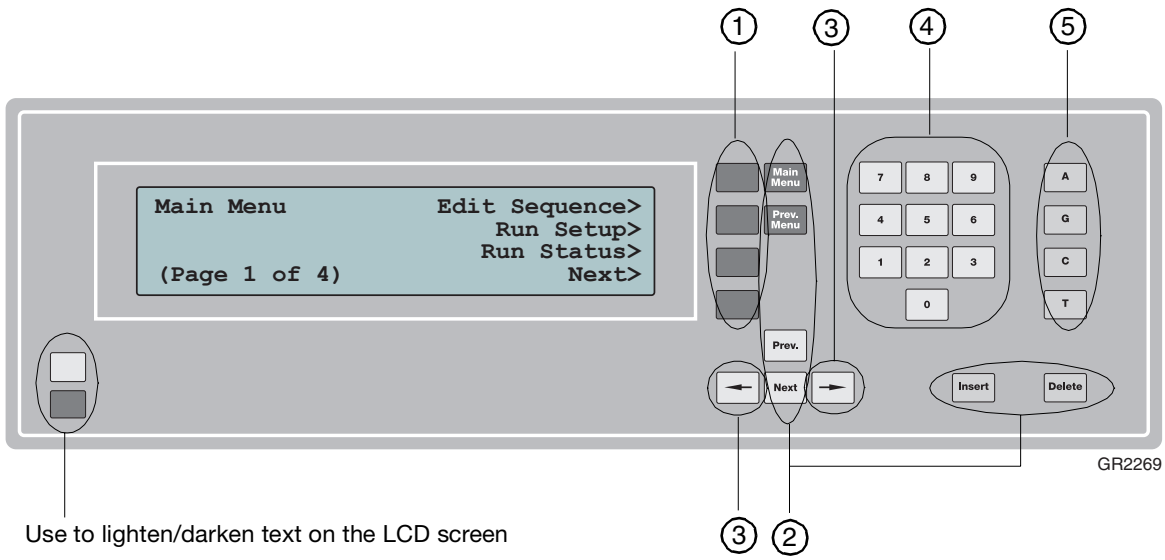
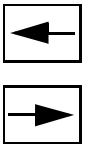


Figure 3-1 The 3400 DNA Synthesizer LCD screen/keypad

Table 3-1 Parts of the keypad

No.	Key Type	Key	Description
1	Soft keys	Four keys, variable designations	The soft keys are used to execute functions or access other menus. Each soft key is designated by the word or phrase shown on the LCD screen, directly to the left of the soft key. The soft key designations change with each menu. Detailed descriptions of each of the soft keys are provided in the menu discussions in Appendix B, “Software Menus.”
2	Command keys	Main Menu Prev. Menu Prev. Next Insert Delete	The command keys are used to execute functions. For a detailed description of each of the six command keys, see “Command Key Functions” on page 3-4.

Table 3-1 Parts of the keypad *(continued)*

No.	Key Type	Key	Description
3	Left- and right-arrow keys		The left- and right-arrow keys are used to control the cursor position. To make entries or deletions, the cursor must be in the correct position and often must be moved. Pressing the left- or right-arrow keys moves the cursor one position in the arrow's direction.
4	Numeric keys	0 to 9	The numeric keys are used to enter a numeric value at the cursor position.
5	Base keys	A, G, C, T	The base keys are used to enter bases A, G, C, and T in a sequence. The base keys are only active when you are creating/editing a sequence.

Command Key Functions

Table 3-2 Command Keys functions

Command Key	Function
Main Menu	Pressing **Main Menu** from any other another menu always returns the display to Page 1 of the Main Menu.
Prev. Menu	Pressing **Prev. Menu** returns the display to the parent of the menu currently displayed.
Prev. Next	In most menus, pressing **Prev.** or **Next** moves to the previous or next page of the current menu. In some menus, however, pressing **Prev.** or **Next** moves the cursor up or down.
Insert	While editing a cycle script within the front panel cycle editor, pressing **Insert** adds a step at the current line. In the front panel sequence editor, pressing **Insert** switches between the overwrite and insert mode: • The overwrite mode is indicated by a block cursor. In this mode, any character typed replaces the character at the current cursor position. • The insert mode is indicated by an underscore cursor. In this mode, any character typed is inserted; all other characters remain, moving forward one position.
Delete	In the sequence editor, pressing **Delete** erases an entry at the cursor position, or removes the last entry in the sequence if the current cursor position is at the end of the sequence. While editing a cycle script in the cycle editor, pressing **Delete** removes the current line/step. The last step is removed if the cursor is positioned beyond the end of the cycle script.

Entering Text

Text Menu

When you are required to enter text on the instrument front panel, the following menu is displayed:

```
Prompt: "input"                    Pick "a">
Pick letters  |abcdefghijklm|       Case>
or use 01-26  |nopqrstuvwxyz|
to enter A-Z  |0123456789-. |        Set>
```

Prompt = Save as:, Host Name:, etc.

input = entered text

Action = Save, Set, etc.

Entering Text

To enter text in the input field:

1.	Press the →, ←, **Prev.**, and **Next** keys to navigate to each desired letter, then press the **Pick "X"** soft key, where X is the letter you want to enter. OR Press the numeric keys **0** to **9** to enter a two-digit number from 01 to 26, representing the position of the desired letter in the alphabet. Use the code 00 to represent a space character, where applicable.
2.	Press the **Delete** key to remove the last character from the input.
3.	Press the **Case** key to switch the available letters from upper- to lowercase, and vice versa. **Note:** The Case key is available only for some types of input. For instance, a host name is always entered using lowercase letters, but a Windows® workgroup is always entered using uppercase letters.
4.	Press the **Clear** soft key to remove all letters from the text input. **Note:** The Clear soft key is available after one or more letters are entered.
5.	Press the **Action** soft key (where Action is Set, Save, or similar) to accept the text input and perform the corresponding action. **Note:** Depending on the input type, the Action soft key may not be available until there is text input.
6.	To cancel the operation (that is, to return to the previous menu without performing the appropriate action), press the **Prev. Menu** command key.

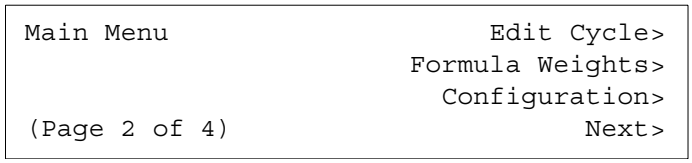
Navigating Through the Main Menu

The Main Menu is the starting menu for the 3400 DNA Synthesizer software. Use the Main Menu to access the 11 major procedural menus:

- Edit Sequence
- Run Setup
- Run Status
- Edit Cycle
- Formula Weights
- Configuration
- Autodilution
- Change Bottle
- Shut Down
- Manual Control
- Diagnostics

Navigating Through the Main Menu

To navigate through the Main Menu:

1.	If you are not already at Page 1 of the Main Menu, press the **Main Menu** command key. Page 1 of the Main Menu is displayed. 
2.	Press the **Next** soft key to move to Page 2 of the Main Menu. 
3.	Press the **Next** soft key to move to Page 3 of the Main Menu. 

To navigate through the Main Menu: ***(continued)***

4.	Press the **Next** soft key to move to Page 3 of the Main Menu.
	
	`Main Menu  Manual Control>`
	`  Diagnostics>`
	
	`(Page 4 of 4)  Next>`

5.	Press the **Next** soft key to return to Page 1 of the Main Menu.
6.	Press the appropriate soft keys on Pages 1 to 4 to access the following menus: • Edit Sequence • Run Setup • Run Status • Edit Cycle • Formula Weights • Configuration • Autodilution • Change Bottle • Shut down • Manual Control • Diagnostics **Note:** For a detailed description of each of the Main Menu soft keys, see Appendix B, “Software Menus.”
7.	To return to Page 1 of the Main Menu at any time, press the **Main Menu** command key.

Setting the Time and Date

Accessing the Time/Date Menu

To access the Time/Date Menu:

1.	From Page 1 of the **Main Menu**, press the **Next > Configuration > Time/Date** soft keys. The Time/Date Menu is displayed.
	
	`Time/Date`
	`[Factory] Time Zone>`
	`[time.nist.gov] Time Server>`
	`[YYYY-MM-DD hh:mm:ss] Set Clock>`

2.	To exit the Time/Date Menu, press the **Prev. Menu** or **Main Menu** command keys.

Selecting the Time Zone

To select the time zone for your area:

1.	Press the **Time Zone** soft key. The Select Timezone Menu is displayed.
	
	`Select Timezone US>`
	`[US/Pacific] Canada>`
	`Americas>`
	`Page 1 of 5 Next>`

2.	Press the **Next** soft key to scroll through general time zones. The general time zones are listed (roughly) in a west-to-east order.

3.	When you see the appropriate general time zone for your area, press the corresponding soft key to select it. Specific time zones for that area are displayed.
	
	`Select Timezone [None]>`
	`Canada/ Atlantic>`
	`Central>`
	`Page 1 of 4 Next>`

4.	Press the **Next** soft key to scroll through the specific time zones. The specific time zones for each general time zone area are listed alphabetically.
5.	When you see the specific time zone for your area, press the corresponding soft key to select it.
6.	To select a different general time zone at any time, press the **Prev. Menu** command key.

Setting the System Time

The Applied Biosystems 3400 DNA Synthesizer by default obtains its system time from a network time server, using the Network Time Protocol (NTP). However, if the instrument is not networked or if a firewall blocks access to the configured timer server, you may need to manually set the system time.

To set the system time:

1.	Press the **Set Clock** soft key. The Set Clock Menu is displayed.
	
	`Enter new date/time (XXX)  Set Clock>`
	`Year (>2000): [YYYY] Hour (0-23): [hh]`
	`Month (1-12): [MM] Minute (0-59): [mm]`
	`Day (1-31): [DD] Second (0-59): [ss]`
	
	XXX = an abbreviated time zone; for example, UTC, CET, PST

2.	Fill in each field: a. Move to the appropriate field using the cursor command keys (left- and right- arrows, Prev., Next). The cursor is placed under the first digit of that field. b. Type in a new value. If necessary, you can erase entries by pressing the **Delete** command key. **Note:** The time values in each field are not automatically updated. To give yourself time to complete the entire date/time setting, it is a good idea to enter values that are a few seconds ahead.
3.	Press the **Set Clock** soft key to update the system time.
4.	To return to the Time/Date Menu without making any changes, press the **Prev. Menu** command key.

Networking the Instrument

Networking the instrument is optional. However, doing so allows you to:

- Access an Ethernet-capable printer to print sequences, cycle scripts, and run reports
- Transfer sequences, cycle scripts, and run reports to or from a computer
- Use a computer and a Web browser to access sequences, cycle scripts, and run reports stored on the instrument
- Update the instrument software

Using the Supplied NAT Router

The 3400 DNA Synthesizer is shipped with a Network Address Translation (NAT) router (sometimes labeled "Cable/DSL router" or "Broadband router") to facilitate a small network consisting of:

- One or more instruments
- An Ethernet printer (optional)
- A computer (optional)

Applied Biosystems recommends that you use this router whether or not you are using a Local Area Network (LAN). This router provides:

- 4 or 8 downstream Ethernet ports (jacks) to which one or more instruments, a computer, and/or a printer can be connected.
- One upstream Ethernet port, which can be connected to your existing LAN (if you have one).
- Basic firewall security using a NAT router. Although the instrument and the computer can transmit data to your LAN through the router (for example, to access an already installed Ethernet printer), incoming data from the outside to the instrument is blocked.
- Automatic network configuration of the instrument, the printer (if any), and the computer (if any), using the Dynamic Host Configuration Protocol (DHCP).

Connecting the Instrument to the Router

To connect the instrument to the supplied NAT router:

1.	To access your existing LAN, connect the "upstream" (sometimes labeled "network" or "WAN") port on the router to an available Ethernet port on your network.
2.	Connect your 3400 DNA Synthesizer to one of the available "downstream" (sometimes labeled "local" or "LAN") ports on the router. If you have more than one instrument, repeat this step for each of the remaining instruments.
3.	Power on (or power-cycle) the router.
4.	Power on the instrument. If the instrument is already running, leave it on.

To connect the instrument to the supplied NAT router: ***(continued)***

5. From the **Main Menu** on the instrument front panel, navigate to: **Next > Configuration > Network > Ethernet**.

```
Ethernet Status: Link OK        Refresh>
Link Speed     : 100baseTx-FD
Autonegotiation: Yes
MAC Address    : XX:XX:XX:XX:XX:XX
```

Note: Verify that the Ethernet Status is Link OK, indicating that there is connectivity between the instrument and the NAT router. If the Ethernet Status is No Link, the NAT router may not yet be ready. In this case, wait 1 to 2 min, then press the **Refresh** soft key.

6. Press the **Prev. Menu** key to return to the Network Menu, then select: **TCP/IP > Change > Automatic**.

```
Select IP Configuration
                          [*] Automatic>
                                Manual>
                              Disabled>
```

Note: This command instructs the instrument to obtain or renew its network settings (DHCP lease) from the router. This can take up to 1 min, during which time the instrument keypad is not responsive. Do not use the keypad during the operation; any keypad entries will be queued for processing after the operation is complete.

Note: Some advanced users may want to use the Manual IP configuration to enter static IP address information. This may be the case if you will need to access the instrument by its IP address rather than by its host name. (See "Setting an Instrument Host Name (Optional)" on page 3-12 and "Connecting a Computer (Optional)" on page 3-14. Refer to the documentation supplied with the NAT router to determine what IP address ranges are available for static configuration.

7. If the operation is successful, the TCP/IP status screen shows the word Automatic on the first line, followed by actual IP configuration on the following lines.

```
TCP/IP Settings - Automatic      Change>
IP Address   : 192.168.2.100
Netmask      : 255.255.255.0
Gateway      : 192.168.2.1         Next>
```

The instrument is now networked.

To connect the instrument to the supplied NAT router: *(continued)*

8.	Refer to the sections that follow for information on: • Setting up the instrument host name • Setting up a printer • Using a computer to access the web-based sequence editor

Setting an Instrument Host Name (Optional)

Setting an instrument host name is optional. However, doing so allows you to:

- Access the instrument by its name, rather than its IP Address (for example, when accessing the instrument with a Web browser).
- Identify each instrument in sequence printouts or run reports in cases where several instruments are available.

To set a host name:

1.	Press the **Main Menu** button on the 3400 DNA Synthesizer front panel, then navigate to: **Next > Configuration > Network > Host Name**.
	
	`Host Name: "ab3400-001"  Pick "a">`
	`  Pick letters  |abcdefghijklm|`
	`  or use 01-26  |nopqrstuvwxyz|  Clear>`
	`  to enter A-Z  |0123456789-. |  Set>`

2.	If a name has already been configured, press the **Clear** soft key to erase it.
3.	Enter the new host name. See "Entering Text" on page 3-5 for information on naming files.

4.	Press the **Set** soft key. The new host name should now appear next to the Host Name soft key inside the Network Configuration menu.
	
	`Network  [ab3400-001] Host Name>`
	`  [XX:XX:XX:XX:XX:XX]  Ethernet>`
	`  [192.168.2.100]  TCP/IP>`
	`(Page 1 of 2)  Next>`

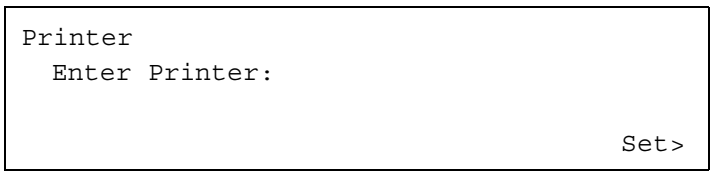
Setting up a Printer (Optional)

Using a printer with the 3400 DNA Synthesizer is optional. However, doing so allows you to print:

- Sequences
- Cycle scripts
- Run reports

To print from the 3400 DNA Synthesizer, you need an Ethernet-capable printer that supports the Line Printer Daemon (LPD) protocol. Nearly all commercially available Ethernet printers support this protocol.

To set up a printer:

1.	Ensure that the instrument is powered on and configured for networking (See “Connecting the Instrument to the Router” on page 3-10).
2.	Determine how to connect the instrument to a printer. You have the following options: • If you have a dedicated printer for your instrument(s), you can connect the printer directly to an available “downstream” (sometimes labeled “local” or “LAN”) port on the supplied NAT router. • You can use a printer already connected to your Local Area Network (LAN) with an instrument networked through the supplied NAT router. Ensure to connect the router to your LAN by its “upstream” (sometimes labeled “network” or “WAN”) port. See “Networking the Instrument” on page 3-10 for details. • If the instrument is connected directly to your LAN, ensure that the printer, too, is available on the LAN.
3.	Determine the printer's IP address. Most Ethernet printers have a front panel where the IP address can be determined directly or where a configuration page containing the printer's current network settings can be printed. **Note:** Due to the vast array of different printer models on the market, Applied Biosystems cannot provide detailed instructions on how to perform this step. **Note:** You achieve the most stable setup if your printer is configured with a static IP address. If the printer's IP address is dynamic, you need to repeat the following steps of the printer setup each time the IP address changes. a. From the Main Menu on the instrument front panel, press **Next > Configuration > Network > Next > Printer > Set by IP Address**.  b. If an IP address is already entered, press the **Clear** soft key to remove it. c. Enter the printer's IP address using the numeric keypad and the . (period) soft key. This soft key is available when applicable. d. Press the **Set** soft key.

You can now use the **Print** soft key in the Edit Sequences, Edit Cycles, and Run Status menus to print stored sequences, cycle scripts, and run reports respectively.

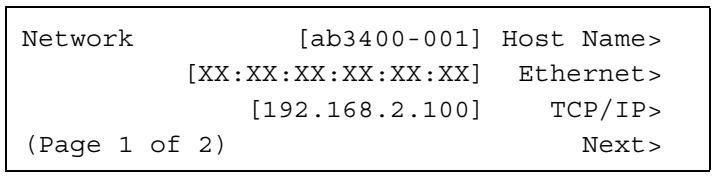
Connecting a Computer (Optional)

Connecting a computer to the 3400 DNA Synthesizer is optional. However, doing so allows you to:

- Use a Web browser to edit (create, modify, and delete) sequences on the instrument. See "Creating a Custom Cycle Script" on page 5-27 for more information.
- Transfer sequences, cycle scripts, and run reports to and from shared network folders on the instrument.
- Perform instrument software updates.

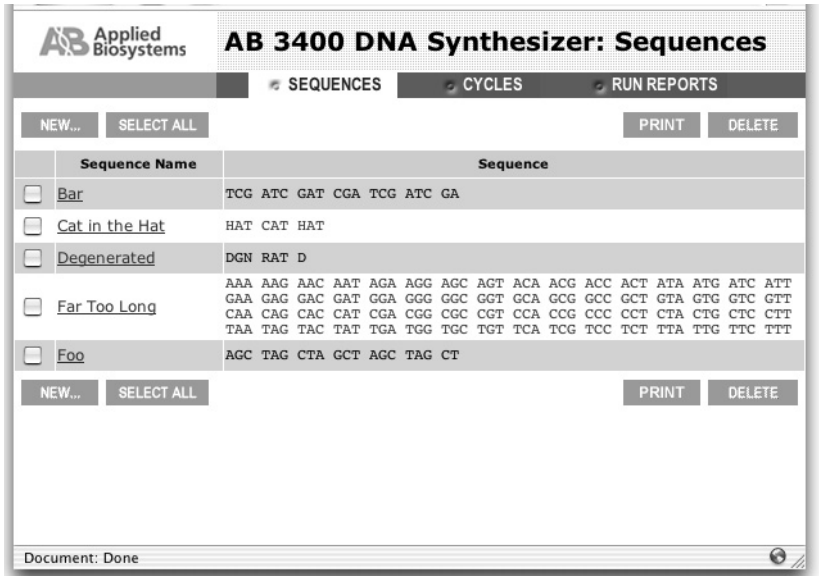
The computer must have an Ethernet interface to communicate with the instrument.

To connect a computer to the instrument:

1.	Ensure that the instrument is powered on and configured for networking. See "Connecting the Instrument to the Router" on page 3-10.
2.	Determine how the computer will be linked to the instrument. a. If the instrument is networked using the supplied NAT router, connect the computer into an available "downstream" (sometimes labeled "LAN) port on the router, in parallel with the instrument. b. If the instrument is connected directly to your LAN, ensure that the computer is also connected to your LAN.
3.	Determine the instrument's host name or IP address. From the Main Menu on the instrument front panel, press the **Configuration > Network** soft keys.  The host name (if any) is displayed next to the **Host Name** soft key and the IP address is displayed next to the **TCP/IP** soft key. On a computer running a variant of the Microsoft® Windows® operating environment, the host name is the preferred way to access the instrument, because the instrument's IP address may periodically change. See "Setting an Instrument Host Name (Optional)" on page 3-12. However, in other cases, it may be necessary to access the instrument by its IP address.
4.	Start your computer, if it is not already running.
5.	Open a Web browser. In the location bar, type **http://*instrument*/** (where *instrument* is the host name or IP address of the instrument), then press **Enter**.

To connect a computer to the instrument:

6. If the connection is successful, the Web page titled *AB 3400 DNA Synthesizer* is displayed.



For information on how to use the Web-based sequence editor, see “Creating a Sequence” on page 5-15.

Run Preparation: Setting Up the Instrument Hardware

4

This chapter covers:

Overview

Setup Checklist

Before beginning a synthesis run, go through the checklist below to ensure the 3400 DNA Synthesizer is properly prepared.

✓	Procedure	For further instructions, see...
	Check the argon cylinder. Change the cylinder when the high-pressure gauge drops below 300 psi.	"Checking the Argon Cylinder" on page 4-3
	Check the waste containers. When a waste container is full, it must be emptied and the waste disposed of properly.	"Checking the Waste Containers" on page 4-4
	Check the fluid levels of all ancillary reagent bottles. If necessary, replace with bottles of fresh reagents.	"Checking the Ancillary and External Reagent Bottles" on page 4-6
	Check the fluid levels of the external bottles (acetonitrile, DCM, TCA).	"Checking the Ancillary and External Reagent Bottles" on page 4-6
	Prepare the phosphoramidite bottle positions. Before installing the new phosphoramidite bottles, the delivery lines must be rinsed. To collect the rinse, an empty phosphoramidite bottle must be attached at each position where you plan to install a new bottle.	"Preparing the Phosphoramidite Bottle Positions" on page 4-9
	Install new phosphoramidites. You can install the phosphoramidites manually or with the Autodilution function.	• "Installing Phosphoramidites Using Autodilution" on page 4-11, or • "Diluting and Installing Phosphoramidites Manually" on page 4-14
	Install the columns.	"Installing Columns" on page 4-18
	Install the oligo collection vials. After cleavage is performed on the instrument, the ammonia solution containing oligo is collected in the oligo collection vials.	"Installing Oligo Collection Vials" on page 4-19

Checking the Argon Cylinder

When to Replace

The low-pressure gauge on the argon cylinder should read about 60 psi. Change the tank when the high-pressure gauge drops below 300 psi.

IMPORTANT! Do not replace an argon cylinder while a synthesis is in progress. If the cylinder becomes empty during a synthesis, stop the synthesis run (see "Pausing or Aborting a Run" on page 6-10).

Equipment Required

You will need the following equipment for this procedure:

√	Item	Supplier	Part Number
	1A cylinder of 2500-psi argon	Major Laboratory Supplier (MLS)	–
	Teflon® tape	MLS	–

Handling Precautions

WARNING **EXPLOSION HAZARD**. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

CAUTION **CHEMICAL HAZARD**. **Argon** is a nonflammable high-pressure gas. Released argon gas reduces the oxygen available for breathing. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Replacing an Argon Cylinder

To replace an argon cylinder:

1.	Shut off the argon cylinder at the cylinder *and* at the needle valve on the gas regulator.
2.	Remove the gas regulator from the empty cylinder.
3.	Clean the threads on the gas regulator fittings.
4.	For maximum gas lifetime, wrap the threads with Teflon tape.
5.	Install the gas regulator on a full argon cylinder.
6.	Turn on the argon cylinder.
7.	Check for leaks at the connection of the argon cylinder to the gas regulator.
8.	Open the needle valve.

Checking the Waste Containers

When to Empty

Empty the waste containers whenever they become full.

IMPORTANT! Do not empty the waste containers while a synthesis is in progress. If a waste container becomes full during a synthesis, stop the synthesis run (see "Pausing or Aborting a Run" on page 6-10).

Equipment Required

You need the following equipment for this procedure:

√	Item	Supplier	Part Number
	10-L (2.5 gal) polyethylene container, for nonchlorinated waste	Applied Biosystems	4304141
	6-L (1.5 gal) polyethylene container, for chlorinated waste		140040
	Cap assemblies for 6- and 10-L waste containers		602544

Handling Precautions

WARNING **CHEMICAL WASTE HAZARD**. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- Read and understand the material safety data sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste container in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Emptying a Waste Container

To empty a waste container for disposal:

1.	Unscrew the cap assembly from the 6- or 10-L waste container to disconnect it from the instrument.
2.	Pour the waste into a separate waste container and seal this container.
3.	Recap the empty 6- or 10-L waste container.
4.	Dispose of the waste following applicable government regulations.

Reattaching a Waste Container

To reattach a waste container to the instrument:

1.	Remove the cap from the emptied 6- or 10-L waste container and replace it with the cap assembly (PN 602544).
2.	Place the 6- or 10-L waste container near the 3400 DNA Synthesizer on the floor or on a bench that is *lower* than the instrument.
3.	Be sure the waste line slopes downward toward the 6- or 10-L waste container and has no troughs that can collect waste and block the line.
4.	Be sure the vent line is properly routed to a fume hood. See the ventilation drawing in the *Applied Biosystems 3400 DNA Synthesizer Site Preparation and Safety Guide* (PN 4334679) to verify proper ventilation requirements. **CAUTION** The waste container is the low-pressure side of the chemical delivery system and must always be kept vented to atmosphere. If the vent line is blocked, back pressure is generated and inhibits the deliveries of reagents and solvents.
5.	Prevent condensation from collecting in the vent line by sloping the tubing upward toward the fume hood.

Checking the Ancillary and External Reagent Bottles

When to Replace

Replace an ancillary and/or external reagent bottle with a bottle of fresh reagent when you determine that there is not enough reagent available for the next run. A good practice is to mark the bottles after a typical run to help you gauge the reagent levels for subsequent runs.

Do not continue the synthesis until you replace the reagent bottle.

Setting Pause Aheads

You can also set Pause Aheads (programmed pauses) for particular reagent bottles at a point prior to reagent depletion. For instructions, see "Pausing or Aborting a Run" on page 6-10.

Equipment Required

You need the following equipment for this procedure:

√	Item	Supplier	Part Number
	180-mL bottles, as required (ancillary reagent bottles: Tetrazole, Ammonia, Acetic Anhydride, N-Methylimidazole, Auxiliary, Iodine)	Applied Biosystems	See Appendix E
	2- or 4-L bottles, as required (external reagent bottles: ACN, DCM, TCA)		
	Polyethylene inserts for the 180-mL bottles		400790

Handling Precautions

WARNING **CHEMICAL HAZARD.** Before handling the reagents needed for synthesis, read the safety warnings on the reagent bottles and in the manufacturers' Material Safety Data Sheets (MSDSs), and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Dispose of waste in accordance with all local, state/provincial, and national environmental and health regulations.

Using the Change Bottle Procedure to Change Reagent Bottles

To change a reagent bottle using the Change Bottle procedure:

1.	From Page 1 of the Main Menu, press the **Next > Next > Change Bottle** soft keys. Page 1 of the Change Bottle Menu is displayed:
	
	`Bottle Change: A Start>`
	`  Please keep the old bottle`
	`  in the instrument until you Prev>`
	`  are prompted to remove it. Next>`

2.	Select the bottle you want to change by pressing the **Prev.** and **Next** soft keys repeatedly. The following bottles are supported: • Amidite Bottles A, G, C, T, 5, 6, 7, 8 • Tet • Ammonia • CapA • CapB • Iodine • Aux • TCA • ACN • DCM

3.	Press the **Start** soft key to begin the Bottle Change procedure. The screen below is displayed.
	
	`Bottle Change: B Stop>`
	`  Valve Operation NNs Hold>`
	
	B = The selected bottle
	Valve Operation = Currently open valve groups
	NN = Remaining step time

To change a reagent bottle using the Change Bottle procedure:

4.	The procedure pauses and prompts you to remove the old bottle, wipe the line clean with a lint free tissue, and insert the new bottle.
	
	`Bottle Change: B Stop>`
	`Remove the old bottle, Continue>`
	`wipe line with a lint free`
	`tissue, and insert new bottle.`
	
	B = The selected bottle
	a. Remove the old bottle containing the rinse: unscrew it, turning counterclockwise, then remove the polyethylene insert (if present) and discard it.
	
	Note: The polyethylene insert forms an airtight seal between each cap assembly and bottle. It is designed for single use and should be replaced with each bottle change.
	
	b. Wipe the instrument lines with a lint-free tissue.
	c. Attach a new ancillary reagent bottle: open the bottle (for ancillary bottles, place a new polyethylene insert (PN 400790) inside the bottle neck), then screw the bottle snugly into its threaded cap on the instrument, turning clockwise.

5.	Press the **Continue** soft key.

6.	After the procedure is complete, the following screen is displayed:
	
	`Bottle Change: B Start>`
	
	`Prev>`
	`Procedure completed. Next>`

7.	Repeat steps 2 to 6 for each of the new reagent bottles you want to install.
8.	Dispose of the contents of the old bottles per your laboratory practices.

Preparing the Phosphoramidite Bottle Positions

When to Prepare the Positions

IMPORTANT! Prepare the phosphoramidite bottle positions before installing any new phosphoramidite bottles.

Before installing the new phosphoramidite bottles, rinse the delivery lines with ACN. Collect the rinse in an empty phosphoramidite bottle attached at each phosphoramidite bottle position.

Removing Phosphoramidite Bottles

To remove phosphoramidite bottles:

1.	Remove the old bottle by firmly pulling it straight down while pressing the black button above its receptacle. If the bottle sticks, carefully move it side to side while pulling down.
2.	Wipe the delivery line with a lint-free tissue.

Attaching Phosphoramidite Bottles

To attach phosphoramidite bottles:

1.	Firmly push the bottle up around its receptacle while pressing the black button. If necessary, maneuver the bottle into place by carefully moving it side to side while pushing.
2.	When the bottle is correctly engaged, release the black button to return it to its out position. If the black button remains in, the bottle is not seated properly and must be repositioned.
3.	Repeat steps 1 and 2 for each phosphoramidite bottle position. **IMPORTANT!** All the phosphoramidite bottles are pressurized simultaneously with a single valve, which requires bottles to be attached to all eight phosphoramidite positions, not just the positions where you plan to install new phosphoramidites.

Choosing Autodilution or Manual Installation

You can install phosphoramidites on the 3400 DNA Synthesizer manually or you can use the Autodilution feature. See the table below for the advantages and disadvantages of each installation type.

Installation Type	Advantages	Disadvantages	For procedures, see ...
Autodilution	You do not have to prepare the phosphoramidites first (that is, you can install the phosphoramidites in powdered form).	The delivery volumes are limited to 0.5, 1.0, and 2.0 g.	page 4-11.
Manual	You can select your own delivery volumes.	You must prepare the phosphoramidites first (that is, you must dissolve the phosphoramidites and install them in liquid form).	page 4-14.

Installing Phosphoramidites Using Autodilution

Autodilution Menu

Use the Autodilution Menu in the 3400 DNA Synthesizer software to install phosphoramidites automatically. For detailed menu information, see "Autodilution Menu (Bottles Selected)" on page B-31.

Delivery Volumes

Autodilution is intended for use with phosphoramidite bottles A, G, C, and T at delivery volumes of 0.5, 1.0, or 2.0 g. If you want to change the delivery volumes, use the manual procedure on page 4-14.

Handling Precautions

The phosphoramidites are atmosphere sensitive. After opening a bottle, quickly place it on the instrument to prevent water contamination.

Autodilution Procedure

To autodilute the phosphoramidites:

1.	Be sure bottles containing old phosphoramidite solution or empty bottles are attached to all eight phosphoramidite positions on the instrument (A, G, C, T, 5, 6, 7, and 8). See "Preparing the Phosphoramidite Bottle Positions" on page 4-9.

2.	From Page 1 of the Main Menu, press the **Next > Next > Autodilution** soft keys. Page 1 of the Autodilution Menu is displayed:
	
	`Autodilution Bottle A>`
	`No bottles selected Bottle G>`
	`Bottle C>`
	`(Page 1 of 4) Next>`

To autodilute the phosphoramidites: *(continued)*

3. Select the bottle(s) you want to dilute by pressing the corresponding soft keys.

   a. For bottles A, G, C, and T, you are prompted for the corresponding bottle size. Based on your selection, the software automatically determines the acetonitrile delivery volume (that is, how much ACN needs to be delivered to the bottle).

```
Select Bottle B Size                [None]>
                                     0.5 g>
                                     1.0 g>
                                     2.0 g>
```

   B = A, G, C, or T

   b. For custom bottles 5, 6, 7, and 8, you are asked to enter the desired ACN delivery volume instead:

```
Bottle B Delivery Volume
  ACN delivery volume:       mL
                                     Clear>
                                       Set>
```

   B = 5, 6, 7 or 8

   To deselect a previously selected custom bottle, press the **Clear** key to remove the previously entered ACN delivery volume, then press **Set.**

   Note: The Clear key is visible only when there is something to clear (that is, when a partial or complete delivery volume has been entered).

4. After selecting each bottle, the bottle size (if applicable) and acetonitrile delivery volume are indicated next to the corresponding soft key in the Autodilution Menu:

```
Autodilution      [1.0g/13.2mL] Bottle T>
N bottles              [12.3mL] Bottle 5>
                                Bottle 6>
(Page 2 of 3)                       Next>
```

   N = Number of bottles selected

To autodilute the phosphoramidites: *(continued)*

5.	After you are done selecting bottles, press the **Start** soft key on page 3 of the Autodilution Menu. The Autodilution: Preparation Menu is displayed:
	
	`Autodilution: Preparation Start>`
	`  Please keep the old bottles`
	`  in the instrument until you Prev>`
	`  are prompted to remove them. Next>`
	
	The old reagent is rinsed out of the lines, then purged into the phosphoramidite bottles.

6.	You can monitor, stop or hold the Autodilution procedure.
	
	`Autodilution: Bottle B Stop>`
	`  Valve Operation NNs Hold>`
	
	B = Each selected bottle, in turn.
	Valve Operation = Currently open valve groups
	NN = Remaining Step Time

7.	For each selected bottle, the procedure pauses and prompts you to remove the old bottle, wipe the line clean with a lint free tissue, and insert the new bottle.
	
	`Autodilution: Bottle B Stop>`
	`  Remove old amidite bottle, Continue>`
	`  wipe line with a lint free`
	`  tissue, and insert new bottle.`

8.	Press the **Continue** soft key to continue the Autodilution procedure. The instrument automatically: • Fills the new bottles with the proper amount of acetonitrile • Mixes the acetonitrile and phosphoramidite in each bottle • Fills the lines with the new phosphoramidite.

9.	After the procedure is complete, the following screen is displayed:
	
	`Autodilution: Cleanup Start>`
	
	`Prev>`
	`Procedure completed. Next>`

Diluting and Installing Phosphoramidites Manually

IMPORTANT! The prepackaged phosphoramidites are bottled as powders. Before installing the phosphoramidites manually on the 3400 DNA Synthesizer, you must dissolve the powdered phosphoramidites.

Equipment Required

You need the following equipment for this procedure:

√	Item	Vendor	Part Number
	Glass syringe with a needle	MLS	–
	Needle without a syringe, any gauge (for venting)	MLS	–
	Phosphoramidites, as required	Applied Biosystems	See Appendix D
	Anhydrous acetonitrile with less than 100 ppm water, one of the following:		
	• 30-mL bottle	Applied Biosystems	400060
	• 100-mL bottle		401445
	Rubber septum	MLS	–

Guidelines for Dissolving Phosphoramidites

Because the phosphoramidites are extremely sensitive to acid, oxygen, and water, you must take special care when dissolving them. Follow the guidelines below to help avoid contamination, prevent degradation, and ensure high coupling yields.

- Use anhydrous acetonitrile with less than 100 ppm water to dissolve the phosphoramidites.
- When transferring the acetonitrile to a phosphoramidite bottle, use a clean, dry, glass syringe with a needle. Follow these precautions:
  - Store the syringe in a 110 to 120 °C oven to prevent atmospheric moisture contamination.
  - Keep a syringe dedicated to acetonitrile transfer.
  - Use acetonitrile to rinse the syringe. Do not use water.
  - Do not contaminate the acetonitrile bottle with traces of phosphoramidites (that is, do not allow the syringe needle to contact the phosphoramidites).
- Add the correct amount of acetonitrile to each phosphoramidite. See “Acetonitrile Volumes” on page 4-15.

Acetonitrile Volumes

When preparing phosphoramidites, add the correct amount of acetonitrile to each phosphoramidite as shown in the table below. Both standard and FastPhoramidite® phosphoramidites are diluted with the same volume of acetonitrile.

ß-Cyanoethyl Phosphoramidite	Volume of Acetonitrile (mL)	Weight of Phosphoramidite (g)	Molarity (M)
A^{bz}	5.6	0.50	0.10
	11.2	0.50	0.05
	11.2	1.00	0.10
	22.4	2.00	0.10
G^{ibu} G^{dmf}	5.8	0.50	0.10
	11.6	0.50	0.05
	11.6	1.00	0.10
	23.2	2.00	0.10
C^{bz}	5.9	0.50	0.10
	11.8	0.50	0.05
	11.8	1.00	0.10
	23.6	2.00	0.10
T	6.6	0.50	0.10
	13.2	0.50	0.05
	13.2	1.00	0.10
	26.4	2.00	0.10

Dissolving the Phosphoramidites

To dissolve phosphoramidites:

1.	Heat a syringe/needle in an oven at 110 to 120 °C to dryness (about 1 h).
2.	Remove the syringe/needle from the oven and allow it to cool to room temperature (preferably in a desiccator).
3.	Prepare the phosphoramidite bottle: a. Pull back the aluminum tab in the direction of the arrow to expose the septum. b. Place a needle (any gauge) without a syringe into the rubber septum. This procedure vents the pressure in the bottle when the anhydrous acetonitrile is added. Venting also prevents accidental splashing when the phosphoramidite bottle is opened and placed on the instrument.

To dissolve phosphoramidites: *(continued)*

4.	Unscrew the cap from the anhydrous acetonitrile bottle and quickly replace it with a clean rubber septum. **WARNING** **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
5.	Pierce the septum of the acetonitrile bottle with the needle/syringe and remove the correct amount of acetonitrile. See "Acetonitrile Volumes" on page 4-15. **WARNING** **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6.	Tap on the phosphoramidite bottle to settle the phosphoramidite powder at the bottom of the bottle.
7.	Pierce the septum of the phosphoramidite bottle a few millimeters with the venting needle, then slowly add the acetonitrile. **IMPORTANT!** Make sure the needle does not touch the phosphoramidite powder or solution.
8.	When you finish adding the acetonitrile, remove both the venting needle and the needle/syringe.
9.	Gently swirl the bottle to dissolve the phosphoramidite powder.
10.	Once dissolved, the phosphoramidites can be installed on the instrument. Continue with "Manually Installing Phosphoramidite Bottles" on page 4-16.

Manually Installing Phosphoramidite Bottles

To change a reagent bottle using the Change Bottle procedure:

1.	From Page 1 of the Main Menu, press the **Next > Next > Change Bottle** soft keys. Page 1 of the Change Bottle Menu is displayed:
	
	`Bottle Change: A Start>`
	`Please keep the old bottle`
	`in the instrument until you Prev>`
	`are prompted to remove it. Next>`

2.	Select the bottle you want to change by pressing the **Prev** and **Next** soft keys repeatedly.

To change a reagent bottle using the Change Bottle procedure:

3.	Press the **Start** soft key to begin the Bottle Change procedure. The following screen is displayed.
	
	`Bottle Change: B  Stop>`
	`  Valve Operation  NNs Hold>`
	
	B = The selected bottle
	Valve Operation = Currently open valve groups
	NN = Remaining step time

4.	The procedure pauses and prompts you to remove the old bottle, wipe the line clean with a lint free tissue, and insert the new bottle.
	
	`Bottle Change: B  Stop>`
	`  Remove the old bottle,  Continue>`
	`  wipe line with a lint free`
	`  tissue, and insert new bottle.`
	
	B = The selected bottle
	a. Remove the old bottle containing the rinse.
	b. Wipe the instrument lines with a lint-free tissue.
	c. Attach the new bottle.

5.	Press the **Continue** soft key.

6.	After the procedure is complete, the following screen is displayed:
	
	`Bottle Change: B  Start>`
	
	`  Prev>`
	`Procedure completed.  Next>`

7.	Repeat steps 2 to 6 for each phosphoramidite bottle.
8.	Dispose of the contents of the old bottles per your laboratory practices.

Installing Columns

Equipment Required

You need the following equipment for this procedure:

√	Item	Vendor	Part Number
	Columns, as required	Applied Biosystems	See pages D-5 to D-7

Installing a Column

To install a column:

1.	Look at your sequence to determine which base is at the 3' end.
2.	The columns are color-coded, as shown in the table below. Make sure your column matches the base is at the 3' end of your sequence.

Support-Bound Nucleoside	Column Color Code
A	Green
G	Yellow
C	Red
T	Blue

3.	Tap the ends of the synthesis column on a dark surface to check for leaks. **⚠ CAUTION** If support falls out, do not use the column. Using a leaky column could damage the instrument.
4.	Firmly push either end of the column straight down onto the lower luer fitting on the instrument. **Note:** Since the column is symmetrical, it can be attached in either direction.
5.	Firmly push the upper luer fitting straight down onto the top of the column. The column should fit securely. **IMPORTANT!** Do not twist the fittings.

Installing Oligo Collection Vials

After cleavage is performed on the instrument, the ammonia solution containing oligo is collected in the oligo collection vials.

Equipment Required

You need the following equipment for this procedure:

√	Item	Vendor	Part Number
	4-mL oligo collection vials with caps, as required for your run	Applied Biosystems	400048
	Teflon-lined caps (size 13-425), as required for your run **IMPORTANT!** Use Teflon-lined caps with the vials because the rubber-lined caps can leach contaminants into the DNA-ammonium hydroxide solution.	MLS	–

Installing an Oligo Collection Vial

To install an oligo collection vial:

1.	Screw the vial snugly into its threaded cap on the instrument, turning clockwise.

Run Preparation: Setting Up the Instrument Software

5

This chapter covers:

Section 5.1 Creating Sequences

This section covers:

Overview

Creating Sequences

When you set up a synthesis run, you select the sequences to be synthesized on each column (page 6-3). Before you can do that, however, you must add sequences to the 3400 DNA Synthesizer software.

This section describes how to create, edit, and delete sequences using:

- The instrument front panel (Edit Sequence Menu)
- An external computer and a Web browser

Before You Begin

Before you begin creating sequences in the 3400 DNA Synthesizer software, Applied Biosystems recommends that you read:

- "Understanding the LCD Screen/Keypad" on page 3-3
- "Edit Sequence Menu" on page B-6

Using the Instrument Front Panel

Creating a Sequence

Two Ways to Create Sequences

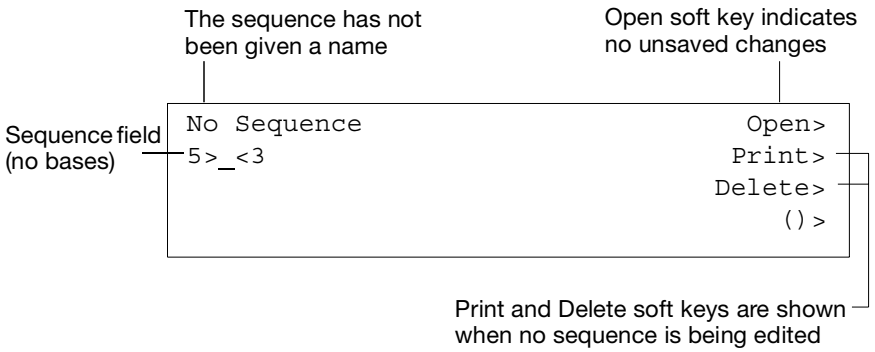
When you use the instrument front panel, you can create a sequence in two ways:

- Create a *new* sequence. In this method, you enter bases in the empty sequence field, then save the sequence.
- Start with an *existing* (previously created) sequence. In this method, you modify the bases in the sequence field, then save the sequence under the same or a new name.

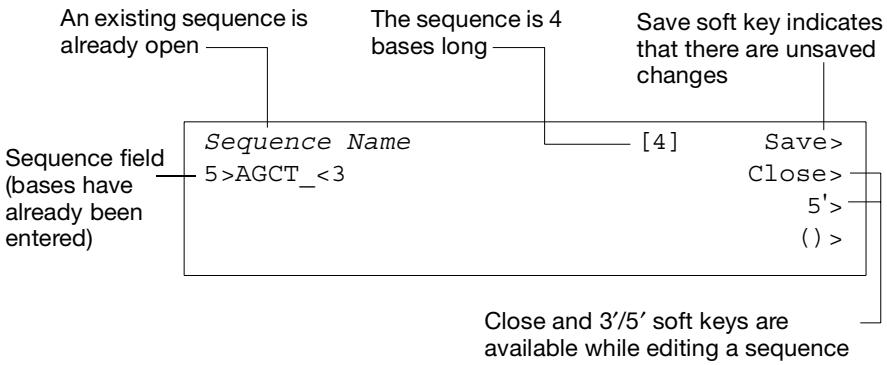
Creating a New Sequence

To create a new sequence:

1. From Page 1 of the Main Menu, press the **Edit Sequence** soft key.
   - If no sequence is currently being edited (no bases are entered), the page is displayed as follows:



   - If a sequence is already open, some or all of the items on the page appear as follows:



   Sequence Name = the name of the an existing sequence, or the text "(Untitled)" if you are creating a new sequence from scratch.

To create a new sequence: ***(continued)***

2.	If bases are already entered in the sequence field, remove the sequence by pressing the **Close** soft key. If the previous sequence was not saved, you are asked for confirmation. Press the **Yes** soft key. The sequence is removed from the sequence field and the sequence name changes to No Sequence.
3.	Continue with "Editing a Sequence" on page 5-7.

Modifying an Existing Sequence

To modify an existing sequence:

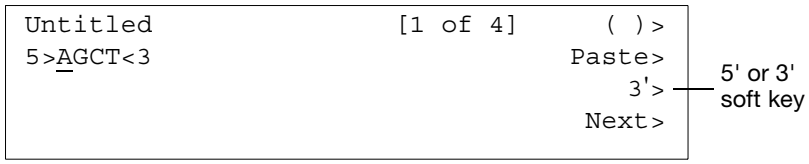
1.	To modify an existing sequence, press the **Open** soft key. The Open Sequence Menu is displayed. `Open Sequence` *`Sequence Name>`* *`Sequence Name>`* *`Sequence Name>`* `(Page 1 of` *`n`*`)` `Next>` *Sequence Name* = the name(s) of the existing sequences in the 3400 DNA Synthesizer software *n* = the total number of pages in the menu, which varies depending on the number of sequences that are stored in the software.
2.	Press the **Next** soft key to browse through all the existing sequences.
3.	When you find the desired sequence, press the appropriate ***Sequence Name*** soft key to return to Page 1 of the Edit Sequence Menu, where the selected sequence is now displayed.
4.	Continue with "Editing a Sequence" on page 5-7.

Editing a Sequence

Note: The menus in the following procedures show "New Sequence" in the sequence name field. If you are starting from an existing or non-empty sequence, then the name of the sequence you are currently modifying is displayed in this field.

Editing a Sequence

To edit a sequence:

1.	To move around in the sequence field: • Press the ← or → (left- or right-arrow key), which moves the cursor one base at a time • Press the **Prev.** or **Next** command key, which moves the cursor up or down a line. • Press the **5′** or **3′** (switch) soft key, which moves the cursor to either end of the sequence  **Note:** If a sequence is too long to fit on the LCD screen, it automatically scroll up or down until the current base position is visible.
2.	To enter new base positions in the sequence field, press the: • Base keys (**A**, **G**, **C**, and **T**) and/or • Numeric keys (**5**, **6**, **7**, and **8** only) • **( )** soft key, which allows you to enter or select bases to be transformed into a degenerated base letter. See "Using the ( ) [Select Region] Menu" on page 5-8. **IMPORTANT!** A sequence is entered 5′ to 3′, according to convention. Likewise, the menu displays the sequence in a 5′ to 3′ orientation. However, the sequence is actually synthesized 3′ to 5′.
3.	To delete bases from the sequence field: • Press the **Delete** command key, which deletes bases one at a time. • Press the **( )** soft key and use the ← , → , **Prev**., and **Next** keys to select a region of the sequence, then press the **Cut** soft key to move the selection to the clipboard. See "Using the ( ) [Select Region] Menu" on page 5-8. • Press the **Close** soft key, which clears any unsaved changes. **Note:** Pressing the **Next** soft key opens Page 2 of the Edit Sequence Menu. After you press the **New** soft key, you are automatically returned to Page 1 of the Edit Sequence Menu.

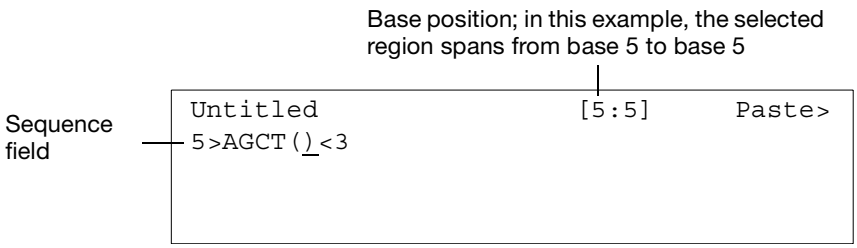
To edit a sequence: ***(continued)***

4.	To select or create a region of the sequence and perform certain operations in this region, press the **( ) [Select Region]** soft key. The ( ) Menu is displayed.
	
	`Untitled [5:5] Cancel>`
	`5>AGCT()<3 Copy>`
	`Cut>`
	`Next>`
	
	You can perform the following operations:
	• Cut/copy the bases in the selected region to the clipboard.
	• Change the bases in the selected region to an IUB (degeneracy) code.
	• Change the bases in the selected region to their complement.
	• Change the bases in the selected region from alphabetic characters to numeric characters and vice versa.
	
	See "Using the ( ) [Select Region] Menu" on page 5-8 for further instructions.

5.	When you finish editing the sequence and are ready to save it, continue with "Saving Your Changes" on page 5-11.

Using the ( ) [Select Region] Menu

To use the ( ) [Select Region] Menu:

1.	From Page 1 of the Edit Sequence Menu, press the **( ) [Select Region]** soft key. The ( ) Menu is displayed, and a set of parentheses is inserted into the sequence at the current base position. 

To use the ( ) [Select Region] Menu: ***(continued)***

2.	In this menu, the bases enclosed in parentheses represent the region on which you wish to operate. Use the cursor command keys (left- and right-arrows, Prev., Next) to move the parentheses, thereby expanding or collapsing the region. Alternatively, you can enter new bases into the region by pressing the: • Base keys (**A**, **G**, **C**, and **T**) And/or • Numeric keys (**5**, **6**, **7**, and **8** only) 
3.	To cut the bases from the selected region to the clipboard, press the **Cut** soft key. The bases are cut from the selected region and copied to the clipboard. You are returned to the Edit Sequence Menu. **Note:** The Cut soft key is available only if the selected region covers one or more bases.
4.	To paste bases from the clipboard into the sequence, press the **Paste** soft key. The bases are pasted from the clipboard into the sequence at the current base position. **Note:** The Paste soft key is available only if the selected region is empty, for example, immediately after pressing the **( )** soft key.
5.	To change the bases in the selected region to an IUB (degeneracy) code, press the ***(bases)*** soft key. The corresponding degenerated base letter is inserted into the sequence. For example, if you select bases **AGC** in step 2, the IUB code **V** is entered into the sequence. **Note:** The ***(bases)*** soft key is available only when the selected region covers a valid IUB code consisting of two to four unique bases. See Table 5-1 on page 5-10 for a list of valid degeneracies.
6.	To change the bases in the selected region to their complement, press the **AGCT/TCGA** soft key. The complement of the selected bases is inserted into the sequence. For example, if you select bases **AGCT** in step 2, the complement **TCGA** is entered into the sequence. **Note:** The AGCT/TCGA soft key is available only if the selected region covers one or more bases.

To use the ( ) [Select Region] Menu: ***(continued)***

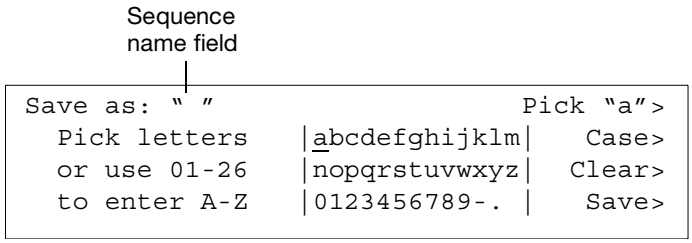
7.	To change the bases in the selected region from alphabetic characters to numeric characters and vice versa, press the **AGCT/5678** soft key. The base designations are changed from alphabetic characters to numeric characters and vice versa. For example, if you select bases **ACGT** in step 2, **5678** is entered into the sequence. **Note:** The AGCT/5678 soft key is available only if the selected region covers one or more bases.
8.	To deselect the region and return to the Edit Sequence Menu at any time, press the **Prev. Menu** command key. You are returned to the Edit Sequence Menu.

Table 5-1 IUB (Degeneracy) Code Table

Original Bases	IUB (Degeneracy) Code
AG	R
AC	M
AT	W
GC	S
GT	K
CT	Y
AGC	V
AGT	D
ACT	H
GCT	B
AGCT	N

Saving Your Changes

To save your changes and create a new sequence:

1.	From the Edit Sequence Menu, press the **Save** soft key. The Save As Menu is displayed. 
2.	Enter a name in the sequence name field: a. Use the left- and right-arrow keys to move the cursor to the desired alphanumeric character on the LCD screen. b. Press the **Pick "n"** soft key. The selected character appears in the sequence name field. **Note:** The **"n"** designates the currently selected alphanumeric character. For example, in step 1 above, the cursor is on the letter **a** and the soft key reads **Pick "a"**.
3.	To switch the alphabetic characters between upper and lower case, press the **Case** soft key.
4.	To delete characters from the sequence name field: • Press the **Delete** command key to delete characters one at a time. • Press the **Clear** soft key to delete all characters at once.
5.	Repeat steps 2 to 4 as necessary until you have entered the desired sequence name.
6.	Press the **Save** soft key. The new sequence is entered into the 3400 DNA Synthesizer software.
7.	Press the **Main Menu** command key to return to Page 1 of the Main Menu.

Deleting a Sequence from the Instrument Software

IMPORTANT! Deleting a sequence removes it permanently from the 3400 DNA Synthesizer software.

To delete sequence(s) from the instrument software:

1.	From Page 1 of the Main Menu, press the **Edit Sequence** soft key. The Edit Sequences Menu is displayed.
	
	`No Sequence Open>`
	`5>_<3 Print>`
	`Delete>`
	`()>`
	
	Note: If a sequence is currently open, it needs to be closed first. In this case, a Close soft key will be available; use it to close the sequence.

2.	Press the **Delete** soft key. The Delete Sequences Menu is displayed.
	
	`Delete Sequences [ ] Sequence Name>`
	`<A> = Select All [ ] Sequence Name>`
	`<C> = Clear All [ ] Sequence Name>`
	`(Page 1 of n) Next>`
	
	Sequence Name = the name(s) of the existing sequences in the 3400 DNA Synthesizer software
	
	n = the total number of pages in the menu, which varies depending on the number of sequences that are stored in the software.

3.	Press the **Next** soft key to browse through all the existing sequences.
4.	When you find the desired sequence(s), press the appropriate ***Sequence Name*** soft key. An "X" appears within the square brackets next to the sequence name, indicating that the sequence is selected for removal. To select all sequences stored in the instrument, press the **"A"** base key.

5.	Press the **Prev. Menu** command key. The confirmation prompt below is displayed.
	
	`Confirm Delete Operation Yes>`
	`Are you sure you want to delete`
	`the n selected sequences?`
	`No>`
	
	n = the total number of sequences selected.

To delete sequence(s) from the instrument software: ***(continued)***

6.	Press the **Yes** soft key. The sequence is deleted from the 3400 DNA Synthesizer software and the message below is displayed.
	
	`Delete operation successful.`
	`  The n selected sequences have`
	`  been removed.`

7.	Press any key to return to the Edit Sequence Menu.
8.	Press the **Main Menu** command key to return to Page 1 of the Main Menu.

Printing a Sequence

Printing Sequences

Note: To print sequences from the Applied Biosystems 3400 DNA Synthesizer, a printer must be configured. See "Setting up a Printer (Optional)" on page 3-12.

To print sequence(s) from the 3400 DNA Synthesizer software:

1.	From Page 1 of the Main Menu, press the **Edit Sequence** soft key. The Edit Sequences Menu is displayed.
	
	`No Sequence  Open>`
	`5>_<3  Print>`
	`  Delete>`
	`  ()>`
	
	Note: If a sequence is currently open, it needs to be closed first. In this case, a Close soft key will be available; use it to close the sequence.

2.	2. Press the **Print** soft key. The Print Sequences Menu is displayed.
	
	`Print Sequences  [ ]  Sequence Name>`
	`<A> = Select All  [ ]  Sequence Name>`
	`<C> = Clear All  [ ]  Sequence Name>`
	`(Page 1 of n)  Next>`
	
	Sequence Name = the name(s) of the existing sequences in the 3400 DNA Synthesizer software
	
	n = the total number of pages in the menu, which varies depending on the number of sequences that are stored in the software.

3.	Press the **Next** soft key to browse through all the existing sequences.

To print sequence(s) from the 3400 DNA Synthesizer software: *(continued)*

4.	When you find the desired sequence(s), press the appropriate ***Sequence Name*** soft key. An "X" appears within the square brackets next to the sequence name, indicating that the sequence is selected for printing. To select all sequences stored in the instrument, press the **"A"** base key. To deselect all sequences stored in the instrument, press the **"C"** base key.

5.	Press the **Prev. Menu** command key. The confirmation prompt below is displayed.
	
	`Confirm Print Operation Yes>`
	`Are you sure you want to print`
	`the n selected sequences?`
	`No>`
	
	n = the total number of sequences selected.

6.	Press the **Yes** soft key. The sequence(s) are printed and the message below is displayed.
	
	`Sequences printed.`
	`The sequences were successfully`
	`Sent to the print spool at`
	`printer.`
	
	printer = the host name or IP address of the configured Ethernet printer.

7.	Press any key to return to the Edit Sequence Menu.
8.	Press the **Main Menu** command key to return to Page 1 of the Main Menu.

Using a Web Browser

You can create or modify sequences using an external computer and a Web browser. This provides a better view of the sequence contents and allows you to copy and paste sequences from other applications and sources.

Web Browser Requirements

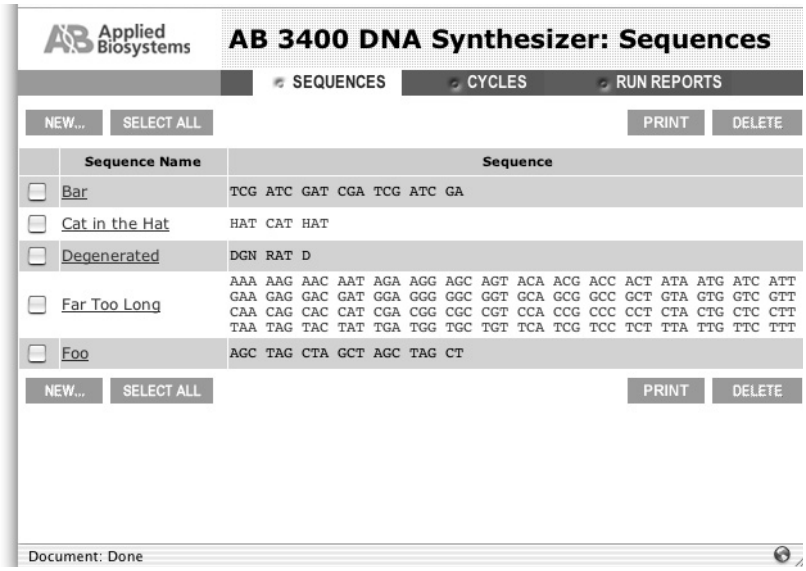
To use a Web browser to work with sequences, your 3400 DNA Synthesizer must:

- Be connected to a computer with a Web browser
- Have the TCP/IP connection correctly configured

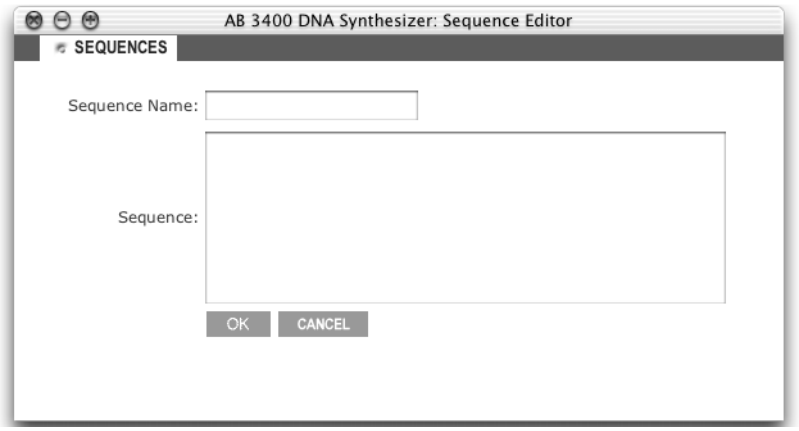
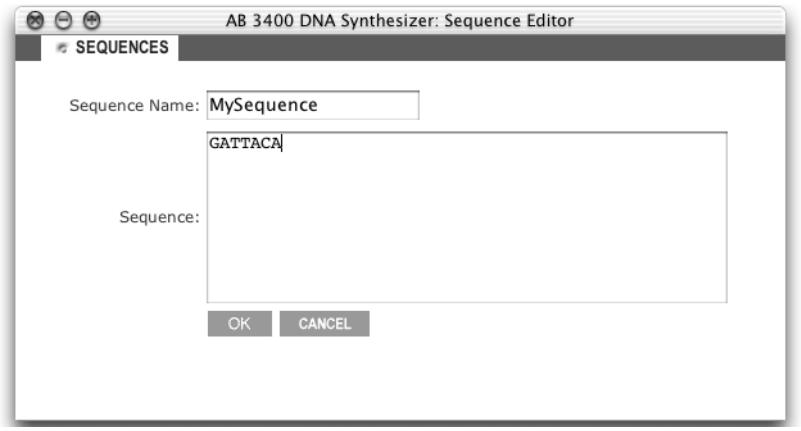
For more information, see "Connecting a Computer (Optional)" on page 3-14 and "Connecting the Instrument to the Router" on page 3-10.

Creating a Sequence

To create a new sequence using a Web browser:

1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12 The Sequences window opens, displaying a list of the sequences currently stored in your 3400 DNA Synthesizer software. 

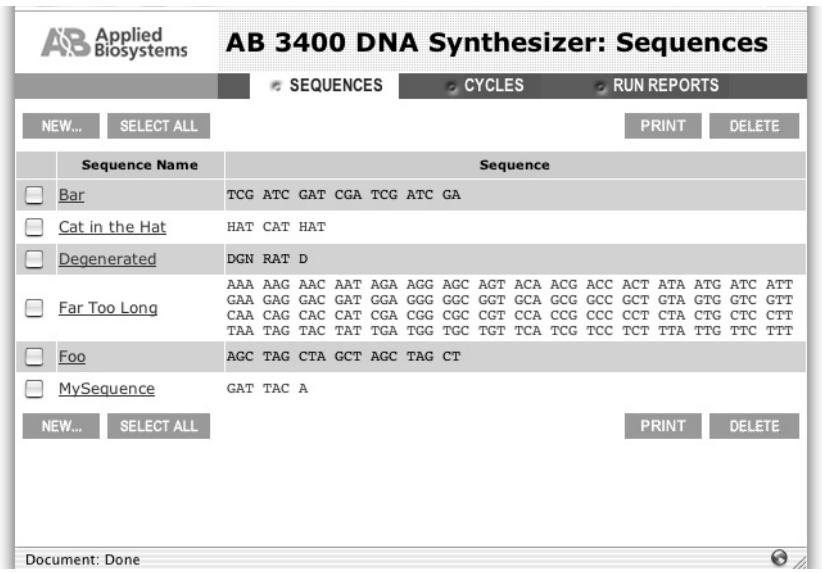
To create a new sequence using a Web browser: ***(continued)***

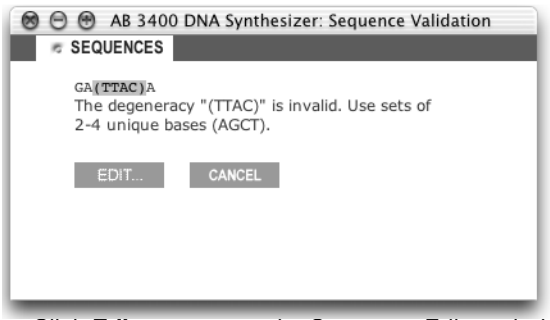
3.	Click **New**. The Sequence Editor window opens. 
4.	Create a new sequence: a. In the Sequence Name field, enter a name for the new sequence. b. In the Sequence field, type or copy/paste to enter the new sequence. Use the copy/paste function in your operating environment to import sequences from other programs or documents. 

To create a new sequence using a Web browser: *(continued)*

5. Click **OK**.

If you ...	Then ...
Entered a valid sequence	You are returned to the Sequences window. The new sequence is added to this window and to your 3400 DNA Synthesizer software.

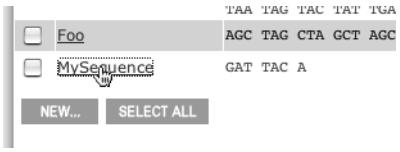
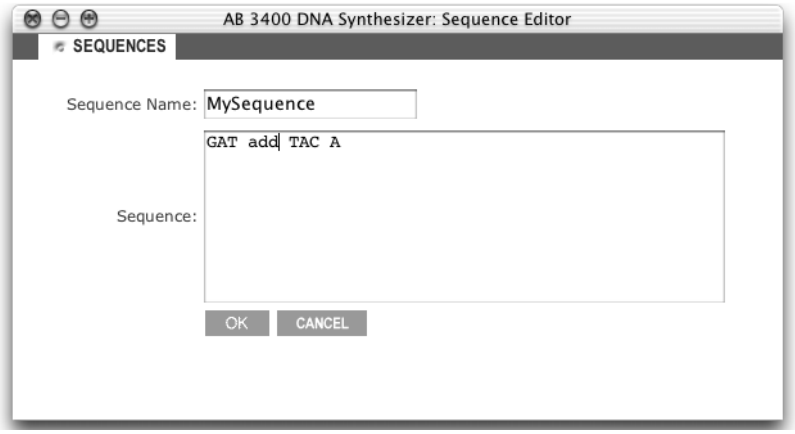


Did not enter a valid sequence	The following error message is displayed:  a. Click **Edit** to return to the Sequence Editor window. b. Correct the error, then click **OK**.

Editing a Sequence

Edit a sequence to change the name of the sequence and/or insert and delete bases in the sequence.

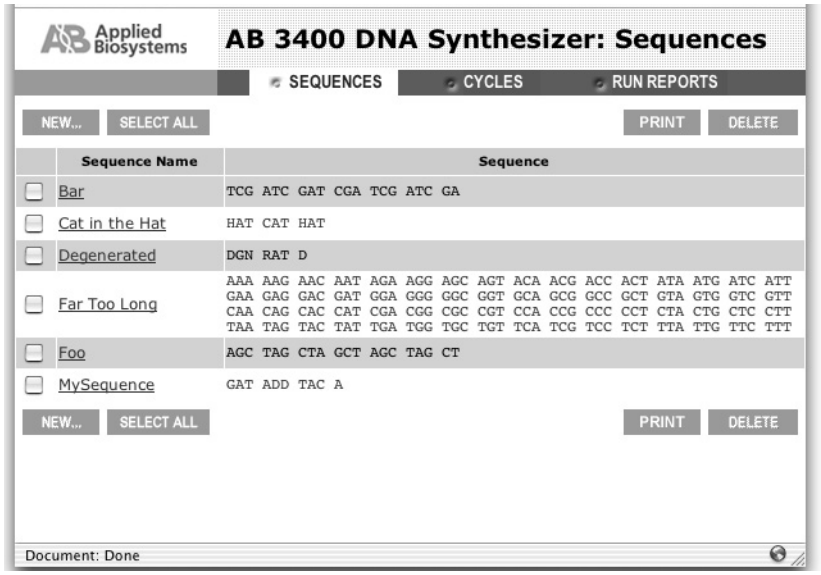
To edit a sequence using a Web browser:

1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12. The Sequences window opens, displaying a list of the sequences currently stored in your 3400 DNA Synthesizer software.
3.	Select the sequence to edit.  The Sequence Editor window is displayed the selected sequence.
4.	Edit the sequence: a. In the Sequence Name field, type a new name for the sequence, if desired. **Note:** The new name overwrites the existing one. b. In the Sequence field, insert or delete base(s) from the sequence. 

To edit a sequence using a Web browser: ***(continued)***

5. Click **OK**.

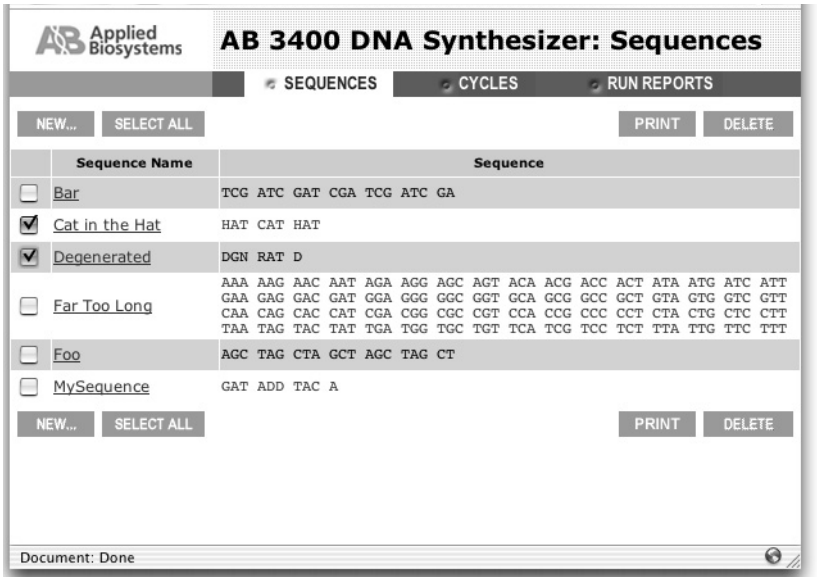
   You are returned to the Sequences window. The new sequence is added to this window and to your 3400 DNA Synthesizer software.



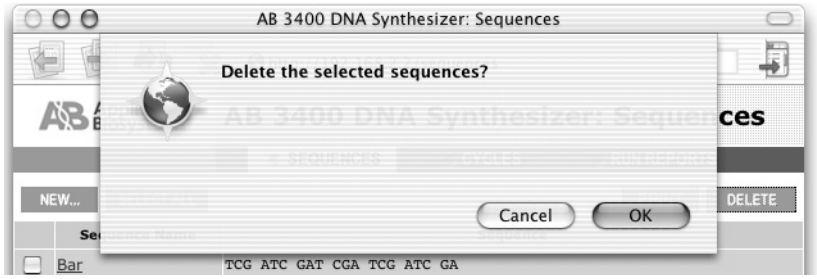
Deleting a Sequence

IMPORTANT! Deleting a sequence removes it permanently from the 3400 DNA Synthesizer software.

To delete a sequence using a Web browser:

1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12 The Sequences window opens, displaying a list of the sequences currently stored in the your 3400 DNA Synthesizer's software. 
3.	Select the checkbox next to the sequence(s) you want to delete.

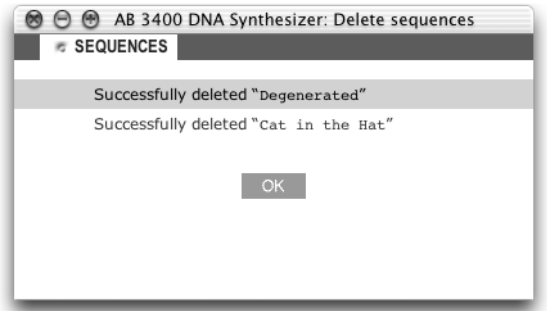
To delete a sequence using a Web browser: ***(continued)***

4.	Click **Delete**. A confirmation prompt appears. 

To delete a sequence using a Web browser: *(continued)*

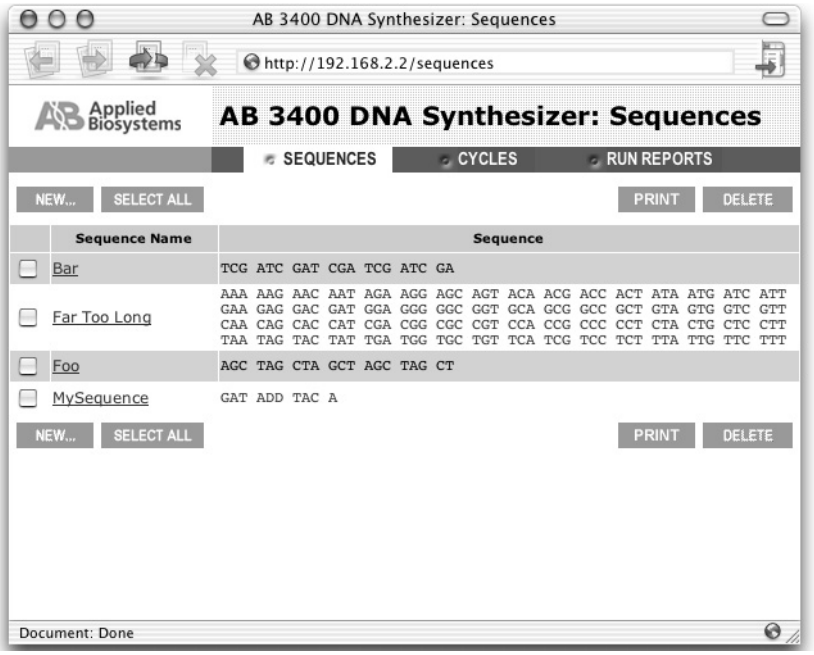
5. Click **OK**.

   The Delete sequences dialog box opens.



6. Click **OK**.

   You are returned to the Sequences window. The selected sequences are deleted from this window and from your 3400 DNA Synthesizer software.



Section 5.2 Creating Custom Cycle Scripts

This section covers:

Overview

Creating Custom Cycle Scripts

When you set up a synthesis run, you select a cycle script for the run (page 6-4). The 3400 DNA Synthesizer software comes with 12 cycle scripts (see "Cycle Scripts Provided" on page 5-25). Although you cannot modify or delete the cycle scripts, you can use them as a basis to create your own custom cycle scripts.

Note: Creating custom cycle scripts is optional.

Three Ways to Create Custom Cycle Scripts

There are three ways to create custom cycle scripts:

- Using an external computer to access or upload cycle script files remotely.

  Cycle scripts can be edited in a text editor (for example, Microsoft Windows® Notepad), then stored in the Cycles folder on the 3400 DNA Synthesizer. This method provides the greatest flexibility for advanced users and for users who want to control the cycle script files externally for distribution onto several instruments.
- Using the Edit Cycle Menu on the 3400 DNA Synthesizer front panel to access the built-in cycle editor.

  The built-in cycle editor provides the advantage of guided command building, which makes it less error-prone than using a text editor. This method may be most convenient for making simple changes while at the instrument because it does not require an external computer.
- Using an external computer and a Web browser to access the built-in Web-based cycle editor.

  This method provides guided command building similar to the instrument front panel cycle editor. However, the Web-based cycle editor also provides a greater overview of the cycle script contents while you edit. If you have an external computer and Web browser, this may be the preferred way to edit cycle scripts.

This section describes how to create and delete custom cycle scripts using:

- The instrument front panel (Edit Cycle Menu)
- An external computer and a Web browser

Before You Begin

Before you begin creating cycle scripts in the 3400 DNA Synthesizer software, Applied Biosystems recommends that you read:

- "Understanding the LCD Screen/Keypad" on page 3-3
- "Overview of the Cycle Scripts" on page 2-16
- "Edit Cycle Menu" on page B-15

Cycle Scripts Provided

The 3400 DNA Synthesizer software has 12 cycle scripts, each of which includes nine cycle procedures.

Note: For a list of the cycle procedures, see page 2-16. For detailed information on each of the 3400 DNA Synthesizer cycle scripts, see Appendix A.

Cycle Script		Cycle Script		Cycle Script	
0.2 µm-PO*	For DNA synthesis	0.2 µm-PS†	For Phos-phorothioate DNA synthesis	0.2 µm-RNA	For RNA synthesis
1 µm-PO		1 µm-PS		1 µm-RNA	
LV40-PO		LV40-PS		LV40-RNA	
LV200-PO		LV200-PS		LV200-RNA	

*PO = Phosphorothioate oxidization
†PS = Phosphorothioate sulfurization

IMPORTANT! The cycle scripts provided with the 3400 DNA Synthesizer are write-protected. Although you cannot modify or delete these cycle scripts, you can use them as a basis to create your own custom cycle scripts.

Cycle Procedure Command Conventions

Each cycle procedure consists of a sequence of instrument commands that control the synthesis by performing valve operations for specified time intervals. The 3400 DNA Synthesizer cycle procedures use four instrument commands:

TRANsfer <*valves*> <*time*> – Transfers reagents by holding open a set of valves and/or valve groups for a specified amount of time.

<*valves*> is a comma-separated list of one or more valves and/or valve groups (described in "Overview of 3400 Valve Operations" on page 2-6).

<*time*> is the step time; that is, how long to perform the reagent transfer (in seconds).

MONitor <*valves*> <*time*> – This command is intended for use during the TCA delivery step inside the detritylation procedure. It is similar to the TRANsfer command. However, during delivery, the conductivity sensor attached to each active column is monitored to get a trityl reading.

Two types of monitoring take place:

- Delivery monitoring – After a trityl baseline (low reading) and trityl peak (high reading) are determined, delivery of reagent to a specified column may terminate prior to the specified step time if the trityl readings approach the baseline within a certain percentage of the peak delta (that is, the difference between the peak height and the baseline).
- Yield monitoring – All trityl readings that were in the upper half of the peak delta are added together to produce a peak area. Cycle after cycle, the peak areas are compared to come up with a trityl yield, or average step-wise yield. If, during the synthesis, the yield of a particular column falls below a specified threshold, that column is terminated.

SLEep <*time*> – Waits for a given number of seconds.

SAFe {Yes | No} – Turns on or off safe mode. While safe mode is on (Yes), the Pause function on the front panel takes effect before the next step. While safe mode is off, the pause request is deferred.

Valve Group Naming Conventions

Some valve groups contain one or more variables, as indicated with a preceding dollar sign ($). When activated during a cycle script, these variables are substituted according to applicable cycle procedure parameters.

For example, valve group ($Base,Tet)ToColumn($Col) contains two variables: $Base and $Col. $Base refers to the currently active base and $Col refers to any currently active columns.

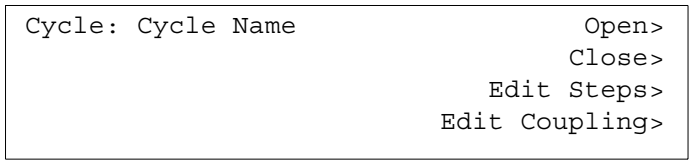
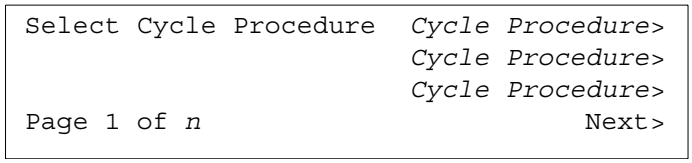
See “Valve Code Listing” on page A-2 for a complete list of valve group names and their code numbers.

Using the Instrument Front Panel

Creating a Custom Cycle Script

Creating a Custom Cycle Script

To create a custom cycle script:

1.	From Page 1 of the Main Menu, press the **Next > Edit Cycle** soft keys. The Cycle Menu is displayed. • If no cycle script is open, the Cycle Menu appears as follows:  • If a cycle script is open, the Cycle Menu appears as follows:  *Cycle Name* = the name of the current cycle script
2.	Press the **Edit Steps** soft key. The Select Cycle Procedure Menu is displayed.  *Cycle Procedure* = the names of the existing cycle procedures in the cycle script that is currently being edited *n* = the total number of pages in the menu, which varies depending on the number of cycle procedures that are stored in the software.
3.	Press the **Next** soft key to browse through all the existing cycle procedures.

To create a custom cycle script: ***(continued)***

4. When you find the desired procedure, press the appropriate ***Cycle Procedure*** soft key. A *Cycle Procedure* Menu containing the steps (or commands) in the selected cycle procedure is displayed.

```
Cycle Procedure                Edit Step>
->TRANsfer Pressure (Amidite, Tet) 15
  TRANsfer AToWaste 3
  TRANsfer GToWaste 3
```

Cycle Procedure = the name of the current cycle procedure. The steps shown in the menu above are examples only; the steps shown on your LCD screen may not be the same.

5. To delete a step:
   a. Press the **Prev.** or **Next** command keys to move to the desired step.
   b. Press the **Delete** command key. The step is deleted from the current cycle procedure.

   Note: If the cursor is past the end of the cycle procedure, pressing the **Delete** command key deletes the last step in the cycle procedure.

6. To insert a new step:
   a. Press the **Prev.** or **Next** command keys to move to the desired location.
   b. Press the **Insert** command key. The Select Command Menu is displayed.

```
Select Command                   Transfer>
                       Monitored Transfer>
                                   Sleep>
                                Safe Mode>
```

   c. See “Inserting a New Cycle Procedure Step” on page 5-29 for further instructions.

7. To modify a step:
   a. Press the **Prev.** or **Next** command keys to move to the desired step.
   b. Press the **Edit Step** soft key. The Edit Cycle Step Menu is displayed.

```
Edit Cycle Step                        Done>
                         [TRANsfer] Command>
          [Pressure (Amidite,Tet)] Valves>
                              [15] Step Time>
```

   c. See “Editing a Cycle Procedure Step” on page 5-32 for further instructions.

To create a custom cycle script: *(continued)*

8.	When you finish creating the new cycle script and are ready to save it:
	a. Press the **Prev. Menu** command key until you are returned to the Cycle Menu.
	
	`Cycle: Cycle Name Save>`
	`Edit>`
	`Edit Steps>`
	`Edit Coupling>`
	
	b. Continue with "Saving Your Changes" on page 5-34.

Inserting a New Cycle Procedure Step

To insert a new step into a cycle procedure:

1.	From the *Cycle Procedure* Menu (see step 4 on page 5-28), press the **Insert** command key. The Select Command Menu is displayed.
	
	`Select Command Transfer>`
	`Monitored Transfer>`
	`Sleep>`
	`Safe Mode>`

To insert a new step into a cycle procedure: *(continued)*

2. Select one of the available instrument commands. Press the:
   - **Transfer** soft key for the TRANsfer command.
   - **Monitored Transfer** soft key for the MONitor command.
   - **Sleep** soft key for the SLEep command.
   - **Safe Mode** key for the SAFe command

   Depending on the command you select, one of the following menus is displayed:

```
Select Valves                          Cancel>
Valve Code: _

Instrument Command
```

```
Set Step Time
Step Time: _

Instrument Command
```

```
Enable safe mode?                      Yes>
   Select Yes to turn safe mode on.
   Select No to turn safe mode off.
                                        No>
```

   Instrument Command = the current Instrument command. See page 5-25 for more information on the Instrument commands.

3. To use the Select Valves Menu:
   a. See "Valve Code Listing" on page A-2 for a complete list of valve group names and their three-digit code numbers.
   b. Using the numeric keys, enter the desired valve group code number.

      The corresponding valve group appears on the last line and a Select soft key is added to the menu. In the example below, the valve group code is 101, the Instrument command is TRANsfer, and the valve group name is ReverseFlush($Col).

```
Select Valves                          Cancel>
Valve Code: 101_

TRANsfer ReverseFlush($Col)            Select>
```

   c. Press the **Select** soft key to confirm your choice. The Set Step Time Menu is displayed, or

      Press the **Cancel** soft key or the **Prev. Menu** command key to exit the Select Valves Menu without selecting (or changing) a valve group. You are returned to the Edit Cycle Step Menu.

To insert a new step into a cycle procedure: ***(continued)***

4.	To use the Set Step Time Menu:
	a. Using the numeric keys, enter the desired step time in seconds. To enter sub-seconds, use real numbers (for example, enter **2.1** for 2.1 sec).
	As you start entering digits, a decimal point (.) soft key and a Set soft key are added to the menu and the generated SCPI command is displayed. In the example below, the step time is 30 seconds and the generated Instrument command is TRANsfer ReverseFlush($Col) 30.
	`Set Step Time`
	`Step Time: 30_ .>`
	`Clear>`
	`TRANsfer ReverseFlush($Col) 30 Set>`
	b. Press the **Set** soft key to confirm your choice. You are returned to the *Cycle Procedure* Menu (see step 4 on page 5-28), OR
	Press the **Cancel** soft key or the **Prev. Menu** command key to exit the Set Times Menu without selecting a time. You are returned to the Edit Cycle Step Menu.

5.	To use the "Enable Safe Mode?" Menu: • If the subsequent steps in the cycle procedure are "safe steps" (that is, it is safe to pause the synthesis at this point), press the **Yes** soft key. • If the subsequent steps in the cycle procedure are not safe steps, press the **No** key. You are returned to the Cycle Procedure Menu.

Editing a Cycle Procedure Step

To edit an existing cycle procedure step:

1. From the Cycle Procedure Menu (see step 4 on page 5-28), press the **Change** soft key. The Edit Cycle Step Menu is displayed.

```
Edit Cycle Step    [TRANsfer]   Command>
         [ReverseFlush($Col)]    Valves>
                         [30] Step Time>
                                 Done>
```

2. Select the part of the command you want to change. (You are prompted for all subsequent parts of the command as well.)

   Depending on which command you are editing and which part of the command you selected, one of the following menus is displayed:

```
Select Command                Transfer>
                    Monitored Transfer>
                                Sleep>
                             Safe Mode>
```

```
Select Valves                    Cancel>
  Valve Code: _

>Instrument Command
```

```
Set Step Time
  Step Time: _

>Instrument Command
```

```
Enable safe mode?                 Yes>
   Select Yes to turn safe mode on.
   Select No to turn safe mode off.
                                   No>
```

   Instrument Command = the current instrument command. See page 5-14 for more information on the instrument commands.

3. To use the Select Command Menu. Press the:
   - **Transfer** soft key for the TRANsfer command.
   - **Monitored Transfer** soft key for the MONitor command.
   - **Sleep** soft key for the SLEep command.
   - **Safe Mode** key for the SAFe command.

To edit an existing cycle procedure step: ***(continued)***

4. To use the Select Valves Menu:

   a. See "Valve Group Listing" on page A-2 for a complete list of valve group names and their three-digit code numbers.

   b. Using the numeric keys, enter the desired valve group code number. The corresponding valve group appears on the last line and a Select soft key is added to the menu. In the example below, the valve group code is 101, the SCPI command is TRANsfer, and the valve group name is ReverseFlush($Col).

```
Select Valves                          Cancel>
  Valve Code: 101_

TRANsfer ReverseFlush($Col)            Select>
```

   c. Press the **Select** soft key to confirm your choice. The Set Step Time Menu is displayed, or

      Press the **Cancel** soft key or the **Prev. Menu** command key to exit the Select Valves Menu without selecting (or changing) a valve group. You are returned to the Edit Cycle Step Menu.

5. To use the Set Step Time Menu:

   a. Using the numeric keys, enter the desired step time in seconds. To enter sub-seconds, use real numbers (for example, enter **2.1** for 2.1 sec).

      As you start entering digits, a decimal point (.) soft key and a Set soft key are added to the menu and the generated SCPI command is displayed. In the example below, the step time is 30 seconds and the generated SCPI command is TRANsfer ReverseFlush($Col) 30.

```
Set Step Time
  Step Time: 30_                            .>
                                          Clear>
TRANsfer ReverseFlush($Col) 30              Set>
```

   b. Press the **Set** soft key to confirm your choice. You are returned to the Cycle Procedure Menu, or

      Press the **Cancel** soft key or the **Prev. Menu** command key to exit the Set Times Menu without selecting a time. You are returned to the Edit Cycle Step Menu.

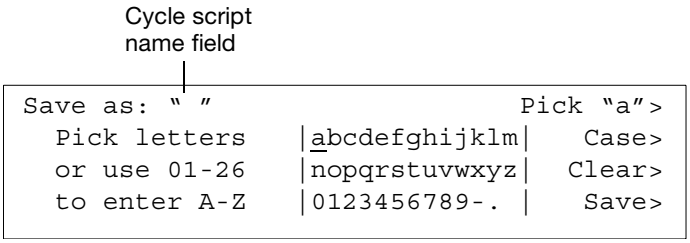
6. To use the "Enable Safe Mode?" Menu:

   - If the subsequent steps in the cycle procedure are "safe steps" (that is, it is safe to pause the synthesis at this point), press the **Yes** soft key.
   - If the subsequent steps in the cycle procedure are not safe steps, press the **No** key.

   You are returned to the Edit Cycle Step Menu.

Saving Your Changes

To save your changes to the custom cycle script:

1.	From the Cycle Menu, press the **Save** soft key. The Save As Menu is displayed. 
2.	Type a name in the cycle script name field: a. Use the left- and right-arrow keys to move the cursor to the desired alphanumeric character on the LCD screen. b. Press the **Pick "n"** soft key. The selected character appears in the cycle script name field. **Note:** The **"n"** designates the currently selected alphanumeric character. For example, in step 1 above, the cursor is on the letter **a** and the soft key reads **Pick "a"**.
3.	To switch the alphabetic characters between upper- and lowercase, press the **Case** soft key.
4.	To delete characters from the cycle script name field: • Press the **Delete** command key to delete characters one at a time. • Press the **Clear** soft key to delete all characters at once.
5.	Repeat steps 2 to 4 as necessary until you have entered the desired cycle script name.
6.	Press the **Save** soft key. The new cycle script is entered into the 3400 DNA Synthesizer software.
7.	Press the **Main Menu** command key to return to Page 1 of the Main Menu.

Deleting a Custom Cycle Script

IMPORTANT! Deleting a custom cycle script removes it permanently from the 3400 DNA Synthesizer software.

Note: The cycle scripts provided with the 3400 DNA Synthesizer software (see page 5-25) cannot be modified or deleted.

To delete a custom cycle script from the 3400 DNA Synthesizer software:

1.	From Page 1 of the Main Menu, press the **Next > Edit Cycle > Delete** soft keys. The Delete Cycle Menu is displayed.
	
	`Delete Cycle  Cycle Name>`
	`  Cycle Name>`
	`  Cycle Name>`
	`Page 1 of n  Next>`
	
	Cycle Name = the name(s) of the existing cycle scripts in the 3400 DNA Synthesizer software
	
	n = the total number of pages in the menu, which varies depending on the number of cycle scripts that are stored in the software.

2.	Press the **Next** soft key to browse through all the existing cycle scripts.

To delete a custom cycle script from the 3400 DNA Synthesizer software:

3. When you find the desired cycle script, press the appropriate ***Cycle Name*** soft key.

If you ...	Then ...

Selected a custom cycle script (that is, one that you created)	The confirmation prompt below is displayed.
	
	`Confirm Deletion Yes>`
	`Are you sure you want to delete the cycle "Cycle Name"? No>`
	
	Press the **Yes** soft key. The cycle script is deleted from the 3400 DNA Synthesizer software and the message below is displayed.
	
	`The cycle "Cycle Name" has been deleted. Press any key...`

Selected one of the cycle scripts provided with the software	The following error message is displayed: `"Cycle Name" is a read-only cycle, which cannot be modified or deleted.`

4. Press any key to return to the Cycle Menu.

5. Press the **Main Menu** command key to return to Page 1 of the Main Menu.

Using a Web Browser

Web Browser Requirements

To use a Web browser to work with cycle scripts, your 3400 DNA Synthesizer must:

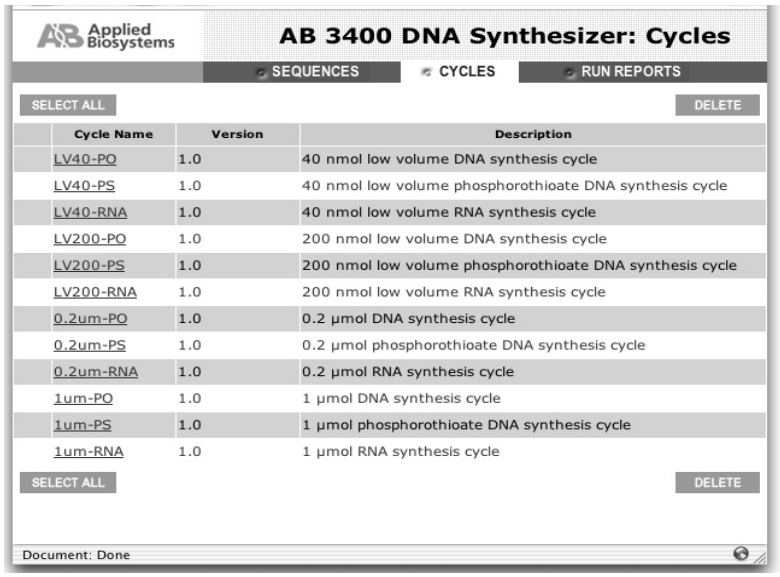
- Be connected to a computer with a Web browser
- Have the TCP/IP connection correctly configured

For more information, see "Connecting a Computer (Optional)" on page 3-14 and "Connecting the Instrument to the Router" on page 3-10.

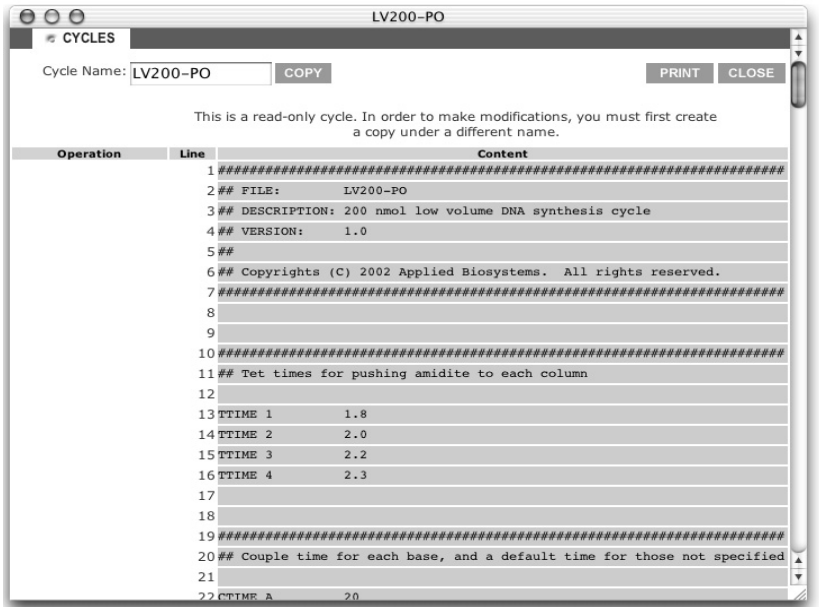
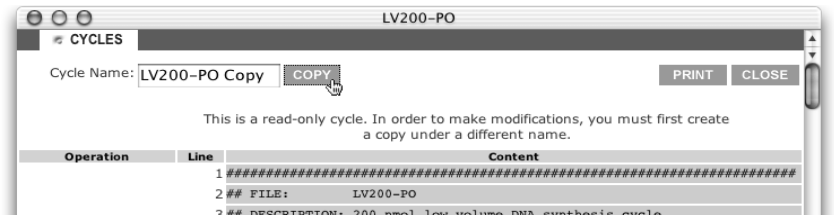
Creating a Custom Cycle Script

Creating a Cycle Script

To create a custom cycle script using a Web browser:

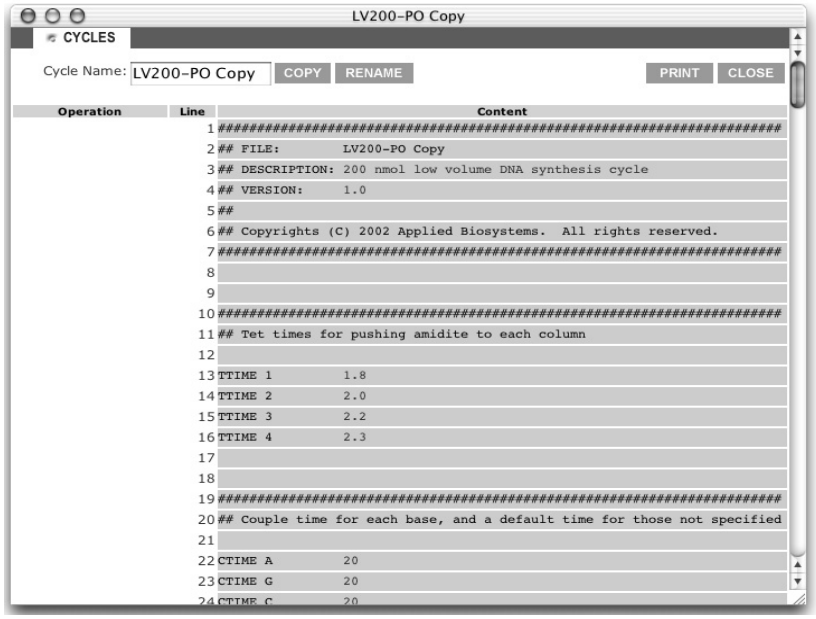
1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12 The Sequences window opens.
3.	Select the **Cycles** tab. The Cycles window opens, displaying a list of the cycle scripts currently stored in your 3400 DNA Synthesizer software. 
4.	Select one of the existing cycle scripts to use as a template for your new custom cycle script.

To create a custom cycle script using a Web browser: *(continued)*

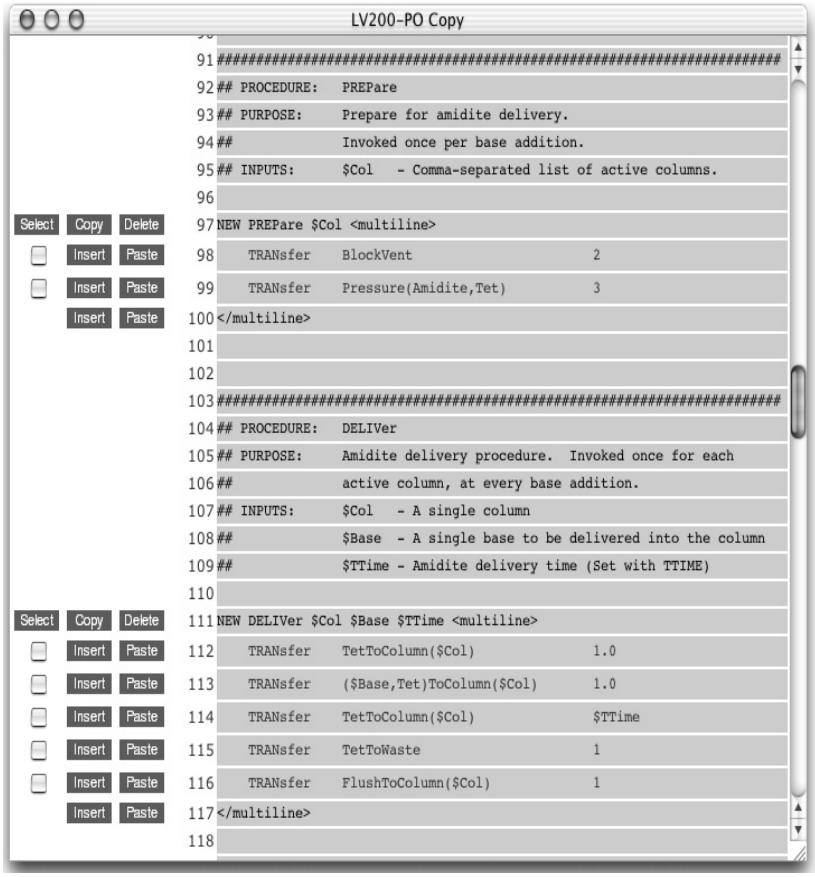
5.	The Cycle Editor window opens, displaying the selected cycle script and its cycle procedures. 
6.	Type a new name in the Cycle Name field. 

To create a custom cycle script using a Web browser: ***(continued)***

7.	Click **Copy** to view the new cycle script.



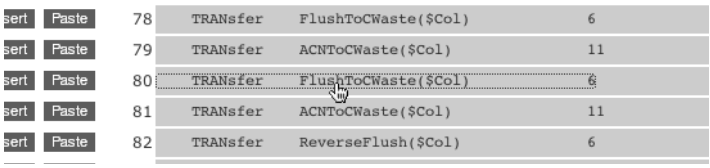
To create a custom cycle script using a Web browser: *(continued)*

8.	Scroll through the window to view the cycle steps. 
9.	To make your changes to the new cycle script, continue with the appropriate procedure below: • Modifying an existing cycle step (see page 5-41) • Copying an existing cycle step (see page 5-43) • Deleting an existing cycle step (see page 5-45) • Inserting a new cycle step (see page 5-46)

Modifying an Existing Cycle Step

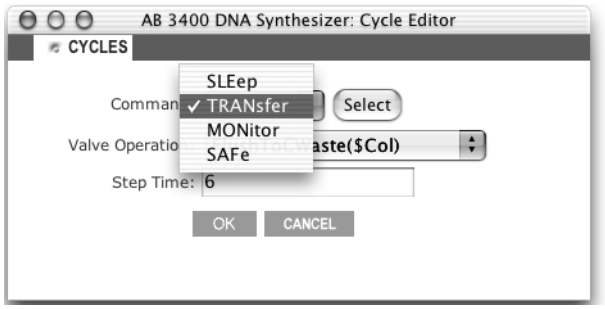
To modify an existing cycle step:

1. Select the cycle step you want to modify.

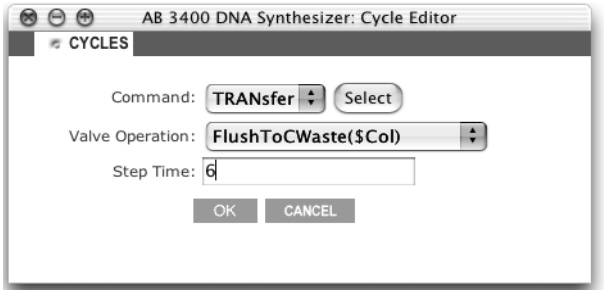
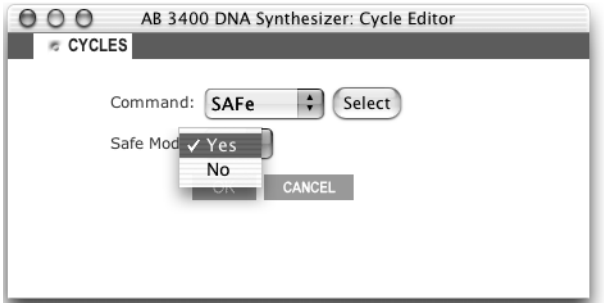
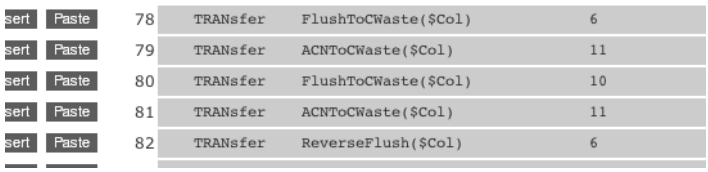


The Cycle Editor is displayed.

2. Select a command from the drop-down menu.



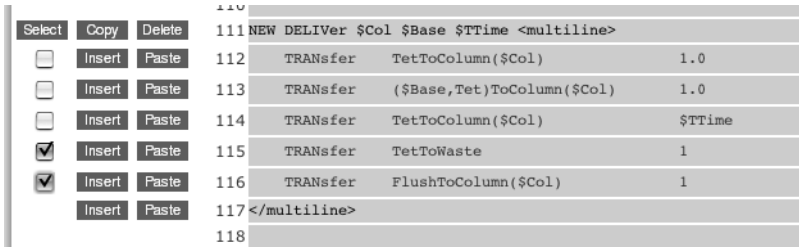
To modify an existing cycle step: ***(continued)***

3.	Select the remaining parameters for the command you selected above. For example, if you selected the: • TRANsfer command, the Cycle Editor displays the Valve Operation and Step Time parameters.  • SAFe command, the Cycle Editor displays the Safe Mode parameter. 
4.	Click **OK**. The Cycle Editor window opens, displaying the modified cycle step. 

Copying an Existing Cycle Step

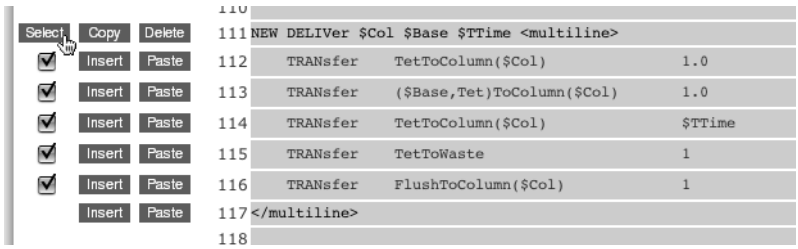
To copy an existing cycle step:

1. Select the cycle step(s) by either of the following methods:
   - Select the checkbox next to the desired cycle step(s).

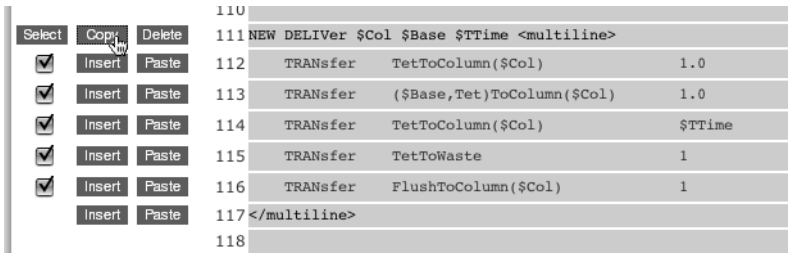


   - Click **Select** to select all cycle steps in the cycle procedure.

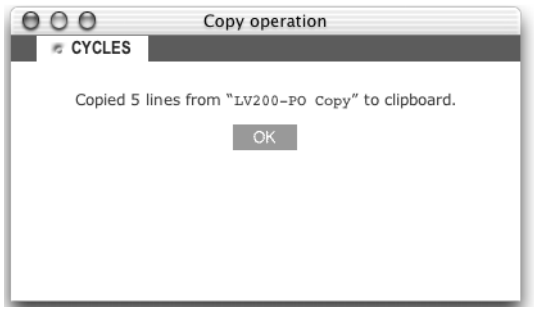
   Note: The Select button is a switch button. Clicking **Select** again deselects all the cycle steps.



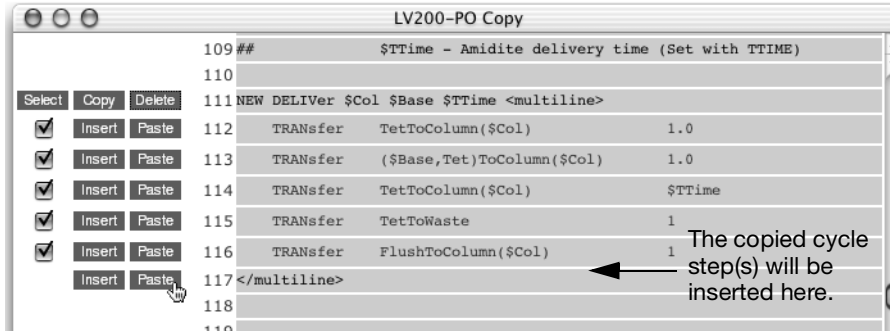
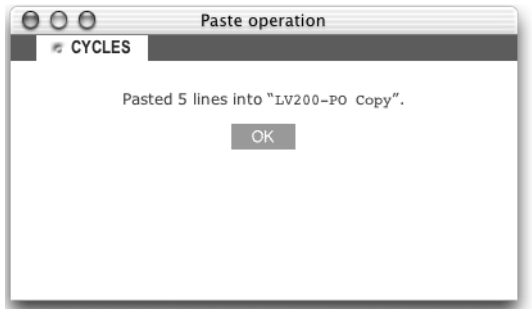
2. Click **Copy**.



   A confirmation prompt is displayed.



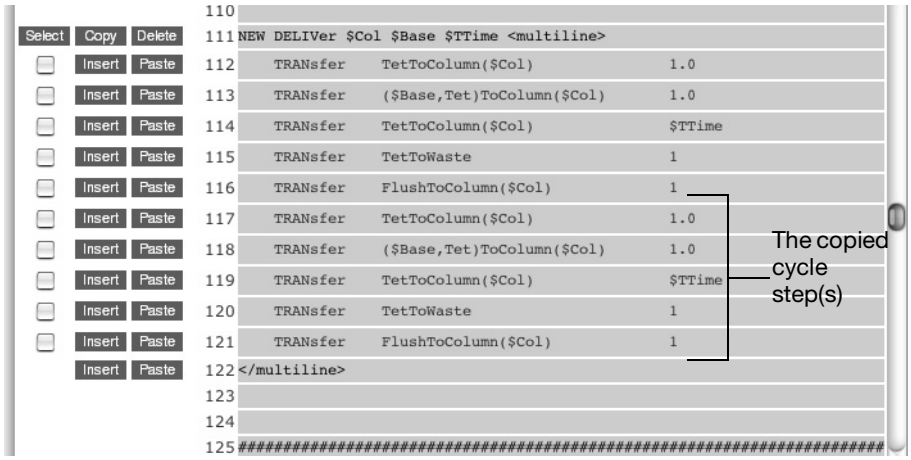
To copy an existing cycle step: *(continued)*

3.	Click **OK**. You are returned to the Cycle Editor window.
4.	Click **Paste** at the position you want the copied cycle step(s) to occur. **Note:** The copied cycle steps are inserted immediately above the line next to the Paste command. In the example below, the Paste command next to </multiline> is selected. The copied cycle steps will appear between TRANsfer FlushToColumn($Col) and </multiline>.  A confirmation prompt is displayed. 

To copy an existing cycle step: *(continued)*

5. Click **OK**.

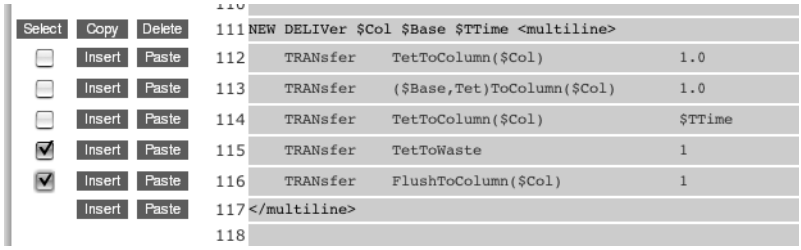
   The Cycle Editor window opens, displaying the pasted cycle step(s).



Deleting an Existing Cycle Step

To delete an existing cycle step:

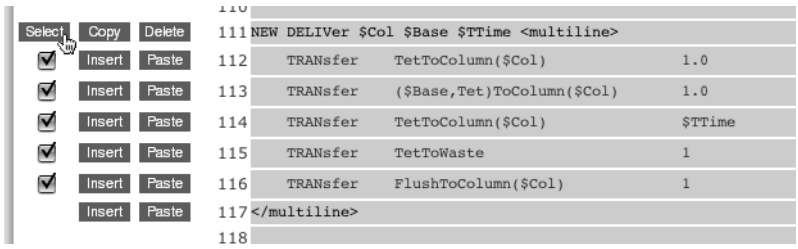
1. Select the cycle step(s) by either of the following methods:
   - Select the checkbox next to the desired cycle step(s).



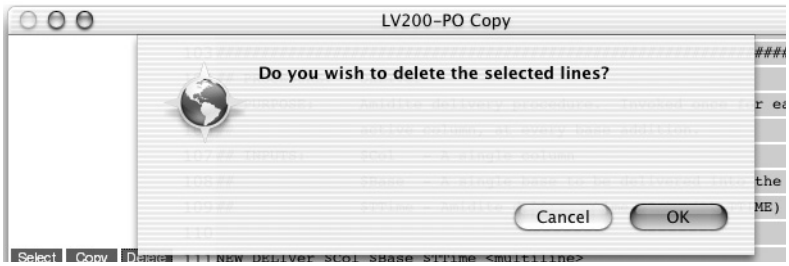
   Or

   - Click **Select** to select all cycle steps in the cycle procedure.

   Note: The Select button is a switch button. Clicking **Select** again deselects all the cycle steps.

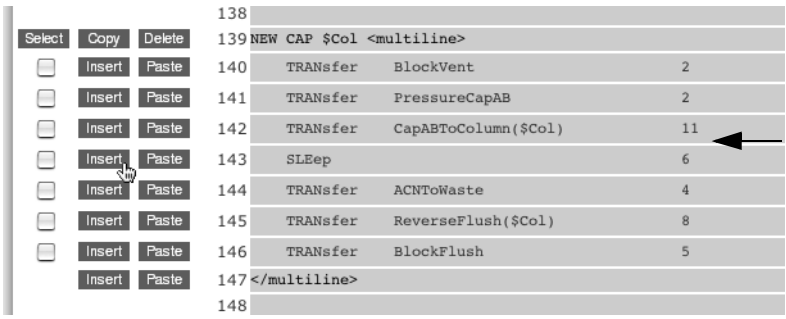
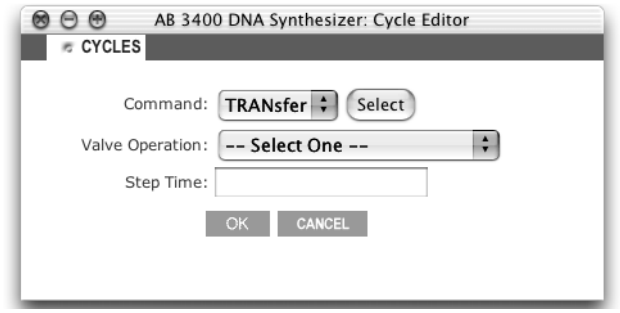


To delete an existing cycle step: *(continued)*

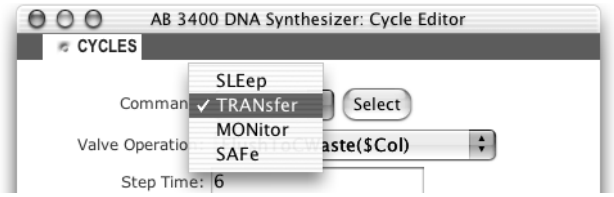
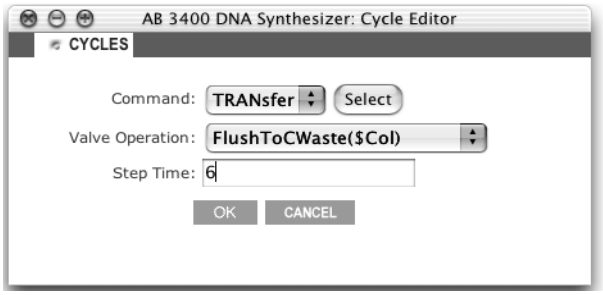
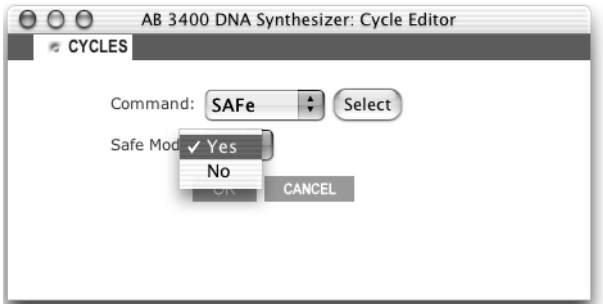
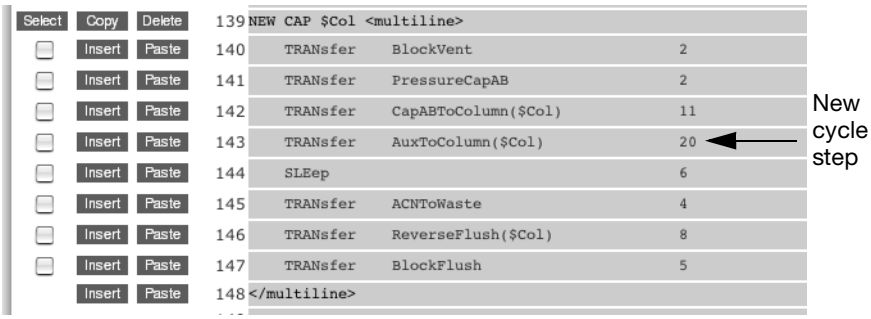
2.	Click **Delete**. A confirmation prompt is displayed. 
3.	Click **OK**. The Cycle Editor window opens, with the selected cycle step(s) removed.

Inserting a New Cycle Step

To insert a new cycle step:

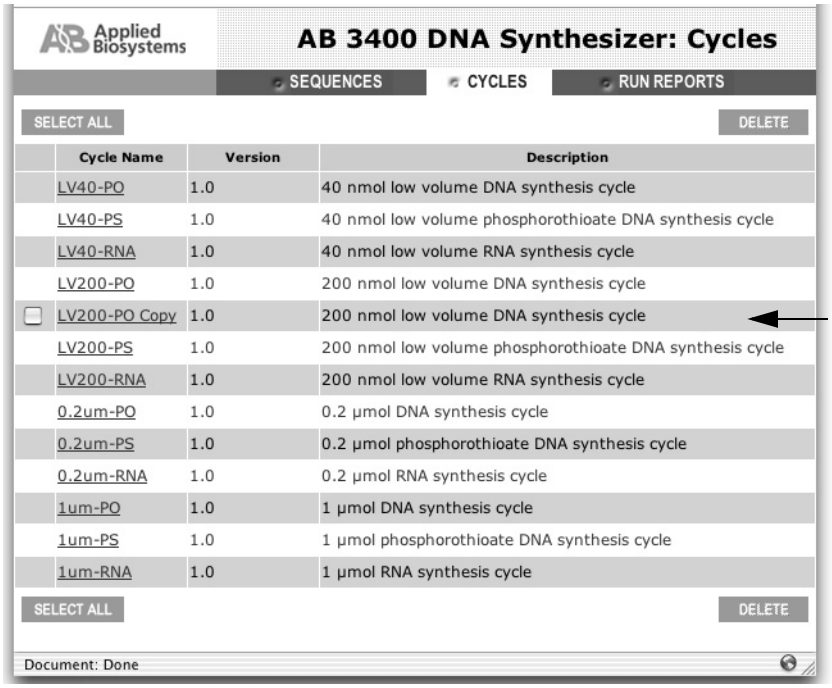
1.	Click **Insert** at the position you want the new cycle step to occur. **Note:** The new cycle step is inserted immediately above the line next to the Insert command. In the example below, the Insert command next to SLEep is selected. The new cycle step will appear between TRANsfer CapABToColumn($Col) and SLEep.  The Cycle Editor is displayed. 

To insert a new cycle step: ***(continued)***

2.	Select a command from the drop-down menu. 
3.	Select the remaining parameters for the command you selected above. For example, if you selected the: • TRANsfer command, the Cycle Editor displays the Valve Operation and Step Time parameters.  • SAFe command, the Cycle Editor displays the Safe Mode parameter. 
4.	Click **OK**. The Cycle Editor window opens, displaying the new cycle step. 

When You Finish

When you finish creating your custom cycle script, click **Close** to return to the Cycles window. The new cycle script is displayed.



Deleting a Custom Cycle Script

IMPORTANT! Deleting a custom cycle script removes it permanently from the 3400 DNA Synthesizer software.

Note: The cycle scripts provided with the 3400 DNA Synthesizer software (see page 5-25) cannot be modified or deleted.

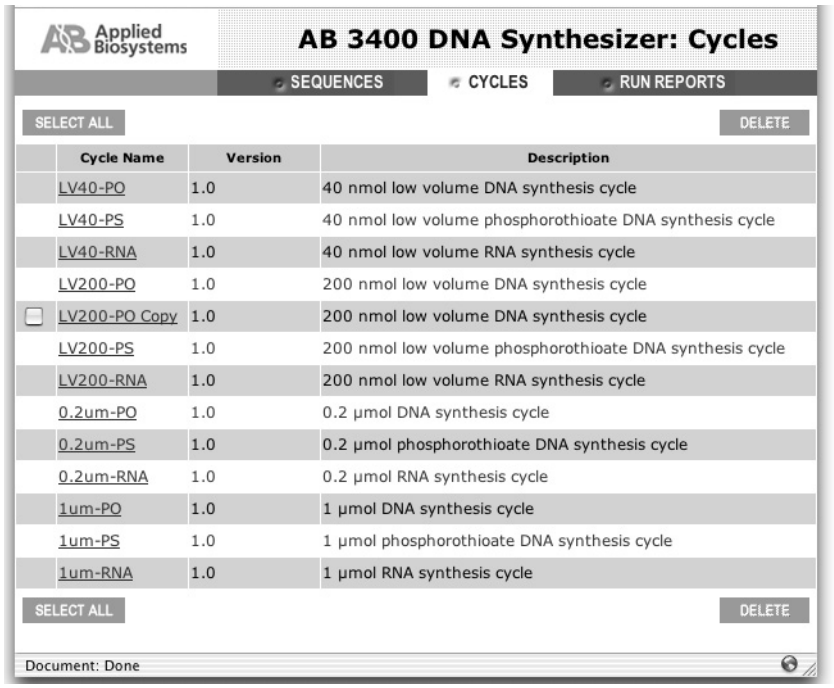
To delete a custom cycle script using a Web browser:

1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** Where *instrument name* = the host name you set on page 3-12 The Sequences window is displayed.

To delete a custom cycle script using a Web browser: ***(continued)***

3. Select the **Cycles** tab.

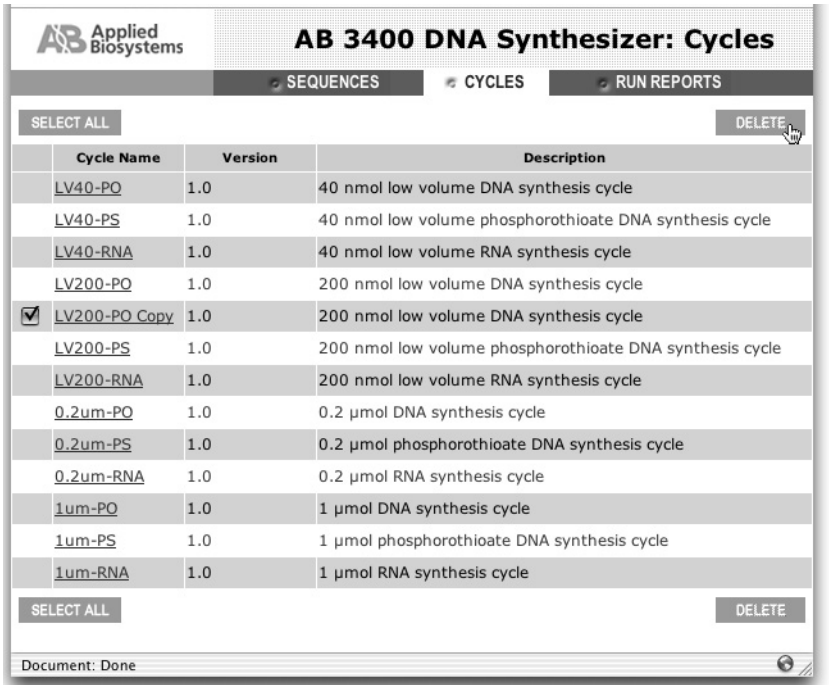
   The Cycles window opens, displaying a list of the cycle scripts currently stored in your 3400 DNA Synthesizer software.



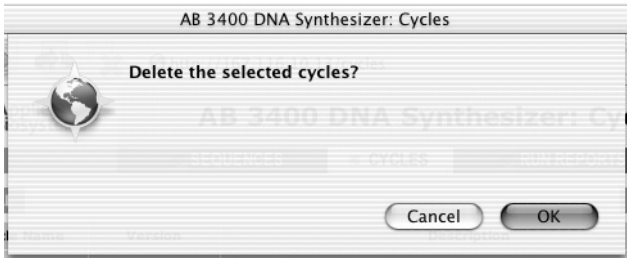
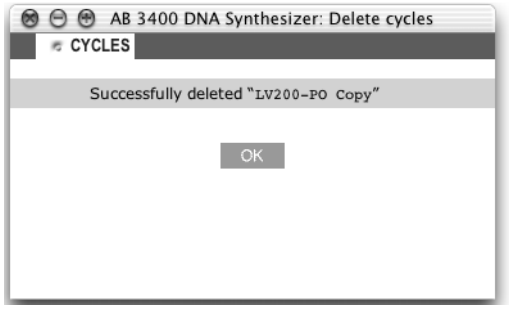
To delete a custom cycle script using a Web browser: ***(continued)***

4. Select the checkbox next to the custom cycle script you want to delete.

   Note: No checkboxes appear next to the cycle scripts provided with the 3400 DNA Synthesizer software (see page 5-25) because these cycle scripts cannot be modified or deleted.



To delete a custom cycle script using a Web browser: ***(continued)***

5.	Click **Delete**. A confirmation prompt is displayed. 
6.	Click **OK**. The Delete Cycles dialog box opens. 
7.	Click **OK**. You are returned to the Cycles window. The selected cycle script is deleted from this window and from your 3400 DNA Synthesizer software.

Performing a Synthesis Run 6

This chapter covers:

Setting Up and Starting a Run

Use the Run Setup Menu to set up and begin a synthesis run. From this menu, you select the following items for your run:

- Run Title
- Sequences to be synthesized in each column
- Cycle script
- Trityl options
- DMT options
- Cleave options

You can also invoke the Run Setup Menu during a synthesis run to:

- View which columns are active
- View which cycle script is selected
- View or change trityl options
- View or change DMT options
- View or change Cleave options

Setting a Run Title (Optional)

You can assign a run title to the synthesis run so you can more easily identify and match it against the subsequent run report. If you do not set a run title, the run report uses the run date and time as a default run title.

To set a run title:

1.	From Page 1 of the Main Menu, press the **Run Setup** soft key. The Run Setup Menu is displayed. Run Setup — Set Run Title> No active columns — Select Sequences> No cycle selected — Select Cycle> (Page 1 of 3) — Next>
2.	Press the **Set Run Title** soft key. The Run Title Menu is displayed. Run Title: “” — Pick “a”> Pick letters |abcdefghijklm| — Case> or use 01-26 |nopqrstuvwxyz| to enter A-Z |0123456789-. | — Set>
3.	Enter the desired run title, as described in “Entering Text” on page 3-5
4.	Press the **Set** soft key to accept the new title and return to the Run Setup menu.

Selecting the Sequences

To select the sequences to be synthesized in each column:

1.	From Page 1 of the Run Setup menu, press the **Select Sequences** soft key. The Select Column Menu is displayed. Select Column — Column 1> / Column 2> / Column 3> / Column 4>
2.	Press the appropriate soft key to select the desired column. The Column *n* Menu is displayed. Column *n* — *Sequence Name*> / Use <Delete> to clear selection — *Sequence Name*> / *Sequence Name*> / Page 1 of *n* — Next> Column *n* = the number of the current column selected (1 to 4) *Sequence Name* = the name(s) of the existing sequences in the 3400 DNA Synthesizer software *n* = the total number of pages in the menu, which varies depending on the number of sequences that are stored in the software.
3.	Press the **Next** soft key to browse through all the existing sequences. **Note:** If you want to add a new sequence to the 3400 DNA Synthesizer software, see Chapter 5, "Run Preparation: Setting Up the Instrument Software."
4.	When you find the desired sequence, press the appropriate ***Sequence Name*** soft key. You are returned to the Select Column Menu; the sequence is listed next to the selected column. **Note:** To make the column inactive, remove any current selection by pressing the **Delete** command key. You are returned to Page 1 of the Select Column Menu, and no sequence is listed next to the selected column.
5.	Repeat steps 1 to 4 for each column you want to include in the synthesis run. **Note:** You can include any combination of Columns 1, 2, 3, and 4

To select the sequences to be synthesized in each column: *(continued)*

6.	When you finish selecting the sequences to be synthesized in each column, press the **Prev. Menu** command key to return to Page 1 of the Run Setup Menu. The Run Setup Menu displays the selected column(s) as active. Run Setup — Set Run Title> Columns: 1, 2, 4 — Select Sequences> No cycle selected — Select Cycle> (Page 1 of 3) — Next>

Selecting a Cycle Script

Note: When more than one column is selected for the synthesis run, the same cycle script is used on all columns.

To select a cycle script:

1.	From Page 1 of the Run Setup Menu, press the **Select Cycle** soft key. The Select Cycle Menu is displayed. Select Cycle — *Cycle Name*> Use <Delete> to — *Cycle Name*> clear selection — *Cycle Name*> (Page 1 of *n*) — Next> *Cycle Name* = the names of the existing cycle scripts in the 3400 DNA Synthesizer software *n* = the total number of pages in the menu, which varies depending on the number of cycle scripts that are stored in the software.
2.	Press the **Next** soft key to browse through all the existing cycle scripts. **Note:** The 3400 DNA Synthesizer software has 12 cycle scripts (see "Cycle Scripts Provided" on page 2-16). To add a new cycle script to the software, see Chapter 5, "Run Preparation: Setting Up the Instrument Software."
3.	When you find the desired cycle script, press the appropriate ***Cycle Name*** soft key. You are returned to Page 1 of the Run Setup Menu, which displays the selected cycle script. Run Setup — Set Run Title> Columns: 1, 2, 4 — Select Sequences> Cycle: 1 um-PO — Select Cycle> (Page 1 of 3) — Next> **Note:** To clear the selection, press the **Delete** command key. You are returned to Page 1 of the Run Setup Menu, and no cycle script is listed. You cannot start a run until a cycle script is selected.

Selecting Trityl Options

To select trityl options:

1. From Page 1 of the Run Setup Menu, press the **Next** soft key. Page 2 of the Run Setup Menu is displayed.

```
Run Setup                          Trityl Options>
Columns: 1, 2, 4                   DMT Options>
Cycle: 1 um-PO                     Cleave Options
(Page 2 of 3)                      Next>
```

2. Press the **Trityl Options** soft key. The prompt below the Trityl Options Menu is displayed.

```
Trityl Options [5%]                Delivery Threshold>
                   [80%]           Yield Threshold>
```

3. To modify the delivery threshold:

   a. Press the **Delivery Threshold** soft key. The following prompt is displayed:

```
TCA Delivery Threshold
  Stop delivery once trityl
  readings approach baseline       Clear>
  within [5]% of peak height       Set>
```

   b. Using the numeric keys, type an appropriate percentage.

      Note: If the delivery threshold is set to zero, delivery monitoring is not in effect and reagent delivery continues in all columns until the designated step time is elapsed.

   c. Press the **Set** soft key. You are returned to the Trityl Options Menu, and the new threshold is shown next to the Delivery Threshold soft key.

Note: If the delivery threshold is modified during a synthesis run, the new setting takes effect immediately.

To select trityl options: ***(continued)***

4.	To modify the yield threshold: a. Press the **Yield Threshold** soft key. The following prompt is displayed: Yield Threshold Terminate a column if its average stepwise yield falls Clear> below [80%] Set> b. Using the numeric keys, type an appropriate percentage. **Note:** If the yield threshold is set to zero, yield monitoring is not in effect. c. Press the **Set** soft key. You are returned to the Trityl Options Menu, and the new threshold is shown next to the Yield Threshold soft key. **Note:** If the yield threshold is modified during a synthesis run, the new setting takes effect immediately.
5.	When you finish selecting the trityl options, press the **Prev. Menu** command key to return to Page 1 of the Run Setup Menu.

Selecting DMT Options

To select DMT options:

1.	From Page 2 of the Run Setup Menu, press the **DMT Options** soft key. The DMT Removal Options Menu is displayed. DMT Removal Options [Yes] Column 1> [Yes] Column 2> Select whether to run [Yes] Column 3> final detritylation [Yes] Column 4>
2.	For each column, switch the desired dimethoxytrityl (DMT) state by pressing the corresponding soft key: a. **Yes**, if you want the DNA automatically detritylated at the end of the run. b. **No**, if you do not want the DNA automatically detritylated at the end of the run. **Note:** If the DMT options are modified during a synthesis run, the new setting takes effect immediately. **Note:** If DMT Removal is not selected (“No”), the DNA can be manually detritylated by a 15-min treatment with 80% acetic acid at room temperature after synthesis and base deprotection.
3.	Press the **Prev. Menu** key to return to Page 2 of the Run Setup Menu.

Selecting Cleave Options

To select cleave options:

1.	From Page 2 of the Run Setup Menu, press the **Cleave Options** soft key. The Cleave Options Menu is displayed. Cleave Options [Yes] Column 1> [Yes] Column 2> Select whether to run [Yes] Column 3> final cleavage [Yes] Column 4>
2.	For each column, switch the desired cleave state by pressing the corresponding soft key: a. **Yes**, if you want cleavage to automatically occur at the end of the run. b. **No**, if you do not want cleavage to automatically occur at the end of the run. **Note:** If the Cleave options are modified during a synthesis run, the new setting takes effect immediately.
3.	Press the **Prev. Menu** key to return to Page 2 of the Run Setup Menu.

Starting the Run

To start the synthesis run:

1.	From the Run Setup Menu, press the **Next** soft key twice. Page 3 of the Run Setup Menu is displayed. Run Setup Start Run> Columns: 1, 2, 4 Cycle: 1 um-PO (Page 3 of 3) Next>
2.	Press the **Start Run** soft key. The **Prepare Columns** menu is displayed. Prepare Columns Start> In column location: 1 2 4 Please place column: A G T
3.	Insert the appropriate columns in the instrument, then press the **Start** soft key. The synthesis run begins and the Run Status Menu is displayed.
4.	Continue with “Monitoring the Run” on page 6-8.

Monitoring the Run

The Run Status Menu displays the status of the instrument during a synthesis run. Use this menu to:

- Determine which cycle procedure is being performed
- Determine which cycle step is being performed
- Access the Trityl Status Menu to view real-time trityl monitor data
- Pause or Abort the run
- Print or Delete run reports for completed runs

Viewing the Run Status

To view the run status:

1. As soon as you press the **Start Run** soft key in the Run Setup Menu (see page 6-7), the Run Status Menu is displayed. If you have exited the Run Status Menu and need to access it again:

   From Page 1 of the **Main Menu**, press the **Run Status** soft key. The Run Status Menu is displayed.

Run Status	Trityl Status>
Real-time	Pause>
run	Abort>
information	Next>

2. Real-time run information is displayed on the lower-left side of the screen. Use this information to determine:
   - The number of bases that have been synthesized
   - The total number of bases to be synthesized
   - Which cycle procedure is being performed
   - Which cycle step is being performed
   - How much time remains in the current step

   The following screen shows sample real-time run information:

Run Status	Trityl Status>
Base 13/25	Pause>
Detritylating	Abort>
TCAToCWaste(1,2,4) 34s	Next>

Viewing the Trityl Status

To view the trityl status:

1.	From the Run Status Menu, press the **Trityl Status** soft key. The Trityl Status Menu is displayed. • If a monitored delivery is not in progress (that is, while the instrument is not delivering TCA to the columns), the Trityl Status Menu looks similar to the following: Not Monitoring PeakArea 102.67 N/A N/A 133.29 Yield 97% 95% 73% 96% Status Active Done Stopped Active • If a monitored delivery is in progress (that is, while the instrument is delivering TCA during the detritylation procedure), the Trityl Status Menu looks like the following: Monitoring Peak 2 25s Baseline 4.21 4.18 4.26 4.32 Peak 12.42 10.88 11.54 13.03 Sensors 6.47 8.91 9.85 7.47
2.	To return to the Run Status Menu, press the **Prev. Menu** button.

Pausing or Aborting a Run

The Run Status Menu displays the status of the instrument during a synthesis run. If necessary, you can use this menu to:

- Pause the run
- Abort the run

Safe Points to Stop a Run

Both the Pause and Abort functions should be used only at points in the cycle procedure that you consider safe.

Although it is safe to pause or abort at the end of each cycle procedure (see page 2-16 for a list of cycle procedures), the best place to stop a run is at the end of the entire cycle script.

IMPORTANT! Failure to interrupt at a safe point could result in clogged valve blocks or lines and a failed synthesis.

Pausing a Run

To pause a synthesis run:

1.	From Page 1 of the Main Menu, press the **Run Status** soft key. The Run Status Menu is displayed. Run Status — Trityl Status> *Real-time* — Pause> *run* — Abort> *information* — Next>
2.	From Page 1 of the Run Status Menu, press the **Pause** soft key. The Pause Menu is displayed. Pause Menu — Hold> *Real-time* — Pause> *run* — Pause Ahead> *information* **Note:** The Pause soft key is available only on the Run Status Menu when there is an active synthesis run.

To pause a synthesis run: ***(continued)***

3. To hold any currently open valves open, press the **Hold** soft key. You are returned to Page 1 of the Run Status Menu, which appears as follows:

Run Status	Trityl Status>
Real-time	[Hold] Cancel>
run	Abort>
information	Next>

After the preset step time has elapsed, "Holding" is displayed instead of remaining step time in the real-time information area:

Run Status	Trityl Status>
Base 13/25	[Hold] Cancel>
Detritylating	Abort>
TCAToCWaste(1,2,4) Holding	Next>

When you are ready to resume the synthesis run, press the **Cancel** soft key.

4. To pause the run after the current cycle procedure, press the **Pause** soft key. You are returned to the Run Status Menu, which appears as follows:

Run Status	Trityl Status>
Real-time	[Pause] Cancel>
run	Abort>
information	Next>

To cancel the pause, press the **Cancel** soft key. If you do not cancel the pause before the current step is completed, the Cancel soft key is replaced by Resume. The menu is displayed as follows:

Run Status	Trityl Status>
	Resume>
Cycle Procedure	Abort>
Paused	Next>

Note: The synthesis does not pause while Safe Mode is off. Instead, the pause operation is deferred until Safe Mode is turned on again. Safe Mode is controlled by the cycle script (see "Cycle Command Conventions" on page 2-16).

To pause a synthesis run: *(continued)*

5. To pause the synthesis run before a particular base is synthesized:

   a. Press the **Pause Ahead** soft key. The Pause Ahead Menu is displayed.

```
Pause Ahead
Pause at base # _
 Enter '0' to pause before      Clear>
 DMT removal and/or cleaving.   Set>
```

   b. Use the numeric keys to type a base in the Pause at base # field.

   c. Press the **Set** soft key to return to the Run Status Menu, which is displayed as follows:

```
Run Status          Trityl Status>
Real-time                    [Pause at base n] Cancel>
run           Abort>
information             Next>
```

   n = the base you entered in the Pause Ahead menu.

   To cancel the pause, press the **Cancel** soft key. If you do not cancel the pause before the selected base is reached, the Cancel soft key is replaced by Resume. The menu is displayed as follows:

```
Run Status          Trityl Status>
                    Resume>
Cycle Procedure           Abort>
Paused                Next>
```

6. When you are ready to resume the synthesis run after setting any type of Pause, press the **Resume** soft key. The run resumes from the point at which you paused it.

Aborting a Run

To abort a synthesis run:

1. From Page 1 of the Main Menu, press the **Run Status** soft key. The Run Status Menu is displayed.

```
Run Status                          Trityl Status>
Real-time                                 Pause>
run                                       Abort>
information                               Next>
```

To abort a synthesis run: ***(continued)***

2.	From the Run Status Menu, press the **Abort** soft key. The Abort Menu is displayed. Abort Menu — Abort immediately> *Real-time* — Abort and clean up> *run* — Abort Ahead> *information* **Note:** The Abort soft key is available only in the Run Status Menu when there is an active synthesis run.
3.	To abort the run immediately, press the **Abort immediately** soft key. The run is aborted and you are returned to the Run Status Menu. **Note:** The Abort immediately soft key is not available while Safe Mode is off. Safe Mode is controlled by the cycle script; see "Cycle Procedure Instrument Command Conventions" on page 2-17. **IMPORTANT!** Be sure you want to immediately abort the run before pressing this soft key. You will not be given a confirmation prompt.
4.	To abort the run immediately and begin cleanup, press the **Abort and clean up** soft key. The run is aborted, the instrument begins automatic cleanup, and you are returned to the Run Status Menu. The status of the cleanup is displayed on the Run Status Menu. **IMPORTANT!** Be sure you want to immediately abort the run before pressing this soft key. You will not be given a confirmation prompt.

To abort a synthesis run: ***(continued)***

5. To abort the synthesis run before a particular base is synthesized:

   a. Press the **Abort Ahead** soft key. The Abort Ahead Menu is displayed.

```
Abort Ahead
  Abort synthesis at base # _
                        Clear>
                          Set>
```

   b. Using the numeric keys, type a base in the Abort synthesis at base # field.

   c. Press the **Set** soft key. You are returned to the Run Status Menu, which appears as follows:

```
Run Status          Trityl Status>
Real-time                        [Abort at base n] Cancel>
run           Abort>
information             Next>
```

   n = the base you entered in the Abort Ahead Menu.

   Note: If desired, you can press the **Cancel** soft key to cancel the abort operation. If you do not cancel before the selected base is reached, the synthesis ends.

   Note: After the selected base is reached, final detritylation and cleavage takes place on those columns for which the DMT Removal and Cleave options have been set. However, you can change these options prior to the actual abort operation. See "Selecting DMT Options" and "Selecting Cleave Options" on page 6-7.

6. After stopping a run with any type of Abort, you should:

   a. Clean the instrument.

   b. Start the run over.

Run Reporting

After synthesis is complete, the instrument generates a run report. The run report is saved on the instrument until it is deleted. At any time, you can view, print, or delete it using an external computer and a Web browser.

Run Report Contents

The run report includes:

- The start time, end time, and elapsed time of the run
- The instrument name (if set)
- The instrument software version
- The name of the cycle script that was used
- The name, content, length, molecular weight, and melting point temperature of the sequence that was synthesized in each column
- Trityl data, including:
  - The delivery threshold
  - The yield threshold
  - The average step-wise yield for each column
  - Peak height and integrated peak area for each monitored delivery

Run Report Name

If a Run Title was set (see "Setting a Run Title (Optional)" on page 6-2), then the name of the run report is the same. Otherwise, the name of the report is based on the time that the run completed. In either case, the name of the run report is shown in a message that is displayed on the front panel LCD screen after the run has completed or is terminated:

```
The synthesis run has completed.
  The total run time was Xh, Xm, Xs.
  A run report has been generated and
  saved as "reportname".
```

Web Browser Requirements

To use a Web browser for run reporting, your 3400 DNA Synthesizer must:

- Be connected to a computer with a Web browser
- Have the TCP/IP connection correctly configured

For more information, see "Connecting a Computer (Optional)" on page 3-14 and "Connecting the Instrument to the Router" on page 3-10.

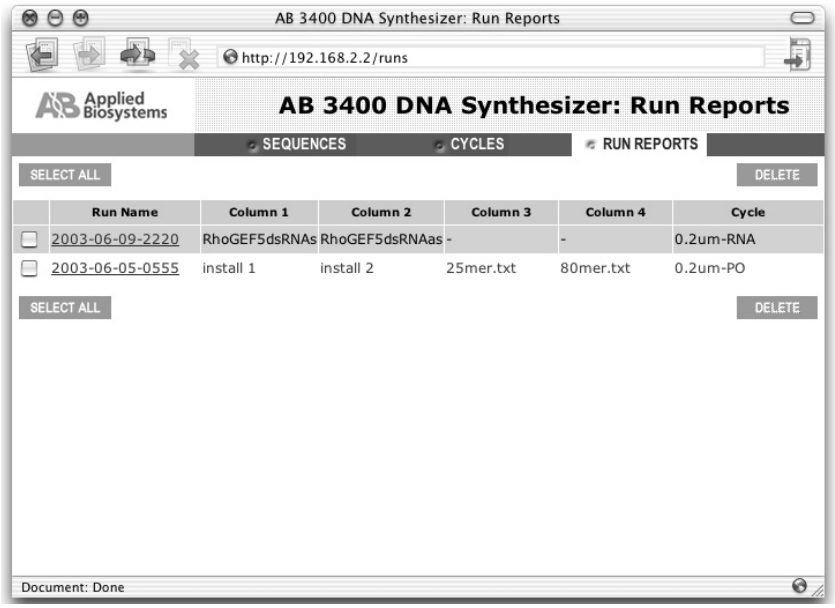
Viewing/Printing a Run Report

To view or print a run report using a Web browser:

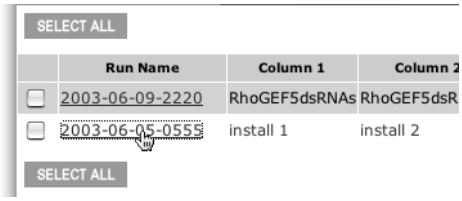
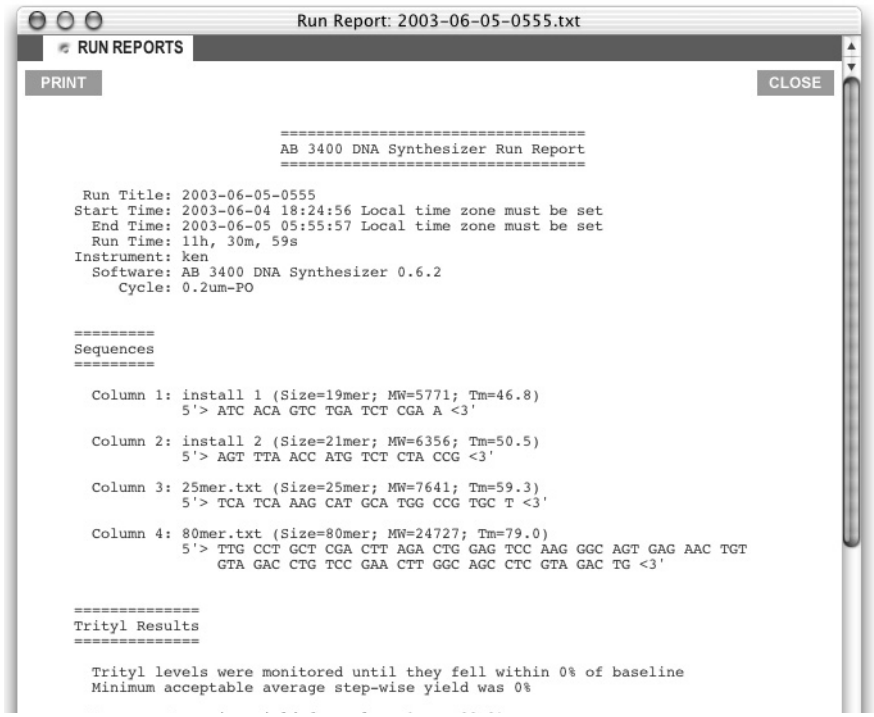
1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12 The Sequences window opens.

To view or print a run report using a Web browser: ***(continued)***

3.	Select the **Run Reports** tab. The Run Reports window opens, displaying a list of the runs currently stored in your 3400 DNA Synthesizer software.



To view or print a run report using a Web browser: *(continued)*

4.	Select the report you want to view.  The selected run report is displayed. 
5.	Click **Print** to print the run report.
6.	Click **Close** to close the run report and return to the Run Reports window.

Deleting a Run Report

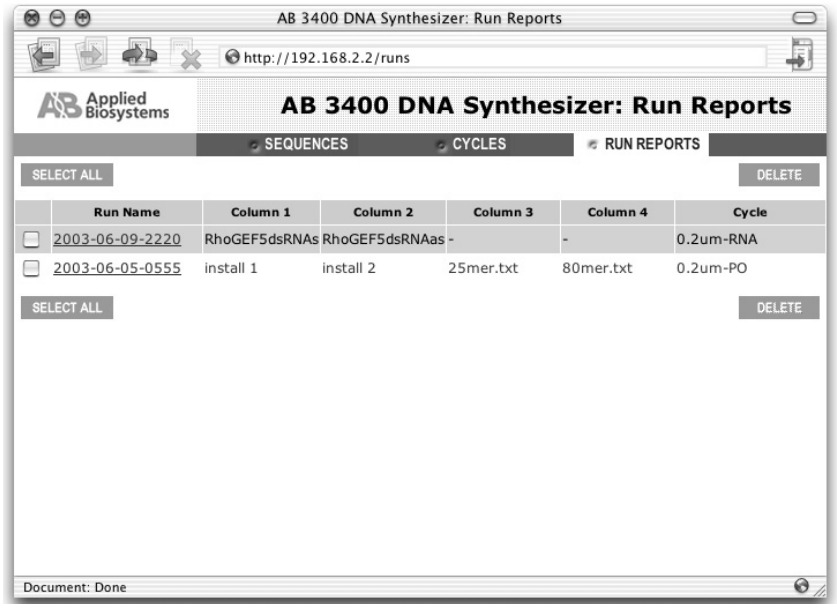
To delete a run report using a Web browser:

1.	Open a Web browser.
2.	In the Web browser, type or select the address for your 3400 DNA Synthesizer: **http://*instrument name*/** *instrument name* = the host name you set on page 3-12 The Sequences window opens.

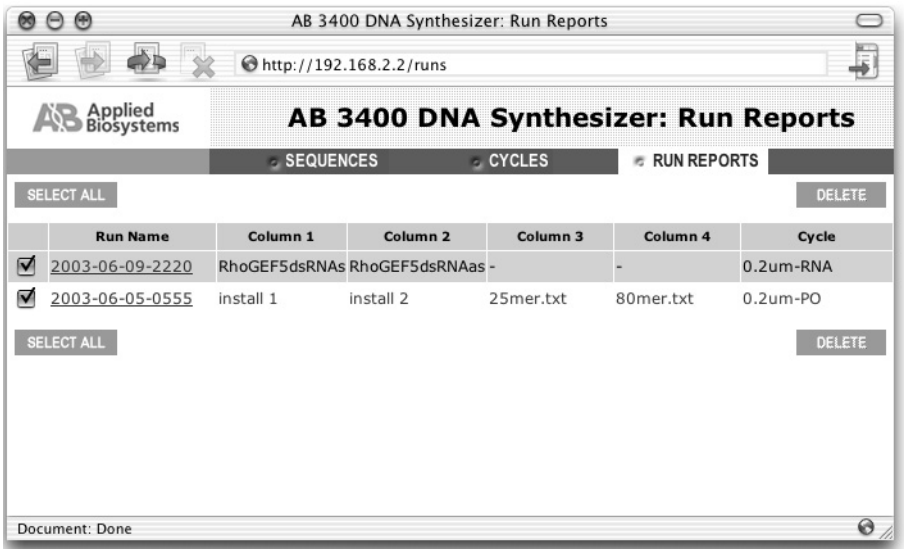
To delete a run report using a Web browser: ***(continued)***

3. Select the **Run Reports** tab.

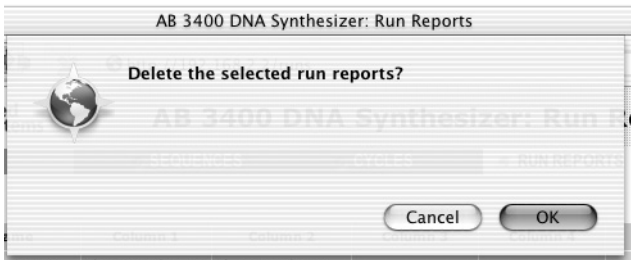
   The Run Reports window opens, displaying a list of the runs currently stored in your 3400 DNA Synthesizer software.



4. Select the checkbox next to the run report(s) you want to delete.



To delete a run report using a Web browser: ***(continued)***

5.	Click **Delete**. A confirmation prompt is displayed. 
6.	Click **OK**. You are returned to the Run Reports window. The selected run report(s) is deleted from this window and from your 3400 DNA Synthesizer software.

Using the Change Bottle Procedure

Changing a Reagent Bottle

To change a reagent bottle using the Change Bottle procedure:

1.	From Page 1 of the Main Menu, press the **Next > Next > Change Bottle** soft keys. Page 1 of the Change Bottle Menu is displayed: Bottle Change: A Start> Please keep the old bottle in the instrument until you Prev> are prompted to remove it. Next>
2.	Select the bottle you want to change by pressing the **Prev** and **Next** soft keys repeatedly. The following bottles are supported: • Amidite Bottles A, G, C, T, 5, 6, 7, 8 • Tet • Ammonia • CapA • CapB • Iodine • Aux • TCA • ACN • DCM

To change a reagent bottle using the Change Bottle procedure:

3.	Press the **Start** soft key to begin the Bottle Change procedure. The following screen is displayed. Bottle Change: *B* Stop> *Valve Operation* *NN*s Hold> *B* = The selected bottle *Valve Operation* = Currently open valve groups *NN* = Remaining step time
4.	The procedure pauses and prompts you to remove the old bottle, wipe the line clean with a lint free tissue, and insert the new bottle. Bottle Change: *B* Stop> Remove the old bottle, Continue> wipe line with a lint free tissue, and insert new bottle. *B* = The selected bottle
5.	Press the **Continue** soft key.
6.	Once the procedure has completed, the following screen is displayed: Bottle Change: B Start> Prev> Procedure completed. Next>
7.	Dispose of the contents of the old bottles per your laboratory practices.

Using the Manual Control Menu

The Manual Control Menu allows you to manually open and close individual valves and valve groups while monitoring the pressure and conductivity sensors installed in the system.

When to Use

The primary uses of the Manual Control Menu are to perform:

- Valve operations (such as flushing reagent delivery lines) that are not covered by automated procedures
- Instrument and plumbing diagnostics

Using the Manual Control Menu

To use Manual Control:

1. From Page 1 of the Main Menu, press the **Next > Next > Next > Manual Control** soft keys. The Manual Control Menu is displayed.

```
Manual Control    0.00 <------ Pressure>
Valves: _
Open Valves: [None]
                              Close All>
```

Note: The Manual Control Menu is not accessible while the instrument is performing a synthesis run.

2. Start entering a list of valve numbers and/or valve codes on which you want to operate. Refer to Appendix E for a plumbing diagram showing valve numbers or Appendix A for a list of valve codes and operations.

Note: As you start entering digits, the Add and Remove soft keys become available.

```
Manual Control    0.00 <------ Pressure>
Valves: 23_                        Add>
Open Valves: [None]              Remove>
                              Close All>
```

Alternatively, you can use the Prev. and Next command keys to scroll through available valve operations.

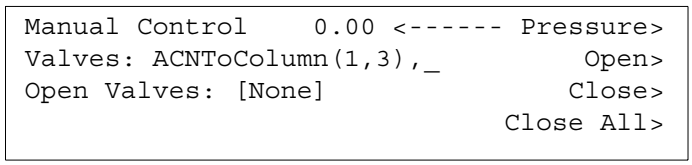
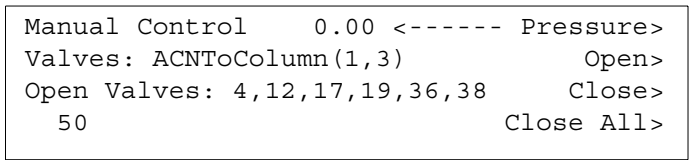
3. If you select a valve operation that requires one or more variable arguments, the cursor is positioned at the location where these arguments should be provided. For instance, if you enter valve code “237” or scroll to the corresponding valve operation, “ACNToColumn($Col),” you need to enter one or more column(s):

```
Manual Control    0.00 <------ Pressure>
Valves: ACNToColumn()              Add>
Open Valves: [None]              Remove>
                              Close All>
```

For instance, if you want to transfer acetonitrile to columns 1 and 3, you would press the **“1”** and **“3”** numeric keys.

```
Manual Control    0.00 <------ Pressure>
Valves: ACNToColumn(1,3)           Add>
Open Valves: [None]              Remove>
                              Close All>
```

To use Manual Control: ***(continued)***

4.	To add this valve operation to the list of valves to open, press the **Add** soft key. To remove this operation from the list (in this case, to clear the list), press the **Remove** soft key. You can also use the **Delete** command key to remove the last entry from the list.
5.	The Add and Remove soft keys are now replaced by Open and Close soft keys.  At this point, you can do either of the following: • Use these soft keys to open and close the selected valves • Enter additional valves/valve codes into the list. The soft keys change back to Add and Remove.
6.	When you complete the list of valves on which you want to operate, press the **Open** soft key. The display changes to indicate that the corresponding valves are open. 

To use Manual Control: ***(continued)***

7.	You can open additional valves by entering the corresponding valve numbers and/or valve codes, each followed by Add.
	
	`Manual Control  0.00 <------ Pressure>`
	`Valves: 8,18_  Add>`
	`Open Valves: 4,12,17,19,36,38  Remove>`
	`50  Close All>`
	
	`Manual Control  0.00 <------ Pressure>`
	`Valves: 8,18,_  Open>`
	`Open Valves: 4,12,17,19,36,38  Close>`
	`50  Close All>`
	
	To open the additional valves, again press the **Open** soft key.
	
	`Manual Control  0.00 <------ Pressure>`
	`Valves: 8,18  Open>`
	`Open Valves: 4,8,12,17,18,19  Close>`
	`36,38,50  Close All>`

8.	At this point, the Open or Close soft keys operate only on the most recently entered list of valves. In other words, valves that were previously open, but not included in the additional list, are kept open even after pressing the **Close** soft key.
	
	`Manual Control  0.00 <------ Pressure>`
	`Valves: 8,18  Open>`
	`Open Valves: 4,12,17,19,36,38  Close>`
	`50  Close All>`

9.	To close all valves, including those that were previously opened, press the **Close All** soft key. The Manual Control Menu returns to its initial view.
	
	`Manual Control  0.00 <------ Pressure>`
	`Valves: _`
	`Open Valves: [None]`
	`Close All>`

To use Manual Control: *(continued)*

10. At any point, the first soft key allows you to scroll through sensor readings for each of the available sensors in the instrument, namely, the valve pressure sensor and each of four conductivity sensors (one for each column).

```
Manual Control   nn.nn <------ Pressure>
Valves: _
Open Valves: [None]
                                Close All>
```

```
Manual Control   nn.nn <- Conductivity1>
Valves: _
Open Valves: [None]
                                Close All>
```

```
Manual Control   nn.nn <- Conductivity2>
Valves: _
Open Valves: [None]
                                Close All>
```

```
Manual Control   nn.nn <- Conductivity3>
Valves: _
Open Valves: [None]
                                Close All>
```

```
Manual Control   nn.nn <- Conductivity4>
Valves: _
Open Valves: [None]
                                Close All>
```

Preparing for Analysis and Purification

After automatic cleavage, you need to remove the columns and oligo collection vials. The collection vial contains the oligonucleotide in ammonium hydroxide.

Removing a Column and Oligo Collection Vial

To remove a column and oligo collection vial:

1.	Unscrew the collection vial and screw on a Teflon® lined-cap.
2.	Pull off the top luer, remove the column, and set the column aside. **IMPORTANT!** Do not discard the column until you have isolated the oligos.

Post-Synthesis Methods

See Chapter 7, "DNA/RNA Synthesis Chemistry," for post-synthesis methods for deprotection, desalting, purification, quantification, and oligo storage.

Method	See Page ...
Deprotection	7-2
Manual Cleavage and Deprotection	7-4
Desalting	7-6
Preparing for Purification	7-7
Purification by the OPC Cartridge	7-8
Oligonucleotide Quantitation	7-13
Storing the Oligonucleotide	7-16

Shutting Down

When to Perform Shutdown

Perform this shutdown procedure if your 3400 DNA Synthesizer will be idle for more than 1 week.

Equipment Required

You need the following equipment for this procedure:

√	Item	Supplier	Part Number
	Column connector tubing (bypass tubing)	Applied Biosystems	225049

Shutting Down the Instrument

To perform shutdown:

1. Replace all four columns with bypass tubing (PN 225049).
2. From Page 1 of the Main Menu, press **Next > Next > Shut Down** soft keys. The Shut Down Menu is displayed:

```
Shut Down                    Start>
  Please keep the old bottles
  in the instrument until you
  are prompted to remove them.
```

3. Press the **Start** soft key to begin the Shut Down procedure

```
Shut Down                    Stop>
  Valve Operation            NNs Hold>
```

Valve Operation = Currently open valve groups

NN = Remaining step time

To perform shutdown: ***(continued)***

4.	The procedure pauses and prompts you to remove all old bottles, wipe lines with a lint free tissue, and insert clean, dry bottles: Shut Down Stop> Remove old bottles, wipe Continue> lines with a lint free tissue, and install clean, dry bottles. Install clean, dry bottles as follows: • Eight 10-mL bottles on the phosphoramidite positions • Six 180-mL bottles on the ancillary reagent positions • One 4-L bottle on the Acetonitrile position • One 2-L bottle on the DCM position • One 2-L bottle on the TCA position
5.	After the procedure is completed, the following screen is displayed: Shut Down Start> Prev> Procedure completed. Next>

DNA/RNA Synthesis Chemistry 7

This chapter discusses the DNA synthesis chemistry that is performed off the 3400 DNA Synthesizer (manually).

This chapter covers:

Deprotection

Phosphate and base deprotection are performed off the 3400 DNA Synthesizer immediately after cleavage.

Materials Required

You need the following materials for this procedure:

√	Item	Vendor	Part Number
	Reagent-grade, concentrated ammonium hydroxide	MLS	–
	Teflon®-lined caps (size 13-425) **IMPORTANT!** Use Teflon-lined caps with the vials because the rubber-lined caps can leach contaminants into the DNA-ammonium hydroxide solution.	MLS	–

Guidelines for Ammonium Hydroxide

Base deprotection is an ammonolysis reaction in which ammonia acts as a nucleophile that attacks the carbonyl of the amide protecting groups. When using ammonium hydroxide:

- Use fresh, concentrated ammonium hydroxide on the instrument.
- To ensure no decrease in ammonia concentration, store the reagent tightly capped in a refrigerator.

Performing Deprotection

To perform deprotection:

1.	After cleavage is performed on the 3400 DNA Synthesizer, remove the collection vials (containing oligonucleotides and ammonium hydroxide) from the instrument. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2.	Place the collection vials in a heat block and heat as follows: • For standard phosphoramidites, 55 °C for at least 8 to 15 h. • For FastPhoramidite® reagents, 65 °C for 1 to 1.5 h.
3.	Cool the ammonium hydroxide/oligonucleotide solution on ice (or put it in a refrigerator) for 10 min to prevent losses from bubbling.

To perform deprotection: ***(continued)***

4.	Remove the ammonia by vacuum. **Note:** Ammonia is much easier to transfer low temperatures than at room temperature. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **CAUTION** When drying down a trityl-on synthesis, 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 50 to 100 mL of saturated sodium bicarbonate. Do not overdry the DMT-on sample Or • Add one drop of distilled triethylamine every 10 min and avoid heating the sample. **DANGER** **CHEMICAL HAZARD**. **Triethylamine** is a flammable liquid and vapor. Exposure causes eye, skin and respiratory tract burns. It is harmful if swallowed or inhaled. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. The oligonucleotide is now dried at the bottom of the collection vial.

Desalting and Purification

The deprotected, detritylated oligonucleotide has free 5'- and 3'-hydroxyls and is biologically active. Desalting and purification may be necessary before use in experiments. See:

- “Desalting” on page 7-6
- “Preparing for Purification” on page 7-7

Manual Cleavage and Deprotection

The information and procedures below are provided as examples for manual cleavage and deprotection of oligonucleotides when the standard 3400 DNA Synthesizer Cleave ending option is not used.

Materials Required

You need the following materials for this procedure:

√	Item	Vendor	Part Number
	Luer tip syringes	MLS	–
	Reagent-grade, concentrated ammonium hydroxide	MLS	–
	4-mL oligo collection vials with a rubber-lined screw cap	Applied Biosystems	400048
	Teflon®-lined caps (size 13-425) **IMPORTANT!** Use Teflon-lined caps with the vials because the rubber-lined caps can leach contaminants into the DNA-ammonium hydroxide solution.	MLS	–

Guidelines for Ammonium Hydroxide

Base deprotection is an ammonolysis reaction in which ammonia acts as a nucleophile that attacks the carbonyl of the amide protecting groups. When using ammonium hydroxide:

- Use fresh, concentrated ammonium hydroxide on the instrument.
- To ensure no decrease in ammonia concentration, store the reagent tightly capped in a refrigerator.

Performing Manual Cleavage and Deprotection

The manual cleavage and deprotection procedure below is called the Double Syringe Method.

To perform manual cleavage and deprotection:

1.	Attach an empty Luer tip syringe, with plunger fully inserted, into one end of a column.
2.	Load 2 to 3 mL of concentrated ammonia in another luer tip syringe and attach it to the other end of the column. **IMPORTANT!** The concentration of ammonia is critical. Use fresh, concentrated ammonium hydroxide that has been opened less than 1 month. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To perform manual cleavage and deprotection: ***(continued)***

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. For optimum results, push the ammonia through the column slowly over 1 h, pushing ~0.5 mL of ammonia through every 10–15 min. **CAUTION** Be careful not to pull the syringes loose from the column.
4.	Drain all the reagent into one syringe and detach it from the column.
5.	Carefully push the ammonium hydroxide/oligonucleotide solution from the syringe into a collection vial and cap it tightly. **IMPORTANT!** Use a tightly sealed oligonucleotide 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. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
6.	The oligonucleotide is now in solution and no longer bound to the support. Save the column until the cleavage is confirmed.
7.	<table><tr><th>If you are using ...</th><th>Then ...</th></tr><tr><td>Standard phosphoramidites</td><td>Remove the exocyclic amine base-protecting groups (benzoyl and isobutyryl) by heating the vial of oligonucleotide at 55 °C for 8 to 15 h.</td></tr><tr><td>FastPhoramidite reagents</td><td>Remove the exocyclic amine base-protecting groups (dimethylformamidine) by heating the vial of DNA at 65 ºC for 1.5 h.</td></tr></table> **IMPORTANT!** Longer treatment is advisable if the ammonium concentration is questionable or if the oligonucleotides are long.
8.	Cool the ammonium hydroxide/oligonucleotide solution on ice (or put it in a refrigerator) for 10 min to prevent losses from bubbling. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Desalting and Purification

The deprotected, detritylated oligonucleotide has free 5'- and 3'-hydroxyls and is biologically active. Desalting and purification may be necessary before use in experiments. See:

- "Desalting" below
- "Preparing for Purification" on page 7-7

Desalting

You can perform desalting using one of the following methods:

- Ethanol precipitation
- C18 RP
- Gel filtration

Only the ethanol precipitation method is described here.

Performing Ethanol Precipitation

To perform ethanol precipitation:

1.	Add 1 M NaCl to the dried-down oligonucleotide as follows: • For a 0.2-µmol scale synthesis, add 150 µL • For a 1.0-µmol scale synthesis, add 250 µL
2.	Vortex until completely dissolved.
3.	Add cold absolute ethanol as follows: • For a 0.2-µmol scale synthesis, add 1 mL • For a 1.0-µmol scale synthesis, add 1.5 mL **WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4.	Vortex. A white precipitate forms.
5.	Store the sample in the freezer for 10 to 15 min, or until cold to the touch.
6.	Remove the sample from the freezer and centrifuge at 9000 rpm for 3 min.
7.	Pour off the supernatant.
8.	Repeat steps 3 to 7 two more times to remove the NaCl.
9.	Redissolve the pellet for quantitation and analysis.

Preparing for Purification

You can perform purification using one of the following methods:

- OPC® cartridge
- High-performance liquid chromatography (HPLC)
- Polyacrylamide gel electrophoresis (PAGE)

Preparation procedures for each purification method are described here.

Preparing for the OPC Cartridge

To prepare for purification by the OPC cartridge:

1.	Dilute the DMT-on oligonucleotide with water: a. Add 100 µL of water and vortex. b. Repeat step a until the oligonucleotide dissolves.
2.	Load the oligonucleotide solution directly onto the OPC cartridge.
3.	No other preparation is needed. For purification procedures, see "Purification by the OPC Cartridge" on page 7-8.

Preparing for PAGE or Ion-Exchange HPLC

If the DMT group was removed previously as a part of the synthesis cycle, the DNA is ready for analysis and/or purification by PAGE or ion-exchange HPLC.

Preparing for Trityl-Specific RPHPLC

To prepare for purification by trityl-specific, reverse-phase HPLC:

1.	Remove the ammonia by vacuum. **Note:** Ammonia is much easier to transfer at low temperatures than at room temperature. **CAUTION** When drying down trityl-on synthesis, 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 50 to 100 mL of saturated sodium bicarbonate. Do not overdry the DMT-on sample Or • Add one drop of distilled triethylamine every 10 min and avoid heating the sample. **DANGER** **CHEMICAL HAZARD**. **Triethylamine** is a flammable liquid and vapor. Exposure causes eye, skin and respiratory tract burns. It is harmful if swallowed or inhaled. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

After RPHPLC Purification

After collection and concentration of the product:

1.	Detritylate the dried sample by dissolving it in 200 µL of 80% acetic acid for 10 to 15 min. **DANGER** **CHEMICAL HAZARD**. **80% Acetic acid** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract burns. It is harmful if inhaled, swallowed, or absorbed through the skin. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. **Note:** Because the acetic acid is an aqueous solution, the trityl cation reacts with water to form tritanol, which does not give an orange color.
2.	Add an equal volume of 95% ethanol and lyophilize the sample until no acetic acid remains. **WARNING** **CHEMICAL HAZARD**. **Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
3.	You can remove the hydrolyzed DMT group and remaining salts from the vial by methods such as: • The OPC cartridge desalting procedure • Ethanol precipitation. See “Performing Ethanol Precipitation” on page 7-6.

Purification by the OPC Cartridge

The Oligonucleotide Purification Cartridge is a rapid purification cartridge used specifically for synthetic DNA. It provides the level of purity required for common applications of synthetic DNA. The oligonucleotide is synthesized with the 5' DMT left on (DMT On).

Advantages of the OPC Cartridge

The OPC cartridge is fast, easy to use, and delivers consistent results. Complete purification, from the deprotected, crude oligonucleotide to its use in an experiment, requires only 20 minutes. If you currently only desalt your synthetic oligonucleotides, you can now use the OPC cartridge to desalt and purify in less time than it takes to desalt alone.

The OPC cartridge has the following features:

- The support material is stable to concentrated ammonia.
- The ammonia solution provides a *denaturing* medium, eliminating secondary structure, hydrogen-bonding, and coelution of partially complementary failure sequences.

- The trityl group is detached and retained in the column.
- The purified, fully deprotected oligonucleotide is eluted in a small volume of 20% acetonitrile in water, completely desalted and ready for use. Many PCR and sequencing reactions are successfully run using DNA primers directly from the 20% acetonitrile/water solution.

Other Purification Methods

Other purification methods include:

- Polyacrylamide gel electrophoresis (PAGE)
- High-performance liquid chromatography (HPLC)

Although the PAGE and HPLC methods can be used for successful purification, they are subject to the following qualifications:

- Both methods can provide a high level of purity, but they require initial capital investment and are labor intensive and time consuming.
- A short oligonucleotide (<30 bases) made with typically high synthesis efficiency (>98% average trityl yield/cycle) may require less stringent purification. Efficient desalting and removal of nonnucleotide synthesis by-products may be sufficient purification.

Using Vacuum Manifold Devices

The OPC cartridge purification process is facilitated by using any one of a number of commercially available vacuum manifold devices. These are designed to process many solid-phase cartridges at one time. You deliver the reagents in reservoirs (syringe barrels) mounted on the OPC cartridge, then direct the device to collect the OPC cartridge effluents or deliver them to waste.

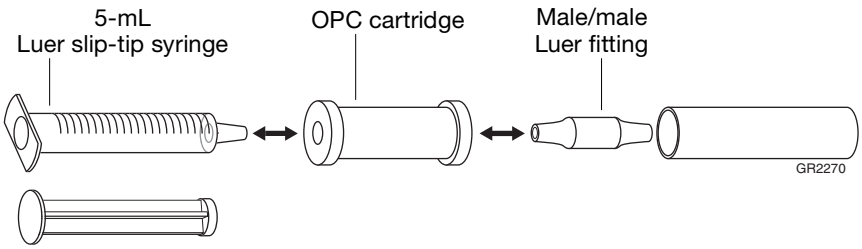
Materials Required

You need the following materials for this procedure:

√	Item	Vendor	Part Number
	Luer slip-tip syringe, all-polypropylene	MLS	–
	OPC® cartridge	Applied Biosystems	400771
	Male/male luer	Applied Biosystems	110127
	HPLC-grade acetonitrile, 5 mL	Applied Biosystems	401087
	2.0 M triethylamine acetate, 5 mL	Applied Biosystems	400613
	Deionized water, 20 mL	MLS	–
	Dilute ammonium hydroxide, 15 mL (1:10 dilution of concentrated ammonium hydroxide in deionized water)	MLS	–
	3% trifluoracetic acid in deionized water, 5 mL	MLS	–
	20% v/v acetonitrile/deionized water, 1 mL	MLS	–

Preparing the OPC Cartridge

To prepare the OPC cartridge:

1.	Make the OPC cartridge connections: a. Connect the Luer slip-tip syringe to one end of the OPC cartridge column. b. Connect a male-to-male Luer tip to the other end. 
2.	Make sure all fittings are snug.
3.	Flush the OPC cartridge with 5 mL of HPLC-grade acetonitrile, keeping the flow rate at 1 to 2 drops/sec. **WARNING** **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4.	Flush the OPC cartridge with 5 mL of 2.0 M triethylamine acetate, keeping the flow rate at 1 to 2 drops/sec. **WARNING** **CHEMICAL HAZARD**. **Triethylamine Acetate** is an irritant if inhaled or absorbed through the skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Loading the OPC Cartridge

To load the OPC cartridge:

1.	Dilute the ammonium hydroxide solution containing the cleaved, deprotected, trityl-on crude oligonucleotide with an equal volume of deionized water. **Note:** Dilution of the ammonia sample with water prior to loading affects the capacity of the OPC cartridge to bind DNA. Although ammonia has a denaturing effect, minimizing secondary structures that hinder purification, it also decreases the capacity of the oligonucleotide to bind to the OPC cartridge medium. For maximum capacity, use an equal volume of deionized water to dilute the ammonia sample. This increases the OPC cartridge capacity up to 10 ODU of purified oligonucleotide. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2.	Load the above solution into the syringe.
3.	Gently push the solution through the OPC cartridge, saving the eluted fraction.
4.	Reload the eluted fraction, then push it through the OPC cartridge again. This loads 1 to 5 ODU of the trityl oligonucleotide onto the OPC cartridge. The ODU value depends on the length, sequence, and synthesis quality.
5.	If desired, store the final eluted fraction at –20 ºC. You can run it through another OPC cartridge until all trityl oligonucleotide is removed.

Detritylating

To detritylate the OPC cartridge-bound oligonucleotide:

1.	Flush the OPC cartridge three times with 5 mL of dilute ammonium hydroxide. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
2.	Flush the OPC cartridge twice with 5 mL of deionized water.

To detritylate the OPC cartridge-bound oligonucleotide: *(continued)*

3.	Detritylate the OPC cartridge-bound oligonucleotide with 5 mL of 3% trifluoroacetic acid (TFA) solution, as follows: a. Gently push ~1 mL of TFA solution through the OPC cartridge. b. Incubate for 5 min. c. Flush the remaining TFA solution through the OPC cartridge. **DANGER** **CHEMICAL HAZARD**. **Trifluoroacetic acid (TFA)** causes eye, skin, and respiratory tract burns. It is harmful if inhaled. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
4.	Flush the OPC cartridge twice with 5 mL of deionized water.
5.	Perform this step only for sequences >40 bases. It removes shorter sequences that had trityl-protecting groups attached and therefore were co-purified with the desired product. If the sequence is ≤40 bases, skip to step 6. a. Flush the OPC cartridge once with 5 mL of dilute ammonium hydroxide. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. b. Flush the OPC cartridge twice with 5 mL of deionized water.
6.	Elute the purified, detritylated oligonucleotide by flushing the OPC cartridge with 1 mL of 20% acetonitrile. **WARNING** **CHEMICAL HAZARD**. **Acetonitrile (ACN)** is a flammable liquid and vapor. Exposure may cause eye, skin, and respiratory tract irritation, central nervous system depression, and damage to the heart, blood system, liver, and kidneys. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
7.	Evaporate an aliquot of the eluate to dryness, then dissolve in water to determine the ODU at 260 nm.
8.	Store any unused OPC cartridge-purified oligonucleotide as a dry solid at –20 °C.

Oligonucleotide Quantitation

UV Spectroscopy

Oligonucleotides, like double-stranded DNA, are most commonly quantified by measuring their absorbance of ultraviolet light at 260 nm. Single-stranded oligonucleotides dissolved in neutral aqueous solution at a concentration of 33 µg/mL have an absorbance at 260 nm (in a 1-cm cuvette) of approximately 1.0 optical density unit (ODU). For comparison, an ODU of 1.0 corresponds to approximately 50 µg/mL for double-stranded DNA.

Nucleic acids of any variety are most easily quantified by UV spectroscopy, measuring at or near their UV absorbance maxima, about 260 nm.

Details	Description
How measured	A dilute aqueous solution ≤1 mL (depending on the cuvette size) is measured by either scanning the region between 200 and 350 nm or using a single 260-nm wavelength measurement.
Characteristics of absorbance	A scan of an oligonucleotide shows broad absorbance with a maxima near 260 nm.

ODU as a Measure of Concentration

A measurement of the absorbance indicates the concentration of the solution, provided the molar extinction coefficient is known.

The following definitions apply to using ODU as the unit of measure:

Term	Definition
ODU and Beer's Law	
ODU	ODU = optical density unit One ODU is the absorbance (typically measured at 260 nm) of a 1-mL solution of oligonucleotide in water or an appropriate buffer at a neutral pH range in a 1-cm pathlength cuvette.
Beer's Law	The measurement uses Beer's Law to allow conversion of the absorbance reading to a molar amount. Beer's Law is $A = \varepsilon Cl$, where: A = Absorbance ε = molar extinction coefficient C = Concentration (mol/L) l = pathlength (cm), typically 1 cm
Molar Extinction Coefficients	
Molar extinction coefficient	The exact molar extinction coefficient of a substance is a constant that depends on the UV-absorbing properties of the chemical structure of that substance.
Major contributors to extinction coefficient	DNA contains four major contributors to the extinction coefficient: the four nucleobases A, G, C, and T. Each of the bases has a different extinction coefficient. A sample of synthetic DNA has (generally) a mixture of all four.

Term	Definition
Criteria for ODU Measurement and Conversion to Mass or Concentration	
Optimum measurement criteria	The ODU of an oligonucleotide is generally measured at the position where the absorbance is at a maximum (typically 260 nm). Oligonucleotides that are rich in either purines or pyrimidines may actually have absorbance maxima above or below 260 nm, depending on the composition.
Criteria for converting ODU to mass or concentration	When the ODU reading is obtained, using the approximation that 1 ODU represents about 33 µg of single-stranded DNA, the mass or concentration of an oligonucleotide can be determined provided the molecular weight of the oligo is known.

Determining Concentration

To determine the concentration of an oligonucleotide stock solution:

1.	Make one to three dilutions of the stock solution (for example, 100 to 500 fold) in distilled water or dilute TE buffer. **Note:** TE buffer is 10 mM Tris-HCl, 0.1 mM EDTA (pH 7.0).
2.	Measure the absorbance at 260 nm.
3.	Calculate the concentration using the formula: [concentration, in µM] = (A × dilution factor × 33,000)/MW of oligo where: • A is the measured absorbance • 33,000 is a conversion factor from mass to concentration • MW is the molecular weight of the oligonucleotide, which can be calculated using the formula: MW = (A × 312.2) + (C × 288.2) + (G × 328.2) + (T × 302.2) − 61 **Note:** If you do not know the actual molecular weight of the particular oligonucleotide, substitute N × 330 for MW: N × 330 = (A × 312.2) + (C × 288.2) + (G × 328.2) + (T × 302.2) − 61 where: • N is the number of bases in the oligo • 330 is the average molecular weight of a nucleotide

Conversion Information

Some useful conversion information is given below.

E260 = 0.89[(A × 15480) + (C × 7340) + (G × 11760) + (T × 8850)]

pmoles/µg = 106/MW

pmoles/OD = 109/E260

µg/OD = MW × 103/E260

GC Content = (#G +#C/Total # bases) × 100

Determining Melting Temperature

Numerous formulas exist to determine the theoretical melting temperature (*Tm*) of nucleic acids (as well as oligonucleotides). These can serve as starting points for determining annealing conditions for PCR applications.

The Tm is the temperature at which half of the potential binding sites in a DNA are thought to have primer molecules bound to them. Longer primers, or ones with higher G+C content (number of guanine and cytosine residues), have higher Tm values because they have a greater number of hydrogen bonds per molecule. However, it is best to determine and optimize (empirically) the annealing conditions by performing the reaction at several temperatures, starting approximately at 5 ºC below any calculated Tm. The following formula can be used to estimate the melting temperature for oligonucleotides:

$$Tm = 81.5 + 16.6\ (\log10[Na+]) + 0.41 \times (\%G+C) - 675/n$$

where:

- [Na+] is the molar salt concentration
- [K+] = [Na+]
- n is the number of bases in the nucleotide

Example

To calculate the melting temperature of a 22-mer oligonucleotide with 60% G+C in 50 mM KCl:

$$Tm = 81.5 + 16.6 \times 9\log10[0.05]) + 0.41 \times (60) - 675/22$$

$$= 81.5 + 16.6 \times (-1.30) + 24.60 - 30.68 = 53.84\ C$$

Storing the Oligonucleotide

Storage for Later Use

Most applications for synthetic oligonucleotides require less DNA than the typical amount produced by the 3400 DNA Synthesizer. Fortunately, oligonucleotides can be stored easily, with little or no degradation, for long periods of time.

Storage Methods

When stored with one of the methods described below, oligonucleotides are stable for over 1 year.

Storage Method	Description
Dry storage	At −20 to 4 °C as a dried pellet in a clean, dry vessel (for example, a microcentrifuge tube). **Note:** The best storage method is dry storage.
Storage in 1X TE buffer	At −20 to 4 °C in 1X TE buffer, in either a crude or purified state.
Storage in ammonia (crudes only)	For oligonucleotides synthesized and collected crude: At −20 to 4 °C in a concentrated ammonia solution. **DANGER** **CHEMICAL HAZARD**. **Ammonium hydroxide** is a corrosive chemical that can burn and cause serious skin or eye damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Storage Guidelines

- Never store oligonucleotides in water because they degrade in water.
- Keep oligonucleotides cold (−20 to 4 °C) to minimize degradation and bacterial growth.

Alternative Chemistries

Other Monomers

In addition to synthesis with the standard phosphoramidite monomers, you can use other monomers on the 3400 DNA Synthesizer. These include:

- Aminolink™ reagents
- Biotin
- Deoxyinosine
- Phosphalink® reagents
- Fluorescent dye amidites

Qualifications

Please note the following qualifications when using other monomers:

- When using Aminolink or Phosphalink reagents, select the DMT ending option (DMT On) for your synthesis run. See “Selecting DMT Options” on page 6-6 for more information.
- Because the DMT-On oligos using Phosphalink reagent synthesis fall off during NH_4OH deprotection, there is no reason to remove it at the end of a synthesis.

For More Information

For more information on the monomers and alternative chemistries listed above, visit the Applied Biosystems Web site:

http://www.appliedbiosystems.com

Valve Groups and Cycle Script Listings

A

This appendix covers:

Valve Code Listing

Sequential List

The table below lists all of the Applied Biosystems 3400 DNA Synthesizer code groups. The are listed in numerical order.

Code	Valve Groups	Code	Valve Groups	Code	Valve Groups	Code	Valve Groups	Code	Valve Groups
100	BlockFlush								
101	ReverseFlush($Col)								
102	FlushToCWaste($Col)								
103	FlushToCollect($Col)								
104	FlushToColumn($Col)								
105	FlushToWaste($Col)								
110	FlushTo($Base)	210	($Base)ToColumn($Col)	310	($Base)ToWaste	410	ACNTo($Base)	510	AuxTo($Base)
111	FlushToA	211	AToColumn($Col)	311	AToWaste	411	ACNToA	511	AuxToA
112	FlushToG	212	GToColumn($Col)	312	GToWaste	412	ACNToG	512	AuxToG
113	FlushToC	213	CToColumn($Col)	313	CToWaste	413	ACNToC	513	AuxToC
114	FlushToT	214	TToColumn($Col)	314	TToWaste	414	ACNToT	514	AuxToT
115	FlushTo5	215	5ToColumn($Col)	315	5ToWaste	415	ACNTo5	515	AuxTo5
116	FlushTo6	216	6ToColumn($Col)	316	6ToWaste	416	ACNTo6	516	AuxTo6
117	FlushTo7	217	7ToColumn($Col)	317	7ToWaste	417	ACNTo7	517	AuxTo7
118	FlushTo8	218	8ToColumn($Col)	318	8ToWaste	418	ACNTo8	518	AuxTo8
119	FlushToBases			319	TetToWaste	419	ACNToBases	519	AuxToBases

Code	Valve Groups	Code	Valve Groups	Code	Valve Groups	Code	Valve Groups	Code	Valve Groups
120	FlushTo($Base,Tet)	220	($Base,Tet)ToColumn($Col)	320	($Base,Tet)ToWaste	420	ACNTo($Base,Tet)	520	AuxTo($Base,Tet)
121	FlushTo(A,Tet)	221	(A,Tet)ToColumn($Col)	321	(A,Tet)ToWaste	421	ACNTo(A,Tet)	521	AuxTo(A,Tet)
122	FlushTo(G,Tet)	222	(G,Tet)ToColumn($Col)	322	(G,Tet)ToWaste	422	ACNTo(G,Tet)	522	AuxTo(G,Tet)
123	FlushTo(C,Tet)	223	(C,Tet)ToColumn($Col)	323	(C,Tet)ToWaste	423	ACNTo(C,Tet)	523	AuxTo(C,Tet)
124	FlushTo(T,Tet)	224	(T,Tet)ToColumn($Col)	324	(T,Tet)ToWaste	424	ACNTo(T,Tet)	524	AuxTo(T,Tet)
125	FlushTo(5,Tet)	225	(5,Tet)ToColumn($Col)	325	(5,Tet)ToWaste	425	ACNTo(5,Tet)	525	AuxTo(5,Tet)
126	FlushTo(6,Tet)	226	(6,Tet)ToColumn($Col)	326	(6,Tet)ToWaste	426	ACNTo(6,Tet)	526	AuxTo(6,Tet)
127	FlushTo(7,Tet)	227	(7,Tet)ToColumn($Col)	327	(7,Tet)ToWaste	427	ACNTo(7,Tet)	527	AuxTo(7,Tet)
128	FlushTo(8,Tet)	228	(8,Tet)ToColumn($Col)	328	(8,Tet)ToWaste	428	ACNTo(8,Tet)	528	AuxTo(8,Tet)
129	FlushToTet	229	TetToColumn($Col)	329	TetToWaste	429	ACNToTet	529	AuxToTet
130	FlushToAmmonia	230	AmmoniaToColumn($Col)	330	AmmoniaToWaste	430	ACNToAmmonia		
131	FlushToCapA	231	CapAToColumn($Col)	331	CapAToWaste	431	ACNToCapA		
132	FlushToCapB	232	CapBToColumn($Col)	332	CapBToWaste	432	ACNToCapB		
133	FlushToCapAB	233	CapABToColumn($Col)	333	CapABToWaste	433	ACNToCapAB		
134	FlushToAux	234	AuxToColumn($Col)	334	AuxToWaste	434	ACNToAux		
135	FlushToIodine	235	IodineToColumn($Col)	335	IodineToWaste	435	ACNToIodine		
136	FlushTo(Aux,Iodine)					436	ACNTo(Aux,Iodine)		
137	FlushToACN	237	ACNToColumn($Col)	337	ACNToWaste				
138	FlushToDCM	238	DCMToColumn($Col)	338	DCMToWaste	438	ACNToDCM		
139	FlushToTCA	239	TCAToColumn($Col)	339	TCAToWaste	439	ACNToTCA	639	DCMToTCA

Code	Valve Groups	Code	Valve Groups	Code	Valve Groups
				900	BlockVent
				910	Vent(&Base)
				911	VentA
				912	VentG
				913	VentC
				914	VentT
				915	Vent5
				916	Vent6
247	ACNToCWaste($Col)			917	Vent7
248	DCMToCWaste($Col)			918	Vent8
249	TCAToCWaste($Col)			919	VentBases
250	AmmoniaToCollect($Col)	820	Pressure(Amidite,Tet)		
		830	PressureAmmonia	930	VentAmmonia
		833	PressureCapAB		
		834	PressureAux		
		835	PressureIodine		
		836	Pressure(Aux,Iodine)		
		837	PressureACN		
		838	PressureDCM		
		839	PressureTCA		

Cycle Script LV40-PO

Cycle script LV40-PO is a 40-nmol, low-volume DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.2
TTIME 2	1.8
TTIME 3	2.2
TTIME 4	2.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	20
CTIME G	20
CTIME C	20
CTIME T	20
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	20

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3

Command	Valve Groups	Time (Sec)
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	2
TRANsfer	PressureACN	2
TRANsfer	PressureDCM	2
TRANsfer	DCMToCWaste($Col)	18
SAFe	No	
MONitor	TCAToCWaste($Col)	45

Command	Valve Groups	Time (Sec)
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	9
TRANsfer	FlushToCWaste($Col)	5
TRANsfer	ACNToCWaste($Col)	9
TRANsfer	ReverseFlush($Col)	5
TRANsfer	ACNToColumn($Col)	9
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	BlockFlush	5
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	0.5
TRANsfer	($Base,Tet)ToColumn($Col)	0.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	6
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	2
TRANsfer	CapABToColumn($Col)	9
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureIodine	2
TRANsfer	IodineToColumn($Col)	9
TRANsfer	BlockFlush	4
SLEep		20

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	5
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script LV40-PS

Cycle script LV40-PS is a 40-nmol, low-volume phosphorothioate DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.2
TTIME 2	1.8
TTIME 3	2.2
TTIME 4	2.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	30
CTIME G	30
CTIME C	30
CTIME T	30
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	30

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15

Command	Valve Groups	Time (Sec)
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureAux	5
TRANsfer	AuxToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	2

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3

Command	Valve Groups	Time (Sec)
TRANsfer	DCMToCWaste($Col)	20
SAFe	No	
MONitor	TCAToCWaste($Col)	45
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToWaste	2
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	9
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column

- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	0.5
TRANsfer	($Base,Tet)ToColumn($Col)	0.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	6
TRANsfer	BlockFlush	5

Sulfurization Procedure

The SULFurize procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureAux	3
TRANsfer	AuxToColumn($Col)	9
TRANsfer	FlushToWaste	5
SLEep		450
TRANsfer	AuxToColumn($Col)	9
SLEep		450
TRANsfer	ACNToWaste	4
SLEep		450

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	6

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	4
TRANsfer	CapABToColumn($Col)	9
TRANsfer	ACNToWaste	4
SLEep		6
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script LV40-RNA

Cycle script LV40-RNA is a 40-nmol, low-volume RNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.2
TTIME 2	1.8
TTIME 3	2.2
TTIME 4	2.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	600
CTIME G	600
CTIME C	600
CTIME T	600
CTIME 5	600
CTIME 6	600
CTIME 7	600
CTIME 8	600
CTIME Default	600

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10

Command	Valve Groups	Time (Sec)
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	20
SAFe	No	
MONitor	TCAToCWaste($Col)	45
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	9

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	0.5
TRANsfer	($Base,Tet)ToColumn($Col)	0.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4

TRANsfer	ReverseFlush($Col)	6
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	9
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	6

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressurelodine	3
TRANsfer	IodineToColumn($Col)	9
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11

Command	Valve Groups	Time (Sec)
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7

Command	Valve Groups	Time (Sec)
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script 0.2 µm-PO

Cycle script 0.2 µm-PO is a 0.2-µmol DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.0
TTIME 2	3.2
TTIME 3	3.4
TTIME 4	3.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	20
CTIME G	20
CTIME C	20
CTIME T	20
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	20

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5

Command	Valve Groups	Time (Sec)
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	2
TRANsfer	PressureACN	2
TRANsfer	PressureDCM	2
TRANsfer	DCMToCWaste($Col)	22
SAFe	No	
MONitor	TCAToCWaste($Col)	60
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	11

Command	Valve Groups	Time (Sec)
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	3
TRANsfer	BlockFlush	5
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.7
TRANsfer	($Base,Tet)ToColumn($Col)	1.5
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	2.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	2
TRANsfer	CapABToColumn($Col)	11
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressurelodine	2
TRANsfer	IodineToColumn($Col)	11
TRANsfer	BlockFlush	4
SLEep		20

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	5
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	5
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	5

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script 0.2 µm-PS

Cycle script 0.2 µm-PS is a 0.2-µmol phosphorothioate synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.0
TTIME 2	3.2
TTIME 3	3.4
TTIME 4	3.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	30
CTIME G	30
CTIME C	30
CTIME T	30
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	30

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3

Command	Valve Groups	Time (Sec)
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureAux	5
TRANsfer	AuxToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	32
SAFe	No	
MONitor	TCAToCWaste($Col)	60

Command	Valve Groups	Time (Sec)
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	14
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	14
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.7
TRANsfer	($Base,Tet)ToColumn($Col)	1.5
TRANsfer	TetToColumn($Col)	1.0

Command	Valve Groups	Time (Sec)
TRANsfer	($Base,Tet)ToColumn($Col)	2.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CoupleTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Sulfurization Procedure

The SULFurize procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureAux	3
TRANsfer	AuxToColumn($Col)	12
TRANsfer	FlushToWaste	5
SLEep		450
TRANsfer	AuxToColumn($Col)	12
SLEep		450
TRANsfer	ACNToWaste	4
SLEep		450
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	10
TRANsfer	BlockFlush	6

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	10
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script 0.2 µm-RNA

Cycle script 0.2 µm-RNA is a 0.2-µmol RNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.0
TTIME 2	3.2
TTIME 3	3.4
TTIME 4	3.6

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	600
CTIME G	600
CTIME C	600
CTIME T	600
CTIME 5	600
CTIME 6	600
CTIME 7	600
CTIME 8	600
CTIME Default	600

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3

Command	Valve Groups	Time (Sec)
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	32
SAFe	No	
MONitor	TCAToCWaste($Col)	60

Command	Valve Groups	Time (Sec)
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	14
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	14
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	14
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
NEW CDELivery $Col $Base $TetTime <multiline>		
TRANsfer	TetToColumn($Col)	1.7
TRANsfer	($Base,Tet)ToColumn($Col)	1.5

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	2.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	6

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressurelodine	3
TRANsfer	IodineToColumn($Col)	12
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToWaste	4
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	14
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	14
TRANsfer	FlushToColumn($Col)	9
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7

Command	Valve Groups	Time (Sec)
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script LV200-PO

Cycle script LV200-PO is a 200-nmol, low-volume DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.8
TTIME 2	2.0
TTIME 3	2.2
TTIME 4	2.3

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	20
CTIME G	20
CTIME C	20
CTIME T	20
CTIME 5	300
CTIME 6	300
CTIME 7	300

Step Name	Time (Sec)
CTIME 8	300
CTIME Default	20

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	2
TRANsfer	PressureACN	2
TRANsfer	PressureDCM	2
TRANsfer	DCMToCWaste($Col)	20
SAFe	No	
MONitor	TCAToCWaste($Col)	60
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	3
TRANsfer	BlockFlush	5
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	1.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	2
TRANsfer	CapABToColumn($Col)	11
SLEep		6
TRANsfer	ACNToWaste	4

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressurelodine	2
TRANsfer	IodineToColumn($Col)	11
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	11
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	11
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6

Command	Valve Groups	Time (Sec)
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script LV200-PS

Cycle script LV200-PS is a 200-nmol, low-volume phosphorothioate DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.8
TTIME 2	2.0
TTIME 3	2.2
TTIME 4	2.3

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	30
CTIME G	30
CTIME C	30

Step Name	Time (Sec)
CTIME T	30
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	30

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureAux	5
TRANsfer	AuxToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	32
SAFe	No	
MONitor	TCAToCWaste($Col)	60
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	1.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Sulfurization Procedure

The SULFurize procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureAux	3
TRANsfer	AuxToColumn($Col)	12
TRANsfer	FlushToWaste	5
SLEep		450
TRANsfer	AuxToColumn($Col)	12
SLEep		450
TRANsfer	ACNToWaste	4
SLEep		450
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	6

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	8
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9

Command	Valve Groups	Time (Sec)
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	8

Cycle Script LV200-RNA

Cycle script LV200-RNA is a 200-nmol, low-volume RNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	1.8
TTIME 2	2.0
TTIME 3	2.2
TTIME 4	2.3

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	600
CTIME G	600
CTIME C	600
CTIME T	600
CTIME 5	600
CTIME 6	600
CTIME 7	600
CTIME 8	600
CTIME Default	600

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3

Command	Valve Groups	Time (Sec)
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	32
SAFe	No	
MONitor	TCAToCWaste($Col)	60
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	FlushToCWaste($Col)	6
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column

- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	$Base+TetToColumn($Col)	1.0
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	TetToWaste	1
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	6

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressureliodine	3
TRANsfer	IodineToColumn($Col)	12
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToWaste	4
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	6
TRANsfer	ACNToColumn($Col)	12
TRANsfer	FlushToColumn($Col)	9
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7

Command	Valve Groups	Time (Sec)
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script 1 µm-PO

Cycle script 1 µm-PO is a 1-µmol DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.9
TTIME 2	4.1
TTIME 3	4.3
TTIME 4	4.5

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	20
CTIME G	20
CTIME C	20
CTIME T	20
CTIME 5	300
CTIME 6	300
CTIME 7	300

Step Name	Time (Sec)
CTIME 8	300
CTIME Default	20

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	2
TRANsfer	PressureACN	2
TRANsfer	PressureDCM	2
TRANsfer	DCMToCWaste($Col)	25
SAFe	No	
MONitor	TCAToCWaste($Col)	110
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	12
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	12
TRANsfer	ReverseFlush($Col)	9
TRANsfer	ACNToWaste	2
TRANsfer	BlockFlush	5
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.3
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	2
TRANsfer	CapABToColumn($Col)	12
SLEep		6

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressureliodine	2
TRANsfer	IodineToColumn($Col)	12
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	13
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	13
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	9
TRANsfer	BlockFlush	5

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Cycle Script 1 µm-PS

Cycle script 1 µm-PS is a 1-µmol phosphorothioate DNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.9
TTIME 2	4.1
TTIME 3	4.3
TTIME 4	4.5

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	30
CTIME G	30
CTIME C	30

Step Name	Time (Sec)
CTIME T	30
CTIME 5	300
CTIME 6	300
CTIME 7	300
CTIME 8	300
CTIME Default	30

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureAux	5
TRANsfer	AuxToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	42
SAFe	No	
MONitor	TCAToCWaste($Col)	110
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	15
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	15
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.3
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Sulfurization Procedure

The SULFurize procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureAux	3
TRANsfer	AuxToColumn($Col)	12
TRANsfer	FlushToWaste	5
SLEep		450
TRANsfer	AuxToColumn($Col)	12
SLEep		450
TRANsfer	ACNToWaste	4
SLEep		450
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	10
TRANsfer	BlockFlush	6

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

$Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	2
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	10
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9

Command	Valve Groups	Time (Sec)
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4

Command	Valve Groups	Time (Sec)
TRANsfer	BlockFlush	8

Cycle Script 1 µm-RNA

Cycle script 1 µm-RNA is a 1-µmol RNA synthesis cycle.

Set Couple Times

TET time for pushing phosphoramidite to each column:

Step Name	Time (Sec)
TTIME 1	3.9
TTIME 2	4.1
TTIME 3	4.3
TTIME 4	4.5

Couple time for each base:

Note: A default time is provided for those not specified.

Step Name	Time (Sec)
CTIME A	600
CTIME G	600
CTIME C	600
CTIME T	600
CTIME 5	600
CTIME 6	600
CTIME 7	600
CTIME 8	600
CTIME Default	600

Begin Procedure

The BEGin procedure primes the delivery lines. It is invoked once at the beginning of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	Pressure(Amidite,Tet)	15
TRANsfer	AToWaste	3
TRANsfer	GToWaste	3
TRANsfer	CToWaste	3
TRANsfer	TToWaste	3
TRANsfer	TetToWaste	5
TRANsfer	PressureCapAB	8
TRANsfer	CapAToWaste	5
TRANsfer	CapBToWaste	5
TRANsfer	PressureIodine	5
TRANsfer	IodineToWaste	5
TRANsfer	PressureTCA	15
TRANsfer	TCAToWaste	10
TRANsfer	PressureDCM	15
TRANsfer	DCMToWaste	10
TRANsfer	PressureACN	15
TRANsfer	ACNToWaste	10
TRANsfer	ACNToColumn($Col)	10
TRANsfer	ReverseFlush($Col)	10
TRANsfer	BlockFlush	10
TRANsfer	BlockVent	3

Detritylate Procedure

The DETRitylate procedure is a deprotection routine. It is invoked at every base addition and (optionally) at the end of the synthesis run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureTCA	3
TRANsfer	PressureACN	3
TRANsfer	PressureDCM	3
TRANsfer	DCMToCWaste($Col)	42
SAFe	No	
MONitor	TCAToCWaste($Col)	110
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	15
TRANsfer	FlushToCWaste($Col)	7
TRANsfer	ACNToCWaste($Col)	15
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	ReverseFlush($Col)	10
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	6
SAFe	Yes	

Preparation Procedure

The PREPare procedure prepares the instrument for amidite delivery. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	Pressure(Amidite,Tet)	3

Amidite Delivery Procedure

The DELIVer procedure delivers the amidite. It is invoked once for each active column, at every base addition.

Inputs:

- $Col = A single column
- $Base = A single base to be delivered into the column
- $TTime = Amidite delivery time (Set with TTIME)

Command	Valve Groups	Time (Sec)
TRANsfer	TetToColumn($Col)	1.3
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	1.0
TRANsfer	($Base,Tet)ToColumn($Col)	2.5
TRANsfer	TetToColumn($Col)	$TTime
TRANsfer	FlushToColumn($Col)	1

Coupling Procedure

The COUPle procedure is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns
- $CTime = Coupling time (Set with CTIME)

Command	Valve Groups	Time (Sec)
SLEep		$CTime
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	5

Capping Procedure

The CAP procedure caps the synthesis columns. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureCapAB	3
TRANsfer	CapABToColumn($Col)	12
SLEep		6

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToWaste	4
TRANsfer	ReverseFlush($Col)	8
TRANsfer	BlockFlush	6

Oxidization Procedure

The OXIDize procedure performs the oxidization routine. It is invoked once per base addition.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	BlockVent	2
TRANsfer	PressureACN	3
TRANsfer	Pressurelodine	3
TRANsfer	IodineToColumn($Col)	12
TRANsfer	BlockFlush	4
SLEep		20
TRANsfer	ReverseFlush($Col)	8
TRANsfer	ACNToWaste	4
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	ReverseFlush($Col)	7
TRANsfer	ACNToColumn($Col)	15
TRANsfer	FlushToColumn($Col)	10
TRANsfer	BlockFlush	6

Cleave Procedure

The CLEave procedure cleaves the active columns. It is invoked (optionally) at the very end of the run.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ACNToColumn($Col)	60

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	6
TRANsfer	PressureAmmonia	15
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	14
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	9
TRANsfer	AmmoniaToCollect($Col)	20
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	7
SLEep		900
TRANsfer	FlushToCollect($Col)	10
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	4
TRANsfer	ACNToColumn($Col)	30
TRANsfer	ReverseFlush($Col)	60
TRANsfer	BlockFlush	10
TRANsfer	VentAmmonia	10

Wash Procedure

The WASH procedure cleans the delivery lines after a run has been terminated.

Inputs:

- $Col = A comma-separated list of active columns

Command	Valve Groups	Time (Sec)
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	ReverseFlush($Col)	15
TRANsfer	BlockFlush	3
TRANsfer	ACNToColumn($Col)	20
TRANsfer	FlushToColumn($Col)	15
TRANsfer	ACNToWaste	4
TRANsfer	BlockFlush	8

Software Menus

B

This Appendix covers:

Main Menu

Accessing the Main Menu

The Main Menu can be accessed at any time.

To access the Main Menu, press the Main Menu command key.

`Main Menu Edit Sequence>`
`Run Setup>`
`Run Status>`
`(Page 1 of 4) Next>`
	← During synthesis: `(Page 1 of 2)`

`Main Menu Edit Cycle>`
`Formula Weights>`
`Configuration>`
`(Page 2 of 4) Next>`
	← During synthesis: `(Page 1 of 2)`

`Main Menu Autodilution>`
`Change Bottle>`
`Shut Down>`
`(Page 3 of 4) Next>`
	← Not available during synthesis (unless paused)
	← Not available during synthesis (unless paused)
	← Not available during synthesis (unless paused)

`Main Menu Manual Control>`
`Diagnostics>`
`(Page 4 of 4) Next>`
	← Not available during synthesis (unless paused)
	← Not available during synthesis (unless paused)

Edit Sequence Menu

No bases entered:	
(No Sequence) Open> 5>_<3 Print> Delete> ()>	← **Open** key because no unsaved changes ← **Print** key because there is no sequence ← **Delete** key because there is no sequence
Bases entered from scratch; not yet saved:	
(Untitled) [6] Save> 5>GATACA_<3 Close> 5'> ()>	← **Save** key if sequence is modified; sequence size ← **Close** key because there is a sequence ← **5'** key because we are at end of sequence
Sequence has just been saved as "Gattaca":	
Gattaca [6] Open> 5>GATACA_<3 Close> 5'> ()>	← **Open** key because no unsaved changes
Inserted base "T" at position 3:	
Gattaca [4 of 7] Save> 5>GATTACA<3 Close> 3'> ()>	← **Save** key because changes are not saved ← 3' to jump to end of sequence

Open Submenu in Edit Sequences Menu

Open Sequence *Sequence Name*> *Sequence Name*> *Sequence Name*> (Page 1 of *n*) Next>

Print Submenu in Edit Sequences Menu

Print Sequences [ ] *Sequence Name*> <A> = Select All [ ] *Sequence Name*> <C> = Clear All [ ] *Sequence Name*> (Page 1 of *n*) Next>	← No sequences selected
Print Sequences [X] *Sequence Name*> <A> = Select All [ ] *Sequence Name*> <C> = Clear All [X] *Sequence Name*> (Page 1 of *n*) Next>	← Selected for printing ← Selected for printing
Confirm Print Operation Yes> Are you sure you want to print the sequence "*Sequence Name*"? No>	Upon leaving the "Print Sequences" Menu: ← A single sequence was selected

Confirm Print Operation Yes> Are you sure you want to print the *n* selected sequences? No>	← Multiple sequences were selected
Sequences Printed. The sequences were successfully sent to the print spool at *printer address*.	Confirmation message.
No printer is configured. The sequences cannot be printed. Please set up a printer under Configuration -> Network -> Printer	Trying to print while no printer is configured
Unable to print sequences: >>*reason*<< Please check printer and settings.	If a printer is configured, but printing failed.

Delete Submenu

Delete Sequences [ ] *Sequence Name*> <A> = Select All [] *Sequence Name*> <C> = Clear All [ ] *Sequence Name*> (Page 1 of *n*) Next>	← No sequences selected
Delete Sequences [X] *Sequence Name*> <A> = Select All [] *Sequence Name*> <C> = Clear All [X] *Sequence Name*> (Page 1 of *n*) Next>	← Selected for deletion ← Selected for deletion
Confirm Delete Operation Yes> Are you sure you want to delete the sequence "*Sequence Name*"? No>	Upon leaving the "Delete Sequences" Menu: ← A single sequence was selected
Confirm Delete Operation Yes> Are you sure you want to print the *n* selected sequences? No>	← Multiple sequences were selected
Delete operation successful. The sequence "*Sequence Name*" has been removed.	Confirmation of a single sequence deletion
Delete operation successful. The *n* selected sequences have been removed.	Confirmation of a multiple sequences deletion

Save Submenu

Save as: "" Pick "a">
  Pick letters |abcdefghijklm| Case>
  or use 01-26 |nopqrstuvwxyz|
  to enter A-Z |0123456789-. |
	No letters entered

Save as: "" Pick "A">
  Pick letters |ABCDEFGHIJKLM| Case>
  or use 01-26 |NOPQRSTUVWXYZ|
  to enter A-Z |0123456789-. |
	← **Case** key was pressed

Save as: "" Pick "D">
  Pick letters |ABCDEFGHIJKLM| Case>
  or use 01-26 |NOPQRSTUVWXYZ|
  to enter A-Z |0123456789-. |
	← **"D"** is highlighted (using ←,→, Prev, Next)

Save as: "D" Pick "D">
  Pick letters |ABCDEFGHIJKLM| Case>
  or use 01-26 |NOPQRSTUVWXYZ| Clear>
  to enter A-Z |0123456789-. | Save>
	← **Pick "D"** was pressed

Save as: "Darren" Pick "n">
  Pick letters |abcdefghijklm| Case>
  or use 01-26 |nopqrstuvwxyz| Clear>
  to enter A-Z |0123456789-. | Save>
	← A name has been entered
	
	← **Save** button saves sequence

Replace existing sequence? Yes>
  The sequence "Darren"
  already exists on this instrument.
  Do you wish to replace it? No>
	If a sequence by the same name already exists, a confirmation prompt appears. (However, if an existing sequence was opened, then saved by the same name, this prompt is skipped).

Darren [7] Open>
5>AGCTATG_<3 Close>
  5'>
  ()>
	← **Open** button shows that all changes are saved.

Close Confirmation

Abandon sequence? Yes>
  There are unsaved changes.
  If you close the sequence,
  these changes will be lost. No>
	If **Close** is pressed while there are unsaved changes, the following prompt appears.

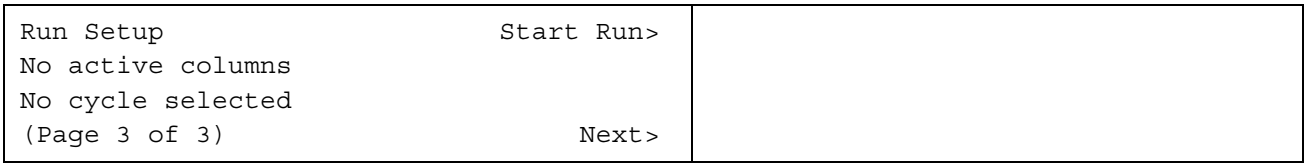
[ ] Submenu inside Edit Sequences Menu

(Untitled) [5 - 5] Paste> 5>AGCT()<3	← The region spans bases 5 through 5 (0 bases)
(Untitled) [5 - 6] Cut> 5>AGCT(A)<3 AGCT/TCGA> AGCT/5678>	← The region spans bases 5 through 6 (1 base) ← A base has been entered between "(" and ")"

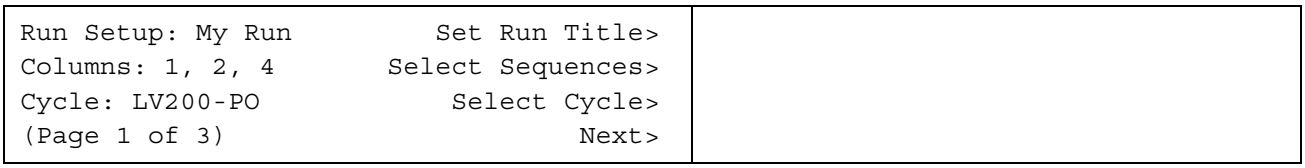
(Untitled) [5 - 7] Cut> 5>AGCT(AT)<3 AGCT/TCGA> AGCT/5678> (AT)>	← Cut is available once there is something to cut ← Complements bases in region ← Translates bases in region; letters ´ numbers ← Inserts an IUB (degeneracy) code at current base position (5)
(Untitled) [4] Save> 5>AGCT_<3 Close> 5'> ()>	The **Cut** key was pressed; we are back to Edit Sequence menu. ← Let's move to the beginning of the sequence
(Untitled) [1 of 4] Save> 5>AGCT<3 Close> 3'> ()>	We are at the beginning of the sequence. ← Let's press the **()** key again.
(Untitled) [1 - 1] Paste> 5>()AGCT<3	← The **Paste** soft key is available after the **()** soft key has been pressed; the region between "(" and ")" does not contain any bases. Let's press it.
(Untitled) [3 of 6] Save> 5>ATAGCT<3 Close> 3'> ()>	The **Paste** key was pressed; we are back to Edit Sequence menu. Bases from the clipboard have been inserted in the beginning of the sequence. ← Let's press **()** once more
(Untitled) [3 - 3] Paste> 5>AT()AGCT<3	We have opened a region; now let us expand it using the > arrow key
(Untitled) [3 - 5] Cut> 5>AT(AG)CT<3 AGCT/TCGA> AGCT/5678> (AG)>	← The region covers bases 3 to (but not including) 5 ← Let's insert the corresponding IUB (degeneracy) code
(Untitled) [4 of 5] Save> 5>ATRCT<3 Close> 3'> ()>	The IUB (degeneracy) code is inserted.

Run Setup Menu (no selections made)

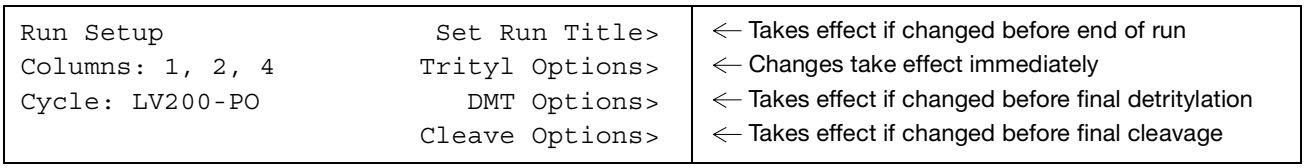
Run Setup Set Run Title> No active columns Select Sequences> No cycle selected Select Cycle> (Page 1 of 3) Next>	 ← Not available during synthesis ← Not available during synthesis
Run Setup Trityl Options> No active columns DMT Options> No cycle selected Cleave Options> (Page 2 of 3) Next>	



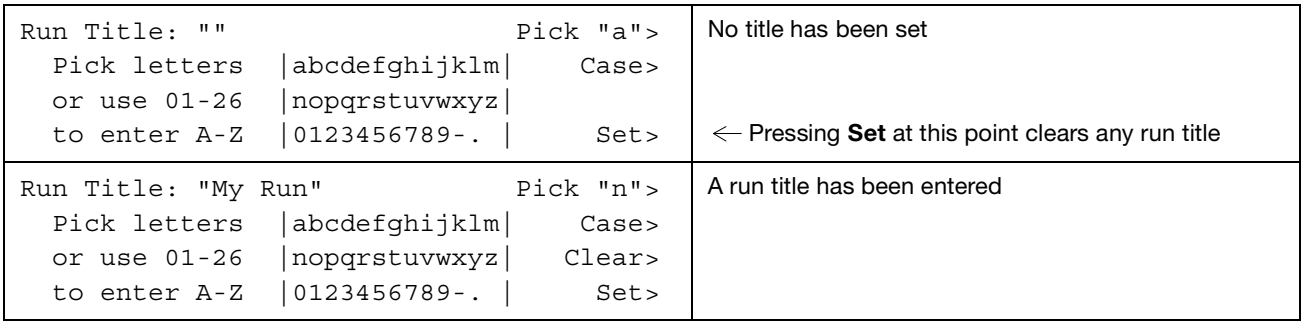
Run Setup Menu (after Run Title, Sequences, and Cycle are selected)



Run Setup Menu (while a synthesis is in progress)

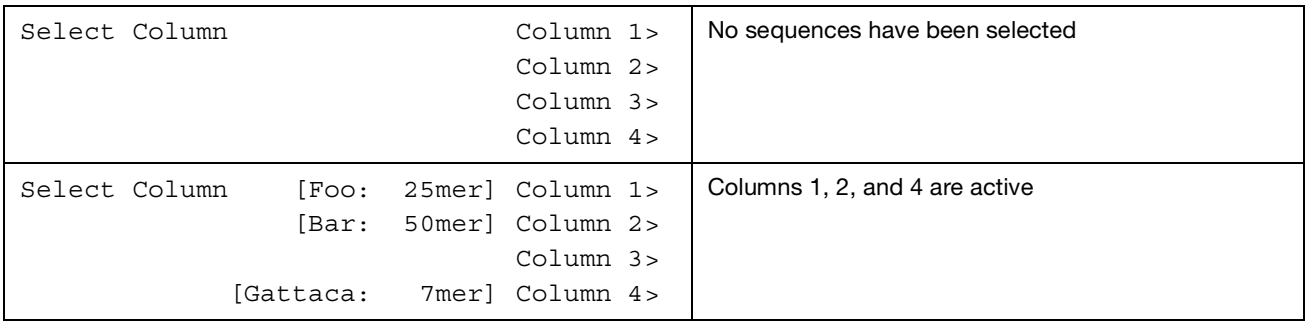


Run Title Menu



Select Column Menu

Invoked by ”Select Sequences” soft key in Run Setup Menu



Select Sequence Menu for a Given Column

Column 1 Bar> Use <Delete> to Degenerated> clear selection Foo> (Page 1 of 2) Next>	Browse through available sequences, or use the Delete key to remove the current selection from a column (make the column inactive).

Select Cycle Menu

Select Cycle LV40-PO> Use <Delete> to LV40-PS> clear selection LV40-RNA> (Page 1 of 4) Next>	Browse through available cycles, or use the Delete key to clear the current selection. If no cycle is selected, the run cannot start.

DMT Removal Options Menu

DMT Removal Options [Yes] Column 1> [Yes] Column 2> Select whether to run [Yes] Column 3> final detritylation [Yes] Column 4>	Pressing any soft key switches corresponding setting between **Yes** and **No**. Setting is saved in instrument; default is **Yes**

Cleavage Options Menu

Cleavage Options [Yes] Column 1> [Yes] Column 2> Select whether to run [Yes] Column 3> final cleavage [Yes] Column 4>	Pressing any soft key switches corresponding setting between **Yes** and **No**. Setting is saved in instrument; default is **Yes**

Trityl Options Menu

Trityl Options [5%] Delivery Threshold> [80%] Yield Threshold>	Trityl Delivery and Yield Monitor thresholds. A ”0” or empty value disables corresponding monitor. Thresholds are saved in instrument; defaults are shown here.

Delivery Threshold Menu

Delivery Threshold Stop delivery once trityl readings approach baseline Clear> within [5]% of peak height Set>	← **Clear** key visible if one or more digits are entered ← Set threshold

Yield Threshold Menu

Yield Threshold Terminate a column if its average stepwise yield falls Clear> below [80]% Set>	← **Clear** key visible if one or more digits are entered ← Set threshold

Prepare Columns Menu

Invoked by ”Start Run” key in ”Run Setup” Menu

Prepare Columns Start> In column location : 1 2 4 Please place column: C G T	
Missing sequences! You have not selected any sequences. In order to start a synthesis run, at least one column must be active.	Shown if no sequences have been selected. Any key will return to Run Setup Menu.
No cycle! You have not selected a cycle. In order to start a synthesis run, you need to do so.	Shown if no cycle has been selected. Any key will return to Run Setup Menu.

Run Status Menu (if no run is in progress)

Run Status Trityl Status> Show Sequences> Print Report> Delete Report>	← Show trityl status from last run ← Show sequences selected for synthesis

Run Status Menu (during a synthesis run)

Run Status: My Run Trityl Status> Base 4 of 50 Pause> Cleaving Abort> ACNToColumn(1,2,4) 41s Next>	← A run title was set (optional) ← Base addition, total # of bases, cycle procedure ← Cycle step; remaining step time
Run Status: My Run Show Sequences> Base 4 of 50 Print Report> Cleaving Delete Report> ACNToColumn(1,2,4) 38s Next>	Second page

Run Status Menu (Hold, Pause or Abort operations selected)

Run Status: My Run Trityl Status> Base 4 of 50 [Hold] Cancel> Cleaving Abort> ACNToColumn(1,2,4) 41s Next>	← Will hold at current step
Run Status: My Run Trityl Status> Base 4 of 50 [Hold] Cancel> Cleaving Abort> ACNToColumn(1,2,4) Holding Next>	← Step time elapsed; still holding

Run Status: My Run Trityl Status> Base 4 of 50 [Pause] Cancel> Cleaving Abort> ACNToColumn(1,2,4) 41s Next>	← Will pause after current step
Run Status: My Run Trityl Status> Base 4 of 50 [Pause at 15] Cancel> Cleaving Abort> ACNToColumn(1,2,4) 41s Next>	← Will pause run before base addition #15
Run Status: My Run Trityl Status> Base 4 of 50 Resume> Cleaving Abort> Paused Next>	← Currently paused ← No current step
Run Status: My Run Trityl Status> Base 4 of 50 [Abort at 15] Cancel> Cleaving Abort> ACNToColumn(1,2,4) 41s Next>	← Will terminate run before base addition #15

Trityl Status Screen (During a Monitored Delivery)

Monitoring Peak 8				22s ← Current base addition; remaining step time
Baseline	4.23	4.12	N/A	N/A ← Baseline reading for each column
Peak	20.41	18.94	N/A	N/A ← Peak reading for each column
Sensors	8.92	6.01	N/A	N/A ← Current sensor readings (updated each second)

Trityl Status Screen (While Not Running or While Not Monitoring Trityls)

Not Monitoring				
PeakArea	220.4	N/A	N/A	N/A ← Sum of readings that are >= 50% of peak delta
Yield	94%	96%	N/A	95% ← Last recorded average step-wise yield
Status	Active	Active	N/A	Done ← Current status

Pause Menu

Pause Menu: My Run Hold> Base 4 of 50 Pause> Cleaving Pause Ahead> ACNToColumn(1,2,4) 41s

Pause Ahead Menu

Pause Ahead Pause at base # 15_ Enter '0' to pause before Clear> DMT removal and/or cleaving Set>

Abort Menu

Abort Menu: My Run Abort immediately> Base 4 of 50 Abort and clean up> Cleaving Abort Ahead> ACNToColumn(1,2,4) 41s	← Only available while Safe Mode is enabled ← Not available if already in cleanup procedure ← Not available if already in cleanup procedure

Abort Ahead Menu

Abort Ahead Abort synthesis at base # 15_ Clear> Set>	

Show Sequences Menu

Sequences [Foo: 25mer] Column 1> [Bar: 50mer] Column 2> [Gattaca: 7mer] Column 4>	

Column *n* Menu (inside Show Sequences Menu)

Column 2: Bar Up> 5>AGC TAG CTA GCT AGC TAG CTA GCT AGC TAG CTA CGT AGC TAG CTA GCT Size=50 MW=15410 Tm=72.0 Down>	

Print Reports Menu

Print Report *Report Name*> *Report Name*> *Report Name*> Next>	
Confirm Print Operation Yes> Are you sure you want to print the run report "*Report Name*"? No>	Upon leaving the Print Report Menu, a confirmation prompt appears.
Run report printed. The run report was successfully sent to the print spool at *printer address*.	Confirmation message
No printer is configured. The run report cannot be printed. Please set up a printer under Configuration -> Network -> Printer	Trying to print while no printer is configured

There is nothing to print!
  The run report you are trying to
  print is empty.
	If there is an empty run report (e.g. an empty file uploaded to the runs directory from a computer)

Unable to print run report: >>*reason*<< Please check printer and settings.	If a printer is configured, but printing failed

Delete Reports Menu

Delete Reports [ ] *Report Name*> <A> = Select All [ ] *Report Name*> <C> = Clear All [ ] *Report Name*> Next>	No reports selected
Delete Reports [X] *Report Name*> <A> = Select All [X] *Report Name*> <C> = Clear All [ ] *Report Name*> Next>	Two reports selected for deletion

Confirm Delete Operation Yes>
  Are you sure you want to delete
  the run report "*Report Name*"?
No>
	Upon leaving the Delete Report Menu, a confirmation prompt appears.
	← A single report was selected

Confirm Delete Operation Yes>
  Are you sure you want to delete
  the *n* selected run reports?
No>
	← Multiple run reports were selected

Delete operation successful.
  The run report "*Report Name*"
  has been removed.
	Single report confirmation message

Delete operation successful.
  The *n* selected run reports
  have been removed.
	Multiple report confirmation message

Edit Cycle Menu

No cycle is open:	
(No Cycle) Open> Print> Delete>	← **Open** key because no unsaved changes ← **Print** key because no cycle is open ← **Delete** key because no cycle is open
A cycle script has been opened, but not yet modified:	
Cycle: LV200-PO Open> Close> Edit Steps> Edit Coupling>	← **Open** key because no unsaved changes

Cycle (steps and/or coupling) has been modified:	
Cycle: LV200-PO Save> [Modified] Close> Edit Steps> Edit Coupling>	← **Save** key because there are changes

Open Cycle Menu

Open Cycle LV40-PO> LV40-PS> LV40-RNA> (Page 1 of 4) Next>	← If custom cycles are added, # of pages increases
Open Cycle LV200-PO> LV200-PS> LV200-RNA> (Page 2 of 4) Next>	
Open Cycle 0.2um-PO> 0.2um-PS> 0.2um-RNA> (Page 3 of 4) Next>	
Open Cycle 1um-PO> 1um-PS> 1um-RNA> (Page 4 of 4) Next>	

Save Cycle Menu

An AB cycle was opened, so the default name is the same:	
Save as: "LV200-PO" Pick "a"> Pick letters |abcdefghijklm| Case> or use 01-26 |nopqrstuvwxyz| Clear> to enter A-Z |0123456789-. | Save>	
Overwriting an AB cycle will fail:	
I am sorry, I cannot let you do that. "LV200-PO" is a read-only cycle, which cannot be modified or deleted.	Any key returns to Edit Cycle Menu.

Close Confirmation Dialog

If "Close" key is pressed while there are changes

```
Abandon current cycle?                Yes>
  Any changes you made
  Will be lost.
                                       No>
```

Print Cycle Menu

Print Cycle *Cycle Name>* *Cycle Name>* *Cycle Name>* (Page 1 of *n*) Next>	← Selecting a cycle prints it immediately.
Cycle printed. The cycle was successfully sent to the print spool at *printer address*.	Confirmation message
No printer is configured. The cycle cannot be printed. Please set up a printer under Configuration -> Network -> Printer	Trying to print while no printer is configured
There is nothing to print! The cycle you are trying to print is empty.	If there is an empty cycle script (e.g. an empty file uploaded to the cycle directory from a computer)
Unable to print cycle: >>*reason*<< Please check printer and settings.	If a printer is configured, but printing failed

Delete Cycle Menu:

Delete Cycle *Cycle Name>* *Cycle Name>* *Cycle Name>* (Page 1 of *n*) Next>	
Confirm Deletion Yes> Are you sure you want to delete the cycle "*Cycle Name*"? No>	Confirmation dialog
Delete operation successful. The cycle "*Cycle Name*" has been removed.	Confirmation message Any key returns to Edit Cycle Menu.
I am sorry, I cannot let you do that. "*Cycle Name*" is a read-only cycle, which cannot be modified or deleted.	If trying to delete an AB cycle

Select Routine Menu – PO + RNA

Invoked by ”Edit Steps” button in Edit Cycle Menu

Select Routine Begin> Detritylate> Prepare> (Page 1 of 3) Next>	← Performed once at beginning of run ← Invoked for each base addition + maybe at end ← Invoked for each base addition
Select Routine Deliver> Couple> Cap> (Page 2 of 3) Next>	← Invoked for each active column each base addition ← Invoked for each base addition ← Invoked for each base addition
Select Routine Oxidize> Cleave> Wash> (Page 3 of 3) Next>	← Invoked for each base addition ← Invoked once at the very end (if selected) ← Invoked by ”Abort and clean up”

Select Routine Menu – PS

Invoked by ”Edit Steps” button in Edit Cycle Menu

Select Routine Begin> Detritylate> Prepare> (Page 1 of 3) Next>	← Performed once at beginning of run ← Invoked for each base addition + maybe at end ← Invoked for each base addition
Select Routine Deliver> Couple> Sulfurize> (Page 2 of 3) Next>	← Invoked for each active column ea base addition ← Invoked for each base addition ← Invoked for each base addition
Select Routine Cap> Cleave> Wash> (Page 3 of 3) Next>	← Invoked for each base addition ← Invoked once at the very end (if selected) ← Invoked by ”Abort and clean up”

Cycle Steps Menu

Invoked by selecting a Cycle Procedure

[*PROCedure $Arguments…*] Change> *->COMMand Arguments…* *COMMand Arguments…* *COMMand Arguments…*	Procedure name and commands are ”SCPI” compliant, and thus capitalized as shown: • Three first letters if the fourth is a vowel • Four first letters if the fourth is a consonant

Edit Cycle Step Menu

Invoked by Change button in Cycle Steps Menu

Edit Cycle Step [*COMMand*] Command> [*Value*] *Argument1*> [*Value*] *Argument2*> Done>	← TRANsfer, MONitor, SLEep, or SAFe ← "Valves", "Step Time", or "Safe Mode", depending on command
Edit Cycle Step [TRANsfer] Command> [Pressure(Amidite,Tet)] Valves> [15] Step Time> Done>	Example screen

Select Command Menu:

Invoked by Command button above, or by Insert key in Cycle Steps Menu

Select Command Transfer> Monitored Transfer> Sleep> Safe Mode>	← TRANsfer *ValveGroups StepTime* ← MONitor *ValveGroups StepTime* ← SLEep *StepTime* ← SAFe Yes or SAFe No

Select Valves Menu:

Invoked after selecting TRANsfer or MONitor, or from Edit Cycle Step Menu if the command is TRANsfer or MONitor

Select Valves Use <Prev> and <Next> to scroll, or enter valve code: _ >*COMMand*	No valve code has been entered yet ← Either "TRANsfer" or "MONitor"
Select Valves Use <Prev> and <Next> to scroll, or enter valve code: 101_ >*COMMand* ReverseFlush($Col) Select>	A valve code has been entered, or located using the **Prev** and **Next** command keys.

Step Time Menu

Invoked after selecting SLEep command, or after the valve code for the TRANsfer or MONitor command, or from the Edit Cycle Step Menu if the command was TRANsfer, MONitor or SLEep

Set Step Time Step Time: _ >*COMMand Arguments*	No step time has been entered yet ← Either "TRANsfer" or "MONitor"

Set Step Time Valve Code: 15_ .> Clear> >*COMMand Arguments* 15 Set>	A step time has been entered

Safe Mode Menu

Invoked after selecting the SAFe command, or from the Edit Cycle Step Menu

Enable safe mode? Yes>
  Select Yes to turn safe mode on.
  Select No to turn safe mode off.
 No>	

Coupling Menu

Invoked by the **Edit Coupling** button in the Cycle Edit Menu

Coupling Menu Tet Delivery Times> Coupling Times>	

Tet Delivery Times Menu

Tet Delivery Times [1.8] Column 1> [2.0] Column 2> [2.2] Column 3> [2.3] Column 4>	

Tet Time: Column *n* Menu

Tet Time: Column *n* Column *n* Tet Time: *n.n* Clear> Set>	

Coupling Times Menu

Coupling Times [30] Default> [30] Base A> [30] Base G> (Page 1 of 3) Next>	← Coupling time for other bases (degeneracies)
Coupling Times [30] Base C> [30] Base T> [300] Base 5> (Page 2 of 3) Next>	

Coupling Times [300] Base 6> [300] Base 7> [300] Base 8> (Page 3 of 3) Next>	

Formula Weights Setup Menu

Formula weights are used for Molecular Weight calculations:

- In sequence printouts
- In run reports
- In the ”Show Sequences” menu inside Run Status

Formula Weight Setup [313.2] Base A> [329.2] Base G> [289.2] Base C> (Page 1 of 3) Next>	
Formula Weight Setup [304.2] Base T> [Not Set] Base 5> [Not Set] Base 6> (Page 2 of 3) Next>	
Formula Weight Setup [Not Set] Base 7> [Not Set] Base 8> (Page 3 of 3) Next>	

Base *X* Formula Weight Menu

Base *X* Formula Weight Please enter the formula weight Of base *X*: *current*_ Clear> Set>	← *X* is A, G, C, T, 5, 6, 7, or 8 ← *current* is a real number, e.g. 313.2 for base A

Configuration Menu

Configuration Reset> Time/Date> Network> Software>	

Reset Menu

Reset Menu Delete All Sequences> Delete Custom Cycles> Delete All Run Reports> (Page 1 of 3) Next>	

Reset Menu Clear Run Settings> Unselect Amidite Bottles> Reset Reagent Bottle Sizes> (Page 2 of 3) Next>	
Reset Menu Reset Formula Weights> Reset Time Zone> Reset Network Configuration> (Page 3 of 3) Next>	

Delete All Sequences Confirmation

Really delete all sequences? Yes> This operation cannot be undone. No>	
Delete operation successful. The *NN* selected sequences have been removed.	

Delete Custom Cycles Confirmation

Really delete custom cycles? Yes> This operation cannot be undone. No>	
Delete operation successful. The *NN* selected cycles have been removed.	

Delete Custom Cycles Confirmation

Really delete all run reports? Yes> This operation cannot be undone. No>	
Delete operation successful. The *NN* selected run reports have been removed.	

Clear Run Settings Confirmation

Really clear run settings? Yes> This operation cannot be undone. No>	

Run settings have been reset. Sequence and cycle selections are cleared. DMT removal, cleavage and trityl options are set to defaults.	
The instrument is currently running! Run settings cannot be cleared until the synthesis run is done.	

Unselect Amidite Bottles Confirmation

Really unselect amidite bottles? Yes> This operation cannot be undone. No>	
Amidite bottles have been unselected. The autodilution procedure will now require bottle sizes to be reselected.	

Reset Reagent Bottle Sizes Confirmation

Really reset reagent bottle sizes? Yes> This operation cannot be undone. No>	
Reagent bottles size has been reset. The Leak Test will now default to using normal size reagent bottles.	

Reset Formula Weights Confirmation

Really reset formula weights? Yes> This operation cannot be undone. No>	
Formula weights have been reset. Any custom formula weights used in molecular weight calculations have been removed.	

Reset Time Zone Confirmation

Really reset time zone? Yes> This operation cannot be undone. No>	

Time zone selection has been reset. The instrument now uses the Factory time zone. Until a new zone is selected, the clock cannot be set.	

Reset Network Configuration Confirmation

Really reset network configuration? Yes> This operation cannot be undone. No>	
Clearing network settings Please wait...	
Network configuration has been reset. Until the instrument is restarted or TCP/IP networking is reenabled, its network operation is suspended.	

Time/Date Menu

Time/Date [Factory] Time Zone> [time.nist.gov] Time Server> [*YYYY-MM-DD hh:mm:ss*] Set Clock>	

Select Timezone Menu

Select Timezone US> [Factory] Canada> Americas> (Page 1 of 5) Next>	← 12 subzones ← 9 subzones ← 121 subzones
Select Timezone Atlantic Ocean> [Factory] Europe> Africa> (Page 2 of 5) Next>	← 11 subzones ← 52 subzones ← 52 subzones
Select Timezone Indian Ocean> [Factory] Asia> Australia> (Page 3 of 5) Next>	← 11 subzones ← 86 subzones ← 21 subzones
Select Timezone Pacific Ocean> [Factory] Posix Format> GMT Offset> (Page 4 of 5) Next>	← 40 subzones ← 45 subzones ← 35 subzones

Select Timezone Factory> [Factory] (Page 5 of 5) Next>	← Unconfigured

Select Timezone Submenu

Example: US

Select Timezone Alaska> US/ Aleutian> Arizona> (Page 1 of 4) Next>	
Select Timezone Central> US/ Eastern> East-Indiana> (Page 2 of 4) Next>	
Select Timezone Hawaii> US/ Indiana-Starke> Michigan> (Page 3 of 4) Next>	
Select Timezone Mountain> US/ Pacific> Samoa> (Page 4 of 4) Next>	

Time (NTP) Server Menu

Time Server Menu Set by host name> Set by IP address> Current Time Server: time.nist.gov	

Time Server By Host Menu

Time Server: "time.nist.gov" Pick "a"> Pick letters |abcdefghijklm| or use 01-26 |nopqrstuvwxyz| Clear> to enter A-Z |0123456789-. | Set>	

Time Server By IP Address Menu:

Time Server Enter Time Server: _ .> Clear> Set>	← Available when applicable ← Available when there is input ← Available when there is a valid IP address

Set Clock Menu

Enter New Date/Time (*Zone*) Year (>2000): [*YYYY*] Hour (0-23): [*hh*] Month (1-12): [*MM*] Minute (0-59): [*mm*] Day (1-31): [*DD*] Second (0-59): [*ss*]	When just entering, no buttons are visible, and display is being updated with current time.
Enter New Date/Time (*Zone*) Set Clock> Year (>2000): [20_] Hour (0-23): [*hh*] Month (1-12): [*MM*] Minute (0-59): [*mm*] Day (1-31): [*DD*] Second (0-59): [*ss*]	← **Set Clock** button is available after valid input is received.

Setting the Clock without a Time Zone

Cannot set clock without timezone! In order to set the clock, you must first select a time zone.	

Network Configuration Menu:

Network [Not Set] Host Name> [*XX*:*XX*:*XX*:*XX*:*XX*:*XX*] Ethernet> [192.168.2.100] TCP/IP> (Page 1 of 2) Next>	
Network [None] Mail Host> [None] Log Host> [None] Printer> (Page 2 of 2) Next>	

Host Name Menu

Host Name: "" Pick "a"> Pick letters |abcdefghijklm| or use 01-26 |nopqrstuvwxyz| to enter A-Z |0123456789-. | Set>	No host name configured
Host Name: "*hostname*" Pick "a"> Pick letters |abcdefghijklm| or use 01-26 |nopqrstuvwxyz| Clear> to enter A-Z |0123456789-. | Set>	A host name is entered

Ethernet Status Menu

Ethernet Status: No Link Refresh> Link Speed : Autonegotiation: No MAC Address : *XX*:*XX*:*XX*:*XX*:*XX*:*XX*	Instrument has no ethernet connection 6 hexadecimal numbers, 00 – FF

Ethernet Status: Link OK Refresh> Link Speed : 100baseTx-FD Autonegotiation: Yes MAC Address : *XX*:*XX*:*XX*:*XX*:*XX*:*XX*	Instrument has ethernet connection

TCP/IP Menu

TCP/IP Settings – Automatic Change> IP Address : 167.116.10.15 Netmask : 255.255.255.0 Gateway : 167.116.10.1 Next>	Status is ”Automatic”, ”Inactive”, ”Manual”, ”Disabled”
TCP/IP Settings – DNS Change> DNS Server 1 : 167.116.75.3 DNS Server 2 : 167.116.75.4 DNS Suffix : pe-c.com Next>	
TCP/IP Settings – WINS WINS Server 1: 167.116.77.153 WINS Server 2: Workgroup : ABIPRISM Next>	

IP Mode Menu (invoked by Change key in TCP/IP Menu)

Select IP Configuration [*] Automatic> Manual> Disabled>	← [*] denotes current mode

IP Config Menu (invoked by Manual key in IP Mode Menu)

IP Config [192.168.2.100] Address> [255.255.255.0] Netmask> [192.168.2.1] Gateway> (Page 1 of 3) Next>	
IP Config [192.168.2.1] First DNS> [None] Second DNS> [None] DNS Suffix> (Page 2 of 3) Next>	
IP Config [None] First WINS> [None] Second WINS> [ABIPRISM] Workgroup> (Page 3 of 3) Next>	

IP Address Menu

Invoked by Address key in IP Config Menu

IP Address
  Enter IP Address: 192.168.2.2_
  Clear>
  Set>	

Netmask Menu

Netmask
  Enter Netmask: 255.255.255.0_
  Clear>
  Set>	

Gateway Menu

Netmask
  Enter Gateway: 192.168.2.1_
  Clear>
  Set>	

Note: First DNS, Second DNS, First WINS, and Second WINS menus follow similar format.

DNS Suffix Menu

DNS Suffix: "applera.net" Pick "t">
  Pick letters |abcdefghijklm|
  or use 01-26 |nopqrstuvwxyz| Clear>
  to enter A-Z |0123456789-. | Set>	

Workgroup Menu

DNS Suffix: "ABIPRISM" Pick "M">
  Pick letters |ABCDEFGHIJKLM|
  or use 01-26 |NOPQRSTUVWXYZ|
  to enter A-Z |0123456789-. | Set>	

IP Configuration Confirmation Prompt

Use automatic network settings? Yes> Do you wish to use the dynamic host configuration protocol to update the instrument network settings? No>	Displayed when user selects ”Automatic” IP config
Apply manual IP Configuration? Yes> Do you wish to update the instrument Network settings using this static IP Configuration? No>	Displayed once user exits ”Manual” config menu

Disable instrument networking? Yes> Do you wish to disable TCP/IP networking on this instrument?	Displayed when user selects ”Disabled”

Mail Host Menu

Mail Host Menu Set by host name> Set by IP address> No Mail Host is configured	No mail (SMTP) host is configured
Mail Host Menu Set by host name> Set by IP address> Current Mail Host: *SMTPserver*	A mail host is configured

Syslog Host Menu

Syslog Host Menu Set by host name> Set by IP address> No Syslog Host is configured	No log (Syslog) host is configured
Syslog Host Menu Set by host name> Set by IP address> Current Syslog Host: *syslog-host*	A log host is configured

Printer Menu

Printer Menu Set by host name> Set by IP address> No Printer is configured	No printer is configured
Syslog Host Menu Set by host name> Set by IP address> Current Printer: *printer*	A printer is configured

Set by Host Name Menu

Invoked by ”Set by host name” key in Time Server, Mail Host, Syslog Host, and Printer Menus

Host Type: "" Pick "a"> Pick letters |abcdefghijklm| or use 01-26 |nopqrstuvwxyz| to enter A-Z |0123456789-. | Set>	*Host Type* is ”Time Server”, ”Mail Host”, ”Syslog Host”, or ”Printer”

Host Type: "hostname" Pick "a"> Pick letters |abcdefghijklm| or use 01-26 |nopqrstuvwxyz| Clear> to enter A-Z |0123456789-. | Set>	A host name is entered

Set by IP Address Menu

Invoked by ”Set by host name” key, as above

Host Type Enter *Host Type*: _ Set>	*Host Type* is ”Time Server”, ”Mail Host”, ”Syslog Host”, or ”Printer” ← With no address, Set will unset/erase the entry
Host Type Enter *Host Type*: *nnn.nnn* .> Clear>	An address is partially entered ← Available when applicable ← Available when there is something to clear
Host Type Enter *Host Type*: *nnn.nnn.nnn.nnn* Clear> Set>	A complete/valid address is entered ← Available when the entry is valid

Software Version Menu

Invoked by Software key in the Configuration Menu

Software Version Details> AB 3400 DNA Synthesizer 1.0	

Details Menu inside Software Version Menu

Single Board Computer User Interface : 1.0 Instrument Control: 1.0 (Page 1 of 6) Next>	
Power Distribution Board Boot Code : 0.51 Application : 0.31 (Page 2 of 6) Next>	
LCD/Keypad Interface Board Boot Code : 0.51 Application : 0.3 (Page 3 of 6) Next>	
Valve Driver Board #1 Boot Code : 0.51 Application : 0.5 (Page 4 of 6) Next>	

Valve Driver Board #2 Boot Code : 0.51 Application : 0.5 (Page 5 of 6) Next>	
Valve Driver Board #3 Boot Code : 0.51 Application : 0.5 (Page 6 of 6) Next>	

Autodilution Menu (No Bottle Sizes Selected)

Autodilution Bottle A> No bottles selected Bottle G> Bottle C> (Page 1 of 3) Next>	
Autodilution Bottle T> No bottles selected Bottle 5> Bottle 6> (Page 2 of 3) Next>	
Autodilution Bottle 7> Bottle 8> (Page 3 of 3) Next>	

Autodilution Menu (Bottles Selected)

Autodilution [1.0g/11.20mL] Bottle A> 5 bottles Bottle G> [0.5g/ 5.90mL] Bottle C> (Page 1 of 3) Next>	← Bottle A size + ACN delivery volume (11.2 mL/g) ← Bottle G size + ACN delivery volume (11.6 mL/g) ← Bottle C size + ACN delivery volume (11.8 mL/g)
Autodilution [2.0g/26.40mL] Bottle T> 5 bottles [12.34mL] Bottle 5> Bottle 6> (Page 2 of 3) Next>	← Bottle G size + ACN delivery volume (13.2 mL/g) ← Bottles 5678 show ACN delivery volume only
Autodilution Bottle 7> 5 bottles [43.21mL] Bottle 8> Start> (Page 3 of 3) Next>	← **Start** button is available if 1+ bottles are selected

Select bottle *N* size Menu

Invoked by Bottle A, Bottle G, Bottle C, and Bottle T buttons above

Select Bottle *N* Size [None]> 0.5 g> 1.0 g> 2.0 g>	

Bottle *N* Delivery Volume Menu

Bottle *N* Delivery Volume ACN delivery volume: _ mL Set>	No volume entered
Bottle *N* Delivery Volume ACN delivery volume: 12.34_mL Clear> Set>	Volume entered

Autodilution Run Menu (While Not Running)

Invoked by Start button in Autodilution Menu

Autodilution: Preparation Start> Please keep the old bottles in the instrument until you Prev> are prompted to remove them. Next>	
Autodilution: Bottle A Start> Please keep the old bottles in the instrument until you Prev> are prompted to remove them. Next>	
… one page for each selected bottle …	
Autodilution: Cleanup Start> Please keep the old bottles in the instrument until you Prev> are prompted to remove them. Next>	

Autodilution Run Menu (While Running)

After each stage/bottle is complete, the procedure automatically advances to the next bottle – finally to the Cleanup stage.

Autodilution: *Stage* Stop> *Valve Operation* *NN*s Hold>	*Stage* is ”Preparation”, ”Bottle *X*”, or ”Cleanup” *NN* is remaining step time in seconds

Autodilution Run Menu (Holding)

Autodilution: *Stage* Stop> *Valve Operation* Continue>	*Stage* is ”Preparation”, ”Bottle *X*”, or ”Cleanup”

Autodilution Run Menu (Paused for New Bottle)

Autodilution: Bottle *N* Stop> Remove old amidite bottle, Continue> wipe line with a lint free tissue, and insert new bottle.	

Autodilution Run Menu (Done)

Autodilution: Cleanup Start> Prev> Procedure Completed :-) Next>	May get nixed by product test. Oh well.

Bottle Change Menu (While Not Running)

Bottle Change: A Start> Please keep the old bottle in the instrument until you Prev> are prompted to remove it. Next>	
Repeat above for bottles: A, G, C, T, 5, 6, 7, 8, Tet, Ammonia, CapA, CapB, Iodine, Aux, TCA, CAN, DCM	

Bottle Change Menu (While Running)

Bottle Change: *Bottle* Stop> *Valve Operation* *NN*s Hold>	*NN* is remaining step time (in seconds)

Bottle Change Menu (Paused for Bottle Change)

Bottle Change: *Bottle* Stop> Remove the old bottle, Continue> wipe line with a lint free tissue, and insert new bottle.	

Bottle Change Menu (Done)

Bottle Change: *Bottle* Start> Prev> Procedure Completed :-) Next>	

Shut Down Menu (While Not Running)

Shut Down Start> Please keep the old bottles in the instrument until you are prompted to remove them.	

Shut Down Menu (While Running)

Shut Down Stop> *Valve Operation* *NN*s Hold>	*NN* is remaining step time (in seconds)

Shut Down Menu (Paused for Bottle Change)

Shut Down Stop> Remove old bottles, wipe Continue> line with a lint free tissue, and insert clean, dry bottles.	

Shut Down Menu (Done)

Shut Down Start> Procedure Completed	

Manual Control Menu

Manual Control *NN.NN* <------ Pressure> Valves: _ Open Valves: [None] Close All>	Displayed upon entering the Manual Control Menu. *NN.NN* is a real-time pressure sensor reading.
Manual Control *NN.NN* <- Conductivity1> Valves: _ Open Valves: [None] Close All>	← The first soft key switches between the 5 different sensors: Pressure, Conductivity1 – Conductivity4.
Manual Control *NN.NN* <--- *Sensor Name*> Valves: 12_ Add> Open Valves: [None] Remove> Close All>	← A valve number has been entered. It can be added to or removed from the list of valves to be opened or closed.
Manual Control *NN.NN* <--- *Sensor Name*> Valves: 12,_ Open> Open Valves: [None] Close> Close All>	← Add key inserts a comma, and makes Open and Close keys available.
Manual Control *NN.NN* <--- *Sensor Name*> Valves: 12,1_ Add> Open Valves: [None] Remove> Close All>	← Another digit was entered; soft keys switch back to Add/Remove.
Manual Control *NN.NN* <--- *Sensor Name*> Valves: 12,FlushTo(C,Tet)_ Add> Open Valves: [None] Remove> Close All>	← A valve code (”123”) was entered. To scroll through available valve operations, use the Prev and Next command keys.

Manual Control *NN.NN <--- Sensor Name*> Valves: 12,FlushTo(C,Tet), Open> Open Valves: [None] Close> Close All>	← Add key was pressed, soft keys are Open/Close.
Manual Control *NN.NN <--- Sensor Name*> Valves: 12,FlushTo(C,Tet) Open> Open Valves: 12,33,35,36,37,41 Close> 44,53 Close All>	← **Open** key was pressed
Manual Control *NN.NN <--- Sensor Name*> Valves: ReverseFlush() Add> Open Valves: 12,33,35,36,37,41 Remove> 44,53 Close All>	← Another valve code (”101”) was entered (or the **Prev/Next** keys were used to locate a desired valve operation).
Manual Control *NN.NN <--- Sensor Name*> Valves: ReverseFlush(1,2) Add> Open Valves: 12,33,35,36,37,41 Remove> 44,53 Close All>	← ”12” was entered to select columns 1 and 2
Manual Control *NN.NN <--- Sensor Name*> Valves: ReverseFlush(1,2) Open> Open Valves: 12,33,35,36,37,41 Close> 44,53 Close All>	← **Add** key was pressed
Manual Control *NN.NN <--- Sensor Name*> Valves: ReverseFlush(1,2) Open> Open Valves: 1,5,12,17,18,26 Close> 33,35,36,37,41,44,53 Close All>	← **Open** key was pressed
Manual Control *NN.NN <--- Sensor Name*> Valves: ReverseFlush(1,2) Open> Open Valves: 12,33,35,36,37,41 Close> 44,53 Close All>	← **Close** key was pressed; ReverseFlush(1,2) closed
Manual Control *NN.NN <--- Sensor Name*> Valves: Open Valves: [None] Close All>	← **Close All** key was pressed.

Diagnostics Menu

Diagnostics Display Test> Keypad Test> Conductivity Test> (Page 1 of 4) Next>	
Diagnostics Ventilation Test> Leak Test> Regulator Test> (Page 2 of 4) Next>	

Diagnostics Ext. Bottles Test> Drip Test> Flow Measurement> (Page 3 of 4) Next>	
Diagnostics Autodilution Setup> Flush Lines> Final Leak Test> (Page 4 of 4) Next>	

Display Test

	Any key returns to Diagnostics Menu

Keypad Test menu

Keypad Test Press each key on the keypad, until every has been tested. Use MainMenu + PrevMenu to abort.	Press any key to enter the test
S1 MainMenu 7 8 9 A S2 PrevMenu 4 5 6 G S3 Prev 1 2 3 C S4 Left Next Right 0 Ins Del T	
S1 <MainMenu> 7 8 9 A S2 PrevMenu 4 5 6 G S3 Prev 1 2 3 C S4 Left Next Right 0 Ins Del T	**MainMenu** key is pressed (but not yet released)
S1 7 8 9 A S2 PrevMenu 4 5 6 G S3 Prev 1 2 3 C S4 Left Next Right 0 Ins Del T	**MainMenu** key is released
S1 7 8 9 A S2 PrevMenu 4 5 6 G S3 <Prev> 1 2 3 C S4 Left <Next> Right 0 Ins Del T	**Prev** and **Next** keys are pressed simultaneously
S4	All keys except **S4** have been pressed and released.
Keypad test completed. All keys passed.	All keys were pressed and released.

Keypad test aborted. Not every key was pressed.	**MainMenu** and **PrevMenu** were pressed simultaneously; test exits.

Conductivity Test Menu

Conductivity Test Start> Idle	Upon entering
Conductivity Test Stop> *Valve Operation* *NN*s Hold>	While running, before Iodine is delivered ← *NN* = remaining step time
Conductivity Test Stop> *Valve Operation* *NN*s Hold> Sensor: Cond1 Cond2 Cond3 Cond4 Value : *nn.nn* *nn.nn* *nn.nn* *nn.nn*	While running, during Iodine delivery ← *NN* = remaining step time ← *nn.nn* = conductivity sensor 1-4 readings
Conductivity Test Stop> *Valve Operation* *NN*s Hold> Min: *nn.nn* *nn.nn* *nn.nn* *nn.nn* Max: *nn.nn* *nn.nn* *nn.nn* *nn.nn*	While running, after Iodine delivery ← Minimum sensor readings ← Maximum sensor readings
Conductivity Test Start> Min: *nn.nn* *nn.nn* *nn.nn* *nn.nn* Max: *nn.nn* *nn.nn* *nn.nn* *nn.nn*	Test completed

Ventilation Test Menu

Upon Entering	
Test 3a: Phosphoramidite Bottles Start> Prev> Idle Next>	
While running first test:	
Test 3a: Phosphoramidite Bottles Stop> Initial Pressure : *nn.nn* *NN*s Hold> Valves 37,36,27,28,41	First Stage ← *nn.nn*=pressure reading; *NN*=remaining step time
Test 3a: Phosphoramidite Bottles Stop> Initial Pressure : *nn.nn* Final Pressure : *nn.nn* Valves 37,36,27,28,41 - 37 + 44	Second Stage
First test Completed	

Test 3a: Phosphoramidite Bottles Start> Initial Pressure : *nn.nn* Final Pressure : *nn.nn* Prev> Test Result : *Result* Next>	← Result is "**Pass**" or "**Fail**"
Last stage of remaining tests	
Test 3b: Ammonia Bottle Stop> Initial Pressure : *nn.nn* Final Pressure : *nn.nn* Valves 45,25,36,41 - 45 + 46	

Leak Test

Upon Entering	
Test 4: Valve Block Test Start> Prev> Idle Next>	
Test 5: Amidite Bottles Start> [Normal] Capacity> Prev> Idle Next>	← **Capacity** keys switches Normal ´ Extended
Test 6: Tetrazole Bottle Start> [Normal] Capacity> Prev> Idle Next>	
Test 7: Ammonia Bottle Start> Prev> Idle Next>	
Test 8: Cap A/Cap B Bottles Start> [Normal] Capacity> Prev> Idle Next>	
Test 9: Auxillary Bottle Start> [Normal] Capacity> Prev> Idle Next>	
Test 10: Iodine Bottle Start> [Normal] Capacity> Prev> Idle Next>	

Test 11: Chlorinated Waste + TCA Start> Prev> Idle Next>	
Test 12: Chlorinated Waste + DCM Start> Prev> Idle Next>	
Test 13: Non-Chlor'd Waste + ACN Start> Prev> Idle Next>	
While running the first test:	
Test 4: Valve Block Test Stop> Initial Pressure : *nn.nn* *NN*s Hold> Final Pressure : *nn.nn* Valves 17,18,19,20,36,41 (37)	← *nn.nn*=pressure reading; *NN*=remaining step time
First test completed	
Test 4: Valve Block Test Start> Initial Pressure : *nn.nn* Final Pressure : *nn.nn* Prev> Drop (max=0.12) : *Result* Next>	← Result is ”Pass”, ”Fail”, or ”Huh?” (if final > initial)

Regulator Test

Upon Entering	
Test 14a: Regulator Gauge 1 Start> Prev> Idle Next>	
Test 14b: Regulator Gauge 2 Start> Prev> Idle Next>	
Test 14c: Regulator Gauge 3 Start> Prev> Idle Next>	
While running the first test:	
Test 14a: Regulator Gauge 1 Stop> Vent Pressure : *nn.nn* *NN*s Hold> Pressure Reading : *nn.nn* Valves 31,36,41,43	← *nn.nn*=pressure reading; *NN*=remaining step time

sFirst test completed	
Test 14a: Regulator Gauge 1 Start> Vent Pressure : *nn.nn* Pressure Reading : *nn.nn* Prev> Compare pressure w/gauge #1 Next>	

External Bottles Test

While not running	
Test 15a: ACN Bottle Connection Start> Prev> Next>	
Test 15b: DCM Bottle Connection Start> Prev> Next>	
Test 15c: TCA Bottle Connection Start> Prev> Next>	
While running the first test:	
Test 15a: ACN Bottle Connection Stop> Valve(s): 50 You should now detect argon Prev> blowing from the delivery line. Next>	

Drip Test

While not running	
Test 16a: ACN to A Start> Prev> Next>	
Test 16b: ACN to G Start> Prev> Next>	
Test 16c: ACN to C Start> Prev> Next>	

Test 16d: ACN to T Start> Prev> Next>	
Test 16e: ACN to 5 Start> Prev> Next>	
Test 16f: ACN to 6 Start> Prev> Next>	
Test 16g: ACN to 7 Start> Prev> Next>	
Test 16h: ACN to 8 Start> Prev> Next>	
Test 16i: ACN to Tetrazole Start> Prev> Next>	
Test 16j: ACN to Ammonia Start> Prev> Next>	
Test 16k: ACN to CAP A Start> Prev> Next>	
Test 16l: ACN to CAP B Start> Prev> Next>	
Test 16m: ACN to Auxillary Start> Prev> Next>	

Drip Test (Continued)

Test 16n: ACN to Iodine Start> Prev> Next>	
Test 16o: ACN to DCM Start> Prev> Next>	
Test 16p: ACN to TCA Start> Prev> Next>	
Test 16q: ACN to Waste (Halogen) Start> Prev> Next>	
Test 16r: ACN to Column + Waste Start> Prev> Next>	
Test 16s: ACN to Waste (Chlor.) Start> Prev> Next>	
While running the first test	
Test 16a: ACN to A Stop> Valves: 50,38,36,31 ACN should now be coming out the Prev> A bottle only. Next>	

Flow Measurement Test Menu

Upon entering	
Test 17a: Aux to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 17b: Aux to Column 2 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	

Test 17c: Aux to Column 3 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 17d: Aux to Column 4 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 18: A to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 19: Ammonia to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20a: G to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20b: C to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20c: T to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20d: 5 to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20e: 6 to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20f: 7 to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	

Test 20g: 8 to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	

Flow Measurement Test Menu (Continued)

Test 20h: Tetrazole to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20i: Cap A to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20j: Cap B to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20k: Aux to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20l: Iodine to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20m: ACN to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20n: DCM to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 20o: TCA to Column 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 21a: Ammonia to Vial 1 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	

Test 21b: Ammonia to Vial 2 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 21c: Ammonia to Vial 3 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 21d: Ammonia to Vial 4 Start> Prepare all bottles for test, Prev> press Start to prime the lines Next>	

Flow Measurement Test Menu (Continued)

While running the first test	
Test 17a: Aux to Column 1 Stop> Valves: 48,23,17 *NN*s Hold> Priming lines; please wait	First stage ← *NN* = remaining step time
Test 17a: Aux to Column 1 Stop> Continue> Tare the balance to zero, then press Continue.	Paused while user sets up scale
Test 17a: Aux to Column 1 Stop> Valves: 48,23,17 *NN*s Hold> Delivering flow to column	Second stage ← *NN* = remaining step time
Test 17a: Aux to Column 1 Start> Repeat> You can now measure the fluid. Prev> The weight should be 1.35 - 1.44 g Next>	First Test Completed

Weight Specifications for Remaining Flow Measurement Tests

Test 17a: Aux to Column 1	1.35 – 1.44 g
Test 17b: Aux to Column 2	1.35 – 1.44 g
Test 17c: Aux to Column 3	1.35 – 1.44 g
Test 17d: Aux to Column 4	1.35 – 1.44 g
Test 18: A to Column 1	1.14 – 1.22 g
Test 19: Ammonia to Column 1	1.95 – 2.10 g
Test 20a: G to Column 1	1.14 – 1.22 g
Test 20b: C to Column 1	1.14 – 1.22 g
Test 20c: T to Column 1	1.14 – 1.22 g
Test 20d: 5 to Column 1	1.14 – 1.22 g
Test 20e: 6 to Column 1	1.14 – 1.22 g
Test 20f: 7 to Column 1	1.14 – 1.22 g
Test 20g: 8 to Column 1	1.14 – 1.22 g
Test 20h: Tetrazole to Column 1	1.14 – 1.22 g
Test 20i: Cap A to Column 1	1.30 – 1.46 g
Test 20j: Cap B to Column 1	1.30 – 1.46 g
Test 20k: Aux to Column 1	1.30 – 1.46 g
Test 20l: Iodine to Column 1	1.30 – 1.44 g
Test 20m: ACN to Column 1	1.30 – 1.44 g
Test 20n: DCM to Column 1	1.30 – 1.44 g
Test 20o: TCA to Column 1	1.30 – 1.44 g
Test 21a: Ammonia to Vial 1	2.60 – 3.10 g
Test 21b: Ammonia to Vial 2	2.60 – 3.10 g
Test 21c: Ammonia to Vial 3	2.60 – 3.10 g
Test 21d: Ammonia to Vial 4	2.60 – 3.10 g

Autodilution Setup Menu

While not running	
Test 22a: ACN to bottle A Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	← Autodilution flow rate was set in manufacturing

Test 22b: ACN to bottle G Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22c: ACN to bottle C Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22d: ACN to bottle T Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22e: ACN to bottle 5 Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22f: ACN to bottle 6 Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22g: ACN to bottle 7 Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
Test 22h: ACN to bottle 8 Start> Last recorded weight: *n.nnn* g Prepare all bottles for test, Prev> press Start to prime the lines Next>	
While running the first test:	
Test 22a: ACN to bottle A Stop> Valves: 50,38,36,31 *NN*s Hold> Priming lines; please wait	First stage ← *NN* = remaining step time
Test 22a: ACN to bottle A Stop> Continue> Tare the balance to zero, then press Continue.	Prompt for user to reset scale
Test 22a: ACN to bottle A Stop> Valves: 50,38,36,31 *NN*s Hold> Delivering flow to column	Second stage

Test 22a: ACN to bottle A Start> Repeat> Measure the fluid, and enter Prev> its weight: *n.nnn*_g Next>	Test completed; prompt for flow rate ← Edit the last recorded flow rate

Flush Lines Menu

Upon entering	
Test 23a: Block Flush Start> Prev> Idle Next>	
While running (once each test completes, the next one is invoked automatically):	
Test 23a: Block Flush Stop> 30s Hold> Prev> Valves: 37,36,26 Next>	
Test 23b: Flush to Column 1 Stop> 30s Hold> Prev> Valves: 37,36,17,4 Next>	
Test 23c: Flush to Column 2 Stop> 30s Hold> Prev> Valves: 37,36,18,8 Next>	
Test 23d: Flush to Column 3 Stop> 30s Hold> Prev> Valves: 37,36,19,12 Next>	
Test 23e: Flush to Column 4 Stop> 30s Hold> Prev> Valves: 37,36,20,16 Next>	
Test 23f: Flush Block 1 Stop> 30s Hold> Prev> Valves: 1,4 Next>	
Test 23g: Flush Block 2 Stop> 30s Hold> Prev> Valves: 5,8 Next>	

Test 23h: Flush Block 3 Stop> 30s Hold> Prev> Valves: 9,12 Next>	
Test 23i: Flush Block 4 Stop> 30s Hold> Prev> Valves: 13,16 Next>	
Test 23j: Flush Vial 1 Stop> 30s Hold> Prev> Valves: 1,2 Next>	
Test 23k: Flush Vial 2 Stop> 30s Hold> Prev> Valves: 5,6 Next>	
Test 23l: Flush Vial 3 Stop> 30s Hold> Prev> Valves: 9,10 Next>	

Flush Lines Menu (Continued)

Test 23m: Flush Vial 4 Stop> 30s Hold> Prev> Valves: 13,14 Next>	
Test 23n: Flush to A Stop> 30s Hold> Prev> Valves: 44,41,36,31,53 Next>	
Test 23o: Flush to G Stop> 30s Hold> Prev> Valves: 44,41,36,32,53 Next>	
Test 23p: Flush to C Stop> 30s Hold> Prev> Valves: 44,41,36,33,53 Next>	
Test 23q: Flush to T Stop> 30s Hold> Prev> Valves: 44,41,36,34,53 Next>	

Test 23r: Flush to 5 Stop> 30s Hold> Prev> Valves: 44,41,36,27,53 Next>	
Test 23s: Flush to 6 Stop> 30s Hold> Prev> Valves: 44,41,36,28,53 Next>	
Test 23t: Flush to 7 Stop> 30s Hold> Prev> Valves: 44,41,36,29,53 Next>	
Test 23u: Flush to 8 Stop> 30s Hold> Prev> Valves: 44,41,36,30,53 Next>	
Test 23v: Flush to Tet Stop> 30s Hold> Prev> Valves: 37,36,35 Next>	
Test 23w: Flush to Ammonia Stop> 30s Hold> Prev> Valves: 37,36,25,46 Next>	
Test 23x: Flush to CapA Stop> 30s Hold> Prev> Valves: 37,36,21 Next>	
Test 23y: Flush to CapB Stop> 30s Hold> Prev> Valves: 37,36,22 Next>	
Test 23z: Flush to Aux Stop> 30s Hold> Prev> Valves: 37,36,23 Next>	

Flush Lines Menu (Continued)

Test 23A: Flush to Iodine Stop> 30s Hold> Prev> Valves: 37,36,24 Next>	

Test 23B: Flush to ACN Stop> 30s Hold> Prev> Valves: 37,38 Next>	
Test 23C: Flush to DCM Stop> 30s Hold> Prev> Valves: 37,39 Next>	
Test 23D: Flush to TCA Stop> 30s Hold> Prev> Valves: 37,40 Next>	

Final Leak Test Menu

Upon Entering	
Test 24a: Valve Block + Columns Start> Prev> Idle Next>	
While running the first test	
Test 24a: Valve Block + Columns Stop> Initial Pressure : *nn.nn* 10s Hold> Valves 17,18,19,20,36,37,41	First Stage
Test 24a: Valve Block + Columns Stop> Initial Pressure : *nn.nn* 10s Hold> Final Pressure : *nn.nn* Valves 17,18,19,20,36,41 (37)	Second Stage
After completing the first test.	
Test 24a: Valve Block + Columns Start> Initial Pressure : *nn.nn* Final Pressure : *nn.nn* Prev> Test Result : *Result* Next>	← Result is ”Pass” or ”Fail”.

Maintaining the 3400 DNA Synthesizer

C

This appendix covers:

Column Flow Restrictors

Reagents flow to the column through the lower luer. Underneath the lower luer is a cylindrical glass flow restrictor. Reagents flow through a small channel in the flow restrictor to the column. Small amounts of reagents can crystallize in the flow restrictor and, over time, may block the flow. This problem may be magnified if reagents other than Applied Biosystems reagents are used.

When to Clean

To prevent clogging, clean the restrictors once a month. Keep sufficient matched sets of restrictors on hand to allow necessary cleaning of each set.

Equipment Required

You may need the following equipment for this procedure:

√	Item	Vendor	Part Number
	Canned compressed air	Major Laboratory Supplier (MLS)	–
	Methanol	MLS	–
	Sonicator	MLS	–
	Glass flow restrictors (6/package)	Applied Biosystems	602190

Removing the Flow Restrictors from the Column

To remove the flow restrictors from the column:

1.	Unscrew the bottom luer from the column.
2.	Locate the flow restrictor and remove it.

Cleaning the Flow Restrictors

Try any one of the following methods:

1. Blow the flow restrictor with canned compressed air.
2. Boil the flow restrictors in water for 15 min, then sonicate in methanol for 15 min.

WARNING **CHEMICAL HAZARD**. **Methanol** is a flammable liquid and vapor. Exposure causes eye and skin irritation, and may cause central nervous system depression and nerve damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

3. If neither method 1 or 2 works, purchase new flow restrictors.

Fuses

The 3400 DNA Synthesizer is shipped with the fuses installed in the power inlet module fuse holder.

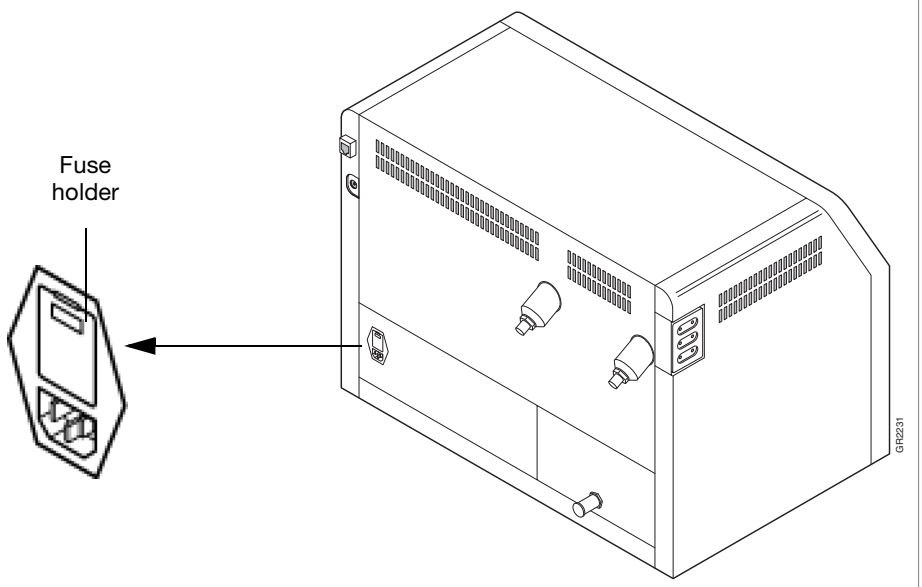
When to Replace

Although the fuses have a long life, they can stop operating. For example, if the instrument has no power (that is, the fan is not working or the LCD screen is blank), a bad fuse may be causing the problem.

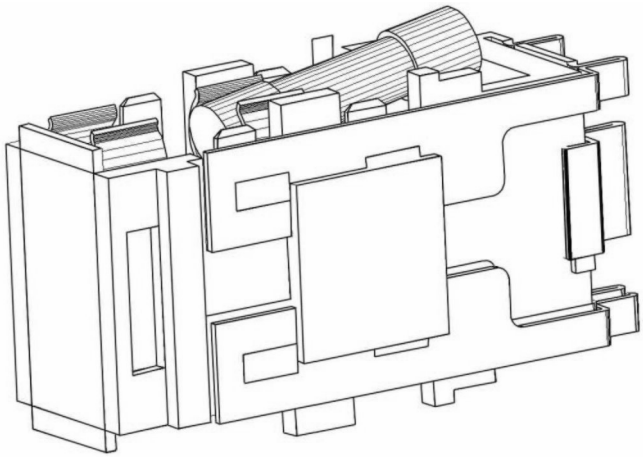
IMPORTANT! Before concluding there is a bad fuse, make sure that the instrument is properly connected to a functional power source.

Replacing a Fuse

To replace a fuse:

1.	Locate the fuse holder on the power inlet module. 
2.	Remove the old fuse.

To replace a fuse: ***(continued)***

3.	Insert the new fuse into the fuse holder so that the back end of the fuse slightly protrudes, as shown below.  **Note:** This allows the fuse to contact the terminal inside the power entry module.
4.	If the instrument still fails to power on, contact an Applied Biosystems service engineer.

Parts List

D

This appendix covers:

3400 DNA Synthesizer Hardware

Instrument

Item	Quantity	Part Number
Applied Biosystems 3400 DNA Synthesizer	1	4334667

Bottle Assemblies

Item	Quantity	Part Number
Assembly, insert tetrazole	1	602184
Assembly, insert ammonium hydroxide	1	602186
Assembly, insert capping	1	602187
Assembly, insert iodine	1	602188
Assembly, insert auxiliary	1	4337118
4-mL oligo collection vials with caps	10/ package	400048
Jerry can, 6-L	1	4304141
Jerry can, 10-L	1	140040
Bottle seals, 180-mL	10/ package	400790
Assembly, cap 4-L 1/8” T (acetonitrile cap assembly)	1	602458
Assembly, waste cap	1	602544
Gasket, EPR 1.38 × 0.88 × 0.060” (4-L bottle)	1	4498
Gasket, Kalrez® 1.38 ×_.775 ×_0.060” (2-L bottle)	1	4297
Kalrez® O-ring, 5/16” ID, 1/2” OD* (phosphoramidite bottle)	1	221014
O-ring, 0.364” ID (deprotect vial)	1	221019

*OD = outside diameter; ID = inside diameter

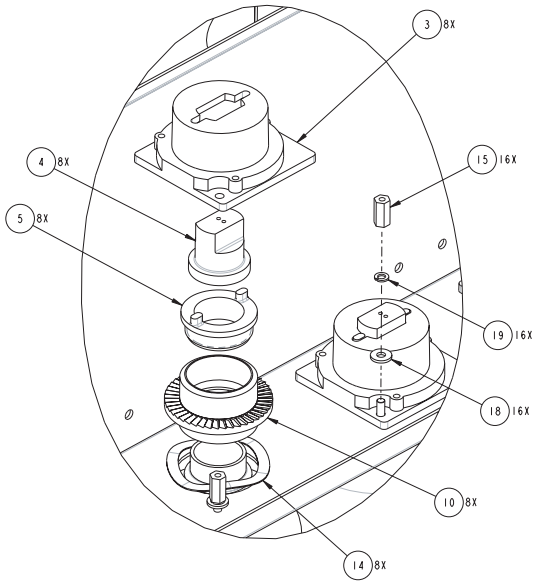
User-Installable Parts

See Figure D-1 on page D-4 for an illustration of the ratchet cap assembly.

Item	Quantity	Part Number
Housing receptacle/ratchet	1	1208
Wave spring, nickel-plated 1.80 OD*	1	2571
Receptacle ratchet, 8-oz	1	3558
Lid, receptacle ratchet	1	3560
Column connector tubing (bypass tubing)	per foot	225049
Assembly, insert R1-R8	1	602183
Matched line R1-R8	1	602189
Glass point restrictor	6/ package	602190
Measuring line tube assembly	1	602278

*OD = outside diameter; ID = inside diameter

Illustration(s)



VIEW SHOWING RATCHET
HOUSING ASSY

SCALE 1/1

ITEM NO.	QTY	PART OR IDENTIFYING NO.	NOMENCLATURE OR DESCRIPTION
19	16	241-0003	WSHR,SPLIT,#6,SST,REGULAR
18	16	223064	WASHER #6 FLAT, SS
17	1	4319930	TRAY, DRIP, REAGENT, 3900
16	1	4319943	SUPPORT, DRIP TRAY, REAGENT, 3900
15	16	200091	STDF,1/4HEX,6-32,F, 1/2 L
14	8	2571	SPRING, WAVE, 1.80 OD, NICKEL PLATED
13	1	4319934	SHROUD, REAGENT, 3900
12	4	P281129	SCR,BTNHD,SKT HEX,6-32X .375L,SST
11	12	212057	SCR,6-32X .25,FLH,SS
10	8	3559	RECEPTACLE, RATCHET 16 OZ (28-400)
9	8	140008	REAGENT, BOTTLE
8	1	4319940	RACK ASSY, REAGENT, 3900
7	1	4319935	PLATE, REAR BEZEL, REAGENT, 3900
6	1	4319928	PANEL ASSY, SIDE, REAGENT, 3900
5	8	3560	LID, RECEPTACLE, RATCHET
4	8	1206	INSERT, RECEPTACLE RATCHET
3	8	1208	HOUSING RATCHET, RECEPTACLE
2	1	4319937	BEZEL ASSY, REAGENT, 3900
1	6	230004	6-32,HEX,KEP,SS

PARTS LIST

Figure D-1 Ratchet cap assembly

3400 DNA Synthesizer Consumables

ABI LV40 Columns

Note: Polystyrene columns cut cycle costs by about 25% when used with a companion cycle. ABI LV40® Column cycles are available for use with the 3400 DNA Synthesizer.

Item	Amount per Package	Part Number
ABI LV40® dA^{bz}	1	401738
ABI LV40® dG^{ibu}	1	401742
ABI LV40® dG^{dmf} *	1	401745
ABI LV40® dC^{bz}	1	401743
ABI LV40® T	1	401744
ABI LV40® dA^{bz} pack	60	4316341
ABI LV40® dC^{bz} pack	60	4316342
ABI LV40® dG^{ibu} pack	60	4316343
ABI LV40® dG^{dmf} pack*	60	4316344
ABI LV40® dT pack	60	4316345

*FastPhoramidite® columns: For use with the fast-deprotecting set of phosphoramidites.

ABI LV200 Columns

Note: Polystyrene columns cut cycle costs by about 40% when used with a companion cycle. ABI LV200™ Column cycles are available for use with the 3400 DNA Synthesizer.

Item	Amount per Package	Part Number
ABI LV200™ dA^{bz}	1	401937
ABI LV200™ dC^{bz}	1	401938
ABI LV200™ dG^{ibu}	1	401939
ABI LV200™ dG^{dmf} *	1	401940
ABI LV200™ T	1	401941
ABI LV200™ dA^{bz} pack	60	4316440
ABI LV200™ dC^{bz} pack	60	4316441
ABI LV200™ dG^{ibu} pack	60	4316442
ABI LV200™ dG^{dmf} pack*	60	4316443

Item	Amount per Package	Part Number
ABI LV200™ dT pack	60	4316444

*FastPhoramidite® columns: For use with the fast-deprotecting set of phosphoramidites.

Controlled Pore Glass (CPG) Columns, 500 Å

Item	Amount per Package	Part Number
dA^{bz}, 0.2 µmol	1	400953
dG^{ibu}, 0.2 µmol	1	400955
dC^{bz}, 0.2 µmol	1	400954
T, 0.2 µmol	1	400956
dA^{bz}, 1.0 µmol	1	400945
dG^{ibu}, 1.0 µmol	1	400947
dG^{dmf}, 1.0 µmol*	1	401346
dC^{bz}, 1.0 µmol	1	400946
T, 1.0 µmol	1	400948

*FastPhoramidite® columns: For use with the fast-deprotecting set of phosphoramidites.

Controlled Pore Glass (CPG) Columns, 1000 Å

Item	Amount per Package	Part Number
dA^{bz}, 0.2 µmol	1	400949
dG^{ibu}, 0.2 µmol	1	400951
dG^{dmf}, 0.2 µmol*	1	401184
dC^{bz}, 0.2 µmol	1	400950
T, 0.2 µmol	1	400952
dA^{bz}, 1.0 µmol	1	401438
dG^{ibu}, 1.0 µmol	1	401439
dC^{bz}, 1.0 µmol	1	401440
T, 1.0 µmol	1	401441

*FastPhoramidite® columns: For use with the fast-deprotecting set of phosphoramidites.

Empty Synthesis Columns

Item	Amount	Part Number
Empty synthesis columns, 1.0 µmol	50/package	400407
Column filters	100/box	400059
Column vials	10/kit	400048

Bulk Controlled Pore Glass (CPG), 500 Å

Item	Size	Part Number
dA^{bz} derivatized CPG	1.0 g	400394
dG^{ibu} derivatized CPG	1.0 g	400396
dC^{bz} derivatized CPG	1.0 g	400395
T derivatized CPG	1.0 g	400397

3400 DNA Synthesizer Chemicals/Reagents

β-Cyanoethyl Phosphoramidites

Note: There is a 2-week phosphoramidite lifetime on instruments using CPG synthesis columns at 0.1 M concentration. There is a 3-week phosphoramidite lifetime on instruments using polystyrene synthesis columns. The standard series of phosphoramidite deprotects in 6 to 8 h at 55 °C. The FastPhoramidite® series deprotects in 1 h at 65 °C.

Item	Size	Part Number
dA^{bz} Phosphoramidite	0.50 g	400330
dG^{ibu} Phosphoramidite	0.50 g	400331
dG^{dmf} FastPhoramidite® reagent*	0.50 g	401182
dC^{bz} Phosphoramidite	0.50 g	400332
T Phosphoramidite	0.50 g	400333
dA^{bz} Phosphoramidite	1.0 g	400326
dG^{ibu} Phosphoramidite	1.0 g	400327
dG^{dmf} FastPhoramidite® reagent*	1.0 g	401183
dC^{bz} Phosphoramidite	1.0 g	400328
T Phosphoramidite	1.0 g	400329
dA^{bz} Phosphoramidite	2.0 g†	401159
dG^{ibu} Phosphoramidite	2.0 g†	401161
dG^{dmf} FastPhoramidite® reagent*	2.0 g†	401165
dC^{bz} Phosphoramidite	2.0 g†	401160
T Phosphoramidite	2.0 g†	401162
Deoxyinosine	0.25 g	400402

*To be used as a component of a fast-deprotecting chemistry set including dA^{bz}, dC^{bz}, and T amidites.
†For use with 3400 DNA Synthesizers equipped with the Extended Capacity Upgrade.

Specialty Phosphoramidite Derivatives

Item	Size	Part Number
TFA Aminolink™ Phosphoramidite	0.25 g	402872
Biotin Amidite	85 mg	401395
Biotin Amidite	250 mg	401396
[6-FAM™] DYE Phosphoramidite	45 mg	403169

Item	Size	Part Number
[6-FAM™] DYE Phosphoramidite	85 mg	401527
[HEX™] DYE Phosphoramidite	55 mg	403170
[HEX™] DYE Phosphoramidite	105 mg	401526
[TET™] DYE Phosphoramidite	50 mg	403171
[TET™] DYE Phosphoramidite	100 mg	401533
Phosphalink® Amidite (for 3' and 5' labeling)	70 mg	401717

Standard

Item	Size	Part Number
Anhydrous acetonitrile, amidite diluent	30 mL	400060
Anhydrous acetonitrile, amidite diluent	100 mL	401445
Tetrazole/acetonitrile, amidite activator solution	180 mL	400606
Acetic anhydride/pyridine/tetrahydrofuran	180 mL	402222
1-Methylimidazole/tetrahydrofuran	180 mL	400785
0.02 M Iodine/water/pyridine/tetrahydrofuran	200 mL	401732
Trichloroacetic acid/dichloromethane, deblock solution	2 L	401272
Dichloromethane, HPLC grade	2 L	402152
Anhydrous acetonitrile, amidite diluent	4 L	401087
Tetraethylthiuram disulfide/acetonitrile	180 mL	401147

Materials for Rapid Purification of Synthetic DNA

Item	Amount/Size	Part Number
OPC® cartridge **Note:** The OPC® cartridge desalts, deprotects, and eliminates trace organic impurities from oligonucleotide synthesis in one step	10/package	400771
Triethylamine acetate, 2.0 M	200 mL	400613
Trifluoroacetic acid, neat liquid	450 mL	400137
20% Acetonitrile in water	200 mL	400314

Documentation

Documentation

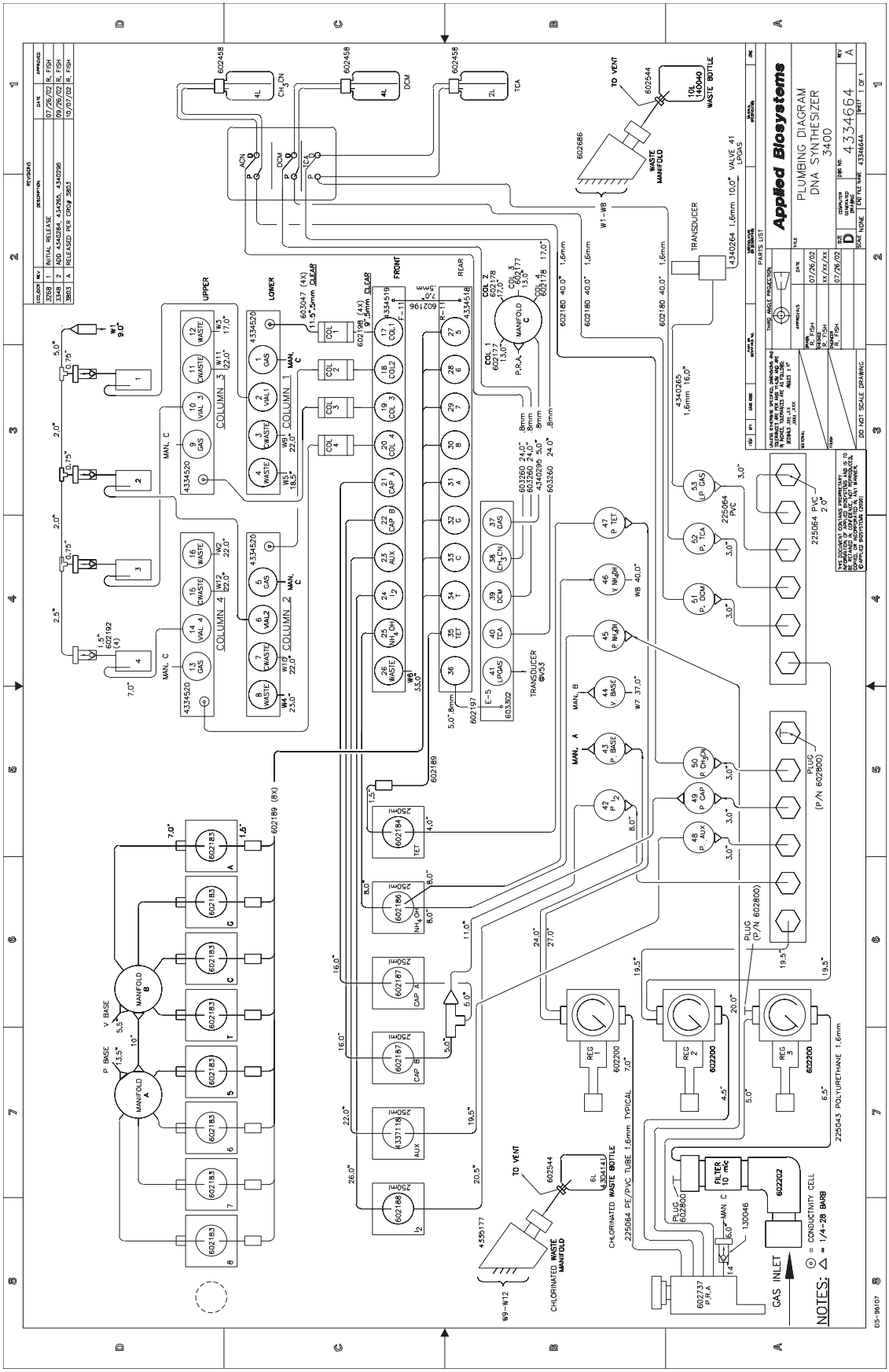
Item	Amount	Part Number
Applied Biosystems 3400 DNA Synthesizer Site Preparation and Safety Guide	1	4334679
Applied Biosystems 3400 DNA Synthesizer User Guide	1	4334680

Instrument Schematic

E

This appendix covers:

Applied Biosystems 3400 DNA Synthesizer



Specifications

F

This appendix contains the following:

Laboratory Environmental Requirements

Altitude

This instrument is for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

Temperature and Humidity

The laboratory temperature should be between 16–22 °C (60–72 °F). The instrument can tolerate maximum relative humidity of 80% for temperatures up to 31 °C, decreasing linearly to 50% relative humidity at 40 °C. Avoid placing the instrument adjacent to heaters, cooling ducts, or in direct sunlight.

Pollution

The installation category (overvoltage category) for this instrument is II, and it is classified as stationary equipment. The instrument has a pollution degree rating of 2 and may be installed in an environment that has nonconductive pollutants only.

Emission/ Immunity Statement

For our European customers, any product marked with the CE label meets the European EMC Directive 89/336/EEC and the Low Voltage Directive 72/23/EEC. This product meets Class B emission limits.

CE

Electrical Requirements

Power

IMPORTANT! You must be able to disconnect the main power supply to the instrument immediately if necessary.

The following table specifies the electrical operating range for the instrument in various parts of the world:

Location	Voltage (VAC)	Frequency	Amperage (A)
Japan	100 ±10%	50/60 Hz ±1%	<6.25
USA/Canada	120 ±10%	50/60 Hz ±1%	<6.25
Europe (pre-1992)	220 ±10%	50/60 Hz ±1%	<6.25
EC	230 ±10%	50/60 Hz ±1%	<6.25
UK (pre-1992)	240 +6%/–10%	50/60 Hz ±1%	<6.25
Australia	240 +6%/–10%	50/60 Hz ±1%	<6.25

Power Line

The electrical receptacle must have a dedicated 1.5 kVA power line and ground or a 1.5 kVA power line with a line conditioner or uninterruptible power supply (UPS).

Electrical Outlets

This instrument requires a Nema 5-15 receptacle in the USA.

The electrical receptacle must be located within 2.5 m (8 ft) of the instrument rear panel. Do not use extension cords.

Power Rating

This instrument is rated for a maximum output of 540 W.

Power Cords

In the USA, Canada, and Japan, the instrument is supplied with a detachable cord equipped with a standard three-prong plug.

In Europe and Australia, the instrument is supplied with an detachable electrical cord equipped with a standard EC plug.

Grounding

Certain types of electrical noise are greatly exaggerated by poor or improper electrical ground connections. To prevent these problems, it is very important to have a dedicated line and ground between the instrument and building main electrical service.

Power Line Regulator

In areas where the supplied power is subject to voltage fluctuations exceeding ±10% of the nominal value (above), a power line regulator may be required. High or low voltages can have adverse effects on the electronic components of the instrument.

Voltage Spikes

Short-duration, high-voltage spikes often cause random failures in microprocessor-controlled instrumentation. These spikes can be caused by other devices using the same power source (refrigerators, air conditioners, and centrifuges) or by outside influences such as lightning. A dedicated line and ground between the instrument and building main electrical service will prevent such problems.

If your environment contains devices that are electrically noisy or you are in an area with frequent electrical storms, a line conditioner with a recommended capacity of at least 1.5 kVA will enhance the reliability of your system.

Power Outages

The instrument has been designed to pause from short periods of power outage (loss). To continue operation you must resume the run from the software, provided that the line voltage did not become excessively noisy before the outage. If you want increased protection during a power outage, install a UPS with a capacity of 2.0 kVA.

Electric Shock Warning

ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high-voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 1 minute before working on the instrument.

Physical Specifications

Dimensions and Weight

The 3400 DNA Synthesizer has the following dimensions:

Component	Width	Depth	Height	Weight
3400 DNA Synthesizer, crated	86.4 cm (34 in)	71.1 cm (28 in)	78.7 cm (31 in)	81.6 kg (180 lbs)
3400 DNA Synthesizer, uncrated	66.0 cm (26 in)	45.7 cm (18 in)	50.5 cm (19.9 in)	42.9 kg 94.6 lbs

Connections and Accessories

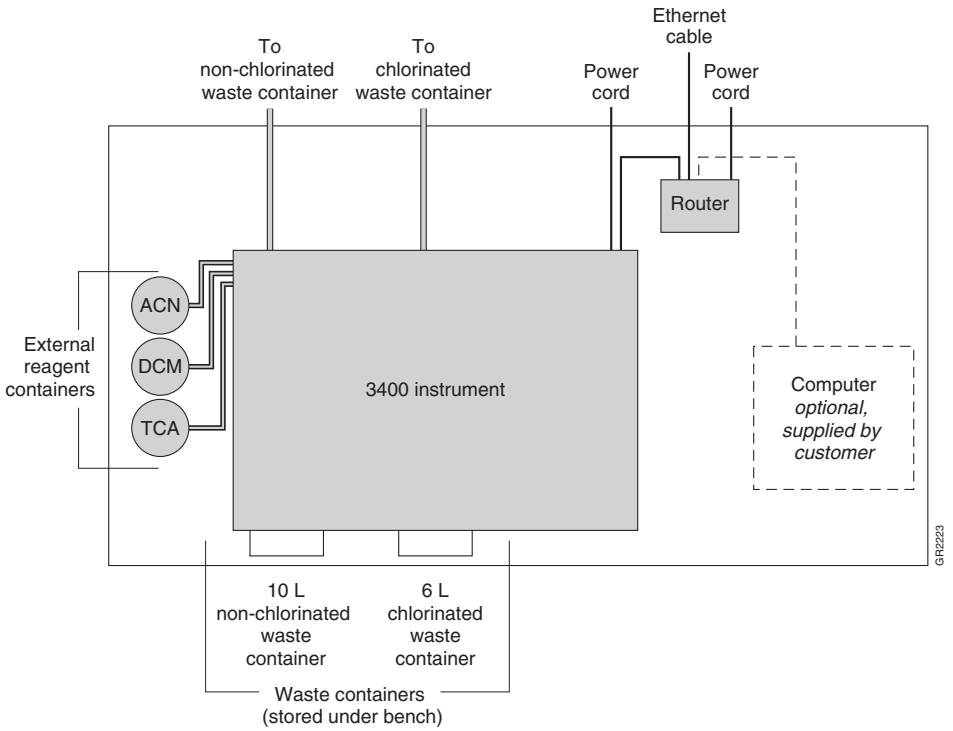


Figure F-1 Connections and accessories in a typical laboratory layout

Instrument Warranty Information

G

This appendix contains the following:

Computer Configuration

Applied Biosystems supplies or recommends certain configurations of computer hardware, software, and peripherals for use with its instrumentation.
Applied Biosystems reserves the right to decline support for or impose extra charges for supporting nonstandard computer configurations or components that have not been supplied or recommended by Applied Biosystems. Applied Biosystems also reserves the right to require that computer hardware and software be restored to the standard configuration prior to providing service or technical support. For systems that have built-in computers or processing units, installing unauthorized hardware or software may void the Warranty or Service Plan.

Limited Product Warranty

Limited Warranty

Applied Biosystems warrants that all standard components of its Applied Biosystems 3400 DNA Synthesizer will be free of defects in materials and workmanship for a period of one (1) year from the date the warranty period begins. Applied Biosystems will repair or replace, at its discretion, all defective components during this warranty period. After this warranty period, repairs and replacement components may be purchased from Applied Biosystems at its published rates. Applied Biosystems also provides service agreements for post-warranty coverage. Applied Biosystems reserves the right to use new, repaired, or refurbished instruments or components for warranty and post-warranty service agreement replacements. Repair or replacement of products or components that are under warranty does not extend the original warranty period.

Applied Biosystems warrants that all optional accessories supplied with its Applied Biosystems 3400 DNA Synthesizer, such as peripherals, printers, and special monitors, will be free of defects in materials and workmanship for a period of ninety (90) days from the date the warranty begins. Applied Biosystems will repair or replace, at its discretion, defective accessories during this warranty period. After this warranty period, Applied Biosystems will pass on to the buyer, to the extent that it is permitted to do so, the warranty of the original manufacturer for such accessories.

With the exception of consumable and maintenance items, replaceable products or components used on or in the instrument are themselves warranted to be free of defects in materials and workmanship for a period of ninety (90) days.

Applied Biosystems warrants that chemicals and other consumable products will be free of defects in materials and workmanship when received by the buyer, but not thereafter, unless otherwise specified in documentation accompanying the product.

Applied Biosystems warrants that for a period of ninety (90) days from the date the warranty period begins, the tapes, diskettes, or other media bearing the operating software of the product, if any, will be free of defects in materials and workmanship under normal use. If there is a defect in the media covered by the above warranty and the media is returned to Applied Biosystems within the ninety (90) day warranty period, Applied Biosystems will replace the defective media.

Applied Biosystems does not warrant that the operation of the instrument or its operating software will be uninterrupted or error free.

Warranty Period Effective Date

Any applicable warranty period under these sections begins on the earlier of the date of installation or ninety (90) days from the date of shipment for hardware and software installed by Applied Biosystems personnel. For all hardware and software installed by the buyer or anyone other than Applied Biosystems, and for all other products, the applicable warranty period begins the date the product is delivered to the buyer.

Warranty Claims

Warranty claims must be made within the applicable warranty period, or, for chemicals or other consumable products, within thirty (30) days after receipt by the buyer.

Warranty Exceptions

The above warranties do not apply to defects resulting from misuse, neglect, or accident, including without limitation: operation with incompatible solvents or samples in the system; operation outside of the environmental or use specifications or not in conformance with the instructions for the instrument system, software, or accessories; improper or inadequate maintenance by the user; installation of software or interfacing, or use in combination with software or products, not supplied or authorized by Applied Biosystems; and modification or repair of the product not authorized by Applied Biosystems.

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THIS WARRANTY IS LIMITED TO THE BUYER OF THE PRODUCT FROM APPLIED BIOSYSTEMS AND IS NOT TRANSFERABLE.

Some countries or jurisdictions limit the scope of or preclude limitations or exclusion of warranties, of liability, such as liability for gross negligence or wilful misconduct, or of remedies or damages, as or to the extent set forth above. In such countries and jurisdictions, the limitation or exclusion of warranties, liability, remedies or damages set forth above shall apply to the fullest extent permitted by law, and shall not apply to the extent prohibited by law.

Damages, Claims, and Returns

Damages

If shipping damage to the product is discovered, contact the shipping carrier and request inspection by a local agent. Secure a written report of the findings to support any claim. Do not return damaged goods to Applied Biosystems without first securing an inspection report and contacting Applied Biosystems Technical Support for a Return Authorization (RA) number.

Claims

After a damage inspection report is received by Applied Biosystems, Applied Biosystems will process the claim unless other instructions are provided.

Returns

Do not return any material without prior notification and authorization.

If for any reason it becomes necessary to return material to Applied Biosystems, contact Applied Biosystems Technical Support or your nearest Applied Biosystems subsidiary or distributor for a return authorization (RA) number and forwarding address. Place the RA number in a prominent location on the outside of the shipping container, and return the material to the address designated by the Applied Biosystems representative.

User Bulletins

H

Use this section to insert User Bulletins for the Applied Biosystems 3400 DNA Synthesizer.

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Printed in the U.S.A., 06/2010
Part Number 4334680 Rev. C