Affymetrix® GeneChip® Scanner 3000 Targeted Genotyping System User Guide

P/N 702164 Rev. 2

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Chapter 1

Getting Started

About Your DNA Samples

SAMPLE CRITERIA

Genomic DNA Sample Criteria

- Concentration

  All genomic DNA samples should be normalized to a single concentration of 150 ng/µL using 1X TE buffer. The Kit Control DNA included in every Affymetrix GeneChip® SNP Kit has already been normalized to a working concentration.

- Minimum volume of DNA required
  - For assay panels that are 5K and lower: 2 µg in approximately 15 µL of 1X TE Buffer
  - For assay panels greater than 5K: 4 µg in approximately 30 µL of 1X TE Buffer
- Buffer: 1X TE buffer
- Extraction and purification methods
  - QIAGEN; QIAamp DNA Blood Maxi Kit
  - Phenol-chloroform extraction
  - Gentra PUREGENE

GENERAL REQUIREMENTS

Genomic DNA samples to be processed using GeneChip SNP Kits must:

- Not be amplified.
- Be free of inhibitors.

  The extraction and purification method should render genomic DNA that is generally salt-free. High concentrations of certain salts can inhibit enzyme reactions.
- Not be contaminated with other human genomic DNA sources or with genomic DNA from other organisms.
- Not be highly degraded.

SOURCES OF GENOMIC DNA

The following sources of genomic DNA have been successfully processed with GeneChip SNP Kits in our laboratories:

- Blood
- Cell line
- Semen
- Tissue including brain, liver and spleen

About the Protocol

PROTOCOL OVERVIEW

One full run of the MIP Assay Protocol (not including data analysis) requires approximately 2.5 days. The protocol is performed in two physically separated labs referred to in this guide as the Pre-Amp Lab and the Post-Amp Lab. Separate rooms are required to prevent contamination of sample DNA with amplified products from previous reactions. To process samples at a throughput of 48 samples per day; 192 samples per 5 day week, at least two full-time employees (Research Assistants) are required.

Recommendations for when to perform the various stages of the protocol are shown in Figure 1.1 and Figure 1.2.

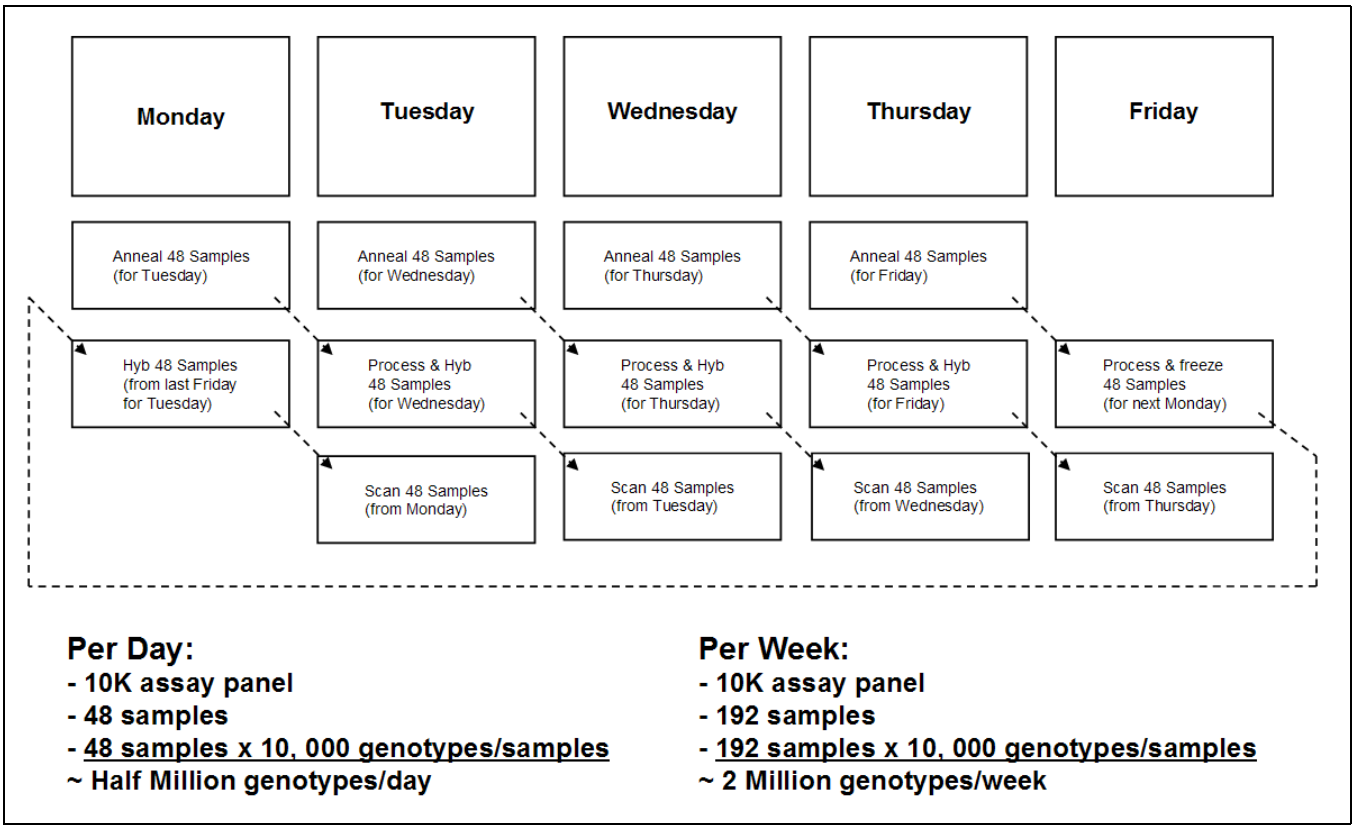


Figure 1.1
Recommended Workflow for Processing 48 Samples Per Day

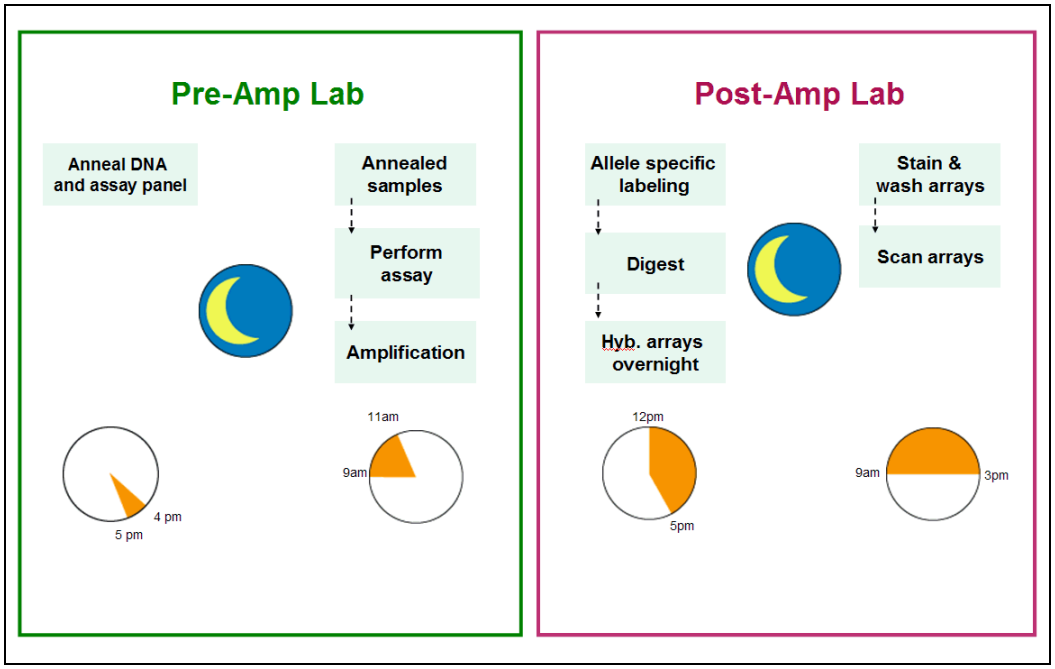


Figure 1.2
Recommended Timing for Stages of the Protocol

GeneChip® SNP Kits

CONTENTS AND STORAGE CONDITIONS

GeneChip SNP Kits consist of the reagents and other components. One kit is sufficient to process 24 reactions (including 1 control).

Kit components are shipped in multiple boxes. Because the various components must be stored under different conditions, be sure to read each label to ensure that reagents are properly stored.

Kit Components Used in the Pre-Amp Lab

The kit components listed in Figure 1.3 and Figure 1.4 are used in the Pre-Amp Lab and should be stored at –20°C.

Assay Panel Box

1 tube each of the following:

- Assay Panel . . . . . 160 µL
- Kit Control DNA . . . . .70 µL

Note: This box will contain two assay panel tubes if you are using a Special Edition panel.

Figure 1.3
Assay Panel Box — Store at –20°C

Pre-Amp Box

1 tube each of the following:

- Enzyme A . . . . . 60 µL
- Buffer A . . . . . 200 µL
- Water . . . . . .2 mL
- Gap Fill Mix . . . . . 130 µL
- Exo Mix . . . . . 530 µL
- Cleavage Tube . . . . . 3000 µL
- Cleavage Enzyme. . . . . .30 µL
- Amp Mix . . . . . 3000 µL
- 1 plate dNTP Mix (20 µL per well)

Figure 1.4
Pre-Amp Box — Store at –20°C

Kit Components Used in the Post-Amp Lab

The kit components listed in Figure 1.5, Figure 1.6 and Figure 1.7 are used in the Post-Amp Lab. Post-Amp Box 1 kit components should be stored at –20°C.

Post-Amp Box 1

1 tube each of the following:

- HY A Allele Tube . . . . . 950 µL
- HY C Allele Tube . . . . . 950 µL
- HY G Allele Tube . . . . . 950 µL
- HY T Allele Tube . . . . . 950 µL
- HY Digest Mix . . . . . 260 µL
- Buffer H . . . . . 330 µL
- Hyb Cocktail . . . . . .900 µL

Figure 1.5
Post-Amp Box 1 — Store at –20°C

The Stain Cocktail (Figure 1.6) is shipped in a box labeled *Stain Kit* .

IMPORTANT !

Even if the Stain Cocktail arrives frozen, it must be stored at 2–8°C.

Stain Kit Box

Stain Cocktail . . . . . 5 mL

Figure 1.6
Stain Kit Box — Store at 2–8°C

Wash Solution Kit contents (Figure 1.7) should be stored in the Post-Amp Lab at room temperature.

Wash Solution Kit Contents

- Wash Solution A . . . . . 500.0 mL
- Wash Solution B . . . . . 500.0 mL

Figure 1.7
Wash Solution Kit — Store at room temperature

Taq Polymerase Required

TAQ POLYMERASE

The MIP Assay Protocol has been optimized with and requires the use of following two Taq polymerases:

Supplier	Description/Part Number	Amount/Vial
Clontech	TITANIUM™ / 639208	100 rxns
(100 µL sufficient to process 24 reactions)	TITANIUM / 639209	500 rxns
Stratagene (sufficient to process 48 reactions)	TAQ Polymerase / 929197	200 µL

For Clontech and Stratagene contact information, refer to Appendix A, *Vendor Contact Information*.

Preventing Sample Contamination

GUIDELINES FOR PREVENTING SAMPLE CONTAMINATION

To prevent sample contamination, we strongly suggest everyone entering the Pre- and Post-Amp Labs follow these guidelines:

- Never bring any items that have been in the Post-Amp Lab into the Pre-Amp Lab. Always assume that all surfaces in the Post-Amp Lab are contaminated with amplified PCR product, and that all items that enter the Post-Amp Lab become contaminated with product.
- Keep dedicated lab coats for the Pre-Amp Lab and the Post-Amp Lab.
- Use proper gowning procedures.
- Always wear gloves when handling equipment or samples in either lab.
- Do not move equipment between the Pre- and Post-Amp Labs. Keep dedicated equipment in both labs.
- Print separate copies of the protocol for each lab.
- If both labs are connected by a door, do not return to the Pre-Amp Lab via the Post-Amp Lab.

About Using Control DNA

ABOUT THE CONTROL DNA

The control DNA supplied in each GeneChip SNP Kit has been normalized to a working concentration. No further dilution or concentration is required.

RECOMMENDATIONS FOR USAGE

To ensure the highest quality data, we strongly recommend running one positive control for every 23 samples. Control DNA is included in every GeneChip SNP Kit.

Our protocol is written such that the control is always placed in column 12 of the PCR plates (Anneal, Assay, Label and Hyb).

BENEFITS OF USING CONTROLS

Running control DNA is important for quality control and troubleshooting purposes. Using controls can help you more quickly resolve issues related to:

- Sample quality
- Assay performance

Chapter 2

Equipment, Supplies, Consumables

Important Notice Regarding Product Support

ABOUT QUALIFYING FOR SUPPORT

The MIP Assay Protocol has been optimized for use on the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System (GCS 3000 TG System) using the equipment, supplies and consumables listed in this chapter.

IMPORTANT !

A double asterisk (**) next to an item indicates that no substitutions can be made. To qualify for support, these particular items must be used. In addition, training will be conducted only when all of the equipment, supplies and consumables are verified as present and properly installed.

About the Equipment, Supplies and Consumables Required

How the Information Is Organized

Information on the equipment, supplies and consumables required is organized as follows:

- *Equipment and Software Supplied by Affymetrix for Standard System Configuration*
- *Equipment, Supplies, and Consumables Required But Not Provided*
  - *Equipment Required But Not Provided*
  - *Supplies Required But Not Provided*
  - *Consumables Required But Not Provided*
  - *Quality Control Gels*

Equipment and Software Supplied by Affymetrix for Standard System Configuration

The following items are shipped to your facility directly from Affymetrix. A double asterisk (**) next to an item indicates that no substitutions can be made.

IMPORTANT ! **Do not unpack any of the equipment supplied by Affymetrix. An Affymetrix representative will unpack, install and verify the operation of the equipment and software.**

IMPORTANT ! **Store GeneChip® Universal Tag Arrays at 4°C.**

Table 2.1
Equipment and Software Supplied by Affymetrix for Standard System Configuration

Item	Quantity	Part Number	Laboratory Location
** Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System including: - GeneChip® Scanner 3000 7G 4C with 7G Instrument Control Workstation - GeneChip® Scanner 3000 Autoloader with 2D barcode reader - GeneChip® Fluidics Station 450 - GeneChip® Hybridization Oven 640 with carriers - GeneChip® Operating Software (GCOS), v1.4 - GeneChip® Targeted Genotyping Analysis Software, v1.5 - TG Pre-Amp Lab Workstation with 2D barcode reader - TG Post-Amp Lab Workstation with SQL Server 2000 Standard Edition license and 2D barcode reader	1	00-0185	Pre and Post-Amp Labs
** The following GeneChip® Universal Tag Arrays as appropriate:			
- GeneChip® Universal 3K Tag Array	As required	900602 (6 pk) 900578 (96 pk)	Post-Amp Lab
- GeneChip® Universal 5K Tag Array	As required	900659 (6 pk) 900660 (96 pk)	Post-Amp Lab
- GeneChip® Universal 10K Tag Array	As required	900604 (6 pk) 900580 (96 pk)	Post-Amp Lab
- GeneChip® Universal 25K Tag Array	As required	900857 (6 pk) 900858 (96 pk)	Post-Amp Lab

Equipment, Supplies, and Consumables Required But Not Provided

VENDOR CONTACT INFORMATION

See Appendix A for vendor contact information.

EQUIPMENT REQUIRED BUT NOT PROVIDED

Table 2.2
Equipment Required but not Provided

Equipment	Quantity	Manufacturer/ Distributor	Part Number (U.S.)	Laboratory Location
** Thermal Cyclers – see Table 2.3 on page 13 for a list of thermal cyclers that have been validated for use with the MIP Assay Protocol				
Eppendorf® Multipurpose Centrifuge 5804	2	VWR Intl.	53513-800	1 Pre-Amp Lab 1 Post-Amp Lab
Eppendorf® Deepwell Swing Bucket Rotor with two buckets	2	VWR Intl.	77888-214	1 Pre-Amp Lab 1 Post-Amp Lab
Mini Vortexer, analog	2	VWR Intl.	58816-121	1 Pre-Amp Lab 1 Post-Amp Lab
Galaxy Mini Centrifuge (interchangeable for microtubes and strip tubes)	4	VWR Intl.	37000-700	2 Pre-Amp Lab 2 Post-Amp Lab
** Aluminum Block, 96-well	4	BioSmith	81001	Pre-Amp Lab
** Pipettes and pipette tips — see Table 2.4 on page 14 for a list of pipettes and tips that have been validated for use with the MIP Assay Protocol				
Carousel Stand for Rainin® Pipettes	4	Rainin	CR-7	2 Pre-Amp Lab 2 Post-Amp Lab
Portable Pipet-Aid (110V)	1	VWR	53498-103	Post-Amp Lab
Freezer, –20°C; deep freeze; manual defrost; 17 cu ft	2	—	—	1 Pre-Amp Lab 1 Post-Amp Lab
Refrigerator, 4°C refrigerator; 6 cu ft	2	—	—	1 Pre-Amp Lab 1 Post-Amp Lab

Thermal Cyclers

To run the MIP Assay Protocol at a throughput of 48 assays/day, you will need 4 thermal cyclers: 2 in the Pre-Amp Lab; 2 in the Post-Amp Lab.

The MIP Assay Protocol has been optimized using GeneAmp® PCR System 9700 Thermal Cyclers (96-well, gold-plated). The additional thermal cyclers listed in Table 2.3 have been validated for use with the protocol in the laboratory location specified.

IMPORTANT !

Note the laboratory location for the thermal cyclers listed in Table 2.3. Some of these instruments can be used in the Post-Amp Lab only.

Table 2.3
Thermal Cyclers Validated for Use With the MIP Assay Protocol

Manufacturer/ Distributor	Item	Part Number (U.S.)	Laboratory Location
Applied Biosystems Thermal Cyclers	GeneAmp® PCR System 9700 Thermal Cycler, 96-well, Silver	N8050001	Use in both the Pre- and Post-Amp Labs
	GeneAmp® PCR System 9700 Thermal Cycler, 96-well, Gold-plated	4314878	
	GeneAmp® PCR System 9700 Thermal Cycler, 96-well, Aluminum	4314879	
	GeneAmp® PCR System 9700 Thermal Cycler, Dual 96-well	4343176	Use in Post-Amp Lab only
BioRad Thermal Cyclers	DNA Engine® Peltier Thermal Cycler chassis only; requires 1 Alpha Unit (see BioRad Alpha Units below)	PTC-0200	Use in Post-Amp Lab only
	DNA Engine Dyad® Peltier Thermal Cycler chassis only; requires 2 Alpha Units (see BioRad Alpha Units below)	PTC-0220	
	DNA Engine Dyad® Peltier Thermal Cycler Disciple chassis only; requires 2 Alpha Units (see BioRad Alpha Units below)	PTC-0221	
	DNA Engine Tetrad® 2 Peltier Thermal Cycler Chassis only; requires 4 Alpha Units (see BioRad Alpha Units below)	PTC-0240	
BioRad Alpha Units	Alpha™ Unit: Holds 96 x 0.2 ml tubes or one 96-well plate	ALS-1296	
	Moto Alpha™ Unit: Holds one 96-well plate with integrated motorized heated lid	ALP-2296	

Pipettes and Pipet Tips

To run the MIP Assay Protocol, you will need the following types of pipettes. The pipettes and tips validated fro use with the MIP Assay Protocol are listed in Table 2.4.

- Single channel pipettes
  - 0.1–2 µL
  - 0.5–10 µL
  - 2–20 µL
  - 20–200 µL
  - 100–1000 µL
- 12-channel pipettes
  - 0.5–10 µL or 1–20 µL
  - 2–20 µL, 5–50 µL, 20–200 µL or 20–300 µL
- 24-channel pipettes, electronic or manual
  - 2–20 µL
  - 10–100 µL

Table 2.4
Pipettes and Pipet Tips Validated for Use With the MIP Assay Protocol

Manufacturer/ Distributor	Item	Quantity	Part Number (U.S.)	Laboratory Location
SINGLE CHANNEL MANUAL PIPETTES				
Rainin®	Pipet-Lite™ LTS™ Pipet, 0.1–2 µL	1	L-2	1 Pre-Amp Lab
	Pipet-Lite LTS Pipet, 0.5–10 µL	2	L-10	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipet-Lite LTS Pipet, 2–20 µL	2	L-20	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipet-Lite LTS Pipet, 20–200 µL	2	L-200	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipet-Lite LTS Pipet, 100–1000 µL	2	L-1000	1 Pre-Amp Lab 1 Post-Amp Lab
Rainin Pipet Tips (use for single, 12-, and 24-channel Rainin pipettes)	LTS™ Pipette Tips, P-1000 barrier (8 racks of 96 tips each; 768 tips per box)	As required	RT-L1000F (1 box)	Pre and Post-Amp Labs
	LTS Pipette Tips, P-200 barrier (10 racks of 96 tips each; 960 tips per box)	As required	RT-L200F (1 box)	
	LTS Pipette Tips, P-10 barrier (10 racks of 96 tips each; 960 tips per box)	As required	RT-L10F (1 box)	

Manufacturer/ Distributor	Item	Quantity	Part Number (U.S.)	Laboratory Location
12-CHANNEL MANUAL PIPETTES				
Rainin Pipettes	Pipet-Lite LTS 12 Channel Pipet, 0.5–10 µL	2	L12-10	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipet-Lite LTS 12 Channel Pipet, 2–20 µL	2	L12-20	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipet-Lite LTS 12 Channel Pipet, 20–200 µL	2	L12-200	1 Pre-Amp Lab 1 Post-Amp Lab
Gilson Pipettes	Pipetman® Ultra, U12-20, P20, 1–20 µL	2	F21041	1 Pre-Amp Lab 1 Post-Amp Lab
	Pipetman Ultra, U12-300, 20–300 µL	2	F21043	1 Pre-Amp Lab 1 Post-Amp Lab
Gilson Pipet Tips	Gilson Diamond® sterilized extra long filter tips, 2-200 µL	As required	D200ST	Pre- and Post-Amp Labs
	Gilson Diamond sterilized filter tips, 30–300 µL	As required	DF300ST	
Eppendorf Pipettes	Research®Series 2100, 0.5–10 µL	2	022453947	1 Pre-Amp Lab 1 Post-Amp Lab
	Research Series 2100, 10–100 µL	2	022453963	
Eppendorf Pipet Tips	epT.I.P.S. Filter, 0.1–10 µL	As required	022491211	Pre- and Post-Amp Labs
	epT.I.P.S. Filter, 2–100 µL	As required	022491237	
24-CHANNEL PIPETTES, MANUAL AND ELECTRONIC Manual or electronic 24 channel pipettes can be used. Electronic recommended for better uniformity when mixing. A minimum and maximum quantity of electronic pipettes is listed. If using the minimum, you will have to reprogram the pipettes throughout the protocol. If using the maximum, you will not have to reprogram the electronic pipettes.				
Rainin	2–20 µL - Pipet-Lite LTS 24 Channel Pipet (manual) - EDP3™ + Elect LTS™ Pipet 24 Channel (electronic)	 2 2 to 4	 L24-20 E24-20	1 Pre-Amp Lab 1 Post-Amp Lab
	10–100 µL - Pipet-Lite LTS 24 Channel Pipet (manual) - EDP3+ Elect LTS Pipet 24 Channel (electronic)	 2 2 to 4	 L24-100 E24-100	1 Pre-Amp Lab 1 Post-Amp Lab
	E3 Rapid Charge Stand	2 or 4	E3-RCS	

SUPPLIES REQUIRED BUT NOT PROVIDED

Table 2.5
Supplies Required but not Provided

Small Supplies	Quantity	Manufacturer/ Distributor	Part Number	Laboratory Location
Cube Rack, polypropylene (4/pack)	2 packs	VWR Intl.	60985-444	1 Pre-Amp Lab 1 Post-Amp Lab
MicroTube Rack, 80-well (5/pack)	2 packs	VWR Intl.	10011-284	1 Pre-Amp Lab 1 Post-Amp Lab
96-place rack for 0.2 mL tubes (5/pack)	3 packs	USA Scientific	2300-9602	Pre- and Post-Amp Labs
Preparation racks for PCR thin-walled tubes (assorted colors)	1 case	Fischer Scientific	05-541-55	Pre- and Post-Amp Labs
MiniAlarm Timer/Stopwatch	2	VWR Intl.	62344-585	1 Pre-Amp Lab 1 Post-Amp Lab
Lab Coats, dedicated	8	—	—	Pre and Post-Amp Labs
Optional: Ice Bucket, red Note: You will need an ice bucket dedicated to the Post-Amp Lab.	1	VWR Intl.	35751-205	Post-Amp Lab
Optional: Ice Container, red (8.5″ x 16″ x 3″) Note: You will need an ice container dedicated to the Post-Amp Lab.	1	VWR Intl.	35751-216	Post-Amp Lab
Optional: Ice Container, green (8.5″ x 16″ x 3″) Note: You will need an ice container dedicated to the Pre-Amp Lab.	1	VWR Intl.	35751-218	Pre-Amp Lab
Calculator	2	—	—	1 Pre-Amp Lab 1 Post-Amp Lab
Scissors	2 pairs	—	—	1 Pre-Amp Lab 1 Post-Amp Lab
Distilled, deionized water supply or carboys (for cleaning the fluidics station)	—	—	—	Post-Amp Lab

CONSUMABLES REQUIRED BUT NOT PROVIDED

Affymetrix GeneChip® SNP Kits

One GeneChip SNP Kit includes reagents sufficient to process 24 reactions. The specific kit required to process your samples is determined by the assay panel you are using.

IMPORTANT !

Kit components are shipped in multiple boxes. Store each box as directed on the label.

Additional Consumables

The quantities listed for consumables are sufficient to process 600 reactions including controls.

Table 2.6
Additional Consumables – Quantities sufficient to process approximately 600 reactions

Item	Quantity	Manufacturer /Distributor	Part Number (U.S.)	Laboratory Location
** Both of the Taq DNA Polymerases listed below are required.				
- Stratagene Taq DNA Polymerase, 200 µL/vial	As needed	Stratagene	929197 (1 vial)	Pre-Amp Lab
- Clontech TITANIUM™ Taq Polymerase 100 rxn size is sufficient for processing 24 reactions (with 10% extra)	As needed	Clontech	639208 (100 rxns) 639209 (500 rxns)	Post-Amp Lab
** PCR Plates (96-well) and barcode labels — see *PCR Plates* on page 18 and Table 2.7 on page 19 for the PCR plate vendors and barcode label supplier validated for use with the MIP Assay Protocol.				
** Clear Film for Arrays (81 labels/sheet; 10 sheets per pack)	1 pack	Affymetrix		Post-Amp Lab
** MicroAmp® Clear Adhesive Films (100 films/pk)	3 pks	Applied Biosystems	4306311 (1 pack)	Pre and Post-Amp Labs
Pipettes, 5 mL, disposable, individually wrapped (200/case)	1 case	VWR	20171-046	Post-Amp Lab
Pipettes, 10 mL, disposable, individually wrapped (200/case)	1 case	VWR	20171-042	Post-Amp Lab
PCR 12-well Tube Strips with Strip Bubble Caps (80/pack)	1 pk	VWR	53509-306 (1 pack)	Pre and Post-Amp Labs
Eppendorf Color-Coded Safe-Lock Microcentrifuge Tubes, amber, 2.0 mL (500/case)	2 cases	VWR	20901-541	Post-Amp Lab
Eppendorf Color-Coded Safe-Lock Microcentrifuge Tubes, natural, 2.0 mL (500/case)	2 cases	VWR	20901-540	Pre and Post-Amp Labs
Reagent Reservoirs, 50 mL (200/case)	1 case	VWR Intl.	53504-035 (1 case)	Pre and Post-Amp Labs
Microtube Tough Spots® for 0.5–2.0 mL tubes, white (1000/roll)	1 roll	USA Scientific	9185-0000 (1 roll)	Post-Amp Lab

Table 2.6
Additional Consumables – Quantities sufficient to process approximately 600 reactions

Item	Quantity	Manufacturer /Distributor	Part Number (U.S.)	Laboratory Location
Corning Conical Tubes, 15 mL (500/case)	1 case	VWR Intl.	21008-670 (1 case)	Pre and Post-Amp Labs
Gloves, 100/box	6 boxes	—	—	Pre and Post-Amp Labs
Bench Pads	1 case	VWR Intl.	56616-026	Pre and Post-Amp Labs
Kimwipes® (12″ x 12″)	2 packs	VWR Intl.	21905-011 (1 pack)	Pre and Post-Amp Labs
Marking pens, one of the following types • Water soluble • Fluorescent	1 pack	Fischer Scientific	13-380-15A or 13-384	Post-Amp Lab
Marking pens, permanent, fine point, assorted colors	12 pens	—	—	6 Pre-Amp Lab 6 Post-Amp Lab
Razor blades	1 pack	—	—	Post-Amp Lab
Aluminum foil	1 roll	—	—	Post-Amp Lab
Compressed air (canned)	2 cans	—	—	Post-Amp Lab
Tacky Floor Mats	1 case	VWR Intl.	12777-112 (1 case)	Post-Amp Lab

PCR Plates

To run the MIP Assay Protocol, you will need a supply of 96-well PCR plates with specific barcode labels. To facilitate sample tracking, the plates are referred to using specific terminology throughout the various stages of the protocol. These designations and the barcode prefix associated with each type of plate is listed below.

- Anneal Plate – ANN barcode
- Assay Plates – ASY barcode
- Label Plates – LBL barcode
- Hyb Plates – HYB barcode

IMPORTANT !

If you purchase plates from a vendor other than Axygen, you must purchase barcode labels from Axygen to apply to your plates. The plates supplied by Axygen come with the barcode labels already affixed to them.

Table 2.7
PCR Plates and Barcode Labels Validated for Use With the MIP Assay Protocol – Quantities sufficient to process approximately 600 reactions

Manufacturer/ Distributor	Item	Quantity	Part Number (U.S.)	Laboratory Location
PCR PLATES				
Axygen distributor	Anneal PCR Plates, 96-well thin-wall (with ANN barcode affixed; 50/case)	1 case	321-63-ANN (1 case)	Pre-Amp Lab
	Assay PCR Plates, 96-well thin-wall (with ASY barcode affixed; 50/case)	1 case	321-63-ASY (1 case)	Pre-Amp Lab
	Label PCR Plates, 96-well thin-wall (with LBL barcode affixed; 50/case)	1 case	321-63-LBL (1 case)	Post-Amp Lab
	Hybridization PCR Plates, 96-well thin-wall (with HYB barcode affixed; 50/case)	1 case	321-63-HYB (1 case)	Post-Amp Lab
Greiner (from E & K Scientific)	PCR Plate, 96 Well, Half Skirt (no barcode; 40/case) Note: If purchasing Greiner plates, you must also purchase barcode labels from Axygen (listed below)	8 cases	EK-19280	Pre- and Post-Amp Labs
BARCODE LABELS				
Axygen distributor	Barcode Labels for Sample Plates	As required	BC-LABEL-SMP	Pre-Amp Lab
	Barcode Labels for Anneal Plates		BC-LABEL-ANN	Pre-Amp Lab
	Barcode Labels for Assay Plates		BC-LABEL-ASY	Pre-Amp Lab
	Barcode Labels for Label Plates		BC-LABEL-LBL	Post-Amp Lab
	Barcode Labels for Hyb Plates		BC-LABEL-HYB	Post-Amp Lab

QUALITY CONTROL GELS

Training Requirements

As part of the training session, quality control (QC) gels are run on each plate of samples processed. Running QC gels helps the trainer determine the source of issues that may arise.

Your laboratory must have gel electrophoresis capability and a gel imaging system. Gel recommendations are listed below.

QC Gel Recommendations

We recommend running two QC gels per assay:

- While learning to run the MIP Assay Protocol
- For troubleshooting purposes

Precast Gel Recommendations

We recommend using one of the following precast gels and gel system:

Biorad Precast Gels and Gel Box System

Table 2.8
Recommended Biorad Precast Gels and Gel Box System

Item	Part Number	For Training	Ongoing
Wide Mini ReadySub-Cell GT Cell	170-4489	Two cells required to concurrently run two gels	Two cells required to concurrently run two gels
ReadyAgarose 96 Plus 3% TBE Gel	161-3062	8 gels	1 gel/24 assays

Invitrogen Precast Gels and Gel System

Table 2.9
Recommended Invitrogen Precast Gels and Gel System

Item	Part Number	For Training	Ongoing
Power supply and gel tray recommendations: • One Mother Base • Three Daughter Bases	Mother Base: EB-M03 Daughter Bases: EB-D03	One Mother Base and Three Daughter Bases	One Mother Base and Three Daughter Bases
E-Gel 48 4% Agarose (8 gels/box)	G8008-04	8 boxes	2 gels/24 assays

Self-Cast Gel Recommendations

If casting your own gels, we recommend following these guidelines:

- Use acrylamide or a high-resolution agarose.
- For agarose gels, we recommend preparing a 4% Agarose SFR gel using Agarose SFR by Amresco, part number J234-100G.

Chapter 3

Running the Protocol

STAGES OF THE PROTOCOL

The MIP Assay Protocol is presented and performed in stages. These stages are:

- Stage 1 – Design an Anneal Plate
- Stage 2 – Anneal
- Stage 3 – Plan and Make Assay Plates
- Stages 4 – Gap Fill, dNTP, Ligate, Invert, First PCR
- Stage 5 – Second PCR
- Stage 6 – Target Digest
- Stage 7 – Sample Hybridization
- Stage 8 – Stain and Wash
- Stage 9 – Scan Arrays

To prevent sample contamination from PCR products, stages 1, 2, 3 and 4 are performed in the Pre-Amp Lab. The remaining stages are performed in the Post-Amp Lab.

NORMALIZE SAMPLES

All genomic DNA samples should be normalized to a single concentration of 150 ng/µL using 1X TE buffer. The Kit Control DNA included in every GeneChip® SNP Kit is already normalized to a working concentration.

Minimum volume of DNA required:

- For assay panels that are 5K and lower: 2 µg in approximately 15 µL of 1X TE Buffer
- For assay panels greater than 5K: 4 µg in approximately 30 µL of 1X TE Buffer

For more information on sample criteria and recommendations, see *Sample Criteria* on page 2.

GENECHIP® SNP KITS AND CONTROL DNA

One GeneChip SNP Kit is sufficient to process 24 reactions (23 samples and 1 control).

IMPORTANT !

To ensure the highest quality data, we strongly recommend running one positive control for every 23 samples. Control DNA is included in each GeneChip SNP Kit.

ENZYMES

The enzymes in GeneChip SNP Kits are temperature sensitive. They must be kept at –20°C until used.

For all mixes and cocktails, always add the enzymes last, and add them right before you plan to use the mix or cocktail.

VOLUMES FOR MIXES AND COCKTAILS

The volumes specified for each mix or cocktail are given for one of the following:

- 24 or 48 reactions
- 24 or 48 arrays

PIPETS AND PIPETTING RECOMMENDATIONS

The types of pipettes used throughout this protocol are:

- Single channel, manual
- 12-channel, manual
- 24-channel, manual or electronic

When the protocol calls for the use of 24-channel pipettes, you can use either a manual or an electronic pipette. Rainin EDP3-Plus with LTS electronic pipettes are recommended for better uniformity when mixing.

General Pipetting Recommendations

To ensure full volume transfer, check pipette tips after each pickup and dispense. To avoid the formation of air bubbles, dispense liquids at the bottom of each well. Always use the type and volume of pipette specified in the protocol.

Electronic Pipetting Recommendations

Follow the instructions provided with the pipettes for the dispense/mix program that:

- Allows reagent to be aspirated and dispensed at a set volume
- Mixes automatically upon dispensing wherein the mix volume can be different from the dispense volume

Two options are available for tracking the number of mixes when using Rainin EDP3-Plus electronic pipettes: the counter option, or the beep option (pipette beeps after each mix). We recommend using the beep option, since the counter does not start at zero with each use. Instead, it counts pipette operations sequentially. Refer to the instructions provided with the pipettes for more information.

QUALITY CONTROL GEL RECOMMENDATIONS

We recommend running quality control gels at two points during the protocol. Knowing in advance that a sample will not provide data will save arrays. The purpose of each gel is described below.

- Gel 1: Run to identify any samples that did not amplify.

  No bands are visible for samples that have not amplified.

- Gel 2: Run to confirm that bands have digested after the target digest stage.

For more information on quality control gels, see:

- *First Quality Control Gel* on page 75
- *Viewing the First Quality Control Gel* on page 75
- *Second Quality Control Gel* on page 81
- *Evaluating the Second Quality Control Gel* on page 82

Create a Project

PREREQUISITES

Before you can create a project and run the protocol, you must import the following into the Affymetrix GeneChip® Targeted Genotyping Analysis Software (GTGS).

- Array definitions

  Array definitions are loaded into the GTGS during system installation. They are also stored on the CD-ROM included with the software. See *Array Definition Management* on page 138 for more information.

- Protocol definitions

  Protocol barcodes are supplied on the MIP Assay Protocol Barcodes quick reference card. See *Adding Protocols* on page 135 for more information.

SUMMARY OF STEPS

To create a project, you will:

1. Optional: Create a Sample Info file that contains sample information such as species and gender. If gender is important to your study, consider creating Sample Info files. See *About Sample Info Files* on page 25.
2. Create at least one Sample Plate file containing sample plate layout information (one file per sample plate)
3. Create a new project in GTGS by importing:
   - Assay panel file – located on the CD-ROM received with your first shipment of GeneChip SNP Kits for a particular assay panel.
   - Genotype settings file – located on the same CD-ROM as the assay panel file
   - Sample info files
   - Sample plate files
4. Add sample plates to your project
5. Design an anneal plate

ABOUT SAMPLE INFO FILES

Creating a Sample Info file is optional. These files can contain the following information:

- Sample name (mandatory)
- Sample gender (optional)
- Population (optional)
- Species (optional)

If your assay panel can report gender, and if sample gender is important, consider creating Sample Info files that include the gender of each sample. This information can help troubleshoot sample tracking errors by comparing the known gender with the

inferred gender. The inferred gender is called by the software during data analysis. Refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software User Guide* for more information.

Once a sample info file has been imported, you can add to or update the existing information. You can also delete sample information that ends up not being used.

Creating Sample Info Files

Sample information must be entered into a tab-delimited text file using an application such as Microsoft® Office Excel® (saved as a .txt file) or Microsoft® Notepad.

If you decide not to create Sample Info files, the sample name only will be appear automatically in the Sample Info table. Gender, population and species will be designated as Unknown (Figure 3.1).

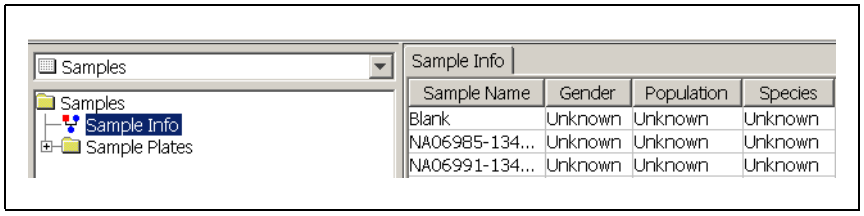


Figure 3.1
Sample Info Icon and Table

Guidelines to follow when creating Sample Info files are listed below.

- Use the column headings and format shown in Figure 3.2.
- Samples names:
  - Maximum length is 50 characters
  - Can appear only once in a Sample Info file
  - Cannot contain the following characters: \ / : ; * ? " < > | ' , { } [ ]

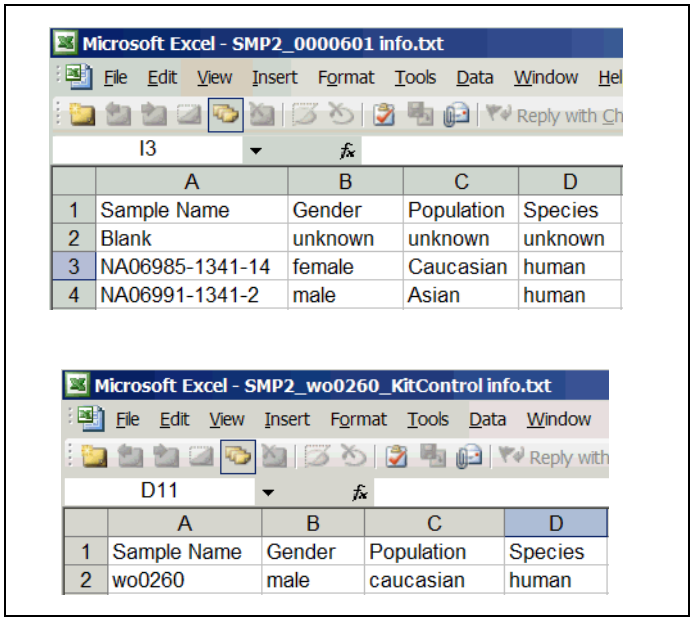


Figure 3.2
Example of Sample Info Files

Adding To or Updating Sample Info Files

You can add to and update information that has already been imported from Sample Info Files.

To add to and update Sample Info File information:

1. Edit the sample info text file.
2. In the left pane of GTGS, open the menu and select **Samples**.
3. Right-click the **Sample Info** icon and select **Add/Update Sample Info**.
4. In the Add/Update Sample Info window, click the **Browse** button.
5. Locate and select the Sample Info filename.
6. Select one or both check boxes to **Add New Sample Info** or **Update Existing Sample Info**.
7. Click **Import**.
8. Click **Save**.

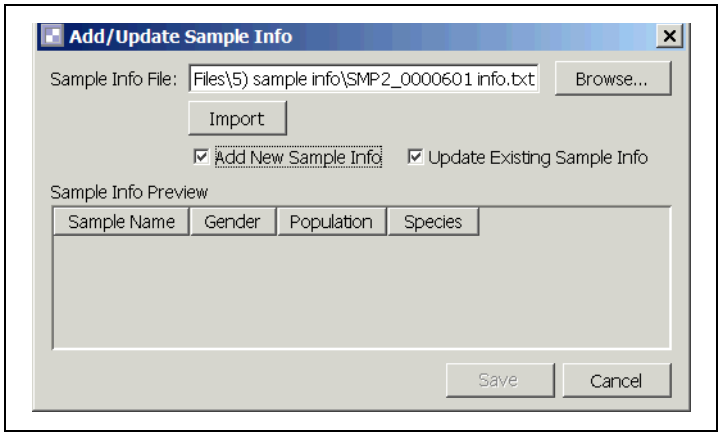


Figure 3.3
Add/Update Sample Info Window

Removing Unused Sample Info

You can remove information that was imported from Sample Info Files but was not used in your study.

To remove unused sample info:

1. In the left pane of GTGS, open the menu and select **Samples**.
2. Right-click the **Sample Info** icon and select **Remove Unused Sample Info**.
3. Click **Yes** when prompted to confirm this action.

ABOUT SAMPLE PLATE FILES

The layout of each sample plate used in the MIP Assay Protocol must be imported into GTGS prior to sample processing. The information in these files is used to design Anneal Plate layouts for the first stage of the protocol.

Sample plate information can be updated until the barcode of an Anneal Plate containing a sample from a particular plate is scanned.

Creating Sample Plate Files

The layout of each sample plate must be entered into a tab-delimited text file using an application such as Microsoft Office Excel (saved as a .txt file) or Notepad.

- Use the column headings and format shown in Figure 3.4 on page 30. Sample types can be sample, control and water.
- Each text file should correspond to one sample plate only.
- Well designations must be based on a 96-well plate format (A1 through H12.)
- The sample plate does not need to be full. Do not include empty wells in the file.
- Samples name guidelines:
  - Must be unique for each unique DNA (repeats should have the same name)
  - Maximum length is 50 characters
  - Cannot contain the following characters: \ / : ; * ? " < > | ' , { } [ ]

IMPORTANT

Assign a unique name to each unique DNA. Repeated samples of the same DNA should have the same name. GTGS compares samples based on sample name. If different DNA samples are assigned the same name, and if those samples are analyzed together, the repeatability metrics calculated by GTGS will be incorrect.

NOTE

For text files containing control DNA information, we recommend including the lot number in the filename. For example, SMP2_wo0260_KitControl.txt. You can use this filename as a the sample plate barcode later in the protocol.

Updating Sample Plate Information

Sample plate information, except for the sample name, can be updated at any time.

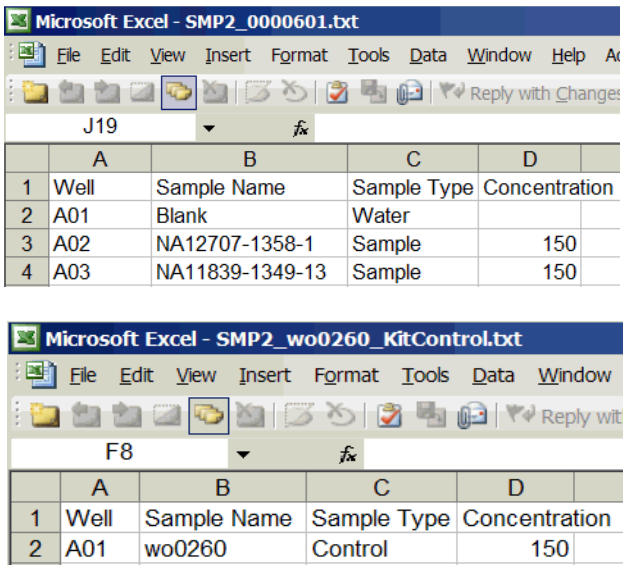
To update sample plate information:

1. Edit the sample plate text file.
2. In the left pane of GTGS, open the menu and select **Samples**.
3. Expand the **Sample Plates** icon.
4. Right-click a sample plate and select **Update Sample Plate …**
5. In the Update Sample Plate window, click the **Browse** button.
6. Locate and select the sample plate filename.
7. Select External or Internal Samples as appropriate.
8. Click **Import**.

9. Click **Save**.

Control DNA Recommendations

To ensure the highest quality data, we strongly recommend running one positive control for every 23 samples. Control DNA is included in each GeneChip SNP Kit.



Microsoft Excel files with sample plate layout and control information.

These files must be saved as tab-delimited text files (.txt).

Figure 3.4
Example of Text Files with Sample Plate Layout and Control DNA Information

CREATE A PROJECT

Import Assay Panel and Genotype Settings Files

You must import the file created for your assay panel before you can run the protocol. The file is located on the CD-ROM that was shipped with the first GeneChip SNP Kits you received.

At the same time, we strongly recommend importing the genotype settings file that is located on the same CD-ROM. Even though the information in this file will not be used until data analysis, the software will not prompt you to load it. The settings in this file were optimized for your particular assay panel. If it is not present when performing data analysis, default genotype settings will be used and your results may not be optimal.

To import assay panel and genotype settings files:

1. In GTGS, import the assay panel and genotype settings files as follows:

   A. Load the CD-ROM containing the files into the hard drive.

   B. Open the drop-down menu and select **Assay Panels**.

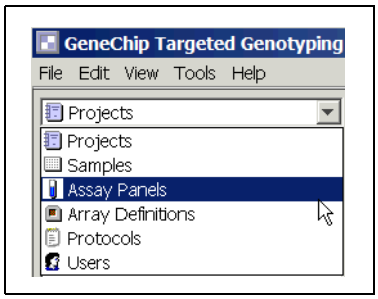


Figure 3.5
Selecting Assay Panels from the Menu

   C. Open **File** → **Import Assay Panel** (or right-click the Assay Panels folder)

   D. In the Import Assay Panel window, click the **Browse** button next to the Assay Panel File field.

   E. Locate and select the assay panel filename, then click **Open**.

   F. Click the **Browse** button next to the Genotyping Settings File field.

   G. Locate and select the genotype settings filename, then click **Open**.

   H. Click the **Import** button.

   Notice that the table in the Import Assay Panel window is now populated (Figure 3.6).

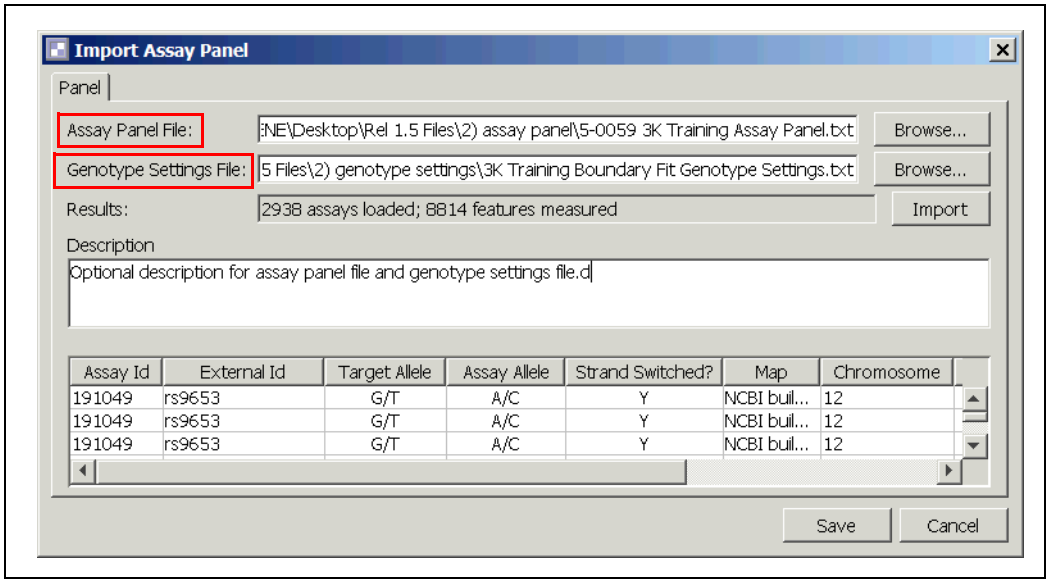


Figure 3.6
Importing the Assay Panel and Genotype Settings Files

I. Optional: Enter a description in the **Description** field.

J. Click **Save**.

If you expand the Assay Panel icon in the left pane, the type of GeneChip® Universal Tag Array that must be used with your assay panel is displayed. In the right pane, a list of the features present on the array is displayed.

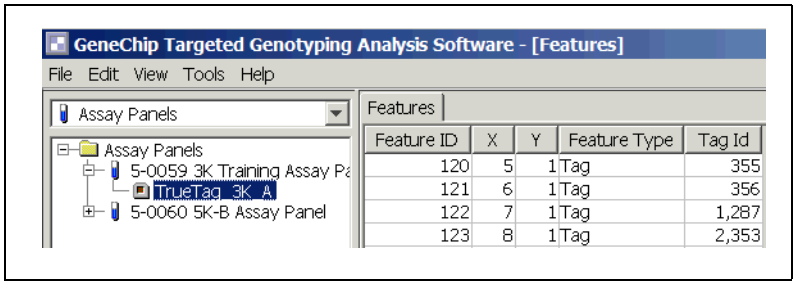


Figure 3.7
Displaying the Type of Array to be Used with a Particular Assay Panel

Import Sample Information

You will now import your sample info files (optional) and sample plate files (required).

To import sample information:

1. Open the drop-down menu and select **Samples**.
2. Optional: Import Sample Info files as follows:

   A. Right-click the Sample Info icon and select **Add/Update Sample Info**.

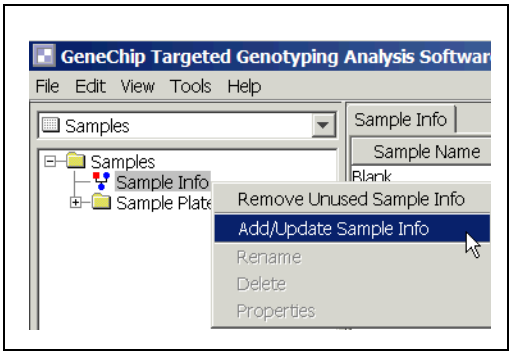


Figure 3.8
Importing Sample Info Files

   B. In the Add/Update Sample Info window, click the **Browse** button next to the Sample Info File field (Figure 3.9).

   C. Locate and select the Sample Info filename, then click **Open**.

   D. Select the **Add New Sample Info** check box.

   E. Click the **Import** button.

   Notice that the Sample Info Preview table is now populated.

   F. Repeat this procedure for all Sample Info files you want to import.

   G. Click **Save**.

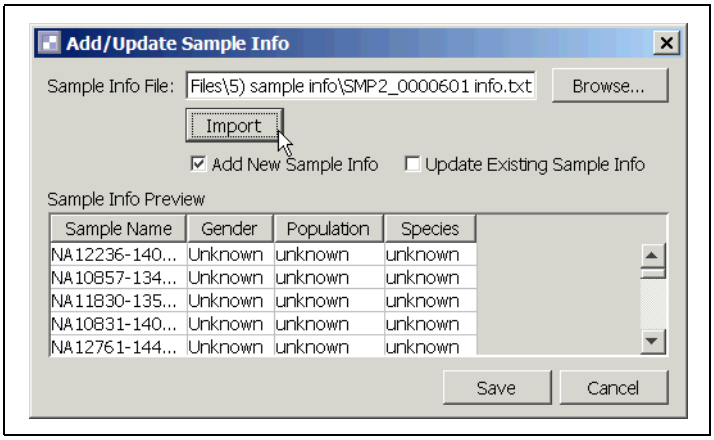


Figure 3.9
Add/Update Sample Info Window

3. Import Sample Plate files as follows:

   A. Right-click the Sample Plates icon and select **Import Sample Plate** (Figure 3.10).

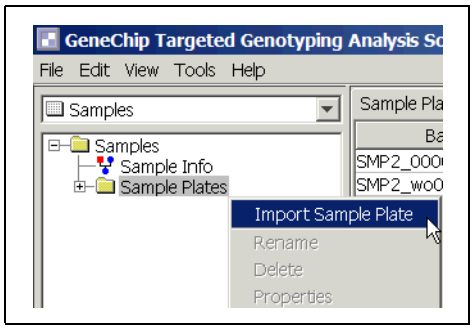


Figure 3.10
Importing Sample Plates

   B. In the Import Sample Plate window (Figure 3.11), click the **Browse** button.

   C. Locate and select a sample plate filename, then click **Open**.

   D. Select Internal or External Samples as appropriate.

   E. Click **Import**.

   Notice that information about the sample plate is now displayed in the Import Sample Plate table (Figure 3.11). The software automatically designates the sample plate filename as the barcode.

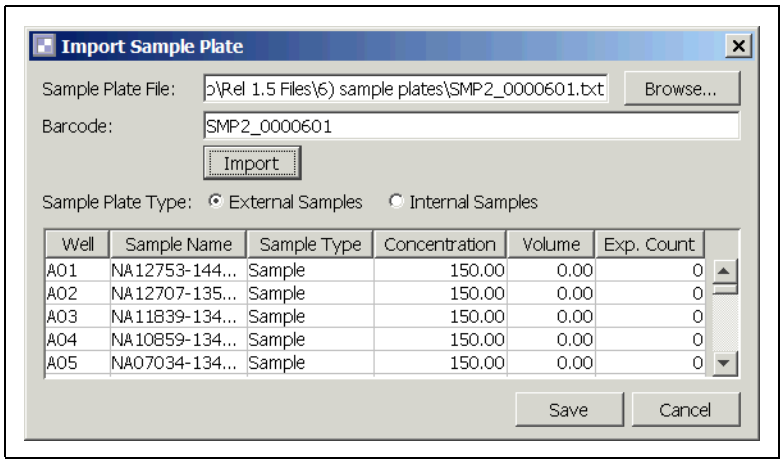


Figure 3.11
Import Sample Plate Window

   F. To change the barcode, either scan the sample plate barcode or type a new barcode into the Barcode field.

   G. Click **Save**.

4. Repeat this procedure for each sample plate you want to add.

About Sample Plate Information

Expand the Sample Plates folder in the left pane to see a list of the sample plates you have imported. Select a sample plate name to see information specific to a particular plate.

In the right pane, you can view:

- General information about each sample plate such as the date it was imported (Sample Plates tab; Figure 3.12).
- A list of samples on a particular plate by selecting the plate name, then clicking the Sample Plate Wells tab (Figure 3.13).
- The Sample Info associated with a particular sample plate by selecting the name of the sample plate and clicking the Sample Info tab (Figure 3.14).

You can fail a sample plate and add a description for a sample plate by modifying the sample plate properties. See *Modifying Sample Plate Properties* on page 36.

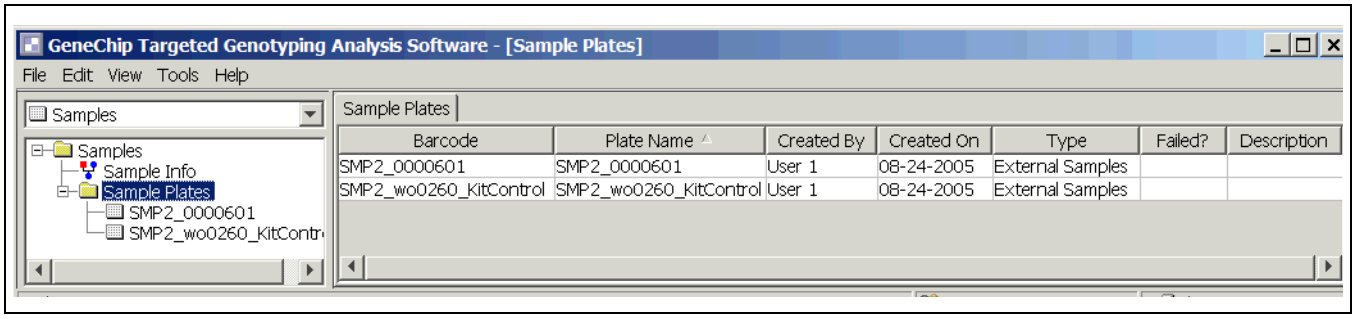


Figure 3.12
Sample Plate Information

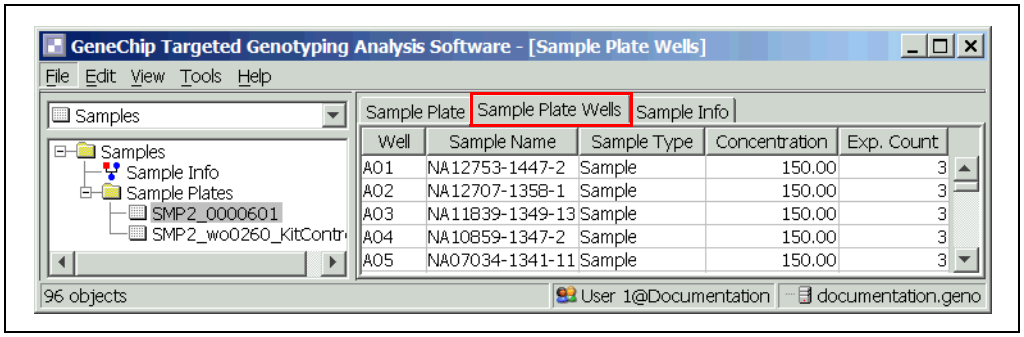


Figure 3.13
Sample Plate Wells Tab

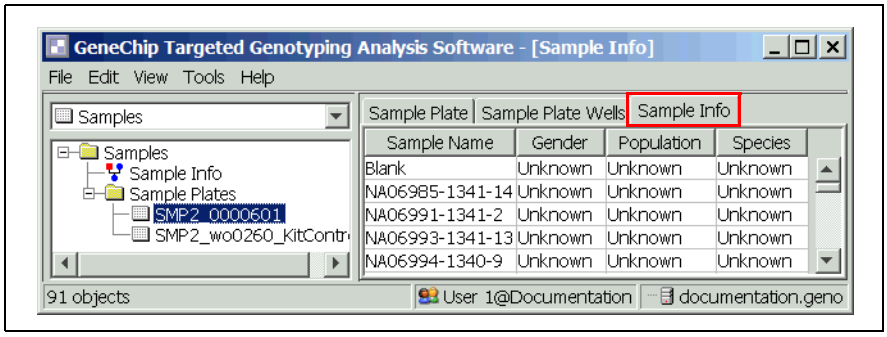


Figure 3.14
Sample Info Tab

Modifying Sample Plate Properties

You can add a description for a sample plate or fail the entire sample plate by modifying the sample plate properties.

To modify sample plate properties:

1. Select a sample plate, then open **Edit** → **Properties** (or right-click the sample plate name).
2. Do one of the following:
   - **A.** Add or modify a description in the Description field (Figure 3.15).
   - **B.** Select or deselect the Failed check box.
3. Click **Save**.

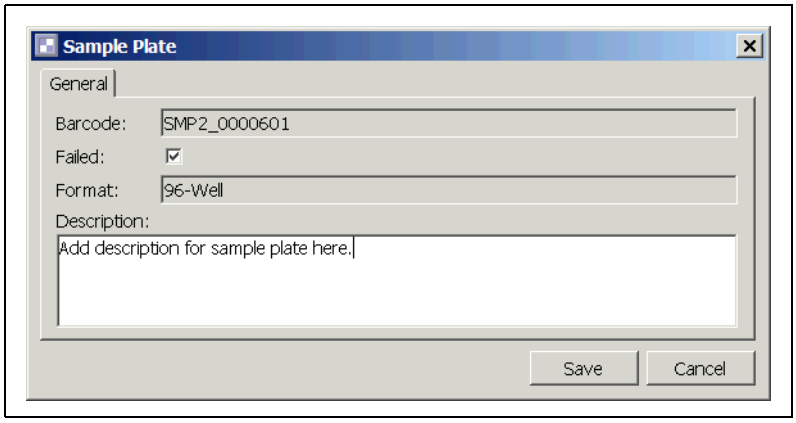


Figure 3.15
Modifying Sample Plate Properties

CREATE A NEW PROJECT

1. Open the drop-down menu and select **Projects**.
2. Right-click the Projects folder and select **New Assay Project**.
3. In the Select an Assay Panel window (Figure 3.16), select the name of an assay panel, then click **OK**.

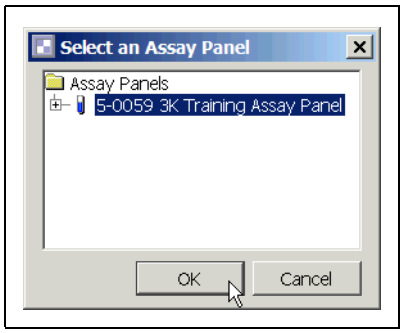


Figure 3.16
Select an Assay Panel Window

4. In the **Project** window (Figure 3.17):
   - **A.** Enter a project name in the Name field.

     Project name constraints are:
     - Maximum length is 50 characters
     - Cannot contain the following characters: \ / : ; * ? " < > | ' , {} [ ]

NOTE **Projects are displayed alphabetically by name. Keep this in mind if you want your projects displayed in a particular order.**

   - **B.** Accept the default values for Scanner Type (GeneChip_Scanner_3000) and Color Format (Four_Color).
   - **C.** Optional: In the **Description** field, enter a description for the project.

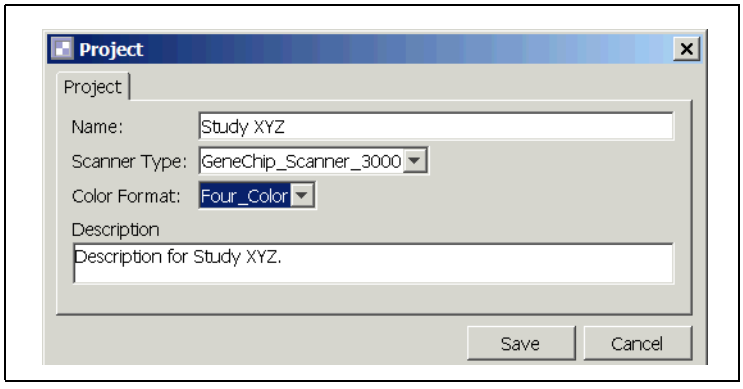


Figure 3.17
Project Window

   - **D.** Click **Save**.

In the left pane, click the + icons next to the project and Tracking icons to expand them (Figure 3.18). Notice the placeholders that have been created under the Tracking icon (Sample Plates, Anneal Plates, Assay Plates, Label Plates, Hyb Plates, and Arrays). Placeholders for array data information and genotype results are also created.

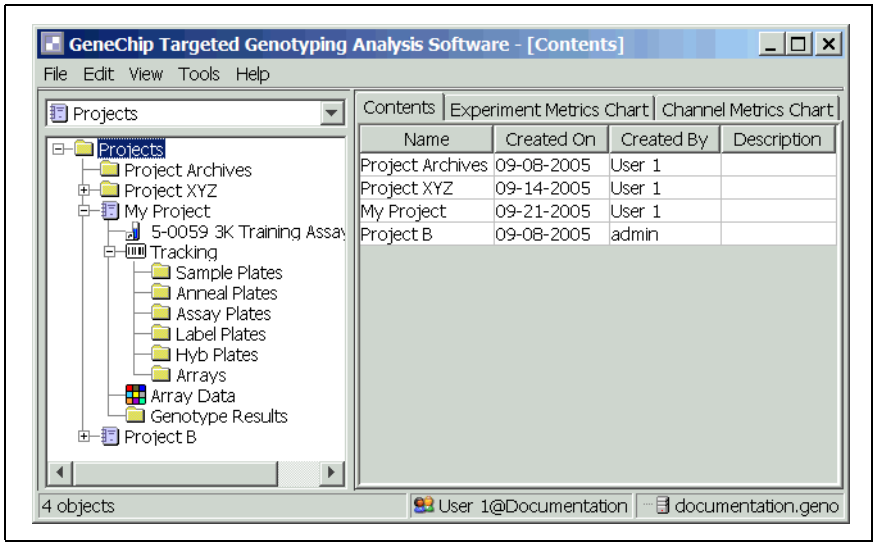


Figure 3.18
Structure of a Project

ADD SAMPLE PLATES TO A PROJECT

To add sample plates to a project, you will:

- Import a Sample Plate file (see *About Sample Info Files* on page 25 for more information)
- Scan the barcode of the sample plate
- Add the sample plate to your project

Add Sample Plates to Your Project

To add sample plates to your project:

1. Expand your project icon, then expand the Tracking icon (Figure 3.19).
2. Right-click the **Sample Plates** folder, and select **Add Existing Sample Plate**.

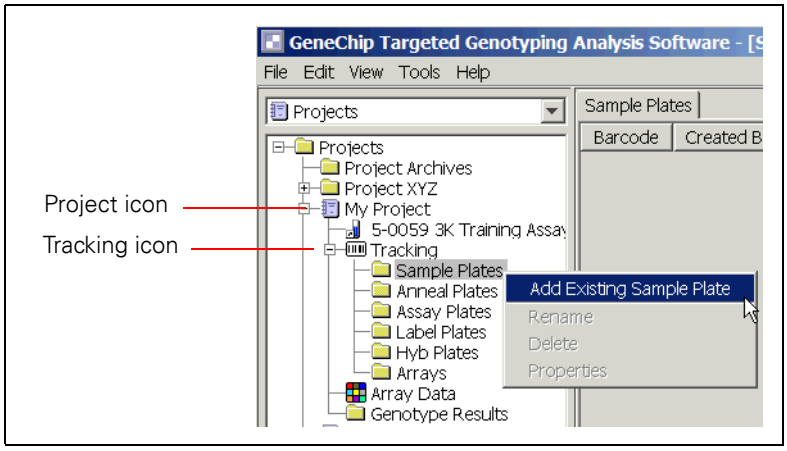


Figure 3.19
Adding Sample Plates to a Project

3. Select a sample plate, then click **OK** (Figure 3.20).
4. Repeat this procedure to add more sample plates to your project.

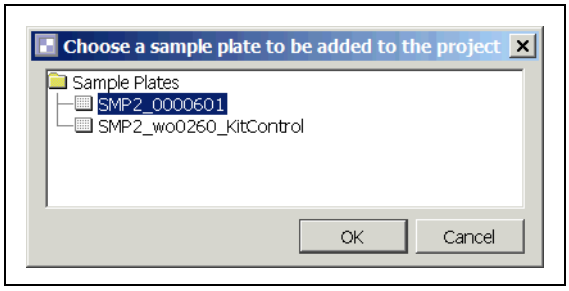


Figure 3.20
Selecting a Sample Plate

To view the sample plates added to your project, expand the Sample Plates icon. Sample plate information is also displayed in the right pane of the window.

Stage 1 – Design an Anneal Plate

Information describing the samples and their well locations on each sample plate is used to design the Anneal Plate. This information is imported into GTGS from tab-delimited text files. If the sample information you require is not yet in GTGS, import it now. Refer to the appropriate sections under *Create a Project* starting on page 25 to create sample files and import them into your project.

For Stage 1, sample plate information is used to design an Anneal Plate. You will designate:

- Which samples are to be transferred from a sample plate to an Anneal Plate.
- Where the samples are to be located on the Anneal Plate.

HOW TO DESIGN AN ANNEAL PLATE

To design an Anneal Plate:

1. Right-click the Anneal Plates icon for your project and select **Add Anneal Plate**.
2. In the Define Sample Plate to Anneal Plate Mapping window, open the **Source** menu and select a sample plate (Figure 3.21).

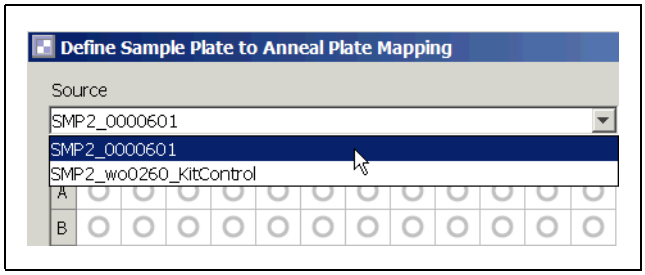


Figure 3.21
Selecting a Sample Plate for Anneal Plate Design

3. Specify which samples are to be transferred from the Sample Plate to the Anneal Plate as follows:
   - **A.** Click and drag your cursor over one or more wells on the Sample Plate.
   - **B.** Click and drag your cursor over the Anneal Plate wells that you want the samples loaded in. (Shown in Figure 3.23 on page 41.)
   - **C.** Click **Set**.

     Notice that the transfers you designate are displayed at the bottom of the window in the Transfers table (Figure 3.22).
4. When finished, click **Save**.
5. To edit the layout after you have clicked Save:
   - **A.** Expand the **Anneal Plates** icon.
   - **B.** Right-click the name of the **Anneal Plate** and select **Edit Sample Mapping**.
   - **C.** Select a line in the **Transfers** table; then click **Clear** (Figure 3.22).
   - **D.** Make your changes.
   - **E.** Click **Save**.

NOTE **Transfers with the status Ready can be changed. Transfers with the status Complete cannot be changed.**

To modify transfers, select the appropriate line in the Transfers table and click Clear.
Transfers with the status Ready can be changed. Transfers with the status Complete cannot be changed

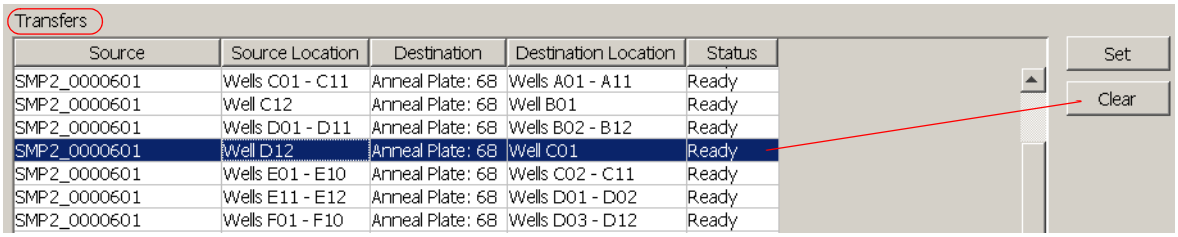


Figure 3.22
Making Changes to Sample Mapping

In this example, DNA from sample plate SMP2_0000601, well A1 to A11, B1 to B12, C1 to C11, and D1 to D12 is to be loaded into Anneal Plate wells A1 to A11, B1 to B12, C1 to C11 and D1 to D12. Kit Control DNA from the tube named SMP2_wo0260_KitControl is to be loaded into Anneal Plate wells A12 and C12.

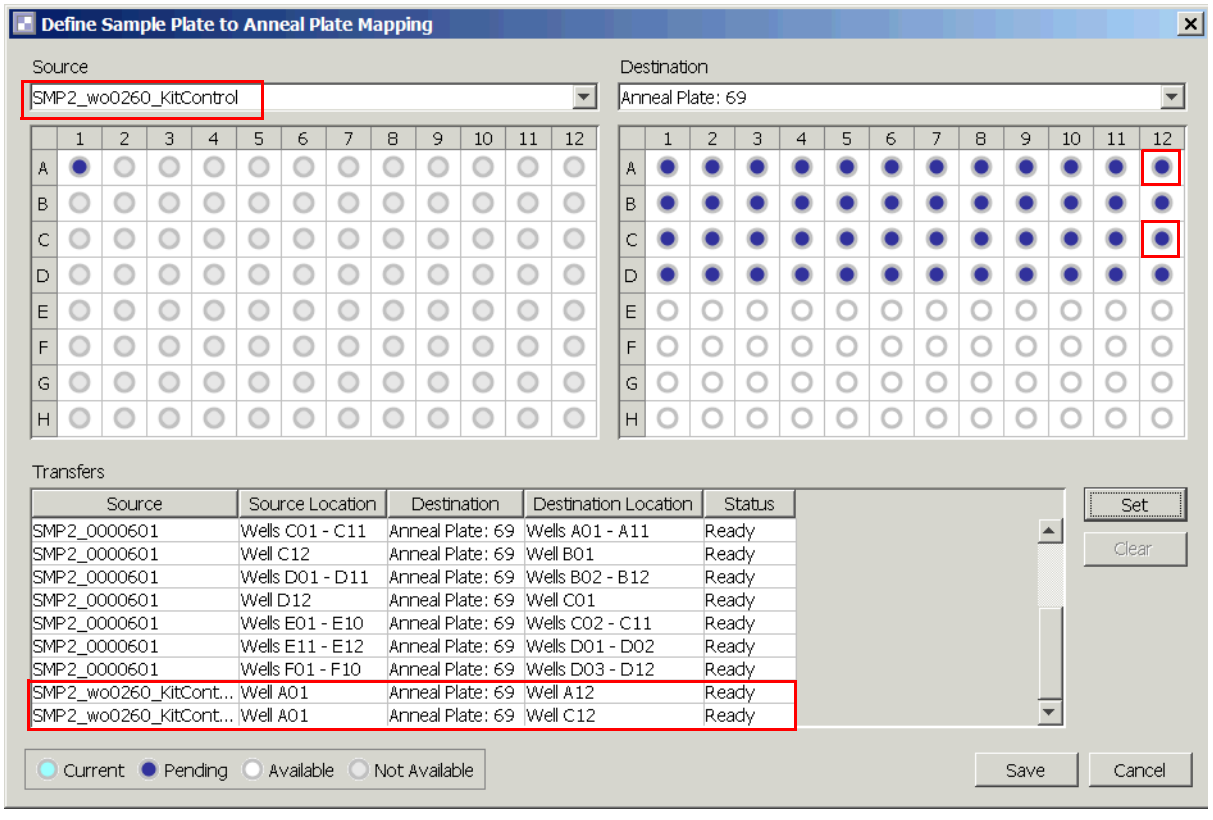


Figure 3.23
Example of Sample to Anneal Plate Mapping

VIEWING ANNEAL PLATE INFORMATION

To view the Anneal Plates you have designed, expand the Anneal Plates icon. To view information about a particular Anneal Plate, select the Anneal Plate name. In the right pane, select the appropriate tab to view:

- Properties – lists properties for an Anneal Plate such as who created it, the date it was created and the barcode.
- Incoming transfers – indicates which samples are to be loaded onto the Anneal Plate from a particular sample plate. Information includes the sample plate well and the Anneal Plate well.
- Outgoing transfers – indicates which Anneal Plate reactions are to be transferred to a specific Assay Plate and where they are to be placed (well location) on the Assay Plate. This table will remain empty until you have run the Anneal Plate and created an Assay Plate.
- All experiments – provides information that is available after the array barcodes are scanned. This information includes the date each sample was hybridized onto an array, as well as the barcode and well for each plate the sample was on during the protocol.

Stage 2 – Anneal

ABOUT THIS STAGE

During this stage, the assay panel probes are annealed to genomic DNA target samples.

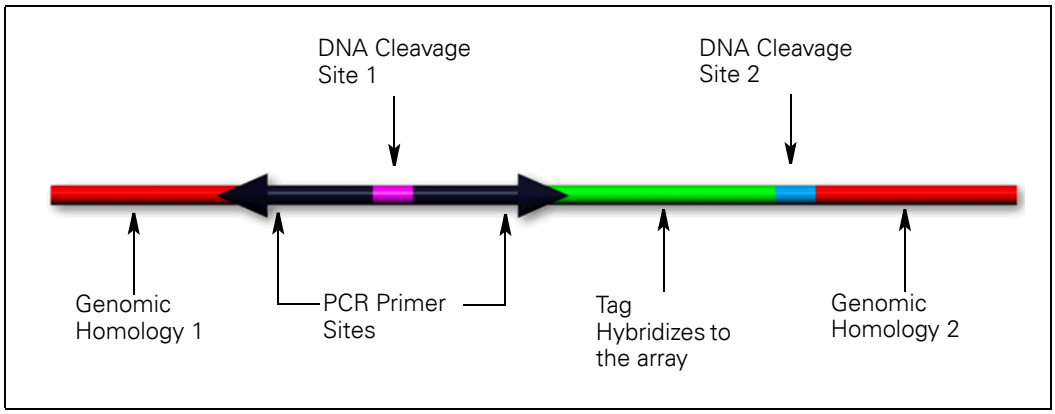


Figure 3.24
Assay Panel Probe

Genomic DNA samples, the assay panel probes, and reagents (Anneal Cocktail) are mixed in an *Anneal Plate*. An Anneal Plate is a 96-well PCR plate with the barcode designation ANN<*barcode*>. The plate is then placed on a thermal cycler and the program, *Meg Anneal*, is run. Because the samples must be left to anneal for ≥ 16 hours, this stage is typically started at the end of the day, and the anneal program is allowed to run overnight.

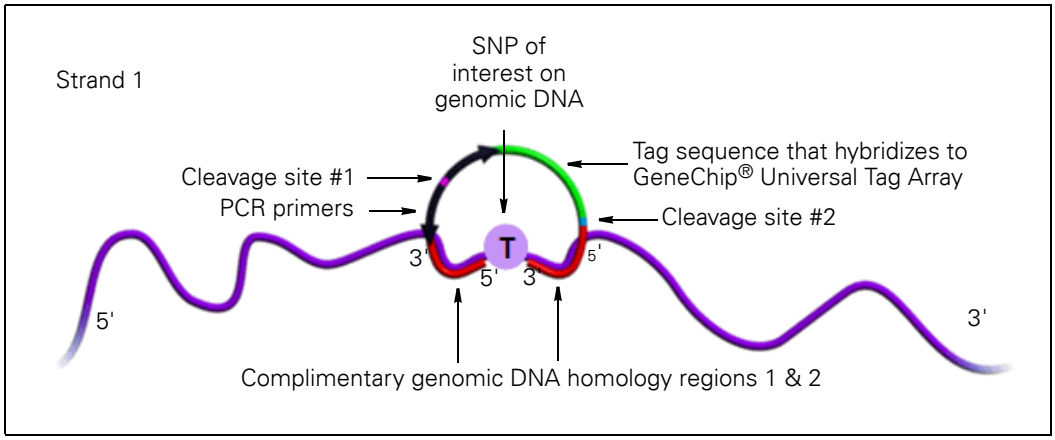


Figure 3.25
Assay Panel Probe Annealed to Genomic DNA Sample

LOCATION AND DURATION

- Pre-Amp Lab
- Hands-on time: approximately 45 minutes

SAMPLE CONCENTRATION REQUIREMENT

Before starting this procedure, all samples should be normalized to a single concentration of 150 ng/µL using 1X TE buffer.

The Kit Control DNA included in every GeneChip SNP Kit has already been normalized to a working concentration. No further dilution or concentration is required. For more information, see *Normalize Samples* on page 22.

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 reactions.

Table 3.1
Equipment and Materials Required for Stage 2 - Anneal

Quantity	Item
1	Centrifuge, plate
1	Cube rack
1	Eppendorf tube, 2 mL
1	Ice container, rectangular, filled with ice
1	Marking pen, extra fine point, permanent
3	MicroAmp Clear Adhesive Films
1	Microfuge
1	PCR plate, 96-well, with ANN barcode
2 – 3	Plate holders (preparation racks)
1	Reagent reservoir, 50 mL
48	Samples of genomic DNA in barcoded plates
1	Thermal cycler, 96-well GeneAmp PCR System 9700
1	Vortexer

KIT COMPONENTS REQUIRED

Two GeneChip SNP Kits are required to anneal 48 reactions (including controls). The following components are required from each kit:

- Water (P/N 5-0004)
- Assay Panel

  For Special Edition panels, there will be two assay panel tubes in the kit:

  - Standard Assay Panel tube (fill size 160 µL)
  - Supplemental Assay Panel tube (fill size 60 µL)
- Buffer A
- Enzyme A
- Kit Control DNA

THAW REAGENTS

To thaw reagents:

1. Place the Water from kit, all Assay Panel tubes, Buffer A, and Kit Control DNA in a cube rack.
2. Allow to warm to room temperature on the bench top.
3. Keep on ice until ready to use.

IMPORTANT ! **Leave Enzyme A at –20°C until ready to use.**

RUN AN ANNEAL PLATE

To run an Anneal Plate:

1. If a project has not been created, create one now.

   Creating a project is typically done once at the beginning of a study. You must create at least one project before you can perform the protocol and track samples using GTGS. For instructions, see *Create a Project* on page 25.

2. Using a permanent marking pen, label the ANN-barcoded PCR plate as shown in Figure 3.26.

   Four full rows (48 reactions) from an Anneal Plate are required to fill two Assay Plates.

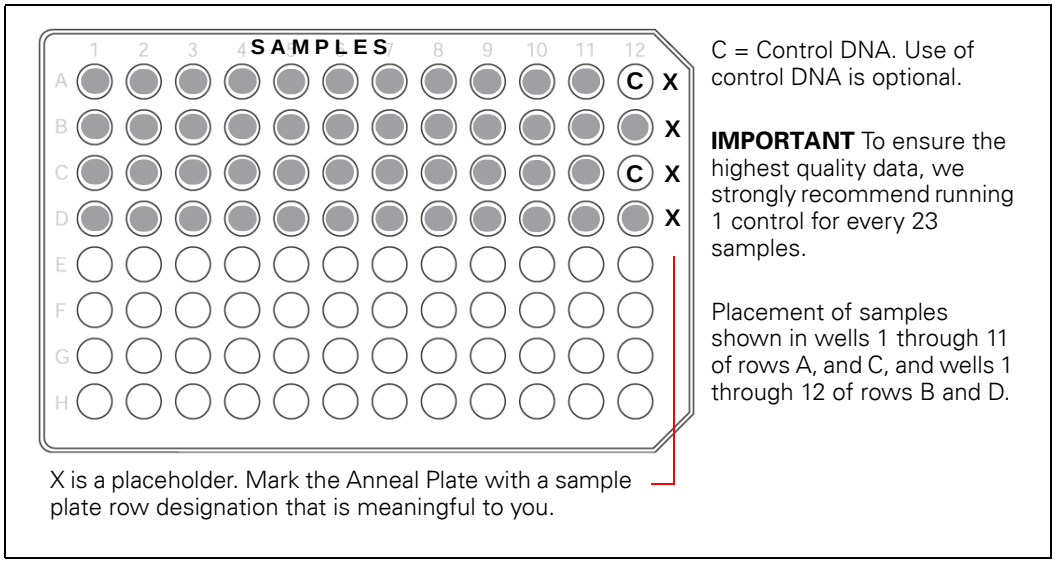


Figure 3.26
Anneal Plate Labeling and Sample Placement

3. In GTGS, expand the Project, Tracking and Anneal Plates icons.
4. Do the following:

   A. Right-click an Anneal Plate and select **Run Anneal Plate**.

   If you are not sure which Anneal Plate to choose, select an Anneal Plate in the left pane. In the right pane, click the Incoming Transfers tab and view the information displayed.

   B. In the Run Anneal Plate window, follow the prompts in the uppermost field and scan the barcode of the first sample plate listed (Figure 3.27).

   C. Press **Enter** or click **Next**.

   D. If more than one sample plate is being used, scan the remaining sample plate barcodes as prompted by the software.

   For the tube of Kit Control DNA, manually enter the barcode you created earlier.

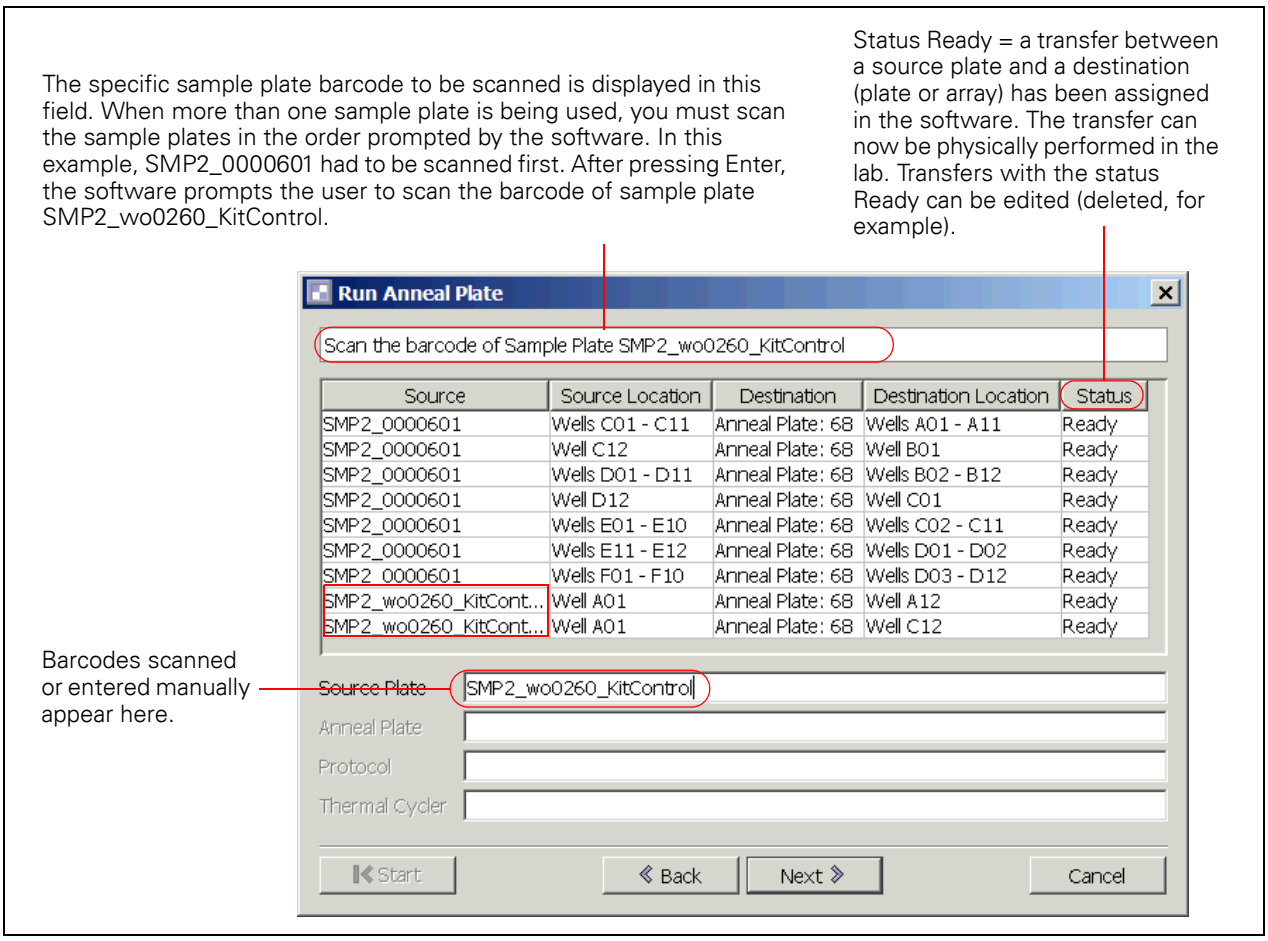


Figure 3.27
Run Anneal Plate Window – Scanning Sample Plate Barcodes

E. Scan the Anneal Plate barcode and press **Enter** or click **Next**.

F. Scan the protocol barcode and press **Enter** or click **Next**.

A list of available protocols is displayed in the upper part of the window.

G. In the Thermal Cycler field, type the number of the thermal cycler the plate will be annealed on and press **Enter** or click **Next**.

H. Click **Save**; then click **Close**.

Notice that the name of the Anneal Plate in the left pane is now the barcode that you scanned. In this example, it changed from Anneal Plate:4 to ANN_0000201. Information about the Anneal Plate is also now displayed in the right pane under the Properties tab (the Anneal Plate name must be selected to see this information; Figure 3.28).

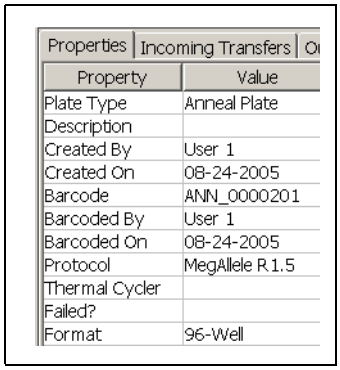


Figure 3.28
Anneal Plate Properties

ALIQUOT SAMPLES FROM SAMPLE TO ANNEAL PLATES

To aliquot samples from a sample plate to an Anneal Plate:

1. Spin down the sample plates at 1000 rpm for 30 seconds.
2. Place the sample and Anneal Plates in plate holders.
3. Spin down the Kit Control DNA.
4. In GTGS, select the Anneal Plate and click the **Incoming Transfers** tab in the right pane.

   Notice that the Status of each transfer has changed to Complete (Figure 3.29.) Once the barcode of the destination plate or array is scanned, the software assumes that the samples have been physically transferred from the source to the destination. You cannot edit transfers with a status of Complete.
5. Transfer samples from the designated sample plate wells (Source and Source Location) to the Anneal Plate (Current and Current Location.)
   - **For 3K and 5K assay panels**, aliquot 13.4 µL of:
     - Genomic DNA sample to wells 1 through 11 of rows A and C, and wells 1 through 12 of rows B and D.
     - Kit Control DNA to well 12 of rows A and C.
   - **For 10K and 20K assay panels**, aliquot 26.7 µL
     - Genomic DNA sample to wells 1 through 11 of rows A and C, and wells 1 through 12 of rows B and D.
     - Kit Control DNA to well 12 of rows A and C.

In this example, sample from well C12 of Sample Plate SMP2_000060 is to be transferred to well B1 of Anneal Plate ANN_2222. Samples C1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11 are to be transferred to wells A1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11, and so on.

Kit Control DNA from the tube called SMP2_wo0260_KitControl is to be aliquoted to wells A12 and C12 of the Anneal Plate ANN_2222.

The assay panel to be used to prepare the Anneal Cocktail is also listed (5-0059 3K Training Assay Panel in this example.) The Anneal Cocktail is to be aliquoted to all wells being used on the Assay Plate.

Status Complete = the Anneal Plate barcode has been scanned and the samples have been transferred from the Sample to the Anneal Plate. Transfers with the Status Complete cannot be changed.

Properties | Incoming Transfers | Outgoing Transfers

Source	Source Location	Current	Current Location	Status
5-0059 3K Training Assay Panel	Tube	ANN_2222	All Wells	Complete
SMP2_wo0260_KitControl	Well A01	ANN_2222	Well A12	Complete
SMP2_0000601	Well C12	ANN_2222	Well B01	Complete
SMP2_0000601	Well D12	ANN_2222	Well C01	Complete
SMP2_wo0260_KitControl	Well A01	ANN_2222	Well C12	Complete
SMP2_0000601	Wells C01 - C11	ANN_2222	Wells A01 - A11	Complete
SMP2_0000601	Wells D01 - D11	ANN_2222	Wells B02 - B12	Complete
SMP2_0000601	Wells E01 - E10	ANN_2222	Wells C02 - C11	Complete
SMP2_0000601	Wells E11 - E12	ANN_2222	Wells D01 - D02	Complete
SMP2_0000601	Wells F01 - F10	ANN_2222	Wells D03 - D12	Complete

Figure 3.29
Sample Plate to Anneal Plate Transfers

PREPARING SPECIAL EDITION ASSAY PANELS

If you are working with a Special Edition assay panel, then your GeneChip SNP Kits contain two tubes of assay panel mix: one tube containing the Standard Panel mix (fill size 160 µL), and one tube containing the Supplemental Panel mix (fill size 60 µL). Before proceeding to the next step, *Prepare Anneal Cocktail* , you must combine the contents of these tubes as directed below.

To prepare the Special Edition assay panel:

1. Combine the standard and supplemental assay panels as follows:
   - **A.** To a 1.5 mL Eppendorf tube, add the appropriate amount of standard assay panel as shown in Table 3.2.
   - **B.** Rinse the pipette tip by pipetting up and down 5 times.
   - **C.** Add the appropriate amount of supplemental assay panel as shown in Table 3.2.
   - **D.** Rinse the pipette tip by pipetting up and down 5 times.
2. Cap the tube and spin down.
3. Mix by vortexing.
4. Spin down again and place on ice until ready to use.

NOTE **The combination of Standard and Supplemental assay panels is now referred to as the *Assay Panel*.**

Table 3.2
Preparing the Special Edition Assay Panel Mix

Tube	1 Reaction	24 Reactions (20% extra)	48 Reactions (20% extra)
Standard Assay Panel Tube (fill size 160 µL)	3.75 µL	144 µL	288 µL
Supplemental Assay Panel Tube (fill size 60 µL)	1.25 µL	48 µL	96 µL
Total	**5 µL**	**192 µL**	**384 µL**

PREPARE ANNEAL COCKTAIL

IMPORTANT **Enzyme A is extremely temperature sensitive. Keep at –20°C until ready to use, and add last to the cocktail. To avoid denaturing, keep the Anneal Cocktail on ice until ready to use. Minimize warming by hand contact.**

To prepare the Anneal Cocktail:

1. Label a 2 mL Eppendorf tube with the letters AC.
2. Vortex and spin down Buffer A and the Assay Panel.

   If using a Special Edition assay panel, the contents of the Standard and Supplemental assay panel tubes should have already been combined. See *Preparing Special Edition Assay Panels* on page 49.
3. Place on ice until ready to use.
4. To the tube labeled AC, add and mix the reagents listed in Table 3.3 in the order shown.

NOTE **Thaw and spin down Enzyme A before adding to the cocktail.**

5. Store the remaining Water at 4°C.
6. Using a P1000 single-channel pipette, mix the Anneal Cocktail by pipetting up and down 5 times.
7. Keep on ice until ready to use.

Table 3.3
Anneal Cocktail

Reagents	1 Reaction	2 Rows (20% extra)	4 Rows (20% extra)
Water (from kit) (3K/5K blue; 10K/20K green)	21.6 µL 8.3 µL	622 µL 239 µL	1244 µL 478 µL
Buffer A	5.00 µL	144 µL	288 µL
Assay Panel	5.00 µL	144 µL	288 µL
Enzyme A (keep at -20°C until addition)	0.0625 µL	1.8 µL	3.6 µL
Total Volume Anneal Cocktail (**3K/5K blue**; **10K/20K green**)	**31.6 µL** **18.3 µL**	**912 µL** **529 µL**	**1824 µL** **1058 µL**

ALIQUOT ANNEAL COCKTAIL AND BEGIN INCUBATION

To aliquot the Anneal Cocktail and begin incubation:

1. Place the Anneal Plates on ice.
2. If using manual 24-channel pipettes, add the Anneal Cocktail as follows:
   - **A.** Place a 50 mL reagent reservoir on ice.
   - **B.** Pipette the Anneal Cocktail into the 50 mL reagent reservoir.
   - **C.** Aliquot the Anneal Cocktail as follows (Table 3.4):
     - *For 3K/5K assay panels*: Use a 12-channel P200 to add 31.6 µL to each reaction
     - *For 10K/20K assay panels*: Use a 12-channel P20 to add 18.3 µL to each reaction
   - **D.** Set a 24-channel P100 to 30 µL, and mix each sample by pipetting up and down 10 times.
3. If using electronic 24-channel pipettes, add the Anneal Cocktail as follows:
   - **A.** Aliquot the cocktail to two strips of 12 tubes each as follows:
     - *For 3K/5K assay panels*: Use a single-channel P200 to aliquot 35 µL (70 µL) to each tube
     - *For 10K/20K assay panels*: Use a single-channel P20 to aliquot 20 µL (40 µL) to each tube
   - **B.** Aliquot the cocktail to each reaction by dispensing/mixing 10 times for each reaction as follows (Table 3.4):
     - *3K/5K assay panels*: Use a P200 set to dispense/mix at 31.6 µL.
     - *10K/20K assay panels*: Use a P20 set to dispense/mix at 18.3 µL.

Table 3.4
Genomic DNA, Kit Control DNA and Anneal Cocktail

Component	3K and 5K Assay Panels	10K and 20K Assay Panels
Genomic DNA (150 ng/µL) or Kit Control DNA	13.4 µL	26.7 µL
Anneal Cocktail	31.6 µL	18.3 µL
Total	**45 µL**	**45 µL**

4. Seal each plate with a MicroAmp adhesive film, covering all wells even if empty.
5. Spin down the Anneal Plate at 1000 rpm for 30 seconds.
6. Start the thermal cycler program, *Meg Anneal* (Figure 3.30).
7. When the temperature on the thermal cycler reaches 20°C, load the Anneal Plate and close the lid.
8. Incubate the samples for 16 to 24 hours.

   Optimal incubation time is 16 to 24 hours. Do not incubate samples for more than 30 hours.

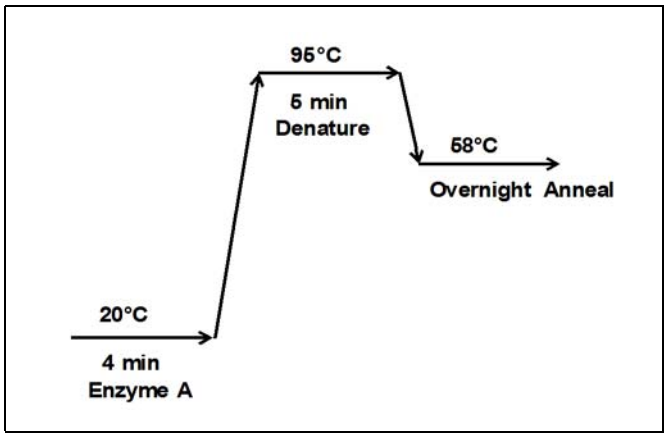


Figure 3.30
Meg Anneal Thermal Cycler Program

PREPARE FOR STAGE 4

To prepare for stage 4:

Place three to four 96-well aluminum blocks in a -20°C freezer overnight.

Stage 3 – Plan and Run Assay Plates

ABOUT THIS STAGE

During this stage, you will plan and prepare what are referred to as Assay Plates. An Assay Plate is a 96-well PCR plate with the barcode designation ASY<*barcode*>. You will use GTGS to perform this step.

LOCATION AND DURATION

- Pre-Amp Lab
- Hands-on time: approximately 10 minutes

EQUIPMENT AND MATERIALS REQUIRED

The following materials are required to perform this stage.

Table 3.5

Quantity	Item
1	Marking pen, extra fine point, permanent
2	PCR plate, 96-well, with ASY barcode

PLAN ASSAY PLATES

To plan Assay Plates:

1. Using a permanent marking pen, label the ASY-barcoded PCR plates as shown in Figure 3.31.

   One Assay Plate can hold up to two full rows of samples from an Anneal Plate. Samples are placed in rows A and E only of the Assay Plate.

IMPORTANT !

To ensure the highest quality data, we strongly recommend running one positive control for every 23 samples. Control DNA is included in each GeneChip SNP Kit.

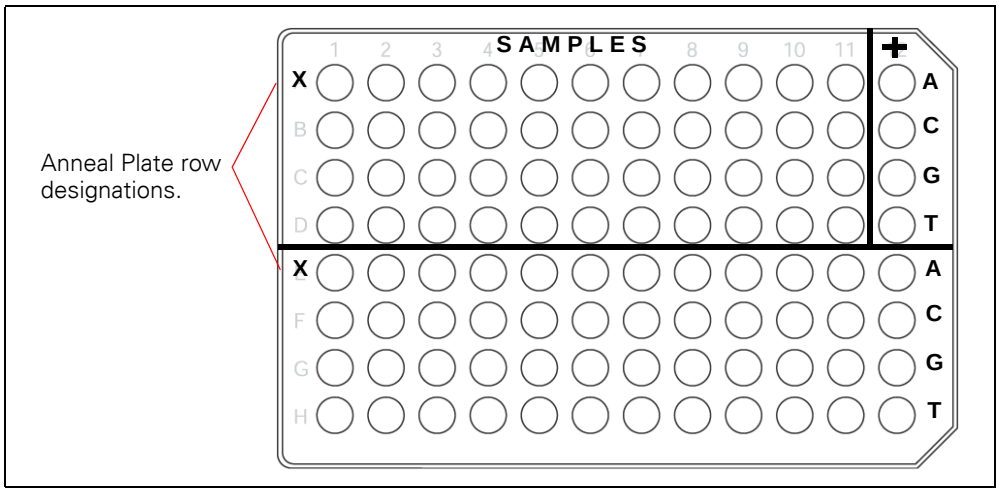


Figure 3.31
Assay Plate Labeling

2. In GTGS, expand the Project and Tracking icons.
3. Right-click the Assay Plates icon and select **Add Assay/Label/Hyb Plate Trio**.
4. Open the **Source** menu and select an Anneal Plate.

   You cannot change the Destination (Assay Plate).
5. Select the samples to be transferred from the Anneal Plate to the Assay Plate using one of these methods:
   - Highlight a row of samples on the Anneal Plate.
   - Select an individual sample on the Anneal Plate, such as sample B3 only.
   - Select a smaller group of samples, such as samples B1 through B4 only.
6. Do one of the following:
   - If you selected an entire row, highlight row A or E on the Assay Plate, then click **Set** (see Figure 3.32.)
   - If you selected one sample, highlight one well in row A or E on the Assay Plate, then click **Set**.
   - If you selected a smaller group of samples, highlight the corresponding number of cells in row A or E on the Assay Plate, then click **Set**.

NOTE **Each Assay Plate can hold a total of 24 reactions (including controls). When planning the Assay Plate in GTGS, the software restricts you to mapping samples from the Anneal Plate to rows A and E only of the Assay Plate.**

7. When all sample transfer designations are complete, click **Save**.

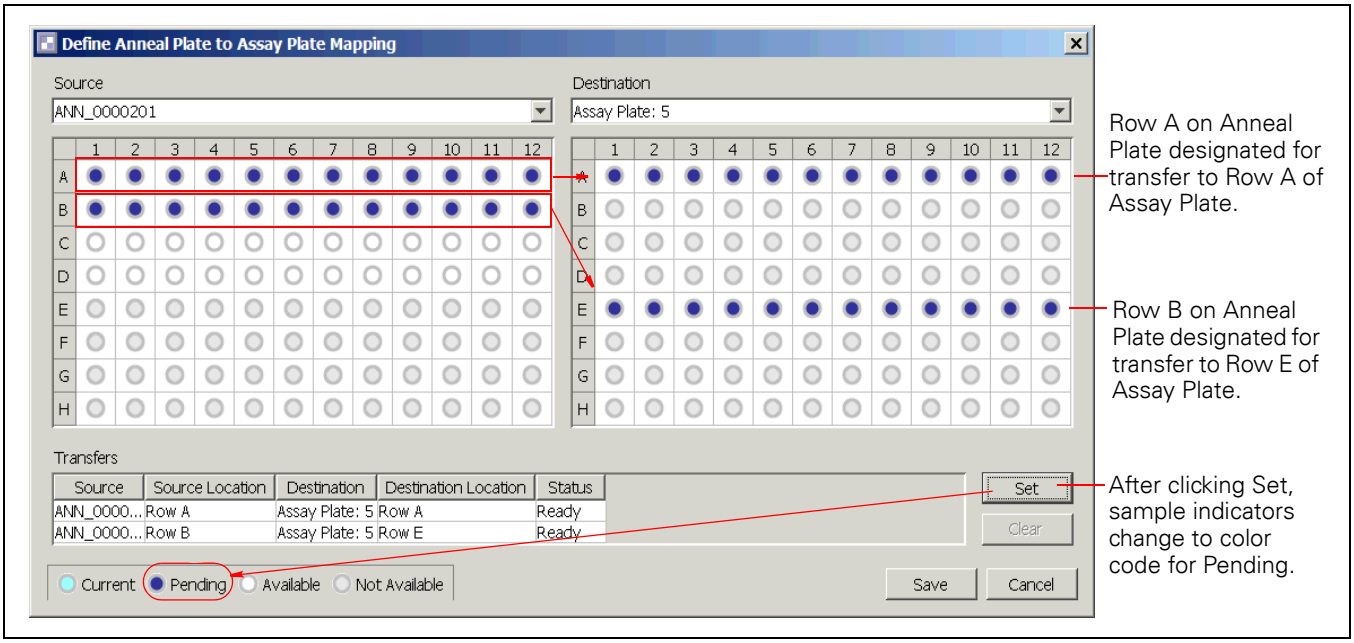


Figure 3.32
Planning an Assay Plate

Expand the Assay, Label and Hyb Plates icons. Notice there is now a placeholder for each type of plate in these folders. These three plates are linked. As you move through the protocol, each one will automatically appear in the plate mapping Source or Destination field as appropriate. Assay Plate samples are transferred to Label Plates, and Label Plate samples are transferred to Hyb Plates.

NOTE

To delete an Assay/Label/Hyb plate trio, you must select and delete the Hyb Plate. This action deletes the associated Assay and Label Plates as well. Once you begin the next stage of the protocol and scan an Assay Plate barcode, you can no longer delete these plates.

RUN ASSAY PLATES

To run Assay Plates:

1. Expand the Assay Plates icon.
2. Right-click an Assay Plate and select **Run** Assay Plate.
3. Scan the Anneal Plate barcode (appears in the Source field) and press **Enter**.
4. Scan the Assay Plate barcode (appears in the Source field) and press **Enter**.
5. Press **Enter** again to proceed past the protocol barcode which is already displayed in the Protocol field.
6. Enter a thermal cycler number and press **Enter**.
7. Click **Save**; then click **Close**.

   Notice that information about the Assay Plate is now displayed in the right pane under each of the tabs (Figure 3.33).

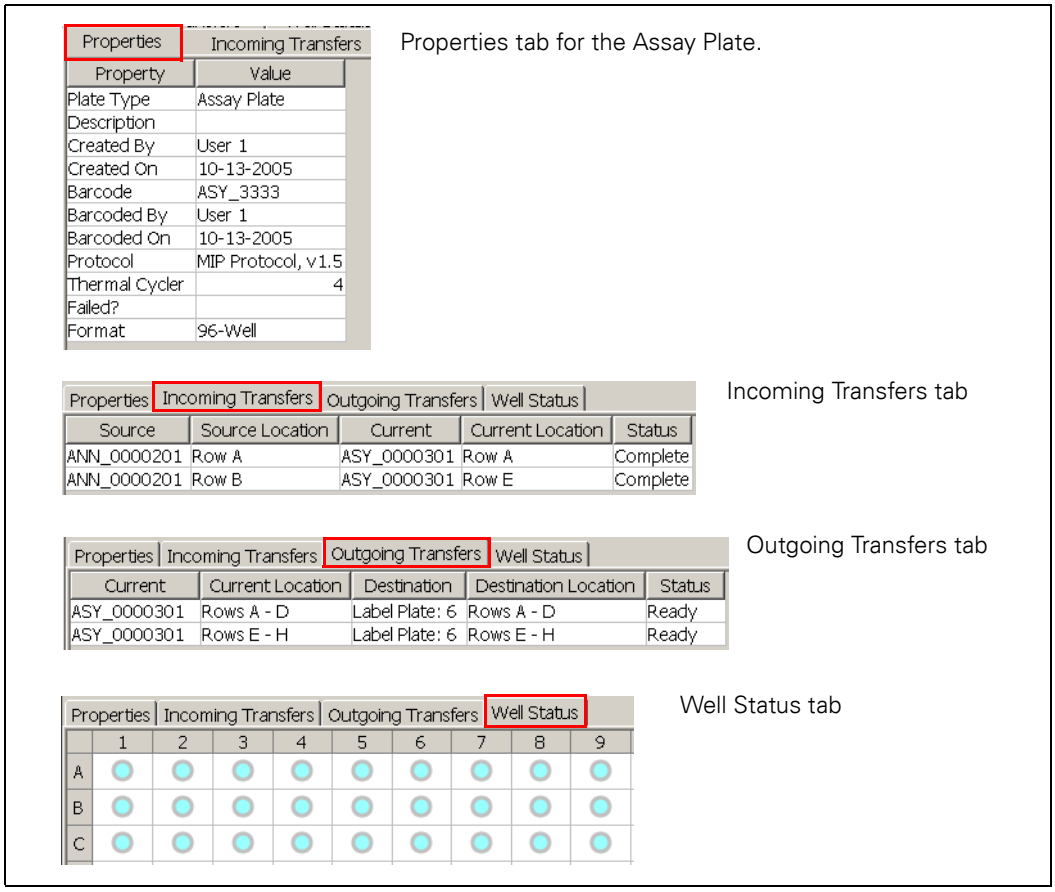


Figure 3.33
Assay Plate Tabs

Stage 4 – Gap Fill, dNTP, Ligate, Invert, First PCR

ABOUT THIS STAGE

During this stage, samples are transferred from the Anneal Plate to two Assay Plates. During the transfer, samples are split into four equal aliquots. Several additions are then made to each sample, one addition at a time, at specific intervals. The additions designated as *cold* are made on ice; the additions designated as *hot* are made while the samples are on a thermal cycler.

The thermal cycler program run for these additions is determined by the size of the assay panel you are using. It will be one of the following: the *Meg 3K 5K 10K* program, or the *Meg 20K* program.

Gap Fill Mix Addition

The first addition, Gap Fill Mix, is a cold addition. Gap fill enzymes find and bind to the single base gap in the assay panel probe. The gap is centered where the SNP of interest is located in the genome.

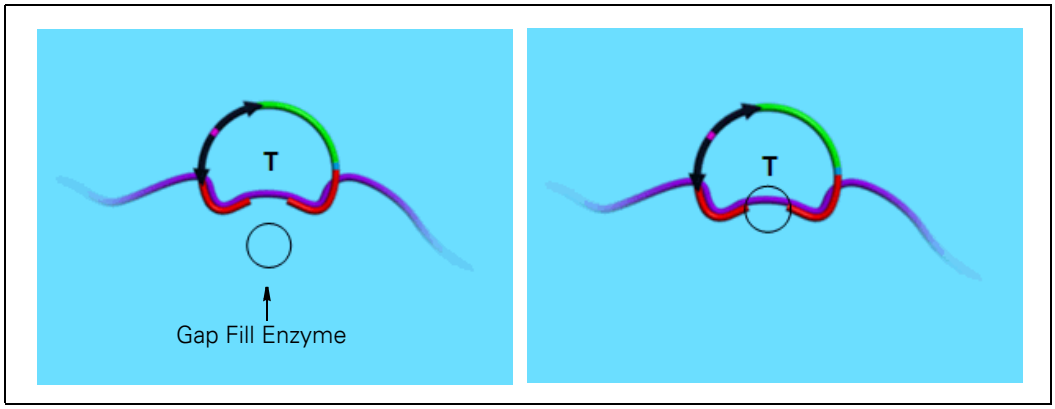


Figure 3.34
Gap Fill Enzyme Addition

dNTP Addition & Ligation

Each sample is now split into 4 equal aliquots in the Assay Plate, followed by the addition of dNTPs. For this cold addition, a different nucleotide is added to each aliquot. In the example shown in Figure 3.35, only the aliquot containing dATP will undergo a reaction, wherein the gap fill enzymes will use dATP to fill the gap in the probe.

NOTE

This example is based on a homozygous SNP locus. If the sample was heterozygous for this locus, two of the four aliquots would have a nucleotide added to the probe.

Once the gap in the probe is filled, the backbone of the assay probe is covalently sealed. The sealed probe is now referred to as a padlocked probe.

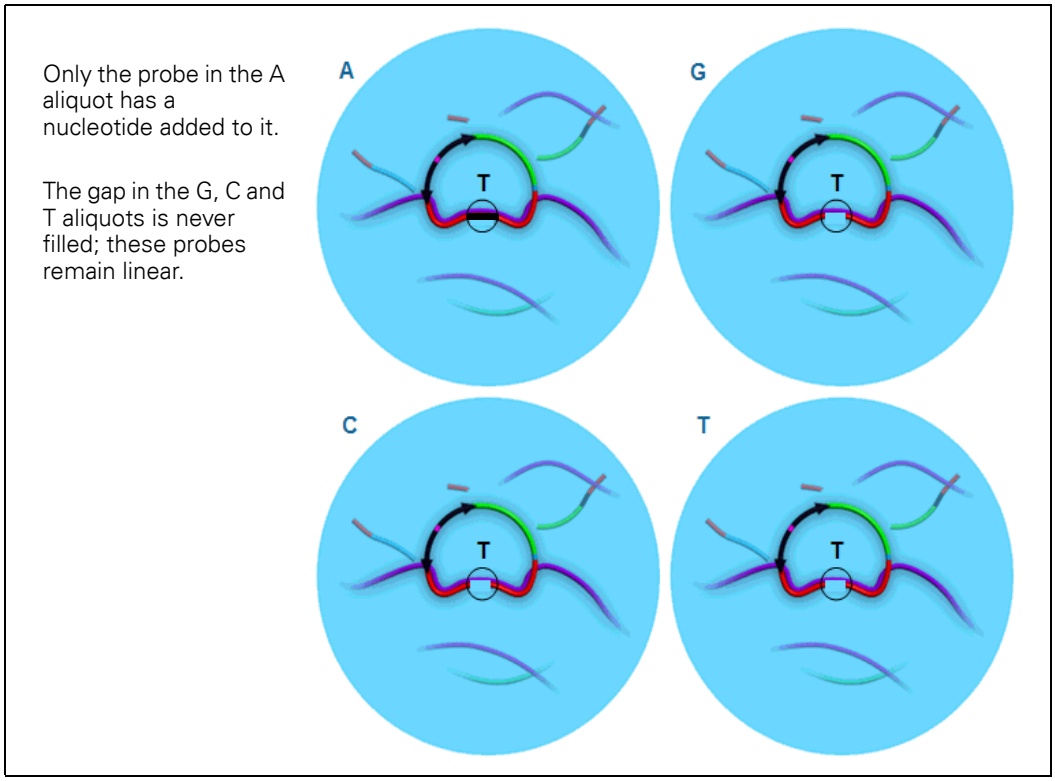


Figure 3.35
dNTP Addition
This example depicts one assay probe only. In reality, thousands of probes are undergoing the same process simultaneously.

Exo Mix Addition

The third addition is the Exo Mix, a cold addition. In this step, exonucleases are added to each aliquot. The exonucleases digest the linear probes and single-stranded DNAs that are present (Figure 3.36). Linear probes are present in the aliquots where the gap was not filled by a dNTP. Single-stranded DNA is present in all aliquots.

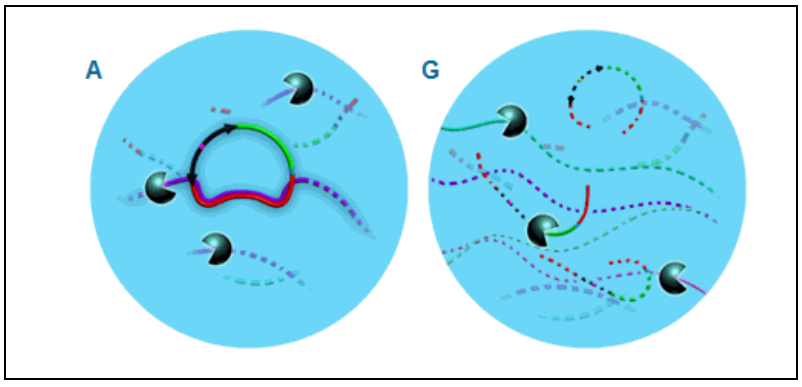


Figure 3.36
Exo Mix Addition Showing Two Aliquots Only

For homozygotes:

After the Exo Mix addition, three of the four aliquots contain digested DNA only for each assay probe. In the fourth aliquot, the genomic DNA is mainly digested away except for the small region where it is double-stranded due to its association with the padlocked probe.

For heterozygotes:

After the Exo Mix addition, two of the four aliquots contain digested DNA only. In the other two aliquots, the genomic DNA is mainly digested away except for the small region where it is double-stranded due to its association with the padlocked probe.

Cleavage Mix Addition and Inversion

The fourth addition is the Cleavage Mix, a hot addition. The padlocked probe is cleaved at cleavage site 1 in the assay probe backbone, thereby releasing it from the genomic DNA.

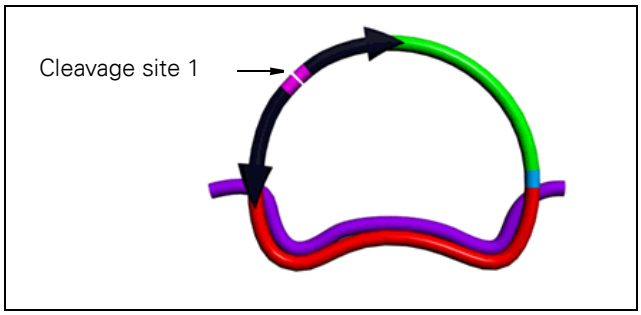


Figure 3.37
Cleavage Mix Addition

Once the assay probe has been cleaved, it releases from the remaining genomic DNA and becomes a linear molecule. Because the orientation of the PCR primer sequences has changed from the original orientation, the probe is now referred to as an *inverted probe*.

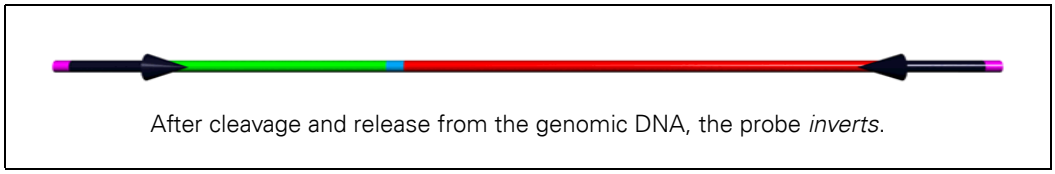


Figure 3.38
Inverted Probe

Amp Mix Addition and First PCR

The fifth addition is the Amp Mix, a hot addition. Once the Amp Mix is added, a PCR reaction takes place and the probe is amplified using common primers. The product from this reaction is referred to as the first PCR product (Figure 3.39).

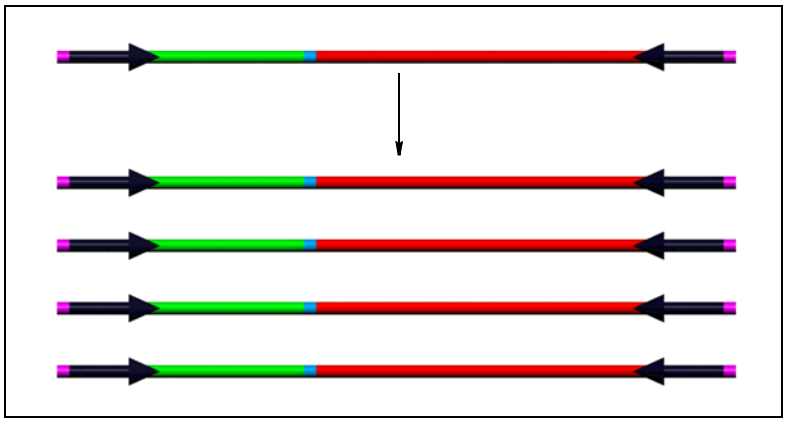


Figure 3.39
Amplified Probes from First PCR

LOCATION AND DURATION

- Pre-Amp Lab
- Hands-on time: 2.5 hours

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform these stages. Quantities shown are for processing 48 reactions.

Table 3.6
Equipment and Materials Required for Stage 4 – Gap Fill, dNTP, Ligate, Invert, First PCR

Quantity	Item
3 or 4	Aluminum blocks, chilled (or 2 aluminum blocks and 1 to 2 plate holders)
1	Anneal Plate from previous stage
2	Assay Plates, prepared and scanned
1	Centrifuge, plate
1	Ice container, rectangular, filled with ice
12	MicroAmp Clear Adhesive Films
1	Microfuge
2	Reagent reservoirs, 50 mL
2	Thermal cyclers, 96-well GeneAmp PCR System 9700
4	Tube strips with caps, PCR 12-well

KIT COMPONENTS REQUIRED

The following components are required from each GeneChip SNP Kit:

- Gap Fill Mix
- Exo Mix
- Cleavage Enzyme
- Cleavage Tube
- Amp Mix
- Plate of dNTPs (located in compartment below reagent tubes in the Pre-Amp Lab box)

OTHER REAGENTS REQUIRED

Stratagene Taq Polymerase (P/N 929197), 134 µL.

For more information, refer to *Taq Polymerase Required* on page 7.

THAW THE REAGENTS

To thaw the reagents:

1. Thaw the plate of dNTPs, the Cleavage Tube, and Amp Mix on the bench top.
2. Keep on ice until ready to use.

IMPORTANT **Leave the Exo Mix, Gap Fill Mix, Cleavage Enzyme, and Taq Polymerase at -20°C until ready to use.**

PREPARE THE GAP FILL MIX, EXO MIX, AND DNTP PLATE

NOTE **Volumes shown are for 24 reactions (and 48 reactions) respectively.**

Prepare the Gap Fill Mix

To prepare the Gap Fill Mix:

1. Place a plate holder or a chilled aluminum block on ice.
2. Spin down the Gap Fill Mix (G).
3. Label two strips of 12 PCR tubes with the letter G.
4. Aliquot 5 µL (10 µL) of Gap Fill Mix to each tube.
5. Cap and spin down the strip tubes.
6. Place the tubes in a plate holder or chilled aluminum block in adjacent rows.
7. Keep on ice until ready to use.

Prepare the Exo Mix

To prepare the Exo Mix:

1. Spin down the Exo Mix (E).
2. Label two strips of 12 PCR tubes with the letter E.
3. Aliquot 21 µL (42 µL) of Exo Mix to each tube.
4. Cap and spin down the strip tubes.
5. Place the tubes in the same plate holder or chilled aluminum block as the Gap Fill Mix.

NOTE **Be sure to keep the two mixes separated and easily identifiable. These mixes are added to the samples at different times. The Gap Fill Mix is added first.**

To save time, you can prepare the Exo Mix during the first or second 58°C incubation.

Prepare the dNTP Plate

To prepare the dNTP Plate:

1. Spin down the plate of dNTPs.
2. Place on ice until ready to use.

ADD GAP FILL MIX – ADDITION #1 ON COLD BLOCK

To add the Gap Fill Mix:

NOTE **These instructions are based on working with 48 reactions.**

1. Remove two aluminum blocks from the –20°C refrigerator and set on ice.
2. Stop the Meg Anneal thermal cycler program.
3. Remove the Anneal Plate from the thermal cycler.
4. Place the Anneal Plate in a chilled aluminum block on ice and cool for 2 min.
5. Spin down the plate at 1000 rpm for 30 seconds.
6. Place an Assay Plate in an aluminum block on ice.
7. Slowly remove the clear film from the Anneal Plate.
8. Working two rows at a time on the Anneal Plate (Figure 3.40), add the Gap Fill Mix using one of the following methods:
   - If using manual 24-channel pipettes:
     1) Use a P20 to add 2.5 µL of Gap Fill Mix to each sample.
     2) Mix up and down 3 times to rinse all of the Gap Fill Mix from the pipette tip.
     3) Set a P200 to 20 µL and mix each sample up and down 10 times.

- If using electronic 24-channel pipettes:

  1) Set a P20 to dispense at 2.5 µL and mix at 19 µL.

  2) Dispense/mix Gap Fill Mix to each sample 10 times.

Figure 3.40
Cold Addition

TRANSFER AND SPLIT SAMPLES

You will now transfer one row of samples from the Anneal Plate to four rows of an Assay Plate, splitting each sample into four aliquots of equal size.

To transfer and split samples:

1. Using a 12-channel P10 pipette, transfer and split the samples from the first two rows of the Anneal Plate to an Assay Plate as follows (Figure 3.41):

A. Transfer 9 µL from each well of the first row (A1 to A12 in the example) of the Anneal Plate to the rows marked A, C, G and T on the top half of the Assay Plate (A1 to D12.)

B. Transfer 9 µL from each well of the next row (B1 to B12 in the example) of the Anneal Plate to the rows marked A, C, G and T on the bottom half of the Assay Plate (E1 to H12.)

Samples from row A of the Anneal Plate are transferred and split between rows A, B, C and D (marked A, C, G and T) of the first Assay Plate. Samples from row B of the Anneal Plate are transferred and split between rows E, F, G and H of the first Assay Plate. C = Kit Control DNA.

NOTE: The samples in rows C and D are transferred to the second Assay Plate and are split in the same manner.

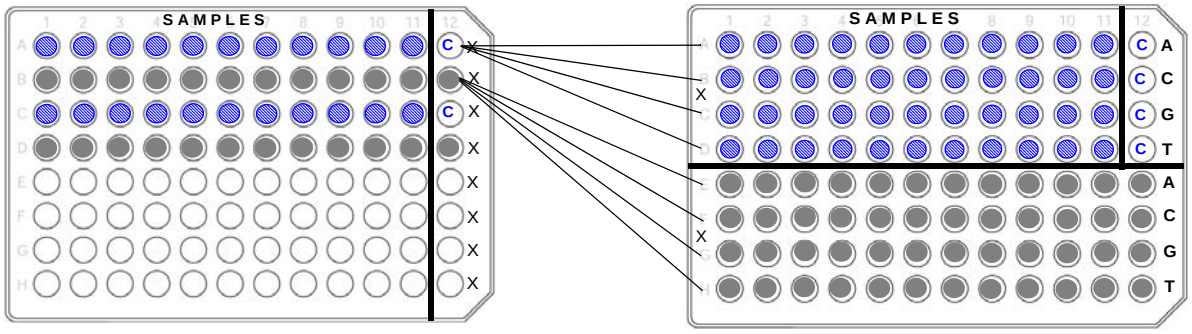


Figure 3.41
Transferring Samples from the Anneal Plate to the Assay Plates

2. Seal all of the wells of the Assay Plate with clear adhesive film.
3. Spin down the Assay Plate at 1000 rpm for 30 seconds.
4. Place in an aluminum block on ice.
5. Start the appropriate thermal cycler program:
   - *For 3K/5K/10K assay panels*: Meg 3-5-10K
   - *For 20K assay panels*: Meg 20K

   See Figure 3.42 on page 65.
6. When the thermal cycler reaches 58°C, load the Assay Plate and close the lid.
7. For 48 reactions, repeat the transfer and split process for the remaining two rows of samples on the Anneal Plate to the second Assay Plate plate.

IMPORTANT **The placement of each Assay Plate onto a thermal cycler should be staggered by approximately 2 minutes.**

8. Leave the aluminum blocks on ice.

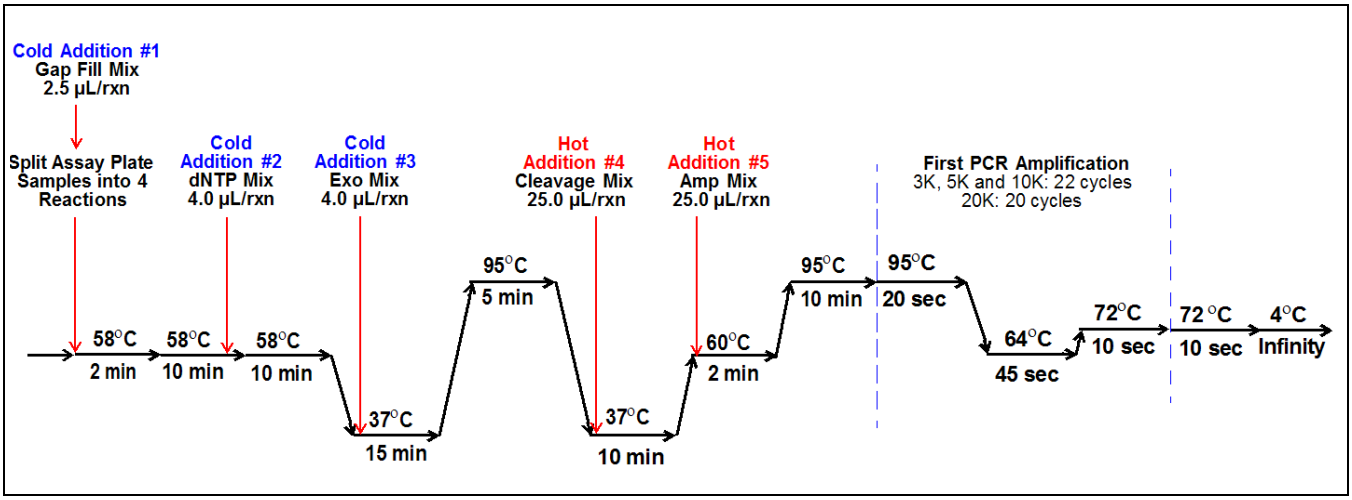


Figure 3.42
Meg 3-5-10K and Meg 20K Thermal Cycler Programs

ADD DNTPS – ADDITION #2 ON COLD BLOCK

To add the dNTPs:

IMPORTANT !

Perform this procedure one Assay Plate at a time.

1. When the thermal cycler timer reads 1:00 minute remaining for the second 58°C period (Figure 3.42), press **Pause**.
2. Remove the Assay Plate and place it in an aluminum block on ice for 2 minutes.

WARNING ▲

PHYSICAL INJURY HAZARD. The thermal cycler lid, sample block and Assay Plate are very hot. Wear protective gloves when opening and closing the lid, and when handling Assay Plates.

3. Remove the seal from the Assay Plate and the dNTP plate.
4. If using a manual 24-channel P20 pipette, add dNTPs to each reaction as follows (Figure 3.43):
   - **A.** Aliquot 4.0 µL dATP to row A, and 4.0 µL dCTP to row B.
   - **B.** Mix by pipetting up and down 20 times, reaching the bottom of all wells.
   - **C.** Aliquot 4.0 µL dGTP to row C, and 4.0 µL dTTP to row D.
   - **D.** Mix by pipetting up and down 20 times, reaching the bottom of all wells.
5. If using an electronic 24-channel P20 pipette, add dNTPs to each reaction as follows (Figure 3.43):
   - **A.** Set the pipette to dispense and mix at 4 µL.
   - **B.** Dispense/mix dNTPs to each sample 20 times.
6. Repeat these additions for the bottom half of the plate.

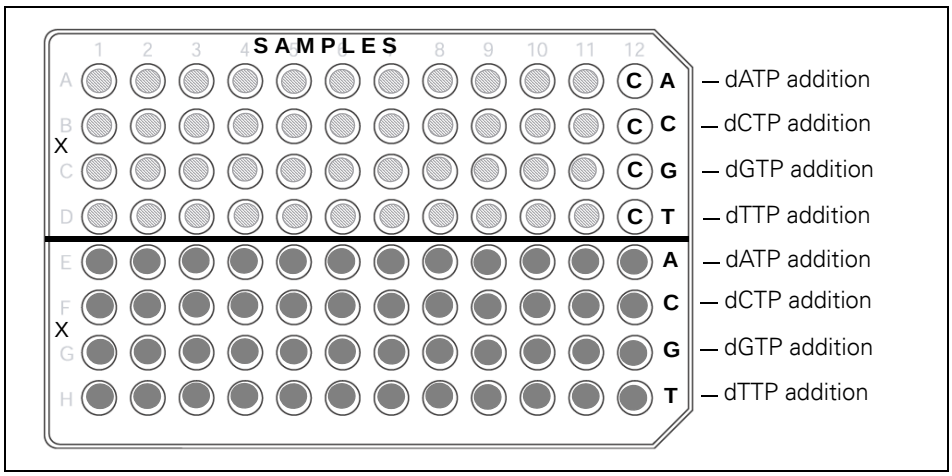


Figure 3.43
dNTP Addition

7. Reseal the plate with a new adhesive film.
8. Spin down at 1000 rpm for 30 seconds.
9. Place the plate back on the thermal cycler and close the lid.
10. Press **Resume** to continue the thermal cycler program.
11. Repeat this procedure for all remaining Assay Plates.

ADD EXO MIX – ADDITION #3 ON COLD BLOCK

To add the Exo Mix:

Perform this procedure one Assay Plate at a time.

1. When the timer reads 14:00 minutes remaining for the first 37°C period (Figure 3.42 on page 65):
   - **A.** Press **Pause** on the thermal cycler.
   - **B.** Remove the Assay Plate and place in an aluminum block on ice for 2 minutes.
   - **C.** If using a manual 24-channel P20 pipette:
     - **1)** Add 4 µL of Exo Mix to each reaction.
     - **2)** Mix by pipetting up and down 20 times, reaching the bottom of all wells.
   - **D.** If using an electronic 24-channel P20 pipette:
     - **1)** Set the pipette to dispense and mix at 4 µL
     - **2)** Add Exo Mix to each reaction, mixing each reaction 20 times.
2. Reseal the plate with a new adhesive film.
3. Spin down at 1000 rpm for 30 seconds.
4. Place the plate back on the thermal cycler and close the lid.
5. Press **Resume** to continue the thermal cycler program.
6. Repeat this procedure for all remaining Assay Plates.

PREPARE THE CLEAVAGE AND AMP MIXES

Prepare the Cleavage Mix

IMPORTANT !

Prepare during first 95°C hold.

To prepare the Cleavage Mix:

1. Spin down the Cleavage Enzyme.
2. Using a P200 single channel pipette, add 24 µL of Cleavage Enzyme to each Cleavage Tube.

   This mixture is now referred to as the Cleavage Mix.
3. Using a P1000 single channel pipette, mix well.
4. Mark a 50 mL reagent reservoir with the letter C.
5. Transfer the Cleavage Mix to the reagent reservoir marked C and place on ice.

Prepare the Amp Mix

IMPORTANT !

Prepare during first 95°C hold.

To prepare the Amp Mix:

1. pin down the Stratagene Taq polymerase.
2. Using a P200 single channel pipette, add 67 µL of Taq polymerase to the Amp Mix tube.
3. Using a P1000 single channel pipette, mix well.
4. Mark a 50 mL reagent reservoir with the letter A.
5. Transfer the Amp Mix to the reagent reservoir marked A and place on ice.

ADD CLEAVAGE MIX – ADDITION #4 ON THERMAL CYCLER

IMPORTANT !

Perform this addition one Assay Plate at a time.

To add the Cleavage Mix:

1. When the thermal cycler timer reads 9:00 minutes remaining for the second 37°C period (Figure 3.42 on page 65):
   - **A.** Press **Pause** on the thermal cycler and open the lid.
   - **B.** Leaving the Assay Plate on the cycler, remove the adhesive film.
   - **C.** Using a 12-channel P200 pipette, aliquot 25 µL of Cleavage Mix to each reaction.
   - **D.** Mix by pipetting up and down 10 times (Figure 3.44).
   - **E.** Reseal the plate with a new adhesive film, and close the thermal cycler lid.
   - **F.** Press **Resume** to continue the thermal cycler program.
2. Repeat this procedure for all remaining Assay Plates.

Figure 3.44
Hot Addition on Thermal Cycler

ADD AMP MIX – ADDITION #5 ON THERMAL CYCLER

IMPORTANT **Perform this addition one Assay Plate at a time.**

To add the Amp Mix:

1. When the thermal cycler temperature reaches 60°C (Figure 3.42 on page 65):
   - **A.** Press **Pause** on the thermal cycler and open the lid.
   - **B.** Leaving the Assay Plate on the cycler, remove the adhesive film.
   - **C.** Using a 12-channel P200 pipette, aliquot 25 µL of Amp Mix to each reaction.
   - **D.** Mix by pipetting up and down 10 times.
   - **E.** Reseal the plate with a new adhesive film.
   - **F.** Close the thermal cycler lid.
   - **G.** Press **Resume** to continue the thermal cycler program.
2. Repeat this procedure for all remaining Assay Plates.

TRANSFER ASSAY PLATES AND KIT COMPONENTS TO POST-AMP LAB

To transfer the Assay Plates and remaining GeneChip SNP Kit components:

WARNING **CONTAMINATION DANGER. Do not remove the adhesive film from the Assay Plates. Assay Plates must remain sealed until they have been transferred to the Post-Amp Lab.**

1. At the end of the program (approximately one hour after the start of the first PCR amplification), remove the Assay Plates from the thermal cyclers.
2. Leaving the plates sealed, transfer the plates and the remaining GeneChip SNP Kit contents to the Post-Amp Lab.
3. Store the kit contents in the appropriate refrigerator or freezer.
4. If not immediately proceeding to Stage 5 – Second PCR, the Assay Plates can be stored at -20°C.

IMPORTANT **We recommend freezing and thawing plates ONE TIME ONLY. For a given set of samples, do not freeze and thaw plates multiple times during the protocol.**

Stage 5 – Second PCR

ABOUT THIS STAGE

During this stage, you will prepare what are referred to as Label Plate*s* (96-well PCR plates with the barcode designation LBL<*barcode*>.) Each sample is transferred from the Assay Plate to a Label Plate.

After the transfer, a different allele-specific primer is added to each reaction as a label, and the second PCR is performed. The thermal cycler program used is determined by the size of the assay panel. For 3K and 5K assay panels, *Meg Hypcr 3-5K* is used. For 10K and 20K assay panels, *Meg Hypcr 10-20K* is used.

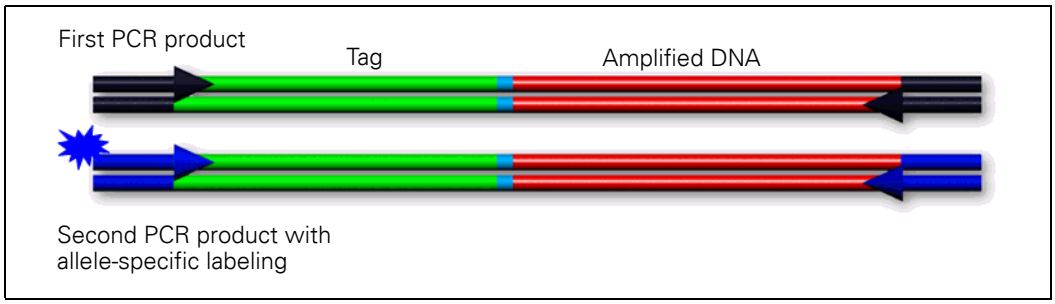


Figure 3.45
Second PCR

LOCATION AND DURATION

- Post-Amp Lab
- Hands-on time: Approximately 25 minutes
- Thermal cycler time: 30 minutes

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 reactions.

Table 3.7
Equipment and Materials Required for *Stage 5 – Second PCR*

Quantity	Item
2	Assay Plates with samples from previous stage
1	Centrifuge, plate
1	Ice container, rectangular, filled with ice
2	PCR plates, 96-well, with LBL barcode
2	Marking pens, extra fine point, permanent, different colors
minimum 4	MicroAmp Clear Adhesive Films
1	Microfuge
4	Plate holders (preparation racks)
4	Reagent reservoirs, 50 mL
2	Thermal cyclers

KIT COMPONENTS REQUIRED

The following components are required from each GeneChip SNP Kit:

- HY A Allele Tube
- HY C Allele Tube
- HY G Allele Tube
- HY T Allele Tube

OTHER REAGENTS REQUIRED

Clontech TITANIUM Taq DNA Polymerase

For more information, refer to *Taq Polymerase Required* on page 7.

THAW REAGENTS

To thaw reagents:

1. Thaw the HY A Allele, HY C Allele, HY G Allele, and HY T Allele tubes on the bench top.
2. Spin down each tube.
3. Keep on ice until ready to use.

RUN LABEL PLATES

To run Label Plates:

1. Using a permanent marking pen, mark the LBL barcoded plates as shown in Figure 3.46.

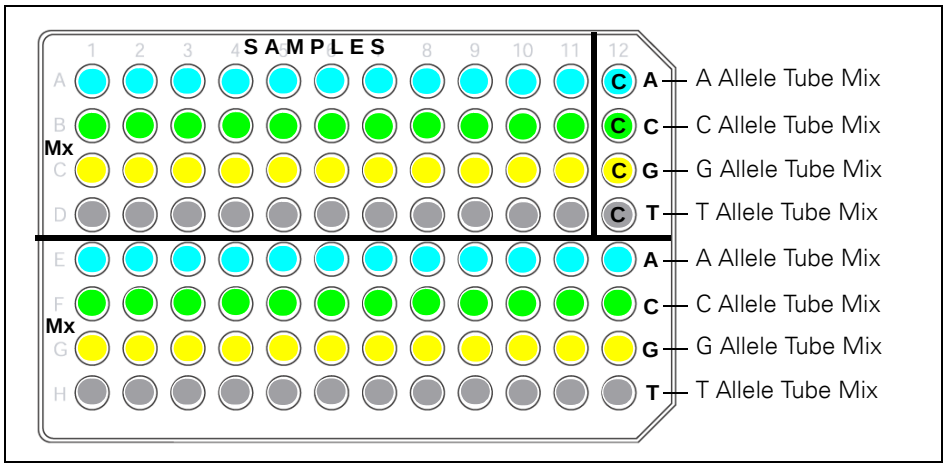


Figure 3.46
Label Plate Labeling and Sample Placement

2. In GTGS, expand the Project, Tracking and Label Plate icons.
3. Right-click the appropriate Label Plate.

   If you are not sure which Label Plate is correct, select a Label Plate. In the right-hand pane, look at the Assay Plate indicated in the Source column under the Incoming Transfers tab.
4. Select **Run Label Plate**.

   The Run Label Plate window appears (Figure 3.47).

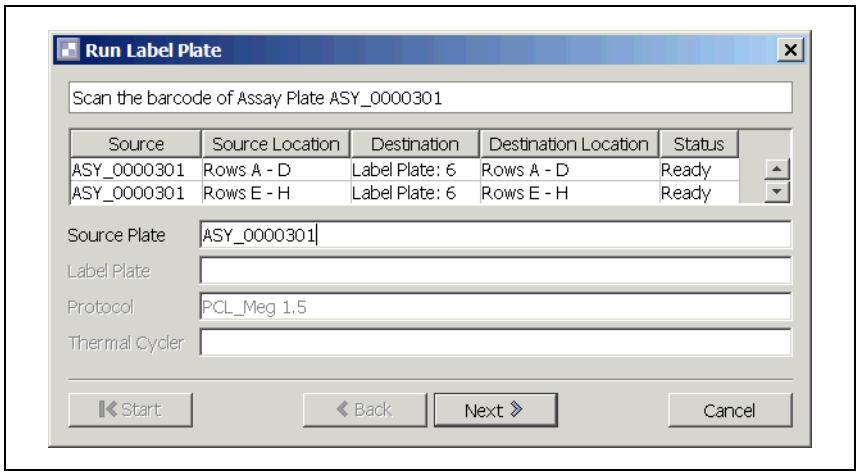


Figure 3.47
Run Label Plate Window

   H. Scan the Assay Plate barcode (Source Plate field) and press **Enter**.

I. Scan the Label Plate barcode (Label Plate field) and press **Enter**.

J. Press **Enter** again to proceed past the protocol barcode which is already displayed in the Protocol field.

K. Enter a thermal cycler number and press **Enter**.

L. Click **Next**; then click **Save**.

5. Using a marking pen, mark the Assay and Label Plates with the same color.

   Use a different color for each set of plates to ensure that samples are transferred from each Assay Plate to the correct Label Plate.

6. Repeat these steps for the next Label Plate.

PREPARE HY ALLELE TUBE MIXES

To prepare the Allele Tube Mixes:

1. Spin down the TITANIUM Taq polymerase.
2. Using a P100 pipette, add 22 µL of Taq polymerase to each allele tube.
3. Set a P1000 pipette to 750 µL and mix by pipetting up and down 10 times.
4. Label one set of 12 strip tubes for each HY Allele mix: A, C, G and T
5. Aliquot 75 µL (150 µL) of each allele tube mix as follows:
   - HY A Allele Tube Mix to the strip tubes labeled A.
   - HY C Allele Tube Mix to the strip tubes labeled C.
   - HY G Allele Tube Mix to the strip tubes labeled G.
   - HY T Allele Tube Mix to the strip tubes labeled T.

TRANSFER REACTIONS TO LABEL PLATES AND ADD ALLELE TUBE MIXES

To transfer reactions to Label Plates and add Allele Tube mixes:

1. Working one row at a time using a 12-channel P200 pipette, aliquot 31 µL of each Allele Tube mix to the corresponding row on the Label Plate (Figure 3.44):
   - HY A Allele Tube mix to rows labeled A (A and E)
   - HY C Allele Tube mix to rows labeled C (B and F)
   - HY G Allele Tube mix to rows labeled G (C and G)
   - HY T Allele Tube mix to rows labeled T (D and H)
2. If using manual 24-channel pipettes:

   A. Set a P20 to 4 µL and transfer 4 µL from each well of the top two rows of the Assay Plate (marked A and C) to the same rows on the Label Plate.

   B. Mix by pipetting up and down 5 times.

   C. Set a P100 to 20 µL, and mix by pipetting up and down 10 times.

   D. Continue transferring and mixing the remaining reactions from the Assay Plate to the Label Plate in the same manner.

3. If using electronic 24-channel pipettes:
   - **A.** Set a P20 to dispense/mix at 4 µL.
   - **B.** Dispense/mix 4 µL from each well of the top two rows of the Assay Plate (marked A and C) to the same rows on the Label Plate 5 times.
   - **C.** Set a P100 set to dispense/mix at 20 µL. Mix each reaction by dispensing/mixing 10 times.
   - **D.** Continue transferring and mixing the remaining reactions from the Assay Plate to the Label Plate in the same manner.
4. Seal each Label Plate with a new adhesive film.
5. Spin down the Label Plates at 1000 rpm for 30 seconds.
6. Place each Label Plate onto a thermal cycler and close the lid.
7. Start the appropriate thermal cycler program:
   - For 3K/5K assay panels, use *Meg Hypcr 3-5K*
   - For 10K/20K assay panels, use *Meg Hypcr 10-20K* (Figure 3.48).

NOTE **This program takes approximately 30 minutes to run.**

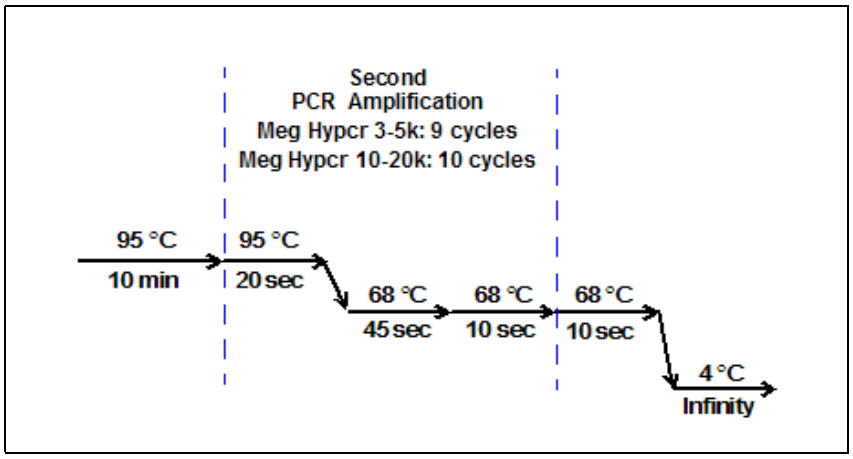


Figure 3.48
Meg Hypcr 3-5K and Meg Hypcr 10-20K Thermal Cycler Programs

8. If running a quality control gel, refer to *First Quality Control Gel* on page 75 now.
9. When the program is finished, remove the plates and spin down at 1000 rpm for 30 seconds.
10. Proceed directly to *Stage 6 – Target Digest* on page 77.

IMPORTANT **Do not freeze samples at this stage of the protocol. Proceed directly to the next stage.**

FIRST QUALITY CONTROL GEL

The first quality control gel is run while the second PCR thermal cycler program is running (Meg Hypcr 3-5K or Meg Hypcr 10-20K). Samples are taken from the Assay Plates. For gel recommendations, see *Quality Control Gels* on page 19.

To run the first quality control gel:

1. Pipette 7 µL of each sample from an Assay Plate to a 96-well PCR plate.
2. Add 3 µL loading buffer and mix well.
3. Load onto a gel.
4. Repeat steps 1 through 3 for each Assay Plate and gel.
5. Seal the Assay Plates with new adhesive films and store at -20°C.
6. Run and inspect the gels.

VIEWING THE FIRST QUALITY CONTROL GEL

A good first quality control gel will look like the one shown below.

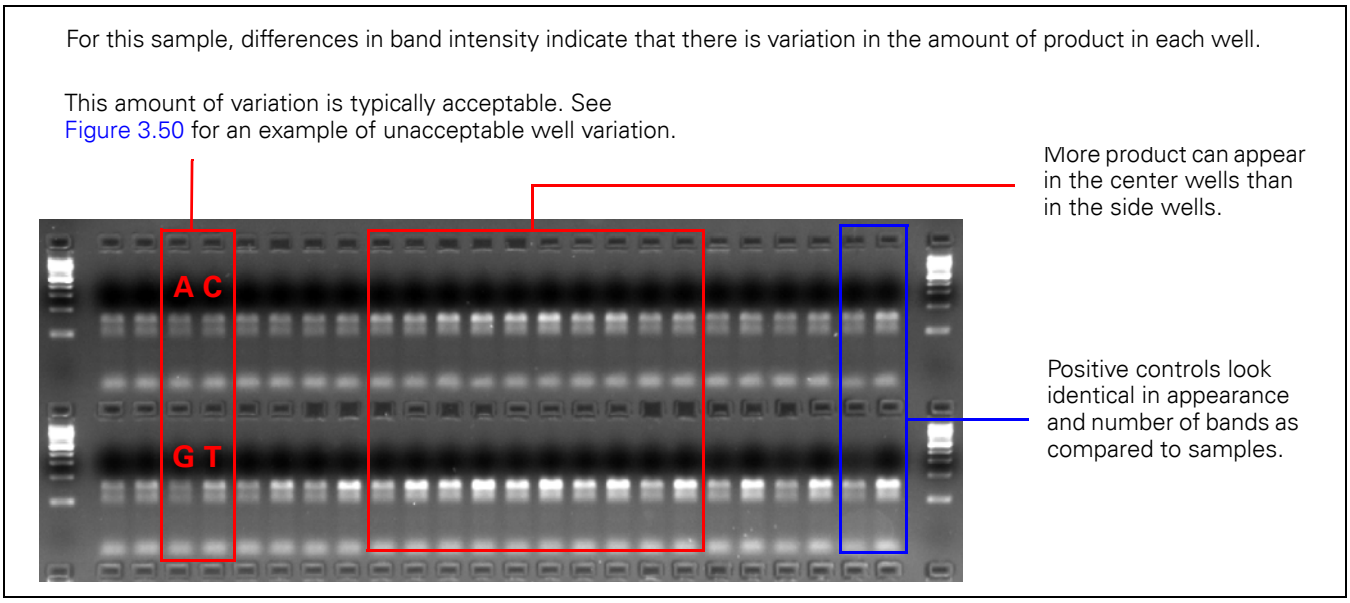
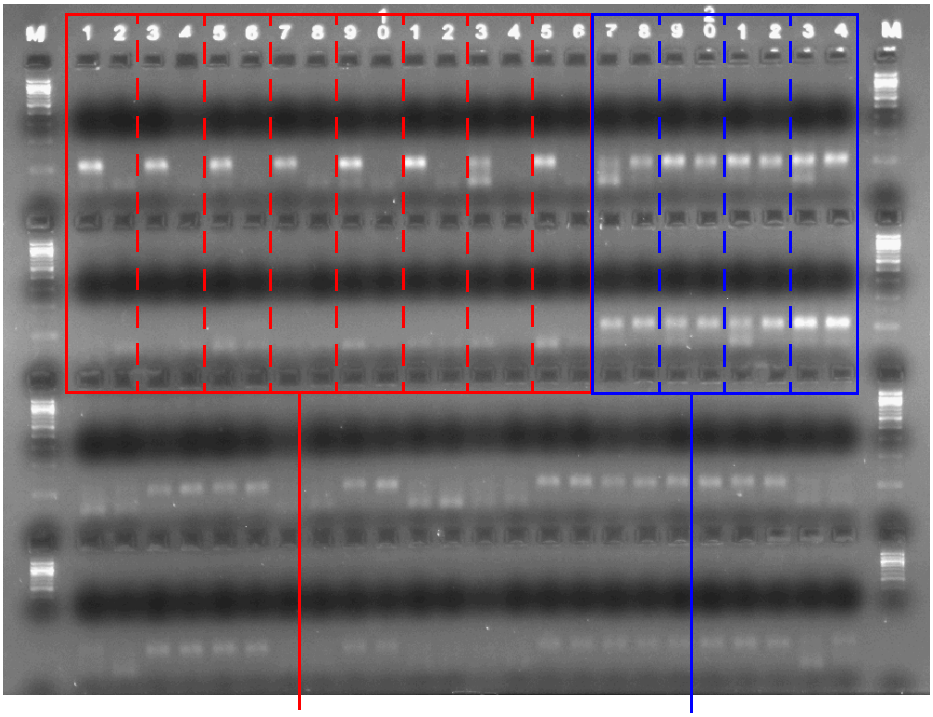


Figure 3.49
Good First Quality Control Gel for Assay Panel run on a 10K Universal Tag Array – Top Half Only



Unacceptable well variation. These samples failed when hybridized to arrays and scanned.

Failure was due to unacceptably low signal strength in several channels. Repeat the experiment from Stage 1.

Samples in wells 17 through 22 passed when hybridized to arrays and scanned. The variation in appearance of these bands is within acceptable limits.

Figure 3.50
First Quality Control Gel with Mixed Results

Stage 6 – Target Digest

ABOUT THIS STAGE

During this stage, the labeled second PCR product is cleaved at cleavage site 2 in the backbone. This process removes the amplified genomic DNA portion of the assay probe from the tag and allele-specific label.

As part of this stage, you will prepare what are referred to as *Hyb Plates* (96-well PCR plates with the barcode designation HYB<*barcode*>).

You will then transfer the four individual reactions for each sample from the Label Plate to a Hyb Plate. At the same time, you will consolidate the four reactions for each sample back to one well. The Hyb Plates are then placed on thermal cyclers and the *Meg Hydigest-a* program is run.

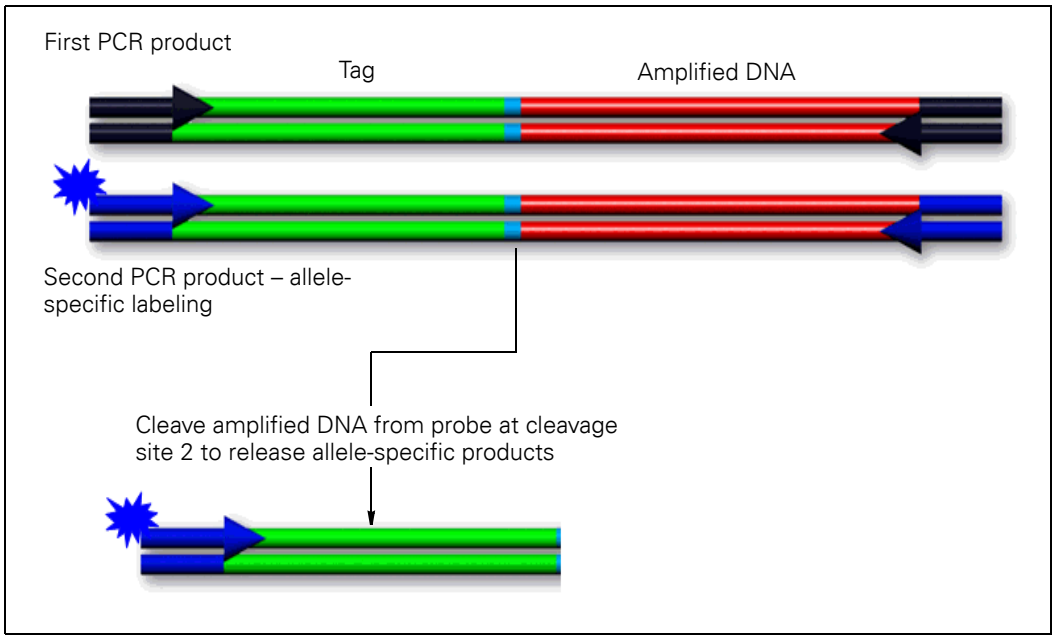


Figure 3.51
Target Digest

LOCATION AND DURATION

- Post-Amp Lab
- Hands-on time: approximately 30 minutes
- Time on thermal cycler: 1 hour and 35 minutes

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 reactions.

Table 3.8

Quantity	Item
1	Centrifuge, plate
1	Ice container, rectangular, filled with ice
2	Label Plates containing samples from previous stage
1	Marking pen, extra fine point, permanent
4	MicroAmp Clear Adhesive Films
1	Microfuge
2	PCR plates, 96-well, with HYB barcode
3	Plate holders (preparation racks)
2	Thermal cyclers
2	Tube strip with caps, PCR 12-well

WARNING ▲ **PHYSICAL INJURY HAZARD. The thermal cycler lid, sample block and PCR plate are very hot. Wear protective gloves when opening and closing the lid, and when handling the PCR plate.**

KIT COMPONENTS REQUIRED

The following component is required from each GeneChip SNP Kit:

- HY Digest Mix

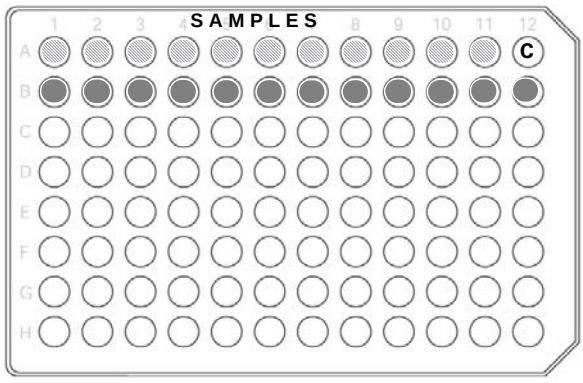
RUN HYB PLATES

To run Hyb Plates:

1. Using a permanent marking pen, label the HYB-barcoded plates as shown in Figure 3.52 on page 79.
2. In GTGS, expand the project, Tracking and Hyb Plates icons.
3. Right-click the appropriate Hyb Plate and select **Run Hyb Plate**.

   To determine the correct Hyb Plate, select a Hyb Plate and click the Incoming Transfers tab. Look at the Label Plate specified in the Source field.
4. Scan the Label Plate barcode (Source Plate field) and press **Enter**.
5. Scan the Hyb Plate barcode (Hyb Plate field) and press **Enter**.
6. Press **Enter** again to proceed past the protocol barcode which is already displayed in the Protocol field.

7. Enter a thermal cycler number and press **Enter**.
8. Click **Save**; then click **Close**.



Rows A through D of the Label Plate are consolidated to row A of the Hyb Plate.

Rows E through H of the Label Plate are consolidated to row B of the Hyb Plate.

Figure 3.52
Hyb Plate Labeling and Sample Placement

TRANSFER REACTIONS TO HYB PLATES

While transferring reactions from Label to Hyb Plates, you will consolidate all four reactions for each sample into one well.

To transfer reactions to Hyb Plates:

1. Spin down each Label Plate at 1000 rpm for 30 sec.
2. Set a 12-channel P20 pipette to 17 µL.
3. Remove the seal from each Label Plate.
4. Transfer 17 µL from each well of Label Plate rows A, B, C and D to Hyb Plate row A (Figure 3.52).
5. Transfer 17 µL from each well of Label Plate rows E, F, G and H to Hyb Plate row B.

Total volume in each well should be 68 µL.

ADD HY DIGEST MIX

NOTE **Volumes shown are for 24 reactions (and 48 reactions) respectively.**

To add the HY Digest Mix:

1. Spin down the HY Digest Mix and keep on ice until ready to use.
2. Into two strips of 12 PCR tubes each, aliquot 9 µL (18 µL) of HY Digest Mix to each tube using a P20 single channel pipette.
3. Cap and spin down the strip tubes.
4. Place the tubes in a plate holder on ice (in 2 adjacent rows) until ready to use.
5. If using manual 24-channel pipettes:

A. Set a P20 to 6 µL.

B. Aliquot 6 µL of HY Digest Mix to each sample, and mix by pipetting up and down 5 times to ensure the tip is thoroughly rinsed.

C. Set a P100 to 50 µL, and mix each reaction by pipetting up and down 15 times.

6. If using electronic 24-channel pipettes:

A. Set a P20 to dispense/mix at 6 µL.

B. Dispense/mix 6 µL of HY Digest Mix to each sample 5 times.

C. Set a P100 to dispense/mix at 50 µL.

D. Mix each sample by dispensing/mixing 15 times.

IMPORTANT !

The full volume must be picked up and dispensed each time to ensure that all of the HY Digest Mix is flushed from the pipette tip.

7. Seal each plate with a new adhesive film.
8. Spin down at 1000 rpm for 30 seconds.

RUN THE MEG HYDIGEST-A PROGRAM

To run the Meg Hydigest-a program:

1. Place each plate on a thermal cycler and close the lid.
2. Start the program, *Meg Hydigest-a* (Figure 3.53).
3. If running a second quality control gel, see *Second Quality Control Gel* on page 81.

   You will remove sample from the plate at a specific point during the thermal cycler program.
4. When the program is finished, remove the plates and proceed immediately to *Stage 7 – Sample Hybridization* on page 84.

IMPORTANT !

Do not freeze samples at this stage of the protocol. Proceed directly to the next stage.

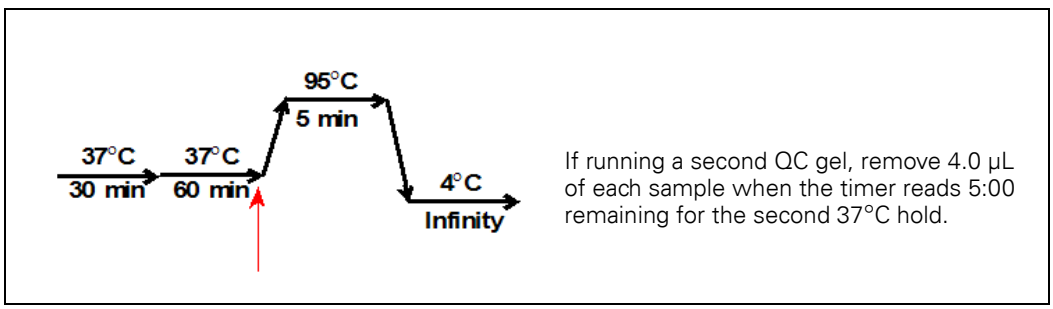


Figure 3.53
Meg Hydigest-a Thermal Cycler Program

SECOND QUALITY CONTROL GEL

NOTE **This operation should be performed quickly and should not exceed 5 minutes.**

To run a second quality control gel:

1. When the timer reads 5:00 minutes remaining for the second 37°C hold (Figure 3.53), press **Pause**.
2. Remove the plate from the thermal cycler and place on the bench top.
3. Remove the adhesive film from the plate.
4. Remove 4.0 µL of each reaction for the gel.
5. Add to each reaction 3 µL of loading buffer and 3 µL of DI water or 1X TE Buffer.
6. Reseal the plate with a new adhesive film.
7. Place the plate back on the thermal cycler and close the lid.
8. Press **Resume** to continue running the program.
9. Combine the 4.0 µL of each reaction with 3 µL loading buffer and 3 µL DI water or 1X TE Buffer.
10. Repeat these steps for each Hyb Plate.
11. Load and run the gels.

EVALUATING THE SECOND QUALITY CONTROL GEL

A good second quality control gel will look like the one shown in Figure 3.54.

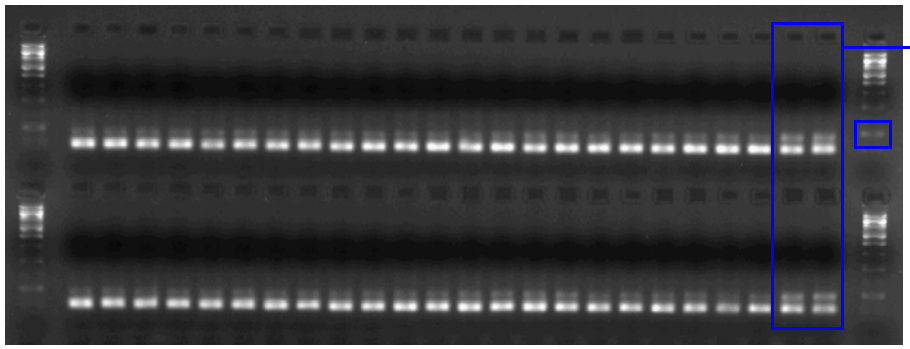


Figure 3.54
Good Second Quality Control Gel – Top Half Only

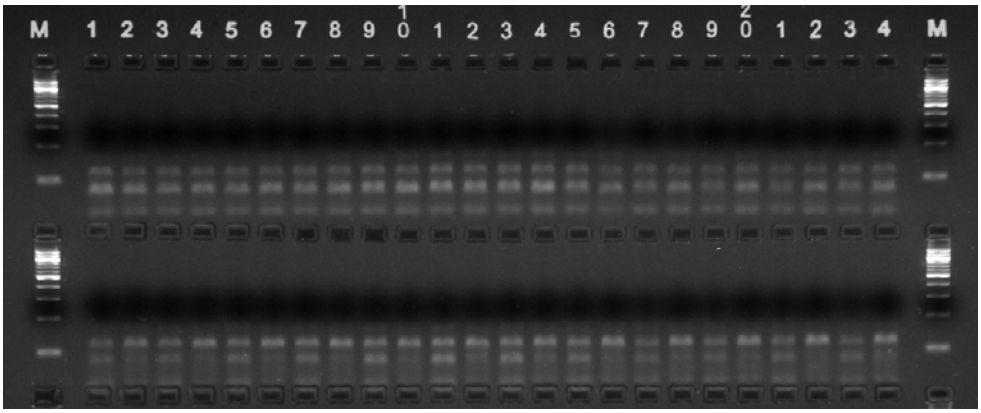


Figure 3.55
Bad Second Quality Control Gel

Three bands are in every well, indicating that incomplete digestion. We recommend going back to the Assay Plate and running the samples again from *Stage 5 – Second PCR*.

MARK EXPERIMENTS THAT WILL NOT BE HYBRIDIZED

At this point, you will want to indicate in GTGS any experiments that are not to be hybridized onto arrays.

To mark experiments that will not be hybridized:

1. In GTGS, expand the project, Tracking and Hyb Plates folders.
2. Select the appropriate Label Plate.
3. Open **Tools** > **Set Well Status**.
4. Right-click the well of each sample you want to fail and select **Failed**.
5. Click **OK**.

Stage 7 – Sample Hybridization

ABOUT THIS STAGE

During this stage, you will add Hyb Cocktail to each sample. The samples are then placed on a thermal cycler and denatured.

After denaturation, each sample is loaded onto a GeneChip® Universal Tag Array (array) – one sample per array. The arrays are placed into a hybridization oven that has been preheated to 39°C. Samples are left to hybridize for 12 to 16 hours.

Each feature on an array contains multiple *complementary tags* (*c-tags*.) If you are using 3K arrays, the array would have > 3000 tags.

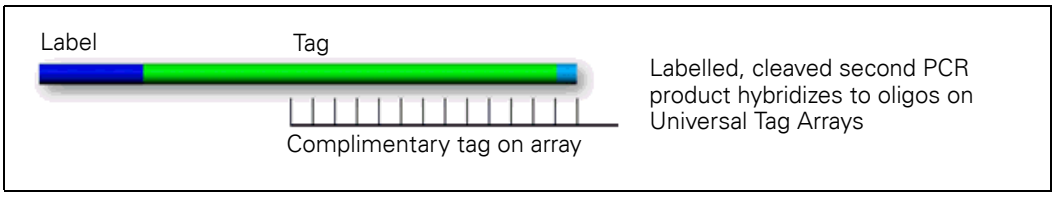


Figure 3.56

The tag in the assay probe hybridizes to the c-tags on the array surface. If the sample is that of a homozygote, only one of the four types of allele-specific labelled probes will hybridize to the c-tags on the feature for the target SNP (for example, probes with the dATP label).

If the sample is that of a heterozygote, two of the four types of allele-specific labelled probes hybridize to the c-tags on the feature for the target SNP (for example, probes with the dATP label and probes with the dCTP label).

LOCATION AND DURATION

- Post-Amp Lab
- Hands-on time: 1 hour
- Time on thermal cycler: 6 minutes
- Hybridization time: 12 to 16 hours

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 reactions (48 arrays).

Table 3.9
Equipment and Materials Required for *Stage 7 – Sample Hybridization*

Quantity	Item
1	Aluminum foil, 1 roll
1	Centrifuge, plate
48	Clear film for arrays (enough for one per array; quantities can vary)
48	Universal Tag Arrays (quantities can vary)
2	Hyb Plates containing samples from the previous stage
1	Hybridization oven with trays
1	Ice container, filled with ice
1	Marking pen, fluorescent or water soluble
2	MicroAmp Clear Adhesive Films
1	Microfuge
2	Plate holders (preparation racks)
1	Reagent reservoir, 50 mL
2	Thermal cyclers, 96-well GeneAmp PCR System 9700

KIT COMPONENTS REQUIRED

The following components are required from each GeneChip SNP Kit:

- Hyb Cocktail

THAW REAGENTS

To thaw the reagents:

1. Wrap the Hyb Cocktail in aluminum foil.
2. Allow to thaw on the bench top.
3. Place on ice until ready to use.

IMPORTANT !

Hyb Cocktail is light sensitive. Do not expose to light.

PREPARE THE GENECHIP® UNIVERSAL TAG ARRAYS

To prepare the arrays:

1. Cover the hybridization oven with aluminum foil to prevent light from entering.
2. Turn the hybridization oven on now and set to 39°C.
3. Remove arrays from the 4°C refrigerator.
4. Unwrap the arrays and place window-side up on a clean bench top.
5. Cover the window of each array with a piece of clear film.

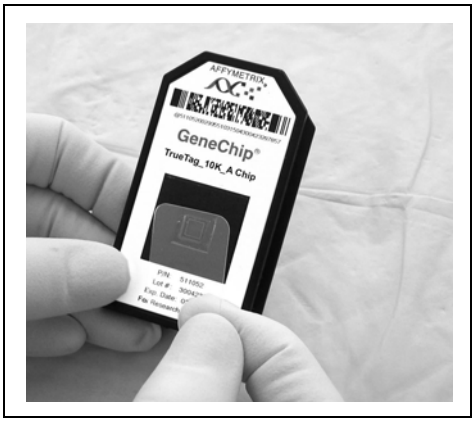


Figure 3.57
Applying Clear Film to Array Window

6. Turn over each array so that the window faces down.

Figure 3.58
Array With Sample Numbering

7. Write a number on each array (Figure 3.58) using a water soluble or fluorescent marking pen, keeping the arrays in numerical order.

   For example, you might number the arrays 1, 2, 3 and so on.

8. Insert a 200 µL pipette tip in the upper right septum of the array.

9. Allow the arrays to warm to room temperature.

WARNING ▲ **To ensure that the data collected during scanning is associated with the correct sample, number the arrays in a meaningful way. It is critical that you know which sample is loaded onto each array.**

ADD THE HYB COCKTAIL

To add the Hyb Cocktail:

1. Using a P200 pipette, aliquot 75 µL (150 µL) Hyb Cocktail to each tube in one set of 12 strip tubes.
2. Using a 12-channel P200 pipette, aliquot 30 µL of Hyb Cocktail to each reaction on the Hyb Plates (final volume approximately 94 µL).

Final Mix Consists of:
~ 64 to 68 µL Sample
+ 30 µL Hyb Cocktail
~ 94–98 µL Total

3. Mix by pipetting up and down 10 times.
4. Seal Hyb Plates with a new adhesive film.
5. Spin down the plates at 1000 rpm for 30 seconds.
6. If not immediately proceeding to *Denature Samples* below, Hyb Plates can be stored at –20°C for up to 2 days.

IMPORTANT ! **Hyb Plates can be stored at –20°C for up to 2 days.**

We recommend freezing and thawing stored plates ONE TIME ONLY. For a given set of samples, do not freeze and thaw plates more than once during the protocol.

DENATURE THE SAMPLES

To denature the samples:

1. Place each Hyb Plate on a thermal cycler, and run the *Meg Denature* program (Figure 3.59.)
2. Immediately after the 6 minute denature:
   - **A.** Remove Hyb Plates from the thermal cycler.
   - **B.** Place on ice.
   - **C.** Cover with aluminum foil to protect from light.
   - **D.** Cool for 2 minutes.
   - **E.** Spin down at 1000 rpm for 30 seconds.

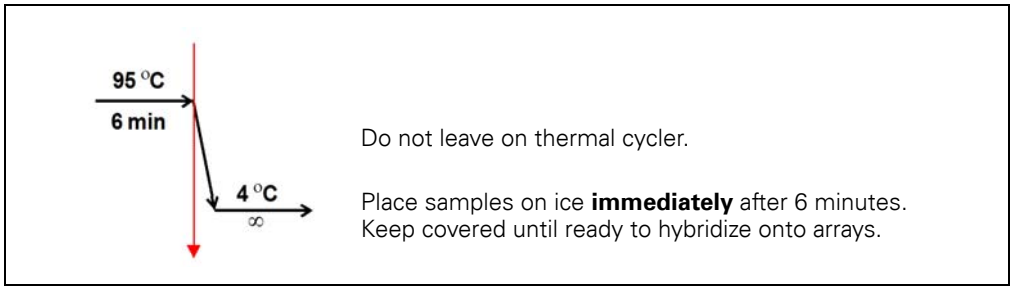


Figure 3.59
Meg Denature Thermal Cycler Program

LOAD REACTIONS ONTO ARRAYS

To load reactions onto the arrays:

1. In GTGS, expand the Project, Tracking and Hyb Plate folders.
2. Right-click the appropriate Hyb Plate and select **Hyb on Arrays**.
3. Scan the Hyb Plate barcode and press **Enter**.

   The Hyb on Arrays window appears (Figure 3.60 on page 88). Notice the top field of this window prompts you as to which reaction to load onto an array. When an array barcode is scanned, the software highlights the next sample to be loaded.

WARNING ▲ **To ensure that the data from the scanner is associated with the correct sample, you must keep track of which sample is loaded onto which array. Follow the transfer instructions displayed in the Hyb on Arrays window (Figure 3.60).**

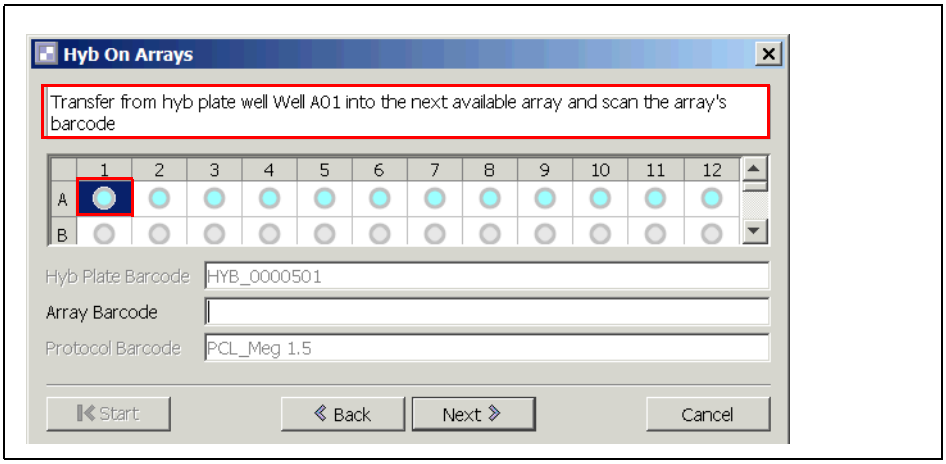


Figure 3.60
Hyb on Arrays Window. The software indicates by well designation which reaction is to be loaded onto an array. After the array barcode is scanned, the software highlights the well of the next reaction to be loaded.

4. Working 24 arrays at a time:

   A. Set a single channel pipette to 90 µL.

   B. Pipette 90 µL of reaction from the plate and well specified in the Hyb on Arrays window, and load it onto an array through the lower left septum (Figure 3.61).

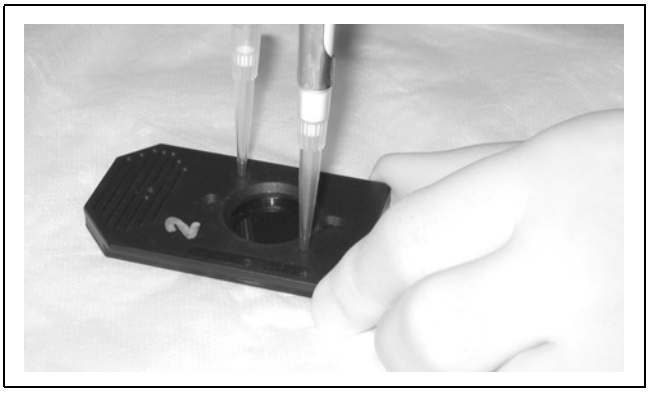


Figure 3.61
Loading Sample Onto an Array

   C. Remove the pipette tip from the upper right septum.

   D. Scan the array barcode.

   E. Place the array in a hybridization oven tray.

   F. When the tray is full, place and secure the tray in the oven.

IMPORTANT !

Place trays in the oven as soon as the arrays are loaded. Do not leave loaded arrays on the bench top for more than 5 minutes.

   G. Repeat this process until all of the reactions have been loaded onto arrays and placed in the hybridization oven.

5. Ensure that the trays are seated properly in the oven carousel.
6. Set the oven speed to 25 rpm.
7. Leave the arrays in the oven for 12 to 16 hours.
8. In GTGS, click **Next**, then click **Save**.

What happens next depends on whether GTGS is configured to communicate with GCOS, or is configured to write sample files (.arr) for AGCC. See the *AGCC and GCOS Compatibility Modes* chapter of the *GeneChip® Targeted Genotyping Software User Guide* for more information about GCOS and AGCC modes.

If you are using AGCC:

1. Select a new or existing folder within the AGCC data root used by the computer that will wash and scan your arrays.

   GTGS will create sample files (.arr) in this folder, which AGCC needs before arrays are washed or scanned. AGCC will create data files in this same folder when the arrays are scanned.

2. Click **Save**.
3. Check the AGCC Portal to ensure that the information from GTGS has been transferred and detected (indexed) by AGCC.

   Indexing may take up to 10 min after files are added to the AGCC data root.

4. Click **Close** to close the Hyb on Arrays window.

IMPORTANT !

If AGCC has not indexed the GTGS-created .arr files by the time the arrays are first washed or scanned, then AGCC will itself create .arr files, which is a problem. When AGCC creates .cel files, the names will be based on the full array barcode.

If this occurs, GTGS will not be able to load the .cel files. The reason is that GTGS expects the .cel files to have a name based on the experiment name used in GTGS. This name is an abbreviation of the full array barcode.

If you accidentally save these files to the wrong location, you can also move your sample files to the correct location before the arrays are washed and scanned.

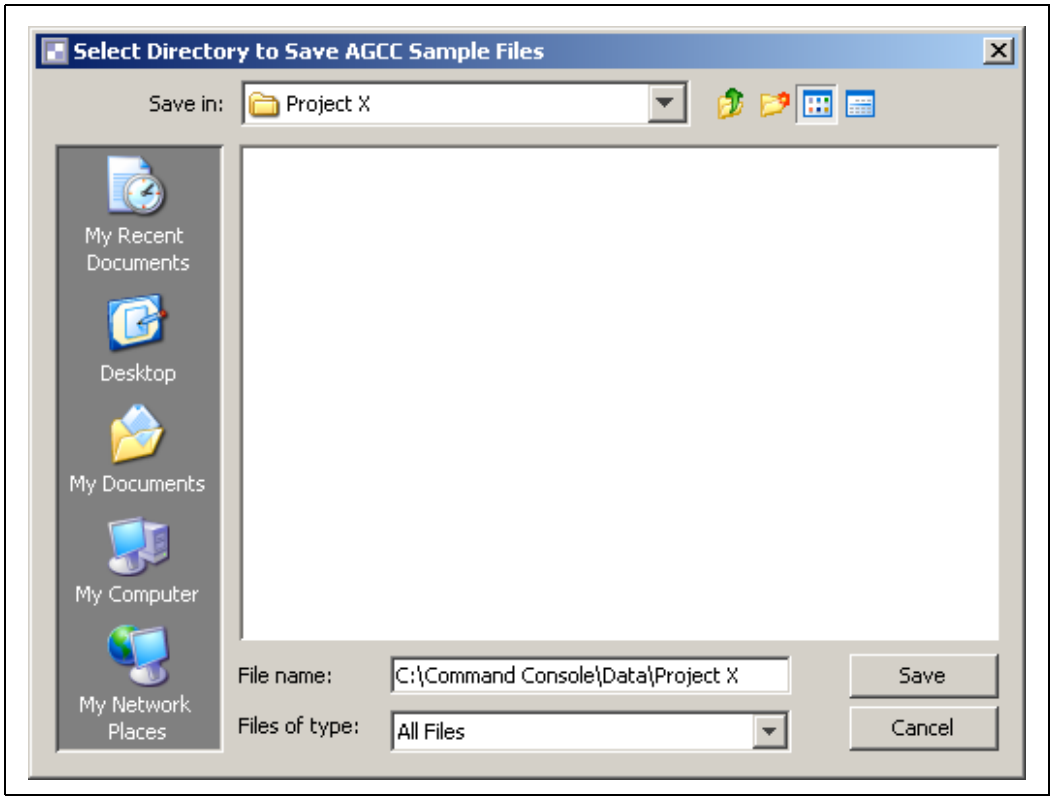


Figure 3.62
AGCC data root

If you are using GCOS:

After several seconds, a message should appear confirming that the experiments have been registered with GCOS.

1. Check GCOS to ensure that the infomration from GTGS has been transferred.
2. Click **Close** to close the window.

For both AGCC and GCOS, you can click the Arrays folder icon to see a list of arrays that have been barcode scanned. You will also see information about each array in the right pane (Figure 3.63).

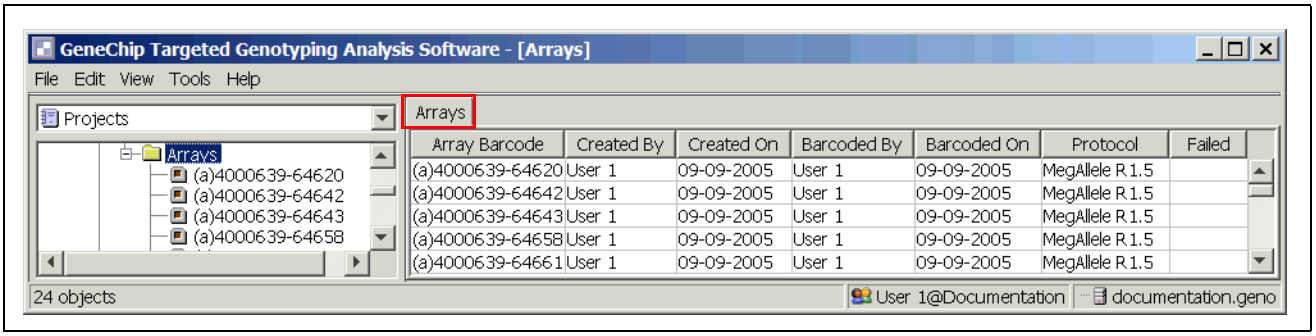


Figure 3.63
Arrays Icon Expanded and Array Tab

Stage 8 – Stain and Wash

NOTE **The instructions for this stage refer to GCOS fluidics station control software. For Affymetrix GeneChip® Command Console users (AGCC), refer to the AGCC user's guide for instructions on controlling the fluidics station.**

ABOUT THIS STAGE

During this stage, the arrays are loaded onto a GeneChip® Fluidics Station (fluidics station). Each array is washed and stained in preparation for scanning.

LOCATION AND DURATION

- Post-Amp Lab
- Hands-on and wash time: approximately 6 hours

NOTE **Stages 8 and 9 are run concurrently. As arrays come off the fluidics station, you will load them onto the GeneChip® Scanner 3000 7G 4C and scan them.**

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 arrays. The number of arrays processed can vary.

Table 3.10
Equipment and Materials Required for Stage 8 – Stain and Wash

Quantity	Item
1	Centrifuge, plate
2	Conical tubes, 15 mL
48	Eppendorf tubes, Amber, 2.0 mL (1 tube per array; actual quantities required may vary)
48	Eppendorf tubes, Natural, 2.0 mL (1 tube per array; actual quantities required may vary)
48	Universal Tag Arrays from the previous stage (quantity can vary)
1	Ice container, rectangular, filled with ice

KIT COMPONENTS REQUIRED

The following GeneChip SNP Kit components are required for this stage:

- Buffer H
- Stain Cocktail
- Wash Soln A
- Wash Soln B

PRIME THE FLUIDICS STATION

To prime the fluidics station:

1. Remove Wash Solutions (Solns) A and B from all kits being used.
1. Turn on the fluidics station and:
   - **A.** Place Wash Solns A and B in the designated positions.
   - **B.** Fill the dH_2O container.
   - **C.** Empty the waste container.
2. Launch GCOS and:
   - **A.** Click the **Fluidics** button. 

   - **B.** Navigate to **Run** → **Fluidics** → **Protocol**.
   - **C.** Open the **Protocol** drop-down list and select **Prime_450**.

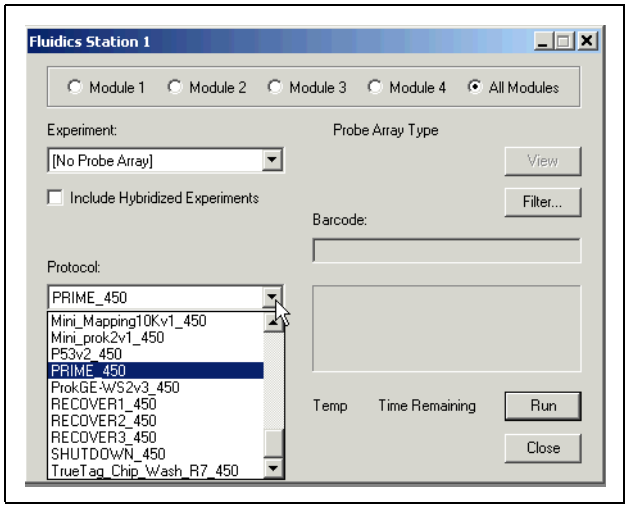


Figure 3.64
Selecting the Prime_450 Protocol

   - **D.** Select the **All Modules** button.
   - **E.** Click **Run**.
   - **F.** Follow the instructions on the monitor.

     The displays on the fluidics station for each module will read *Priming done* when the program is finished.

PREPARE REAGENTS

To prepare the reagents:

1. From each kit being used, remove the Buffer H and Stain Cocktail.
2. Mix the Stain Cocktail by inverting 5 times.
3. Wrap the Stain Cocktail tube in aluminum foil and place on ice.
4. Thaw Buffer H on the bench top.
5. Spin down Buffer H and mix by pipetting up and down.
6. Keep all reagents on ice until ready to use.

IMPORTANT !

Stain Cocktail is light sensitive. Keep protected from light.

PREPARE THE STORAGE COCKTAIL

To prepare the Storage Cocktail:

1. In a 15 mL conical tube, add the reagents listed in Table 3.11 in the order shown. Be sure to dilute Buffer H using Wash Soln B; do not use water.
2. Cap the tube and mix gently by inverting.

Table 3.11
Storage Cocktail

Reagent	1 Array	24 Arrays (2 extra arrays)	48 Arrays (4 extra arrays)
Wash Soln B	179 µL	4.65 mL	9.31 mL
Buffer H	11.4 µL	296 µL	593 µL
Total	190 µL	4.95 mL	9.90 mL

ALIQUOT THE STAIN AND STORAGE COCKTAILS

To aliquot the stain and storage cocktails:

1. For every array, aliquot 190 µL of Stain Cocktail to an amber 2.0 mL Eppendorf tube (one tube per array).
2. Keep the tubes on ice and covered to protect from light until ready to use.

IMPORTANT !

Stain Cocktail is light sensitive. To avoid exciting the dyes, keep tubes protected from light.

3. For every array, aliquot 190 µL of Storage Cocktail to a clear 2.0 mL Eppendorf tube (one tube per array.)
4. Keep the tubes on ice until ready to use.

LOAD ARRAYS ONTO THE FLUIDICS STATION

To load arrays onto the fluidics station:

1. Remove eight arrays from the hybridization oven.

IMPORTANT !

Leave remaining arrays in the hybridization oven until ready to wash.

2. Place each array on a fluidics station, but do **not** engage the wash block.
3. In GCOS, click the **Fluidics** button 

.
4. Select the appropriate module.
5. Open the **Protocol** menu and select **TrueTag_Chip_Wash_R7_450**.

   This step is required when you scan the first array only.

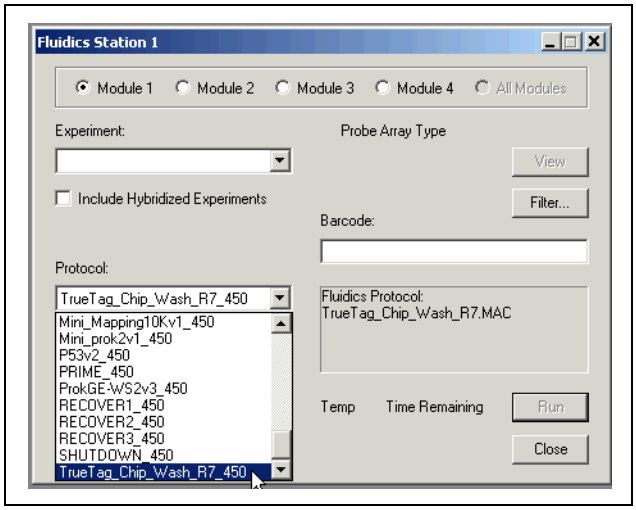


Figure 3.65
Selecting a Wash Protocol on the Fluidics Station

6. To scan the array barcode:

   A. Highlight the barcode field (or click in the field if this is the first run).

   If this is the first run of arrays, the Barcode field is empty. You must still click in the Barcode field before scanning each of the first four arrays.

   B. Scan the array barcode.

   C. Wait for the Experiment field to update (Figure 3.66).

IMPORTANT !

You must wait for the Experiment field to update after scanning the array barcode. If you click Run before the field updates, the scanner will not be able to scan the array. A message stating that the array was not hybridized is displayed.

After the barcode is scanned, GCOS checks the experiment list and automatically updates the Experiment field. The experiment name is typically a truncated version of the array barcode.

An error message is displayed if GCOS cannot find an experiment that corresponds with the array barcode.

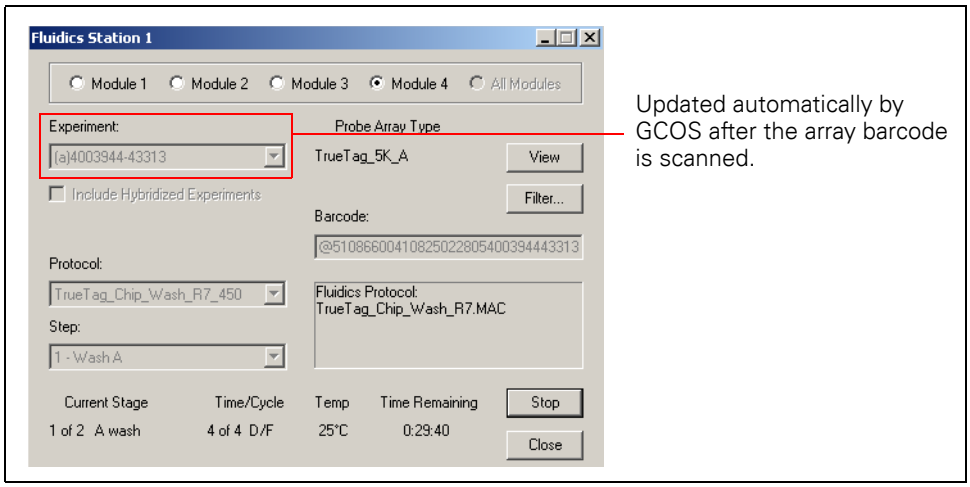


Figure 3.66
Experiment Field

7. Click **Run** (or press Enter) to proceed to the next module and scan the array barcode.
8. Repeat until all of the barcodes have been scanned and entered.
9. Follow the prompts on the fluidics station as follows:
   - **A.** When the display reads *Load Cartridge*, engage the wash blocks.
   - **B.** When the display reads *Load Stain in 1, Store in 2* (Figure 3.67) :
     - **1)** Load a tube of Stain Cocktail in position 1.
     - **2)** Load a tube of Storage Cocktail in position 2.
     - **3)** Load an empty tube in position 3.
   - **C.** Lower the needles to start the program.

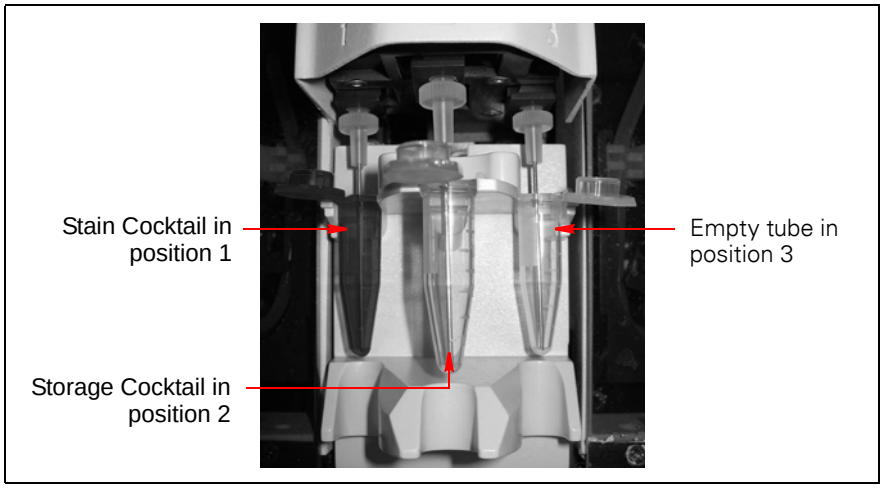


Figure 3.67
Tube Positions on the Fluidics Station

10. If the scanner is not turned on:

A. Launch GCOS first.

B. Turn the scanner on.

The scanner must warm up for 10 minutes before scanning arrays.

REMOVE ARRAYS FROM THE FLUIDICS STATION

To remove arrays from the fluidics station:

1. When the *Remove Cartridge* prompt is displayed, remove the array.
2. Inspect the array for air bubbles. If found:
   - For small bubbles, gently swipe the array on the bench top to move the bubble out of window (Figure 3.68).
   - For larger bubbles:
     1) Place the array back on the fluidics station.
     2) Re-engage the wash block.
     3) **Allow the program to finish.**

        The array is automatically drained and refilled with Storage Cocktail. Repeat this process as many times as necessary.

NOTE **The *TrueTag_Chip_Wash_R7_450* script is designed to pause automatically so that you can check the arrays for bubbles, and reinsert the arrays to drain and refill as many times as necessary.**

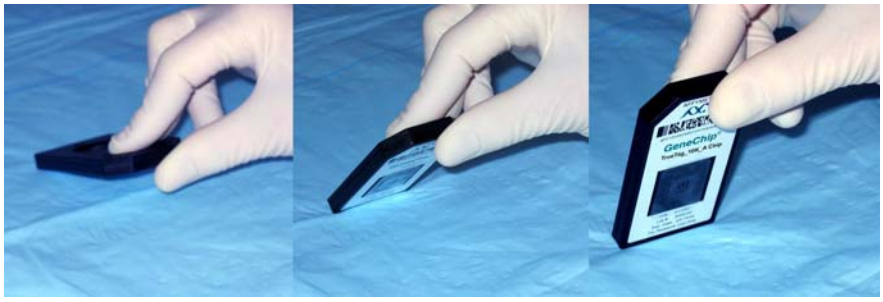


Figure 3.68
Swiping an Array to Dispel Air Bubbles

3. Reinspect the array and repeat the previous step if necessary.
4. Re-engage the washblock to restart the **TrueTag_Chip_Wash_R7_450** script and allow it to finish.
5. At the prompt, remove and discard the stain and storage cocktail vials from positions 1 and 2.

   Protocol Done is displayed on the fluidics station when the script is finished. Leave the empty tube in position 3.
6. Place the arrays face down and cover the septa with Tough Spots.

IMPORTANT **Do not allow edges of Tough Spots to overlap the large center circle of the array. If tags overlap, the array may get stuck in the scanner.**

7. Remove the clear film from each array window.
8. Proceed immediately to *Stage 9 – Scan Arrays* on page 99 and scan the arrays.

PROCESS THE REMAINING ARRAYS

To process the remaining arrays, repeat the steps listed under *Load Arrays onto the Fluidics Station* on page 95 and *Remove Arrays from the Fluidics Station* on page 97.

IMPORTANT **Load new tubes of Stain and Storage Cocktail for each array. Leave the empty tube in position 3.**

SHUT DOWN THE FLUIDICS STATION

To shut down the fluidics station:

1. Remove Wash Solns A and B and replace with distilled H_2O (dH_2O).
2. Place tubing in dH_2O.
3. Place empty tubes in positions 1, 2 and 3.
4. Select the **All Modules** button.
5. Run the protocol called **Shutdown_450** (Figure 3.69).
6. Turn off the fluidics station.

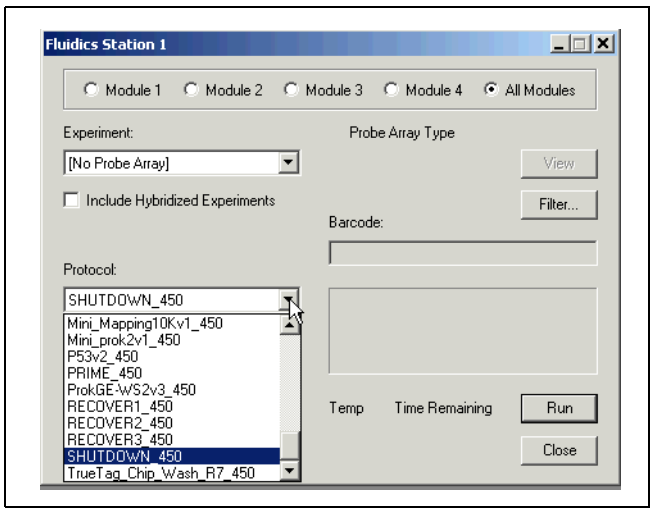


Figure 3.69
Shutting Down the Fluidics Station

Stage 9 – Scan Arrays

NOTE **The instructions for this stage refer to GCOS scanner control software. For Affymetrix GeneChip® Command Console users (AGCC), refer to the AGCC user's guide for instructions on controlling the scanner.**

ABOUT THIS STAGE

During this stage, the arrays are loaded onto the GeneChip® Scanner 3000 7G 4C (Scanner 3000 7G 4C). Each array is scanned individually, and the data collected is stored in four files referred to as .cel files. Each .cel file contains the data for one channel (A, C, G or T). The naming convention for AGCC .cel files created by GTGS is *<abbreviated barcode>_<channel designation>*.cel. The naming convention for GCOS .cel files is *<abbreviated barcode><channel designation>*.cel. The channel designation is A, B, C or D.

AGCC Example: @1234567-12345_A.cel

GCOS Example: @1234567-12345A.cel

Data is generated by collecting light from four different wavelengths – one for each channel. The amount of light emitted by each feature on the array is collected, and the background is subtracted.

If the sample is from a homozygote, only a single wavelength of light is emitted from the feature for the target SNP. If the sample is from a heterozygote, two different wavelengths of light are emitted.

LOCATION AND DURATION

- Post-Amp Lab
- Hands-on and scanning time: approximately 4 to 5 hours

NOTE **Stages 8 and 9 are run concurrently. As arrays come off the fluidics station, they are loaded onto the Scanner 3000 7G 4C and scanned.**

EQUIPMENT AND MATERIALS REQUIRED

The following equipment and materials are required to perform this stage. Quantities shown are for processing 48 arrays:

Table 3.12
Equipment and Materials Required for Stage 9 – Scan Arrays

Quantity	Item
1 can	Compressed air
1	GeneChip Scanner 3000 7G 4C
48	Arrays from the previous stage (quantities can vary)

PREPARE THE SCANNER

If the scanner is not already on and warmed up:

1. Launch GCOS first.
2. Turn on the scanner.
3. Allow the scanner to warm up for 10 minutes before scanning arrays.

LOAD THE ARRAYS

To prepare the arrays for scanning:

1. Inspect the windows for dust, lint or other blemishes.
2. If necessary, clean the array window using compressed air.
3. Load each array the scanner carousel, starting at position 1.

   The carousel holds 48 arrays.

SCAN THE ARRAYS

To scan the arrays:

1. Click the **Start** button. 

   The GeneChip Scanner dialog box is displayed.

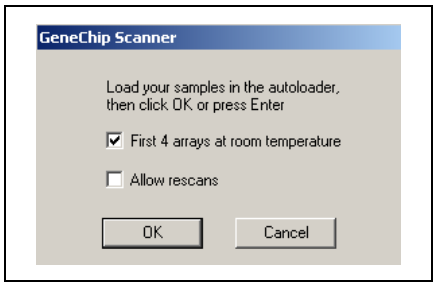


Figure 3.70
GeneChip Scanner Dialog Box

2. If the arrays are:
   - At room temperature, select *First 4 arrays at room temperature*.
   - If arrays are not at room temperature, deselect the check box. Bringing arrays to room temperature takes approximately 5 minutes.
3. If:
   - None of the arrays have been scanned, leave *Allow rescans* deselected.
   - One or more of the arrays have already been scanned, select the *Allow rescans* check box.

   The scanner will identify arrays that have already been scanned. When rescanned, the associated files will have the suffixes such as _2A, _2B, _2C, and _2D added to the filename (A for the first rescan, B for the second rescan, and so on.)
4. Click **OK**.

   The scanner processes approximately 11 arrays per hour.

5. To view the scanning process, open **View → Scan in Progress**.

   The scanner must finish autofocusing before you can view the scan.

6. When scanning is finished, do one of the following:
   - Leave the arrays in the carousel where they are held at 15°C.
   - Remove and store the arrays at 4°C until no longer needed.

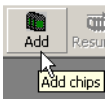
IMPORTANT **We strongly recommend rescanning arrays within 6 to 24 hours of the first scan if necessary. Data quality may be degraded if arrays are rescanned after 24 hours.**

ADDING ARRAYS DURING AN AUTOLOADER RUN

To add arrays while an AutoLoader run is in progress:

1. Do one of the following:

   A. Click the **Add** icon.

   B. Open **Run → Add Chips,** 

.

   The GeneChip Scanner window appears (Figure 3.71).

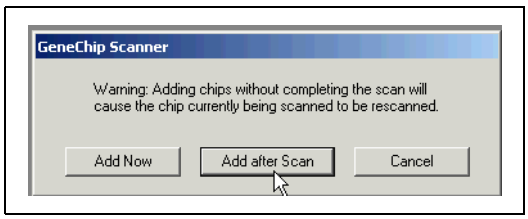


Figure 3.71
GeneChip Scanner Window

2. Click **Add after Scan**.

IMPORTANT **Do not use the Add Now feature. Use only the Add after Scan feature when working with Universal Tag Arrays.**

3. Wait until the scanner is finished scanning the current array.
4. When the status on the scanner reads **Autoloader Unlock**, open the scanner and add the arrays.
5. Close the scanner.
6. When the following message is displayed, click **OK**.

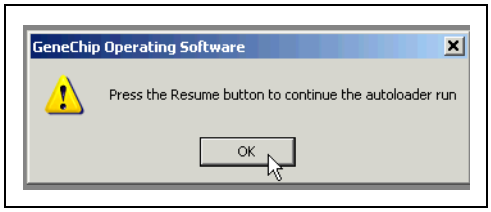


Figure 3.72
Resume Prompt

7. Do one of the following:
   - Click the **Start** icon.
   - Click the **Resume** icon, 

   - Open **Run** → **Resume Scan**.

SHUTDOWN THE SCANNER

To shutdown the scanner:

Turn off the scanner by pushing the power button.

Rescanning Arrays

GUIDELINES FOR RESCANNING ARRAYS

IMPORTANT !

We strongly recommend that arrays be rescanned if necessary within 6 to 24 hours of the first scan. Scanning arrays after 24 hours can result in degraded data.

Guidelines for rescanning arrays are as follows.

Table 3.13
Guidelines for Rescanning Arrays

If …	Then …
there is no image when viewing the .dat file (white screen)	rewash the array with fresh Wash Solution B and rescan. See *Rewash Arrays* below.
lint, dust or bubbles are present	clean the array window or rewash the array as appropriate and rescan. See *Rewash Arrays* below.
data quality is poor	rescan the array.
the image does not grid properly (.cel file is missing)	do not rescan the array. Manually apply the grid to the array image and generate the missing .cel file. See *Manually Regridding Arrays* on page 118.

REWASH ARRAYS

To rewash arrays:

1. Insert a P-200 pipette tip in the upper-right septum of the array.
2. Pipette out the Storage Cocktail from the array.
3. Pipette fresh Wash Solution B through the lower left septum as follows:
   - **A.** Pipette up and down 5 times to fill and drain the array.
   - **B.** Repeat the fill and drain with fresh Wash Solution B two more times.
   - **C.** Remove Wash Solution B from the array.
4. Slowly refill the array with fresh Storage Cocktail (approximately 100 µL), ensure there are no air bubbles and rescan immediately.

RESCAN THE ARRAY

To rescan the array:

1. Load the array into the autoloader.
2. Rescan the array as you would normally.
3. At the prompt *Allow Rescans*, select **Yes**.

   When the AGCC or GCOS software reads the barcode, it will recognize that this array has already been scanned. AGCC or GCOS will add suffixes (_2A, _2B, _2C, and _2D) to the associated filenames to reflect the fact that they are rescans.

Deleting Projects and Project-Related Information

DELETING A PROJECT

To delete a project, you must delete the project components in the reverse order from which they were created.

For example, if there are cluster results in the Genotype Results folder, you must:

1. Delete each cluster genotype that is in the Genotype Results folder.
2. Delete all of the experiments listed under the Array Data icon in the Experiment QC Summary.

   Experiments may be deleted individually or all at once. If you are using GCOS, you may also want to go to GCOS Manager and delete all experiments associated with the project. If you are using AGCC, you may also want to go to the folder containing .arr and .cel files, and move or delete these as well.
3. Delete the arrays from the Arrays folder.
4. Delete each plate in the Hyb Plates folder.
5. Delete each plate in the Label Plates folder.
6. Delete each plate in the Assay Plates folder.
7. Delete each plate in the Anneal Plates folder.
8. Delete each plate in the Sample Plates folder.
9. Delete the project icon.

DELETING CLUSTER GENOTYPE RESULTS

To delete cluster genotype results:

1. Right-click the cluster name.
2. Select **Delete**.
3. Click **Yes**.

DELETING EXPERIMENTS

Experiments can be deleted only if they are not included in any cluster genotyping results.

To delete individual experiments:

1. Click the **Array Data** icon for your project.
2. Click the Experiment QC Summary tab.
3. Right-click anywhere in the row of the experiment you want to delete and select **Delete**.

   If you cannot select Delete, the experiment is included in cluster genotype results. You must delete the cluster genotype results before you can delete the experiment.
4. Click **OK**.

To delete all experiments at once:

1. Right-click the **Array Data** icon for your project.
2. Select **Delete All Experiments**.

The status bar at the bottom of the the application window will show the deletion progress. Do not force quit the application during this operation, or all of the experiments will be restored. Since restoring all experiments takes some time, the application may be unresponsive until the SQL Server has finished this task.

DELETING ARRAYS

You should delete arrays only after the associated Array Data experiments have been deleted.

To delete arrays:

1. Select the arrays folder.
2. In the right pane, right-click the set of arrays to delete and select **Delete**.
3. Click **Yes** to confirm deletion of each selected array.

   To delete multiple arrays, simply hold down the Enter key to quickly accept each confirmation message.

DELETING ANNEAL, ASSAY, LABEL AND HYB PLATES

You can delete plates as long as there are no arrays hybridized with samples that originated from these plates.

To delete these plates, you must delete them in reverse order starting with the Hyb Plate.

To delete plates:

1. Select the relevant plates folder.
2. Right-click the set of plates to delete, and select **Delete**.
3. Click **Yes**.

   To delete multiple plates, simply hold down the Enter key to quickly accept each confirmation message.

DELETING SAMPLE PLATES

You can delete a sample plate as long as it is not included in a project. If referenced by any projects, you must first remove the sample plate from those projects, then delete the sample plate.

To delete a sample plate:

1. Delete any projects that reference the sample plate.

   See *Deleting a Project* on page 104.

2. Open the menu in the left pane of the GTGS window and select **Samples**.
3. Expand the **Sample Plates** folder.
4. Right-click the sample plate name and select **Delete**.

   You can also select **Edit** → **Delete**. If you cannot select Delete, the sample plate has been used in a project that still exists.

5. Click **Yes**.

DELETING SAMPLE INFO

If a sample name in the Sample Info table does not exist in any Sample Plate, you can remove these samples.

To delete Sample Info:

1. Open the menu in the left pane of the GTGS window and select **Samples**.
2. Right-click the Sample Info icon and select **Remove Unused Sample Info**.
3. Click **Yes**.

DELETING ASSAY PANELS

You can delete an assay panel as long as it is not included in a project. If referenced by any projects, you must first delete those projects, then delete the assay panel.

To delete an assay panel:

1. Delete any projects that reference the assay panel.

   See *Deleting a Project* on page 104.

2. Open the menu in the left pane of the GTGS window and select **Assay Panels**.
3. Expand the **Assay Panels** folder.
4. Right-click the assay panel name and select **Delete**.

   You can also select **Edit** → **Delete**.

5. Click **Yes**.

Chapter 4

Troubleshooting

NOTE **This section covers troubleshooting of issues using GCOS fluidics and scanner control software. Affymetrix GeneChip Command Console (AGCC) users should refer to the AGCC User's Guide for how diagnose and recover from fluidics, scanner, or array-related problems.**

FLUID NOT DETECTED

Problem: Fluid not Detected

The GeneChip® Fluidics Station (fluidics station) has detected a problem with either the Stain Cocktail or the Storage Cocktail. This problem can be caused by:

- No solution in a tube
- Solution not prepared correctly
- Wrong solution in a tube

Messages Displayed

On the Instrument Control Workstation, you will see the following messages (the word *position* refers to the fluidics station module in these messages):

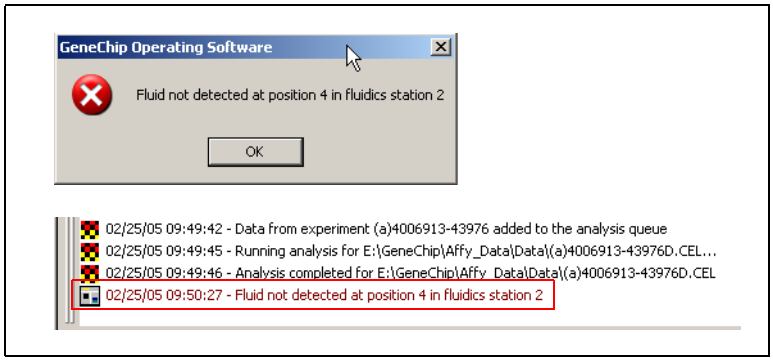


Figure 4.1
Fluid Not Detected

Solution

To resolve the error and resume the wash and stain operation:

1. Determine which cocktail or solution is causing the problem by looking at the Step field.
2. If the stain cocktail is causing the problem:

   A. Run the protocol **RECOVER1_450** (Figure 4.2).

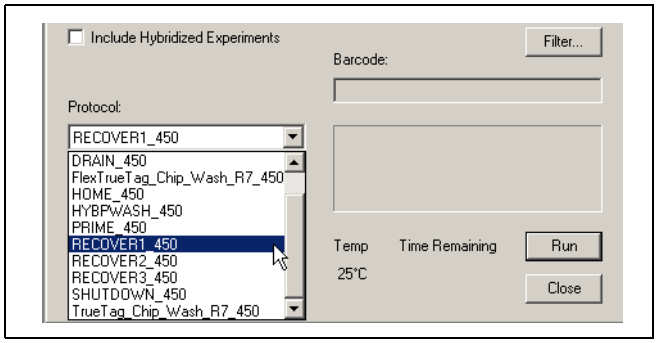


Figure 4.2
Recover1_450 Protocol

B. Follow the prompts on the fluidics station LCD.

C. When finished, a message similar to the one outlined in red (Figure 4.3) appears.

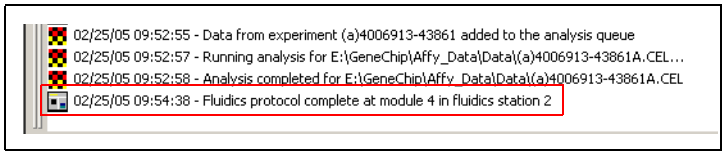


Figure 4.3
Fluidics Protocol Complete

D. At the prompt, remove the array from the wash block.

E. Re-engage the wash block and allow the protocol to finish.

3. If the storage cocktail is causing the problem:

A. Run the protocol **RECOVER2_450.**

B. Follow the same steps used for RECOVER1_450 above.

4. Open the **Protocol** menu and select **TrueTag_Chip_Wash_R7_450**.

5. Highlight the barcode field and scan the barcode of the array that failed.

6. Click **Resume**.

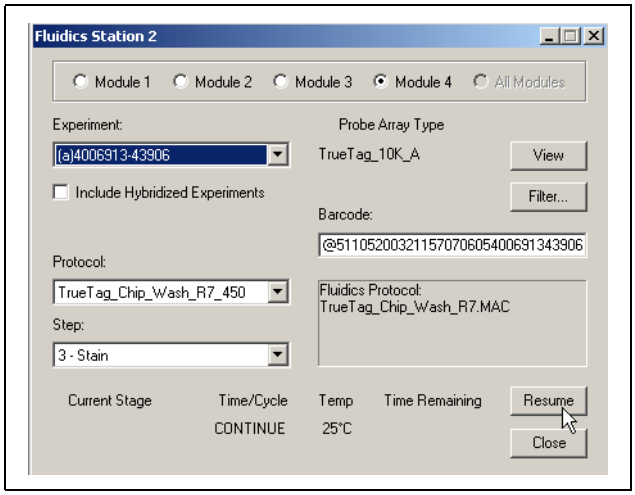


Figure 4.4
Array Barcode in Experiment Field

7. At the prompt, click **OK**.

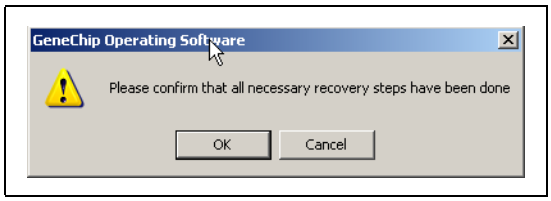


Figure 4.5
Confirm That Recovery Steps Complete

Troubleshooting the GeneChip® Scanner 3000 7G 4C

SCANNING STOPPED PREMATURELY

Problem: Scanning Stopped Prematurely

On the Instrument Control Workstation, if the scanner should be actively scanning but the Scan Status reads Ready, the scanner has lost communication with the workstation.

The upper blue light on the scanner will be illuminated, indicating that the door is locked and that the instrument should be scanning.

Solution

To re-establish communication and continue scanning:

1. On the GeneChip® Operating Software (GCOS) tool bar, click the **Stop** button.

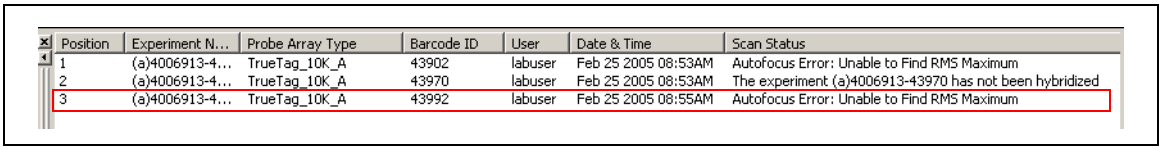
   The upper light on the scanner will turn off indicating the door is unlocked.
2. At the prompt, click **OK**.
3. Open the scanner door, and remove the arrays that have been completely scanned (scan status reads *Scan Complete*).

   If you do not remove the arrays that have already been scanned, those arrays will be scanned again once operation resumes.
4. Reposition the arrays, or add additional arrays so that there is an array in position 1 of the autoloader.
5. Close the scanner door.
6. Click the **Start** button.
7. At the prompt, select **Allow Rescans**.
8. Click **OK**.

AUTOFOCUS ERROR MESSAGE

Problem: Autofocus Error Message

If the clear film was left on the array, you will see the message outlined in red below on the Instrument Control Workstation:



Position	Experiment N...	Probe Array Type	Barcode ID	User	Date & Time	Scan Status
1	(a)4006913-4...	TrueTag_10K_A	43902	labuser	Feb 25 2005 08:53AM	Autofocus Error: Unable to Find RMS Maximum
2	(a)4006913-4...	TrueTag_10K_A	43970	labuser	Feb 25 2005 08:53AM	The experiment (a)4006913-43970 has not been hybridized
3	(a)4006913-4...	TrueTag_10K_A	43992	labuser	Feb 25 2005 08:55AM	Autofocus Error: Unable to Find RMS Maximum

Figure 4.6
Message Displayed in GCOS When Clear Film Left on Array Window

Solution

To fix the problem and continue scanning:

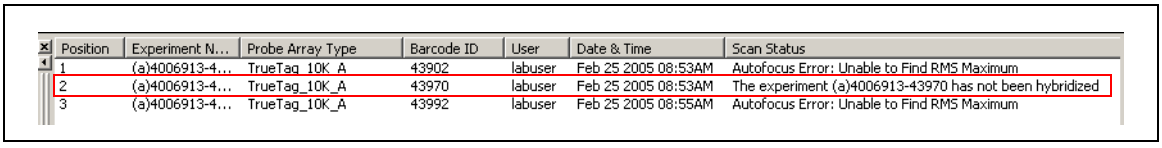
1. On the GCOS tool bar, click the **Stop** button.

   The upper light on the scanner will turn off indicating the door is unlocked.
2. Click **OK** when the prompt appears in GCOS.
3. Open the scanner door, and remove the array.
4. Remove the clear film and place the array back in the autoloader.
5. Close the scanner door.
6. Click the **Start** button.
7. Click **OK** when the prompt appears.

EXPERIMENT NOT HYBRIDIZED MESSAGE

Problem: Experiment not Hybridized Message

On the scanner you will see the message: *The experiment <experiment name> has not been hybridized* (example outlined in red below):



Position	Experiment N...	Probe Array Type	Barcode ID	User	Date & Time	Scan Status
1	(a)4006913-4...	TrueTag_10K_A	43902	labuser	Feb 25 2005 08:53AM	Autofocus Error: Unable to Find RMS Maximum
2	(a)4006913-4...	TrueTag_10K_A	43970	labuser	Feb 25 2005 08:53AM	The experiment (a)4006913-43970 has not been hybridized
3	(a)4006913-4...	TrueTag_10K_A	43992	labuser	Feb 25 2005 08:55AM	Autofocus Error: Unable to Find RMS Maximum

Figure 4.7
Error Message – Experiment Not Hybridized

Cause

This error can occur if you do not allow the Experiments field to update on the fluidics station when scanning array barcodes. When the field is not allowed to update, the fact that the array has been stained and washed is not registered with GCOS (even though the error message says the experiment has not been hybridized).

Even though the array barcode is passed to GCOS, the software does not advance the status of the array to scan. Therefore, when you try to scan the array, GCOS has no record of the barcode and displays this error message.

Solution

To advance the status of an array to scan:

1. Click the **Workflow Monitor** button (or select **Workflow Monitor** from the **Run** menu).

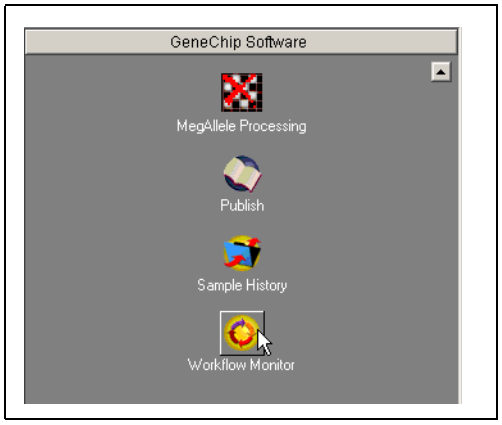


Figure 4.8
Selecting the Workflow Monitor

2. In the window that appears, open the **Assays** menu and select **Universal**.

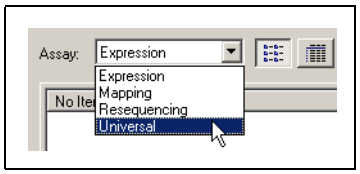


Figure 4.9
Selecting Universal

A list of arrays may appear.

3. If no list of arrays appears, check you filter settings as follows:

   A. Open **Tools** → **Filters . . .**

   B. Reset any incorrect fields and click **Refresh**.

   C. If no list of arrays, deselect the **Date** check boxes and click **Refresh**.

D. Click **OK**.

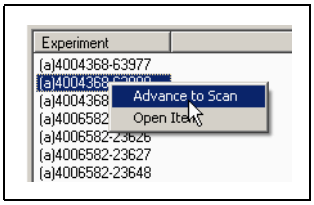


Figure 4.10
Selecting Advance to Scan

4. Locate and right-click the barcode for the array, then select **Advance to Scan**.
5. Put the array back into the scanner, and scan the array.

Troubleshooting and Generating Missing .cel Files

CAUSES OF MISSING .CEL FILES

On occasion, the scanner may generate .dat files but no .cel files for a particular array or group of arrays. Causes are:

- A software error occurred
  When many .cel files are missing, this is typically the cause.
- A gridding error occurred

DETERMINE THAT .CEL FILES ARE MISSING

The first indication that .cel files are missing is when you import experiment data into Affymetrix GeneChip® Targeted Genotyping Analysis Software (GTGS). The Import Experiment Data window in GTGS displays:

- The total number of experiments you attempted to process
- The number of experiments actually processed
- The number of experiments that were skipped

To generate missing .cel files, you must first determine the name of the corresponding .dat files. The two methods we recommend are:

- Viewing the Messages field of the Import Experiment Data window in GTGS

  Use this method if less than 5 experiments were skipped due to missing .cel files. See *Locate .dat Filenames in GTGS* on page 115.
- Using the MegAllele™ Processing tool in GeneChip® Operating Software (GCOS)

  Use this method if more than 5 experiments were skipped due to missing .cel files. See *Locate the .dat File in GCOS* on page 119.

LOCATE .DAT FILENAMES IN GTGS

To identify the .dat files that correspond to missing .cel files in GTGS:

1. In GTGS, look at the Import Experiment Data window.
2. Look for an error similar to the one shown below in the Messages field.

   ERROR DAT file exists but CEL file does not exist:
   \\DB4YGL51\GCLims\Data\(a)4004602-02693D.cel

   Based on the .cel filename, you know that the name of the corresponding .dat file is (a)4004602-02693D.dat.

Refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software User Guide* for more information.

GENERATE MISSING .CEL FILES

Restart the Instrument Control Workstation

If the .cel files are missing due to a software error, restart the Instrument Control Workstation. Restarting the workstation will force the creation of the missing .cel files. Once generated, reimport your experiment data into GTGS.

Manually Grid the .dat Files

If the .cel files are missing due to gridding errors, you must manually regrid the .dat files. Follow the instructions below to locate the .dat filenames and manually apply the grid.

Locating .dat Filenames in GCOS

To locate .dat files that correspond to missing .cel files in GCOS:

Click the **MegAllele Processing** button.

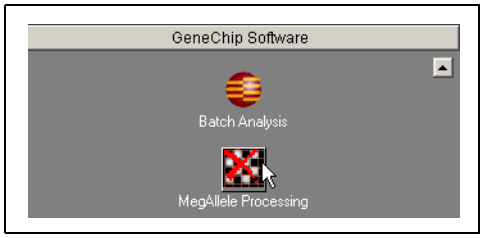


Figure 4.11
MegAllele Processing

The Status window displays the messages "Searching for unprocessed MegAllele files…" and "Reading .dat file headers…" as shown in Figure 4.12.

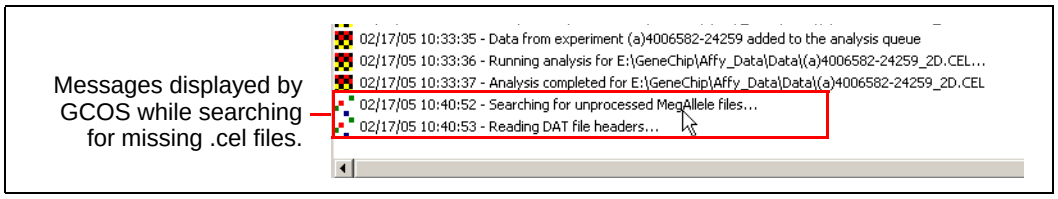


Figure 4.12
Missing CEL File Error Messages

Next, a table listing the .dat files is displayed (Figure 4.13).

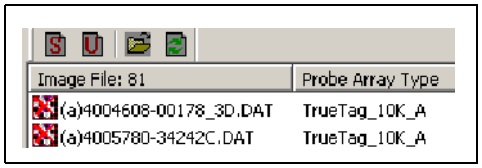


Figure 4.13
List of DAT Files

The software searches the entire list of .dat files in the system; therefore, this procedure can take some time. The search is complete when the Status window displays the message "<*number*> unprocessed MegAllele files found (example shown in Figure 4.14).

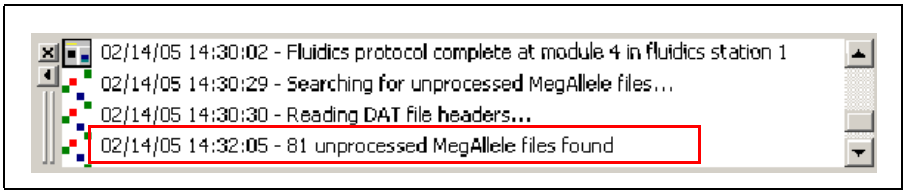


Figure 4.14
Unprocessed MegAllele Files Message

Opening .dat Files to Fix Gridding Errors

To open .dat files:

1. Do one of the following:
   - Select the filename and click the **Open** button.
   - Double-click the filename.
   - Click the **Select All** icon; then click the **Open** button.

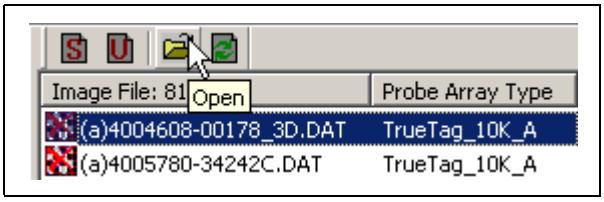


Figure 4.15
Opening a DAT File

2. When you see the message *Cell grid is not aligned in C:\<path to .dat file>*, click **OK**.

   The DAT file opens and is displayed with an un-aligned grid.
3. Manually align the grid and click Calculate CEL Intensity.

   The .cel file is generated automatically.

   For instructions on manually aligning a grid, see *Manually Aligning a Grid* on page 120.

Manually Regridding Arrays

WHAT YOU WILL SEE

When scanning arrays, the gridding step can sometimes fail. When this occurs, a .cel is not generated from the corresponding .dat file. You can determine that a .cel file has not been generated in various ways:

- When importing .cel files to GTGS, an error message is displayed stating that a particular .cel file could not be found.
- In GCOS, the following gridding error message is displayed in the Scan Status column of the lower left window:
  Failed to align grid. Error returned is: Failed to align the grid in Checkerboard Grid Align. Corrective action to manually align the grid.

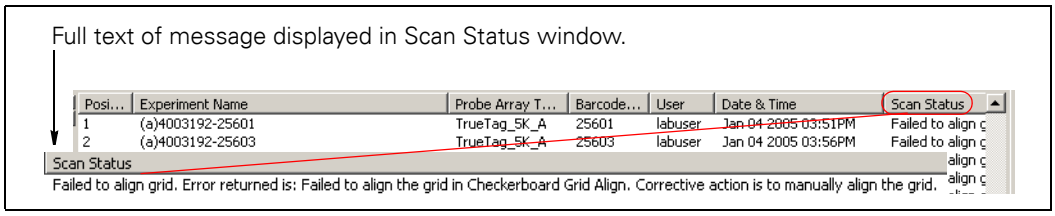


Figure 4.16
Gridding Error Message

- In GCOS in the lower right window, no information is displayed for .cel files that were not generated. In the example below, no .cel file was generated for the A channel for experiment (a)4003192-25600. Only channels B, C and D have .cel files (Figure 4.17).

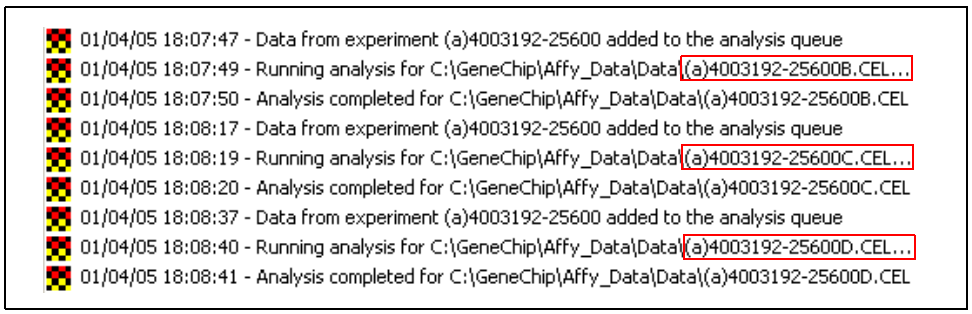


Figure 4.17

Figure 4.18 shows a .dat file that did not grid properly. You can see how the grid is not aligned with the edges of the array image.

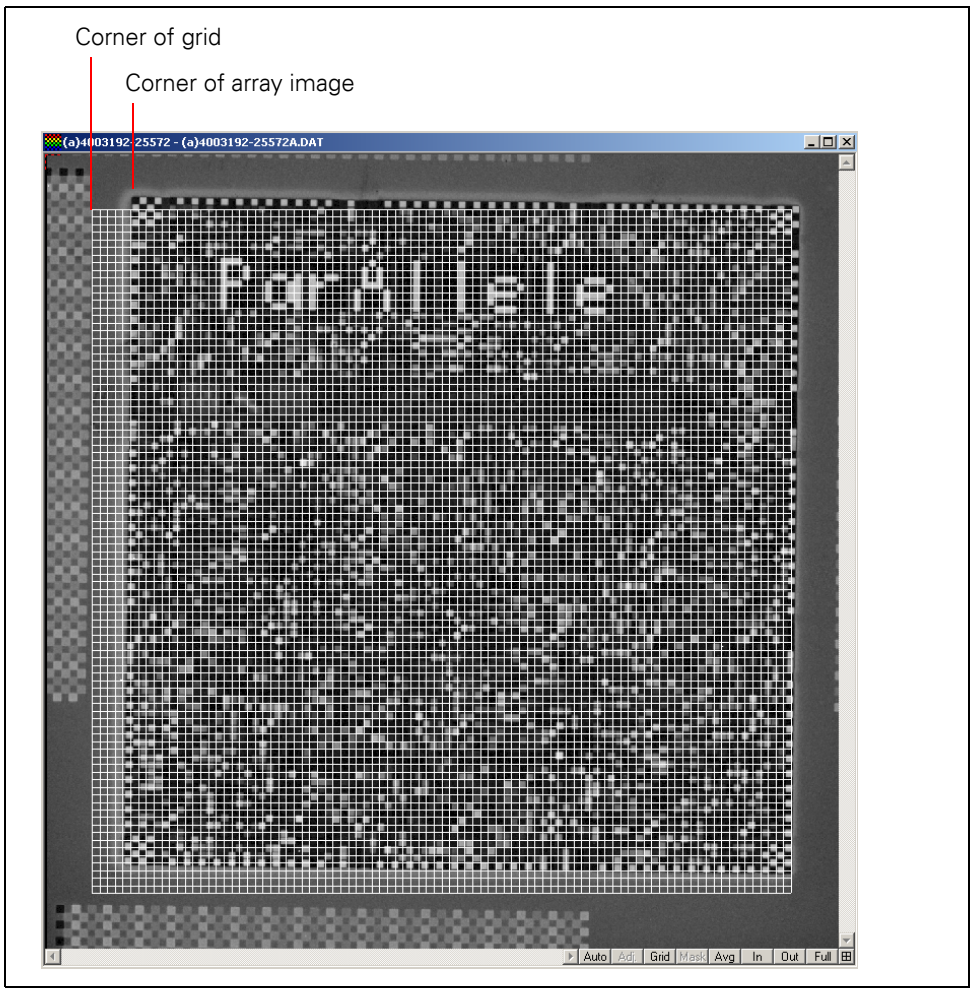


Figure 4.18
Example of .dat File That Did Not Grid Properly

LOCATE THE .DAT FILE IN GCOS

To locate the .dat file on the Instrument Control Workstation:

1. On the workstation, expand the **Image Data** folder.
2. Double-click the .dat file that corresponds to the missing .cel file.

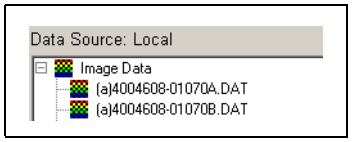


Figure 4.19
Image Data Folder and DAT Files

3. Click **OK**.

   The array image is displayed.

MANUALLY ALIGNING A GRID

To manually align a grid:

1. If a grid is not already displayed over the array image, click the **Grid** button.

   The Grid button is located in the lower right corner of the array image window.

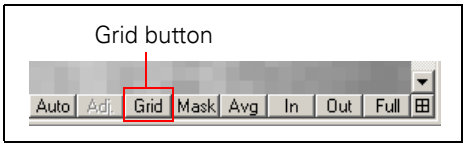


Figure 4.20
Grid Button

The misaligned grid is applied over the array image (Figure 4.21).

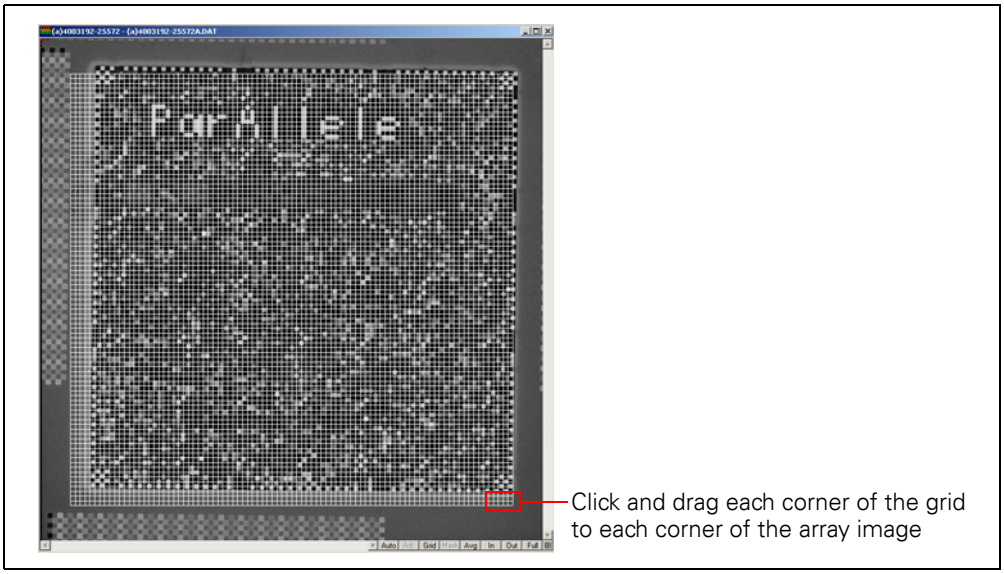


Figure 4.21
Misaligned Grid

2. Perform a preliminary alignment as follows:

   A. Click and hold the left mouse button on a corner of the grid.

   B. Drag and align the grid over the array image.

   C. Repeat this procedure for each corner of the grid.

3. Perform a fine alignment as follows:

   A. Jump to a corner of the grid using one of these methods:

   - Press F5, F6, F7 or F8 on the keyboard
   - Click the corners icon to expand it; then click one of the corners (Figure 4.22).

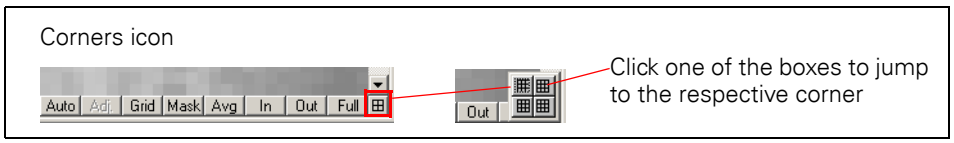


Figure 4.22
Corners Icon

B. Click and drag the corner of the grid over the array image as shown in the following illustration.

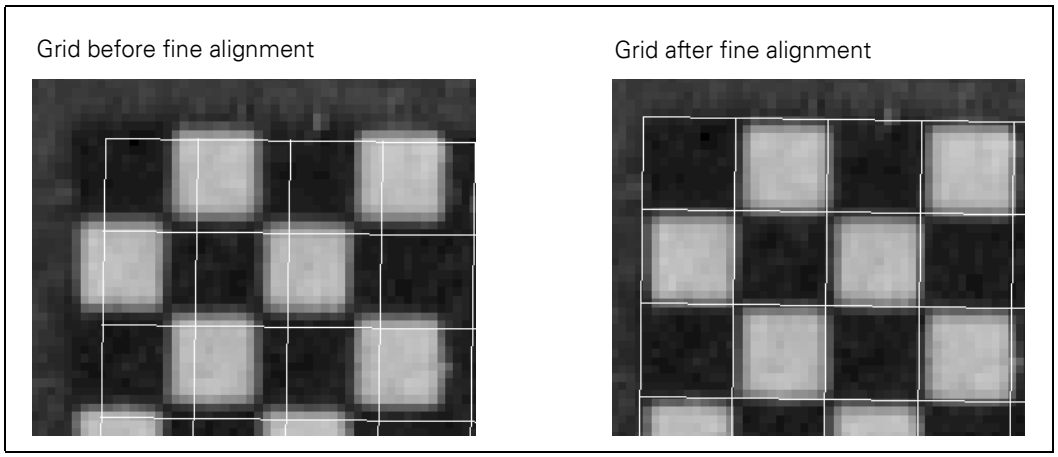


Figure 4.23
Grids Before and After Fine Alignment

C. Click **Recalculate Cel Intensity**.

GENERATE A .CEL FILE

To generate a .cel file:

1. Place the mouse over the image, right-click and choose **Recalculate Cell Intensity**.
2. Update the .dat and .cel files in the Image Data and Cell Intensities folders as follows:

   A. Open **Tools** → **Filters** → **Refresh**.

   B. Click **OK**.

Identifying Skipped Experiments

HOW TO IDENTIFY SKIPPED EXPERIMENTS

Two methods are available for identifying skipped experiments. You can:

- View the Import Experiment Data window as .cel files are being imported and processed.
- View the Experiment QC Summary table.

VIEWING THE IMPORT EXPERIMENT DATA WINDOW

Experiment data is imported to GTGS and processed via the Import Experiment Data window. Once the software is finished reading and processing .cel file data, look at the **Processed/Skipped/Total** field to see if any experiments were skipped. In the example shown in Figure 4.24, one experiment was skipped.

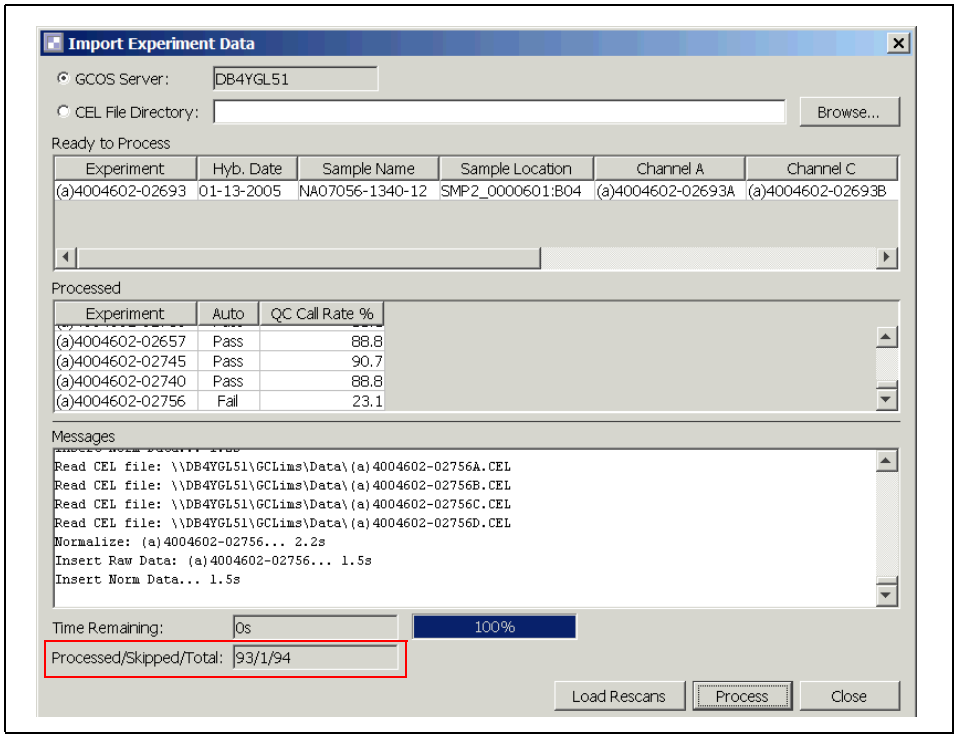
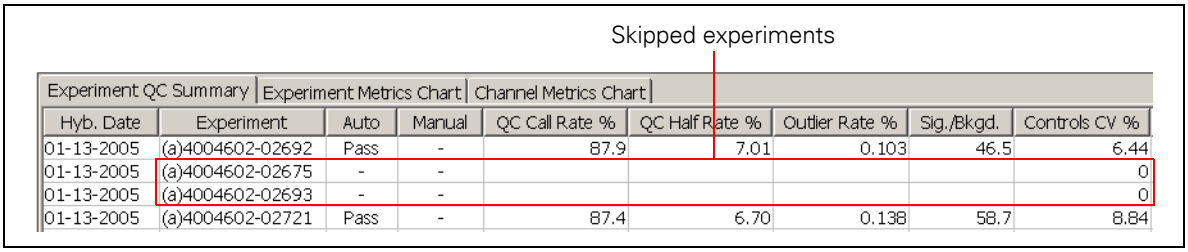


Figure 4.24
Identifying Skipped Experiments in the Import Experiment Data Window

For instructions on troubleshooting skipped experiments via the Import Experiment Data window, refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software User Guide*.

VIEWING THE EXPERIMENT QC SUMMARY WINDOW

If the Import Experiment Data window has already been closed, you can identify skipped experiments by looking at the Experiment QC Summary table. The columns that hold values for the QC metrics (QC Call Rate, QC Half Rate, and so on) will be empty as shown in Figure 4.25. Refer to the *Affymetrix GeneChip ® Targeted Genotyping Analysis Software User Guide* for information on QC metrics.



Hyb. Date	Experiment	Auto	Manual	QC Call Rate %	QC Half Rate %	Outlier Rate %	Sig./Bkgd.	Controls CV %
01-13-2005	(a)4004602-02692	Pass	-	87.9	7.01	0.103	46.5	6.44
01-13-2005	(a)4004602-02675	-	-					0
01-13-2005	(a)4004602-02693	-	-					0
01-13-2005	(a)4004602-02721	Pass	-	87.4	6.70	0.138	58.7	8.84

Figure 4.25
Identifying Skipped Experiments in the Experiment QC Summary Table

To troubleshoot skipped experiments found in the Experiment QC Summary table:

1. Select the **Array Data** icon for your project.
2. In the **Experiment QC Summary** table, look for rows where the QC Metric columns are empty (Figure 4.25).
3. Determine why the data was not imported as follows:

   A. Right-click the **Array Data** icon for the project and select **Import Experiment Data**.

   The Import Experiment Data window appears.

   B. Look at the **Ready to Process** field for experiments that were skipped.

   The names of the experiments you identified in the Experiment QC Summary table should appear in the Ready to Process list.

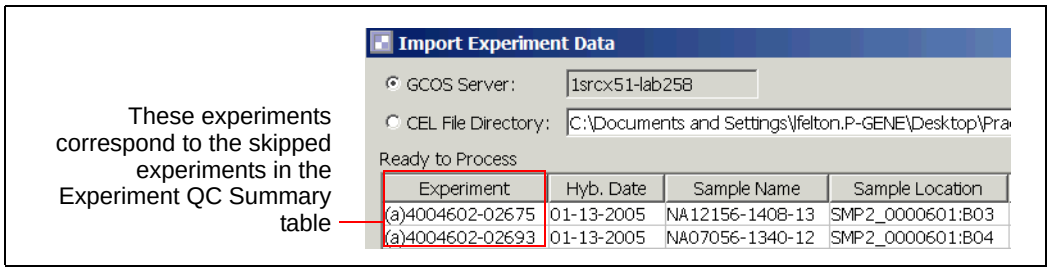


Figure 4.26
Identifying Skipped Experiments

   C. Look at the **Messages** field and do one of the following:

   - If the **Messages** field is empty, click **Process**. The software will run through the list of experiments again and will display error messages related to the skipped experiments.
   - If the Messages field is not empty, scroll through the messages for the ones related to the skipped experiments.

Troubleshooting Failed Experiments

NOTE **For more information on GTGS and reviewing data quality, refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software User Guide*.**

ABOUT DATA PROCESSING

Data processing occurs as .cel files are imported into GTGS. During processing, GTGS calculates and compares the results of each experiment against the following set of quality control metrics ():

- QC Call Rate
- QC Half Rate
- Signal to Background (median ratio)
- Controls Coefficient of Variation (Controls CV %)

Based on this analysis, GTGS automatically assigns the status of Pass or Fail to each experiment. The threshold for each of these metrics must be met for an experiment to be assigned the status Pass. If any metric is not met, the experiment is assigned the status Fail.

Table 4.1
Quality Control Metrics

Quality Control Metric	Description	Threshold
QC Call Rate %	Estimated percentage of assays clearly genotyped.	Must be ≥ 80%
QC Half Rate %	Estimated percentage of assays marginally genotyped.	Must be ≤ 10%
Signal to Background	Median ratio of assay allele/non-allele channel signals (median signal to background ratio)	Must be ≥ 20
Controls CV% (Controls coefficient of variation)	Percent coefficient of variation of control feature signals.	Must be ≤ 30%

IDENTIFYING FAILED EXPERIMENTS

To identify failed experiment in GTGS:

1. Click the **Array Data** icon for your project.
2. In the Experiment QC Summary table, sort on the **Hyb Date** column so that the latest imports are displayed first.

   As experiment data is imported into GTGS, it is appended to the bottom of the list.
3. Look at the Auto column for experiments with the status Fail.

TOOLS AVAILABLE FOR REVIEWING YOUR DATA

The following tools are available in GTGS for reviewing your data prior to cluster genotyping:

- Experiment QC Summary
- Experiment Metrics Chart
- Channel Metrics Chart
- Experiment Details including:
  - Summary
  - Channel Details
  - Array View

For more information on these tools and how to work with them, refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software: User Guide*.

TROUBLESHOOTING FAILED EXPERIMENTS

To troubleshoot failed experiments:

1. Look at the **QC Call Rate** for the failed experiments.
2. Click the **Experiment Metrics Chart** tab.
3. Select these display options:

   Y Axis: **Call Rates %**

   X Axis: **Experiment Name**

Table 4.2
Troubleshooting Failed Experiments

If …	Then …	Suggested Actions
Many experiments have low call rates	compare sample call rates to control call rates by toggling the Controls check box on and off.	If Kit Control data Auto status is Pass, but many experiments were automatically failed, the quality of your sample DNA is suspect. See *About Your DNA Samples* on page 2 for sample criteria. If both samples and controls failed, an error was made while performing the protocol. Rerun the samples.
Individual experiments seem to have randomly failed	Open the Array View and look at the control features.	• If Kit Control data Auto status is Pass, but many experiments were automatically failed, the quality of your sample DNA is suspect. See *About Your DNA Samples* on page 2 for sample criteria. • If both the border control features and the text are clearly seen (Figure 4.27), then the array hybridization and stain/wash steps were correctly performed. Possible cause: assay performed incorrectly prior to Stage 8 – Preparation for Sample Hybridization. - Look at second stage gel. Was there product of correct size in all 4 lanes? If yes, then at some point between target digest and hyb on arrays something went wrong. Solution: rerun samples from stage 5 on. - If the second stage gel looks bad, go to first stage gel. If first stage gel looks good, then rerun samples from stage 5 on. - If first stage gel bad, then rerun the samples from stage 1 on.
If control features are not uniform across the array	• there may be a hybridization problem • the array may be defective	• Look at the Experiment QC Summary and sort on Controls CV %. If the value is over 30%, the experiment is failed automatically. • Look at the .cel file borders in the Array view. Possible causes: • Hybridization oven stopped • Temperature in Hybridization oven too high or too low • Fluidics station error such as wrong buffers used. Look at log for errors. • Excess stain. Solution: • Rewash and rescan the array. See *Rescanning Arrays* on page 103 for instructions. • Rerun the samples. • Arrays may be defective.

Figure 4.27
Controls Features Present; No Signal for Assay Features

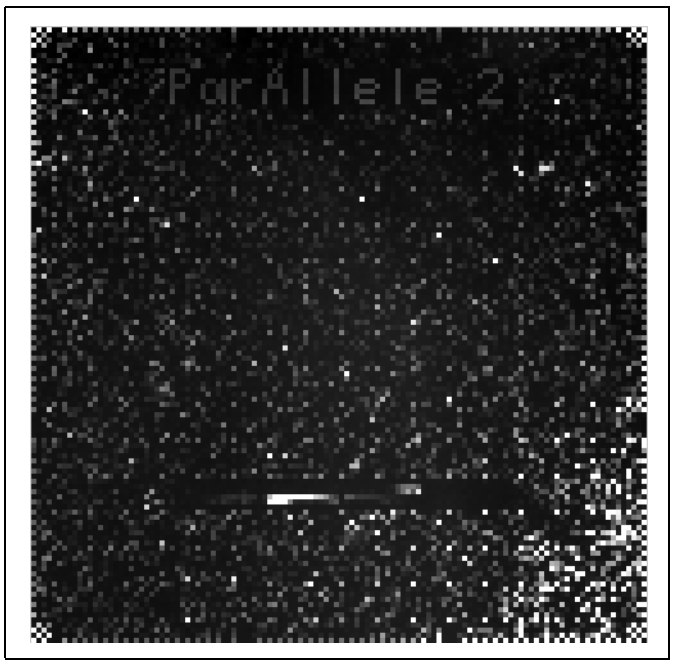


Figure 4.28
Control Features Not Uniform

To understand sample data quality more thoroughly, look at all of the X and Y axis display options in both the Experiment Metrics Chart and the Channel Metrics Chart. For example, in the Channel Metrics Chart the typical signal strength (signal-to-noise) of each nucleotide is displayed.

In the Experiment Metrics Chart, signal-to-background correlates strongly with QC Call Rate. In this context, background is signal in the non-allele channels. When the QC Call Rate is high, signal-to-background is a more sensitive metric for detecting trends in data quality. Low signal-to-background can indicate one of the following:

- Low sample concentration
- Poor amplification
- Sample contamination

High QC half rates can indicate:

- Sample contamination
- Reactions with multiple DNA samples mixed together

Chapter 5

Affymetrix GeneChip® Targeted Genotyping Analysis Software

About Affymetrix GeneChip® Targeted Genotyping Analysis Software

SOFTWARE OVERVIEW

Affymetrix GeneChip® Targeted Genotyping Analysis Software (GTGS) provides a full set of tools to help you generate and manage the highest quality genotypes using the MIP Assay Protocol. This software supports networked client-server environments with multiple users and large experimental data sets.

System management functionality includes:

- User management
- Protocol management
- Array definition management

Project management functionality includes:

- Experiment design
- Sample tracking through the genotyping process.
  - See *Project Management* on page 141 for an overview.
  - Instructions for using the software throughout the various steps of the protocol is presented in Chapter 3, *Running the Protocol*.

  Data analysis functionality includes:
- Generating genotypes from the data collected by the GeneChip® Scanner 3000 7G 4C (Scanner 3000 7G 4C).
- Managing and overseeing the quality of the array data stored in the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System database.

Refer to the *Affymetrix GeneChip® Targeted Genotyping Analysis Software User Guide* for further information and instructions.

System Management

USER MANAGEMENT

User management functions include:

- Adding users
- Activating and deactivating users
- Deleting users
  A user cannot be deleted if the user name is associated with data.
- Modifying user properties
- Changing user names without logging out

Adding Users

You must be logged in as admin to add users.

To add a new user:

1. In the left pane, open the menu and select **Users**.
2. Do one of the following:
   - Open **File** → **New User**.
   - Right-click the **Users** folder and select **New User** (Figure 5.1).

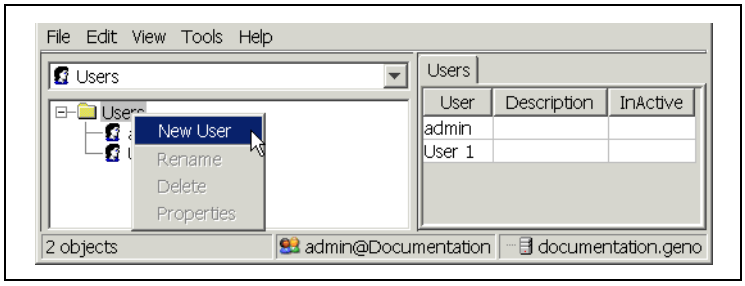


Figure 5.1
Users window

The User window appears.

3. In the **Name** field, enter a user name.

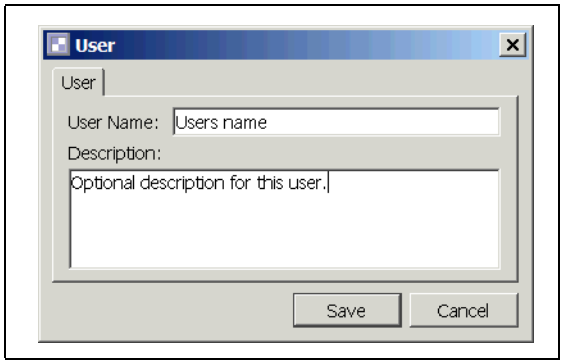


Figure 5.2
Enter User Name and Description

4. Optional: In the Description field, enter a description for the user.
5. Click **Save**.

   When created, the user name and description are added to the table in the right pane of the Users window as shown in Figure 5.3.

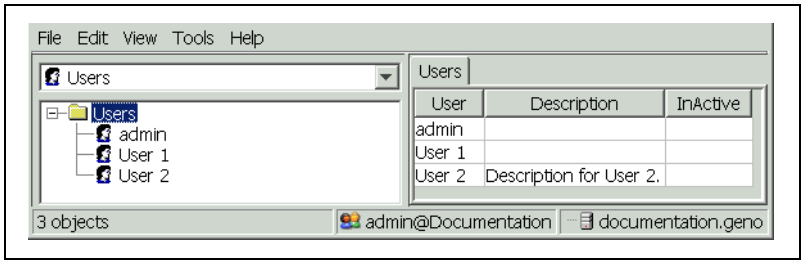


Figure 5.3
New User Displayed in Users Window

Deleting Users

A user cannot be deleted if the user name is associated with any experiments or data. To prohibit a user whose name has been associated with data from logging on to GTGS, you must deactivate them. See *Deactivating Users* on page 133.

To delete a user:

1. Open the menu and select **Users**.
2. Select the user name.
3. Do one of the following:
   - Open **Edit** → **Delete**.
   - Right-click the user name and select **Delete** (Figure 5.4).
4. At the confirmation prompt, click **Yes**.

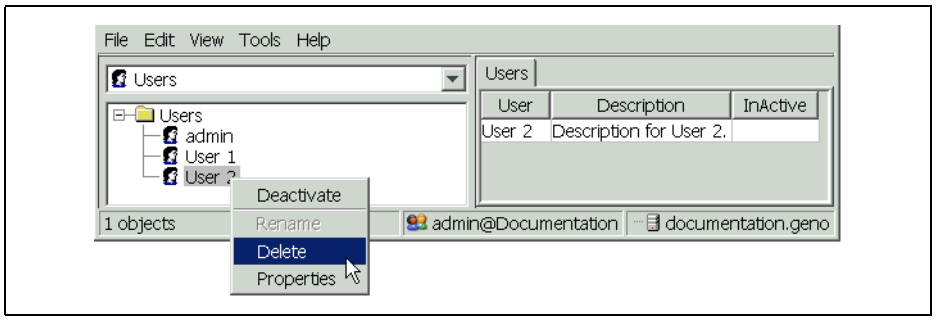


Figure 5.4
Deleting a User

Changing Users

While using the software, you can switch from one user name to another without logging out by using the Set User command.

To change users:

1. Open the menu and select **Users**.
2. Open **Tools → Set User …**
3. Open the User Name menu and select a user.
4. Click **OK**.

Deactivating Users

Users can be deactivated to prohibit them from logging in to GTGS. This functionality is useful when a user has experiments or data associated with their name and therefore cannot be deleted. You must be logged in as admin to deactivate users.

To deactivate a user:

1. Log on to GTGS or Set User as **admin**.
2. Open the menu and select **Users**.
3. Do one of the following:
   - Select the user name and open **Tools → Deactivate**.
   - Right-click the user name and select **Deactivate**.

   As shown below, notice there is now a Y in the InActive column for that particular user.

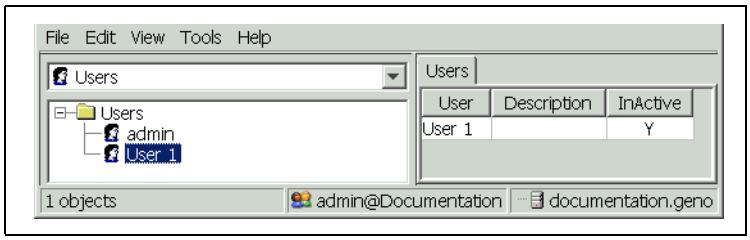


Figure 5.5
Deactivating a User

Activating Users

You must be logged in as admin to activate users.

To activate a user that has been deactivated:

1. Log on to GTGS or Set User as **admin**.
2. Open the menu and select **Users**.
3. Do one of the follow:
   - Select the user name and open **Tools → Activate**.
   - Right-click the user name and select **Activate**.

   Notice that the Y in the InActive column has been removed. The user status is now active.

Modifying User Properties

If you are logged in as admin, you can change the user name and description. If you are logged in as the user, you can modify the user description only.

To modify user properties:

1. Do one of the following:
   - Select the user name and open **Edit → Properties**.
   - Right-click the user name and select **Properties**.
2. Edit the user properties as permitted.
3. Click **Save**.

Exporting a List of Users

You can export the display in the right pane of the Users window as a text file.

To export a list of users:

1. Open **File → Export Table**.
2. Browse to the location where you want the file saved.
3. In the File name field, enter a name for the text file.
4. Click **Save**.

PROTOCOL MANAGEMENT

A protocol designation must be entered into GTGS before samples can be processed using the MIP Assay Protocol. When running the protocol in the lab, users are asked to enter a protocol barcode. Entering the protocol barcode enables future users to see which procedure was followed in the lab when processing a particular set of samples.

Protocol management functions include:

- Adding protocols
- Deleting protocols (if the protocol has not been used)
- Modifying protocol properties

Adding Protocols

To add a protocol:

1. Open the menu and select **Protocols**.
2. Select the Protocols folder and do one of the following:
   - Open **File** → **New Protocol**.
   - Right-click the Protocols folder and select **New Protocol** (Figure 5.6).

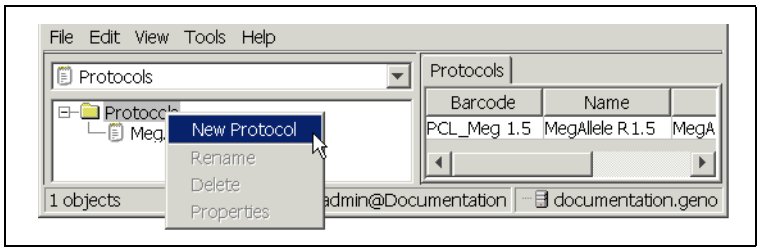


Figure 5.6
Add a New Protocol

3. In the Protocol Creation Dialog window:
   - **A.** In the **Name** field, enter a name for the protocol.
   - **B.** Optional: In the Description field, enter a description for the protocol.
   - **C.** Click the **Barcode** tab.
   - **D.** In the Enter Barcode field, scan or type a barcode for the protocol.

     Barcodes must contain the prefix PCL_. A barcode for the MIP Assay Protocol is provided on the quick reference card, MIP Assay Protocol *Barcodes*. Barcode designations entered here are associated with experiments run in the laboratory using the same protocol.

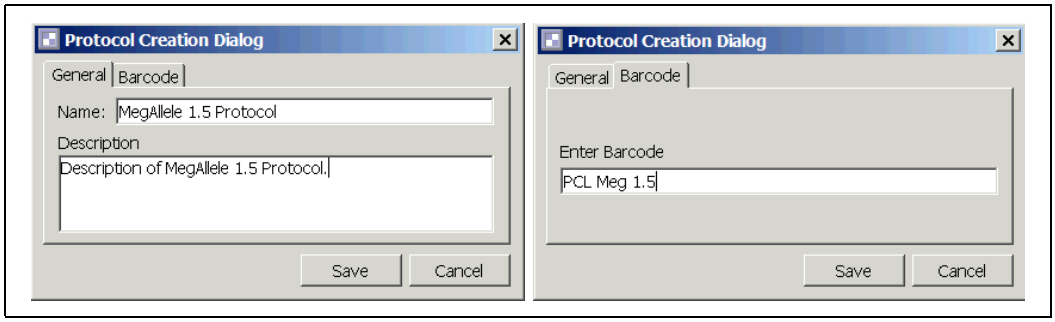


Figure 5.7
Entering New Protocol Information

4. Click **Save**.

   The protocol properties are displayed in the right pane of the Protocols window.

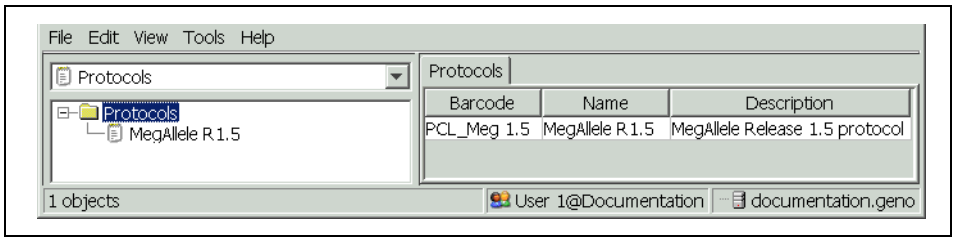


Figure 5.8
New Protocol Displayed in Protocols Window

Deleting Protocols

You can delete a protocol only if it is not associated with any experiments or data.

To delete a protocol:

1. Open the menu and select **Protocols**.
2. Do one of the following:
   - Select the protocol name and open **Edit** → **Delete**.
   - Right-click the protocol name and select **Delete**.
3. At the Confirm Deletion prompt, click **Yes**.

Modifying Protocol Properties

You can modify the name and description of a protocol. You cannot modify the protocol barcode.

To modify protocol properties:

1. Open the menu and select **Protocols**.
2. Select the protocol name and do one of the following:
   - Open **Edit** → **Properties**.
   - Right-click the protocol name and select **Properties**.
3. Modify the protocol name or description.
4. Click **Save**.

Exporting a List of Protocols

You can export the display in the right pane of the Protocols window as a text file.

To export a list of protocols:

1. Open the menu and select **Protocols**.
2. Open **File → Export Table**.
3. Browse to the location where you want the file saved.
4. In the File name field, enter a name for the text file.
5. Click **Save**.

ARRAY DEFINITION MANAGEMENT

An array definition file identifies each feature on an array. Based on this file, the software is able to distinguish control features from genotyping features. You cannot analyze data on an array if the corresponding array definition file is not present in the software.

Array definitions are installed during GTGS installation and configuration. If your assay panel uses an array type not yet defined in the software, you will need to import it before proceeding with the MIP Assay Protocol.

Array definition management functions include:

- Adding array definitions
- Deleting array definitions
- Modifying array definition properties

Adding Array Definitions

1. Load the array definition CD-ROM onto the appropriate computer.
2. In GTGS, open the menu and select Array Definitions.
3. Do one of the following:
   - Open **File → Import Array Definition**.
   - Right-click the Array Definitions folder and select **Import Array Definition**.
4. In the Array Definition File window, locate the filename and select it.
5. Click **Open**, then click **OK**.

   Notice the array definition is now displayed (Figure 5.9).

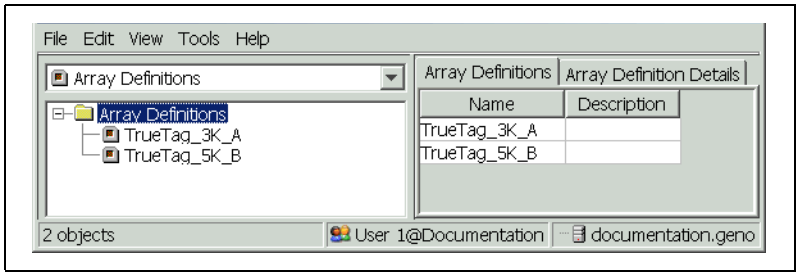


Figure 5.9
Adding an Array Definition

Deleting Array Definitions

An array definition cannot be deleted once an assay panel that references the definition is imported.

To delete an array definition:

1. In the software, open the menu and select **Array Definitions**.
2. Do one of the following:
   - Select an array name and open **Edit → Delete**.
   - Right-click the array name and select **Delete**.
3. Click **Yes**.

Modifying Array Definition Properties

You can modify the description of an array definition.

To modify the description of an array definition:

1. In the software, open the menu and select **Array Definitions**.
2. Select an array name.
3. Do one of the following:
   - Open **Edit** → **Properties**.
   - Right-click the array name and select **Properties**.
4. Enter or modify the description.
5. Click **Save**.

Displaying Array Definition Details and Features

You can display general details about a particular array definition such as the number of features on that type of array. You can also display details about the features of a particular array type such as the Feature ID and Tag ID.

To display array definition details:

1. Open the menu and select **Array Definitions**.
2. Select the Array Definitions folder.
3. Click the **Arrays Definition Details** tab.

Details are displayed in the right pane.

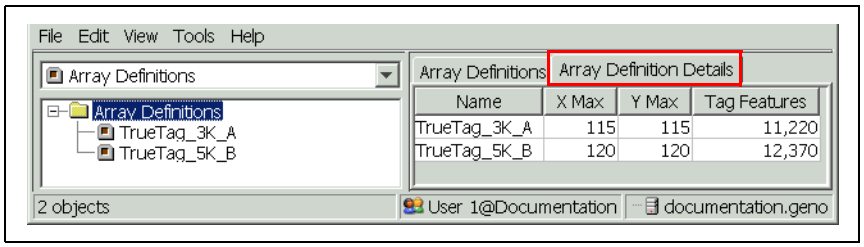


Figure 5.10
Displaying Array Definition Details

To display feature information for a particular type of array:

1. Open the menu and select **Array Definitions**.
2. Select an array definition name.

   As shown below, information for each feature on the array is displayed in the right pane of the window.

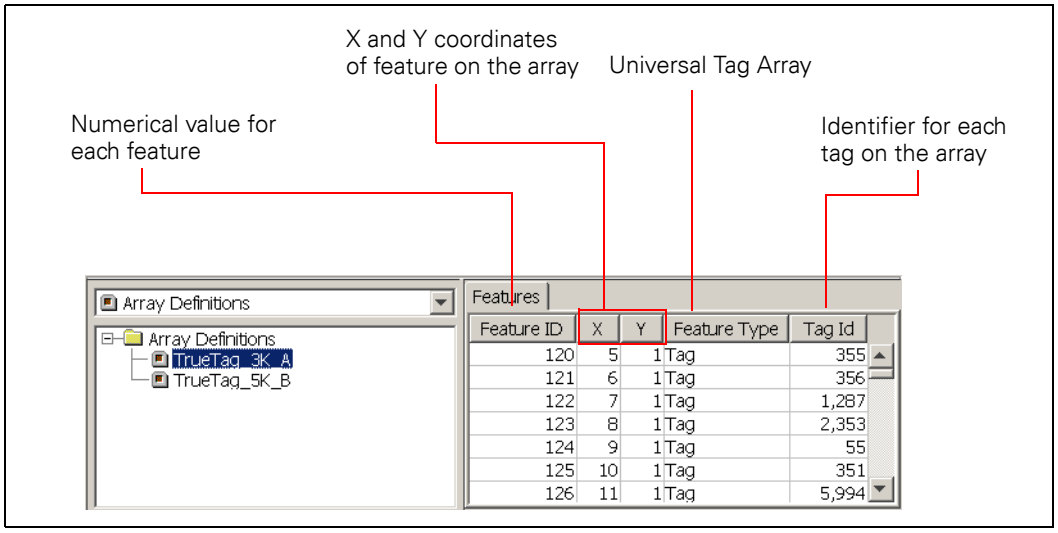


Figure 5.11
Feature Information for a Particular Type of Array

Exporting a List of Array Definitions

You can export the information in the right pane of the Array Definitions window as a text file.

To export a list of array definitions:

1. Open the menu and select **Array Definitions**.
2. Select the **Array Definitions** folder.
3. Open **File** → **Export Table**.
4. Browse to the location where you want the file saved.
5. In the File name field, enter a name for the text file.
6. Click **Save**.

Exporting A List of Array Features

You can export a list of the features for a particular array as a text file.

To export a list of array features:

1. Open the menu and select **Array Definitions**.
2. Select the name of an array.
3. Open **File** → **Export Table**.
4. Browse to the location where you want the file saved.
5. In the File name field, enter a name for the text file.
6. Click **Save**.

Project Management

ABOUT PROJECTS

In GTGS, sample tracking information is organized in folders called projects. Each project contains information about all of the samples that have been tested (or will be tested) using a specific assay panel.

Each project you create will ultimately contain the following information:

- **Sample Plates**
  Contains information on the sample plates to be used for a particular project. Information includes the sample plate barcode, sample names and well contents.
- **Anneal Plates**
  Contains information used to determine which samples are to be transferred:
  - First, from a specific Sample Plate to a specific Anneal Plate.
  - Then from the Anneal Plate to a specific Assay Plate.
    Information includes Sample, Anneal and Assay Plate barcodes, and well or row designations on all plates.
- **Assay Plates**
  Contains information used to determine which samples are to be transferred:
  - First from a specific Anneal Plate to a specific Assay Plate.
  - Then from the Assay Plate to a specific Label Plate.
    Information includes Anneal, Assay, and Label Plate barcodes, and well or row designations on all plates.
- **Label Plates**
  Contains information used to determine which samples are to be transferred:
  - First from a specific Assay Plate to a specific Label Plate.
  - Then from the Label Plate to a specific Hyb Plate.
    Information includes Assay, Label, and Hyb Plate barcodes, and well or row designations on all plates.
- **Hyb Plates**
  Contains information used to determine which samples are to be transferred:
  - First from a specific Label Plate to a particular Hyb Plate.
  - Then from the Hyb Plate to a specific array.
    Information includes Label Plate, Hyb Plate and array barcodes, and well or row designations on all plates.
- **Arrays**
  Contains information on the arrays used for a particular project including the array barcode and the date the barcode was scanned.

ARRAY DEFINITIONS AND ASSAY PANELS

An array definition for the type of array referenced by an assay panel must be imported into GTGS before you can import the panel information.

DELETE FUNCTIONALITY

Project components must be deleted in the reverse order of how they were added.

You must be logged in as admin to delete arrays. See *Deleting Projects and Project-Related Information Deleting Projects and Project-Related Information* on page 104.

EXPORT FUNCTIONALITY

You can export any project information displayed in the right pane as text file. An example of what would be exported from the Sample Plates window is shown in Figure 5.12.

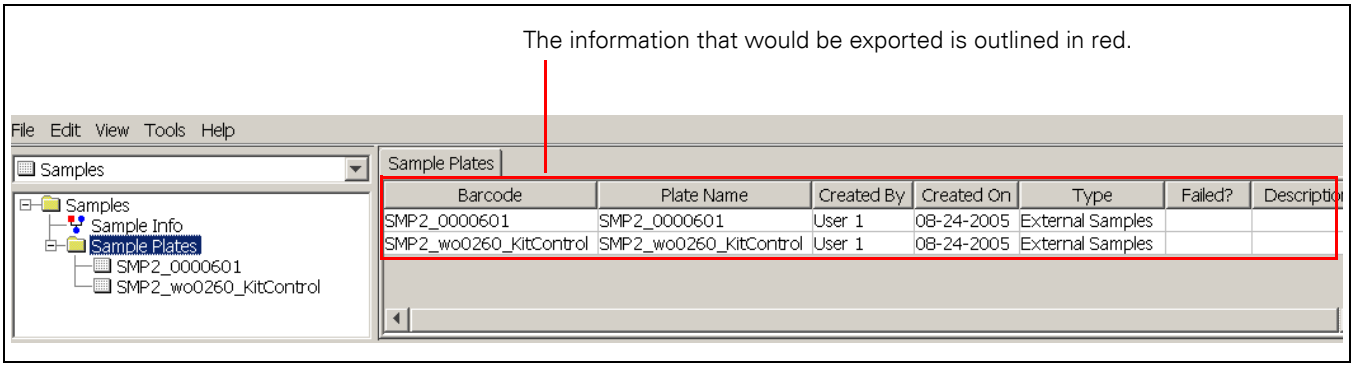


Figure 5.12
Exporting Sample Plate Information

To export project information:

1. Navigate to the window with the desired information.
2. Open **File → Export Table**.
3. Navigate to the location where you want the text file saved.
4. In the File Name field, enter a name for the text file.

   You do not need to add the extension .txt.
5. Click **Open**.

Appendix A

Vendor Contact Information

Vendor Contact Information

NOTE **Customers outside the United States should check each vendor's website for additional contact information.**

Table A.1
Vendor Contact Information

Company	Address	Telephone
Affymetrix www.affymetrix.com	3380 Central Expressway Santa Clara, California 95051	U.S. 1-888-362-2447 U.K. +44 (0)1628 552550 Japan +81-3-5730-8222
Applied Biosystems www.appliedbiosystems.com	850 Lincoln Centre Foster City, California 94404	1-800-327-3002 1-650-638-5800
Axygen Scientific, Inc. www.axygen.com	33210 Central Ave. Union City, California 94587	1-800-4-AXYGEN 1-510-494-8900
Distributors for Barcoded 96-Well PCR Thermal Cycler Plates and Barcode Labels (Anneal, Assay, Label, Hyb) For distributors in countries not listed, refer to:www.axygen.com/Distributors		
	U.S.A. E & K Scientific, Inc. 3575 Thomas Road Santa Clara, California 95054 www.eandkscientific.com	Tel: 1-800-934-8114 or 1-408-378-2013 Fax: 1-408-378-2611
	Australia Radiometer Pacific P/L PO Box 47 Nunawading Vic 3131 oceania1.radiometer.com	Tel: [61] 39-7063-655 Fax: [61] 39-7063-755
	Canada Ultident Scientific 4850 Chemin Bois Franc Suite 100 St. Laurent, Quebec H4R 2G7 www.ultident.com	Tel: 514-335-3433 Fax: 514-335-0992
	Germany G.Kisker GbR Produkte f.d. Biotechnologie Postfach 1329 48543 Steinfurt www. kisker-biotech.com	Tel: [49] 02551-864310 Fax: [49] 02551-864312
	Japan Funakoshi Co Ltd. 9-7. Hongo 2-Chome Bunkyo-Ku Tokyo 113-0033 www.funakoshi.co.jp	Tel: [81] 3-5259-5901 Fax: [91] 3-5259-1005

Table A.1
Vendor Contact Information

Company	Address	Telephone
	Singapore Bio Laboratories 10 Ubi Crescent. Lobby A #06-02. Ubi Techpark 408564 www.biolab.com.sg	Tel: [65] 6846-7577 Fax: [65] 6846-7477
	United Kingdom Thistle Scientific Ltd. DFDS House Goldie Road Uddingston. Glasgow G71 6NZ	Tel: [44] 169-833-8844 Fax: [44] 169-833-8880
Bio-Rad Laboratories www.biorad.com	2000 Alfred Nobel Dr. Hercules, California 94547	Life Science Research Group 1-800-424-6723 1-510-741-1000
BioSmith Biotech www.biosmith.com	3649 Conrad Ave. San Diego, California 92117	1-800-929-7894 1-858-270-8389
Clontech, a TAKARA BIO Company www.clontech.com/clontech	1290 Terra Bella Avenue Mountain View, CA 94043 USA	1-800-662-2566 1-650-919-7300
Coriell Cell Repositories http://locus.umdnj.edu/ccr/	403 Haddon Avenue Camden, NJ 08103	Tel: 1-800-752-3805 Fax: 1-856-757-9737
Eppendorf U.S. only: www.eppendorfna.com Also available through distributors	One Cantiague Road P.O. Box 1019 Westbury, NY 11590-0207	1-800-645-3050
Gilson www.pipetman.com	3000 W. Beltline Hwy. P.O. Box 620027 Middleton, WI 53562-0027	1-800-GILSON1 or 1-608-836-1551
Invitrogen Life Technologies www.invitrogen.com	1600 Faraday Ave. P.O. Box 6482 Carlsbad, California 92008	1-760-603-7200
Rainin Instrument, LLC www.rainin.com	7500 Edgewater Dr. P.O. Box 2160 Oakland, California 94621	1-800-472-4646 1-510-564-1600
Stratagene www.stratagene.com	11011 North Torrey Pines Rd. La Jolla, California 92037	1-858-535-5400
USA Scientific, Inc www.usascientific.com	P.O. Box 3565 Ocala, Florida 34478	U.S.: 1-800-522-8477 International: 1-352-237-6288
VWR International www.vwr.com	1310 Goshen Pkwy. West Chester, Pennsylvania 19380	1-800-932-5000

Appendix B

Thermal Cycler Programs

Thermal Cycler Programs

The MIP Assay Protocol has been optimized for use with the 96-well GeneAmp® PCR System 9700 Thermal Cyclers (silver block) manufactured by Applied Biosystems. Equivalence tests have been run on the other models listed in Chapter 2, *Equipment, Supplies, Consumables*.

Five thermal cycling programs are used throughout the protocol. This appendix briefly describes each of these programs. It also includes step-by-step instructions for programming the Meg 22 (20) cycle programs.

PRE-AMP LAB THERMAL CYCLER PROGRAMS

Only the GeneAmp PCR System 9700 Thermal Cyclers are approved for use in the Pre-Amp Lab. These thermal cyclers must be set up to run the following programs:

- Meg Anneal
- Meg 22 (20) cycle programs
  - Meg 3-5-10k
  - Meg 20k

POST-AMP LAB THERMAL CYCLER PROGRAMS

The thermal cyclers listed under *Thermal Cyclers* on page 13 can be used in the Post-Amp Lab. These instruments must be set up to run the following programs:

- Meg Hypcr programs
  - Meg Hypcr 3-5k
  - Meg Hypcr 10-20k
- Meg Hydigest-a
- Meg Denature

SETTING THE RAMP SPEED AND VOLUME FOR EACH PROGRAM

IMPORTANT !

The first time you run each of these programs, you must change the default ramp speed and volume settings.

Ramp Speeds

Use the following ramp speeds for the thermal cyclers validated for use with the Affymetrix GeneChip® Scanner 3000 Targeted Genotyping System.

- GeneAmp PCR System 9700 Thermal Cycler with a gold or silver block: *Max*
- GeneAmp PCR System 9700 Thermal Cycler with an aluminum block: *Std*
- GeneAmp PCR System 9700 Thermal Cycler with a dual block: *96D*
- DNA Engine® Peltier and Dyad: *Gradient*

Setting Ramps Speeds and Volumes

The following instructions are for programming a GeneAmp PCR System 9700 thermal cycler with a gold or silver block.

To set the ramp speed and volume for each program:

1. Press **Run** (F1).
2. Use the arrow pad to select the program.
3. Press **Start** (F1).
4. Press the down arrow to move to the ramp speed.
5. Press **Max** (F3).

   Max is the ramp speed to use for GeneAmp PCR System 9700 thermal cyclers with a gold or silver block. For other cycler ramp speeds, see *Ramp Speeds* above.
6. Press the up arrow to move to the reaction volume and enter the volume appropriate volume:
   - Meg Anneal: 45
   - Meg 3-5-10k and Meg 20k: 67
   - Meg Hypcr 3-5k and Meg Hypcr 10-20k: 35
   - Meg Hydigest-a: 75
   - Meg Denature: 90
7. Press **Start** (F1) to start the program.

Meg Anneal Thermal Cycler Program

ABOUT THE MEG ANNEAL PROGRAM

The *Meg Anneal* program consists of three holds and no cycles.

Ramp speed and volume:

- **Ramp speed**
  - GeneAmp PCR System 9700 with a gold or silver block = Max
  - GeneAmp PCR System 9700 with an aluminum block = Std
- **Volume**: 45 µL

IMPORTANT !

The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.

Table B.1
Stages of the Meg Anneal Thermal Cycler Program

Stage	Temperature	Time
Enzyme A	20°C	4 minutes
Denature	95°C	5 minutes
Anneal	58°C	Infinity

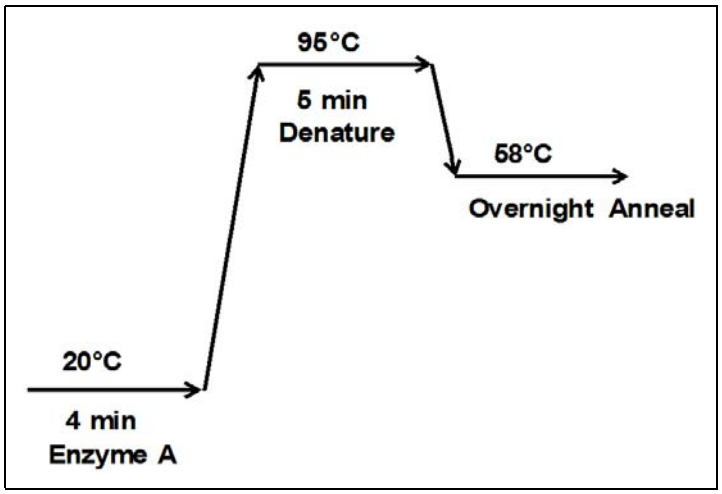


Figure B.1
Meg Anneal Thermal Cycler Program

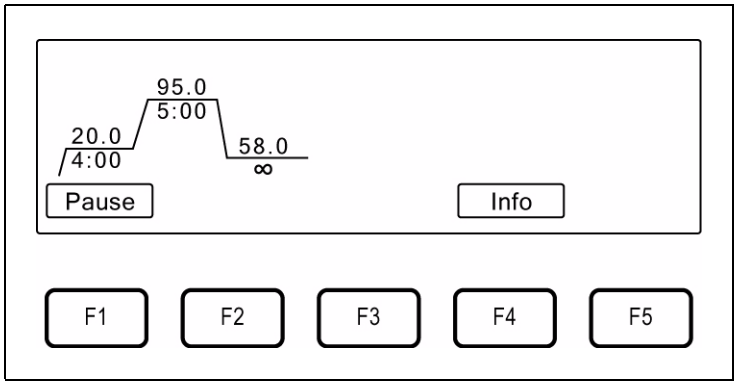


Figure B.2
Thermal Cycler Display for the Meg Anneal Program

Meg 22 (20) Cycle Thermal Cycler Programs

ABOUT THESE PROGRAMS

Meg 3-5-10k and Meg 20k are two variations of the same thermal cycling program. The difference between these programs is the number of cycles used for the denature, anneal and extend stages at the end of the program (Table B.2). The version that you will use is determined by the assay panel size. The only difference between these programs is the number of amplification cycles performed.

- For 3K, 5K and 10K assay panels: use *Meg 3-5-10k* (22 cycles)
- For 20K assay panels: use *Meg 20k* (20 cycles)

Ramp speed and volume for both programs:

- **Ramp speed**
  - GeneAmp PCR System 9700 with a gold or silver block = Max
  - GeneAmp PCR System 9700 with an aluminum block = Std
- **Volume**: 67 µL

IMPORTANT !

The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.

Table B.2
Stages of the Meg 22 (20) Cycle Thermal Cycler Programs

Stage	Temperature	Time	Cycles
Gap Fill/Split	58°C	2 minutes	
dNTP Mix/Anneal	58°C	10 minutes	
Ligate	58°C	10 minutes	
Exo	37°C	15 minutes	
Denature	95°C	5 minutes	
Cleavage Mix	37°C	10 minutes	
Amp Mix	60°C	2 minutes	
Denature	95°C	10 minutes	
Denature	95°C	20 seconds	3K/5K/10K: 22 cycles 20K: 20 cycles
Anneal	64°C	45 seconds	
Extend	72°C	10 seconds	
Finish	72°C	10 seconds	
	4°C	Infinity	

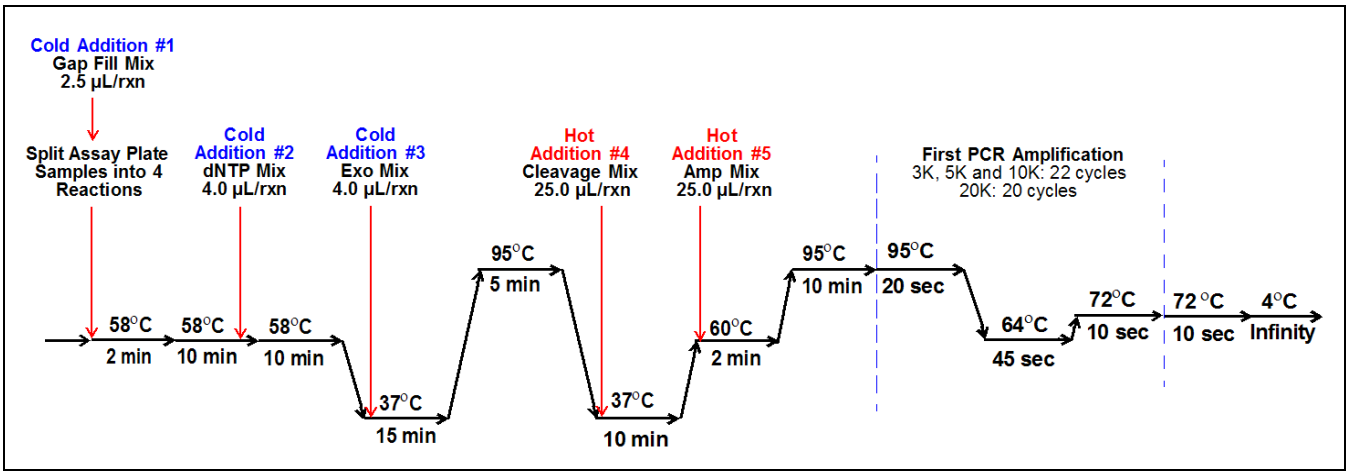


Figure B.3
Meg 22 (20) Cycle Thermal Cycler Programs

TO SET UP THE MEG 22 (20) CYCLE PROGRAMS

The GeneAmp PCR 9700 thermal cyclers accept six holds only. Because these programs include 10 holds, the instrument must be programmed using an unconventional method. Essentially the thermal cycler is programmed to accept two high-level hold programs: one with 6 holds; the other with 2 holds.

To set up the Meg 22 (20) cycle programs, you will:

- Insert an additional cycle program
- Change one of the cycles to a hold and create the first 8 holds
- Set the temperatures and times for the first 8 holds
- Set the temperatures and times for the amplification cycle
- Set the temperatures and times for the last 2 holds
- Store the program

Insert Addition Cycle Program

To insert an additional cycle program:

1. Turn on the thermal cycler.
2. Press **F2** to select **Create**.

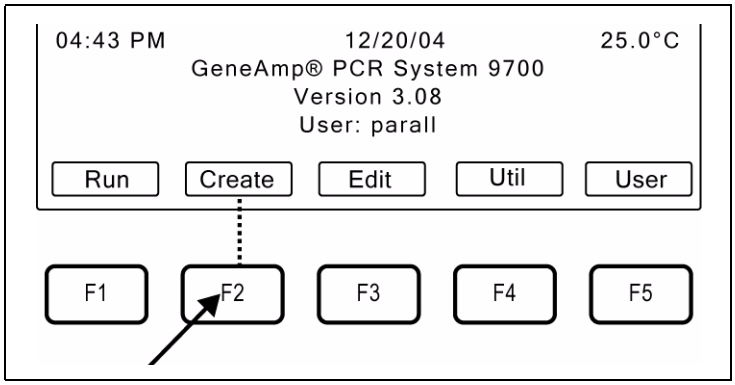


Figure B.4

The generic program appears. The 1 of 1 **Hld** is highlighted.

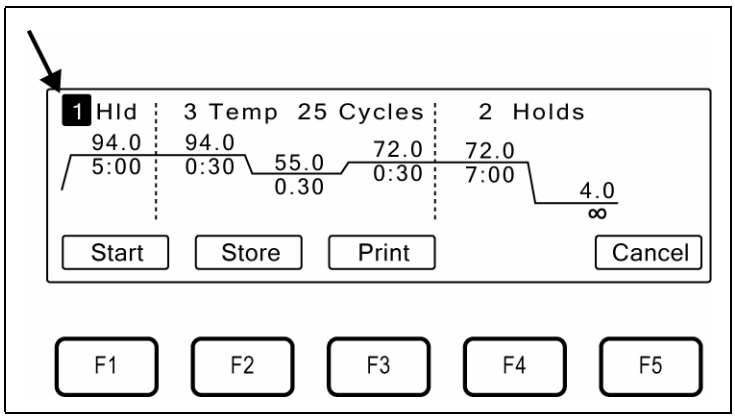


Figure B.5

3. Press the down arrow to highlight 94.0 and display different options above the function keys.

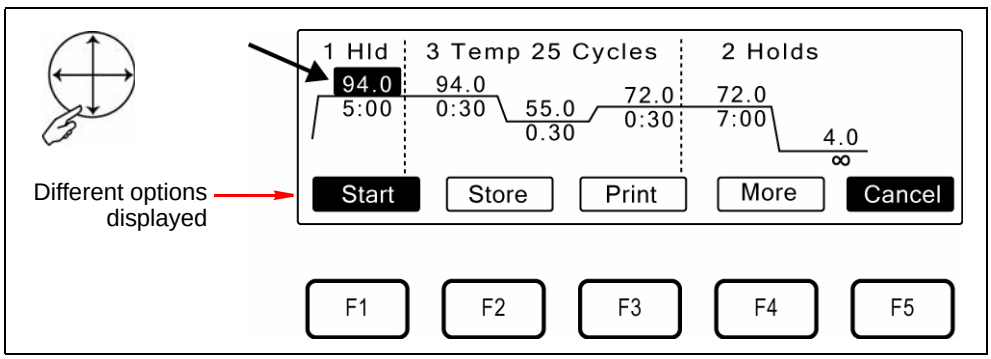


Figure B.6

4. Press F4 to select **More**.

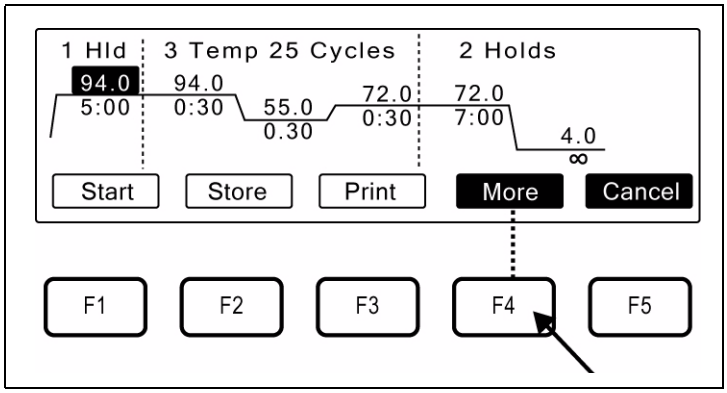


Figure B.7

5. Press F2 to select **Insert**.

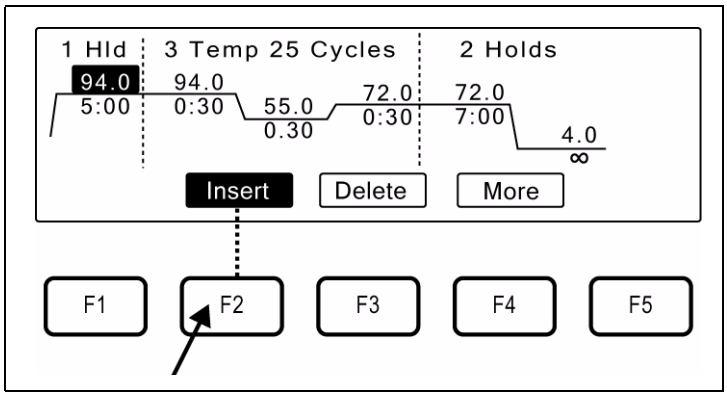


Figure B.8

6. Press F2 to select **Cycle**.

IMPORTANT !

Do not choose Hold. Selecting Hold will not insert an additional hold step into the program.

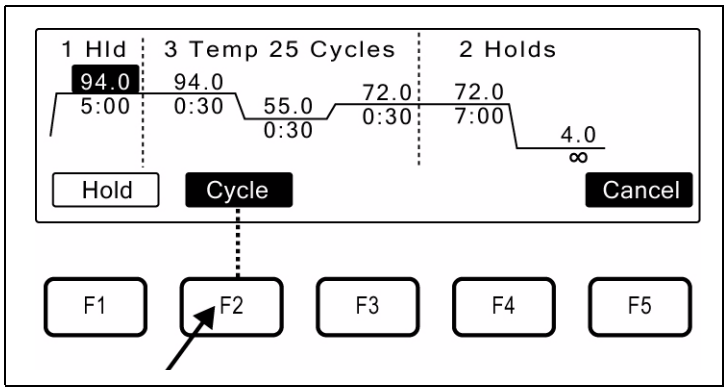


Figure B.9

Now the generic program displays two cycle programs (3 Tmp 25 Cycles) separated by one hold (1 Hld).

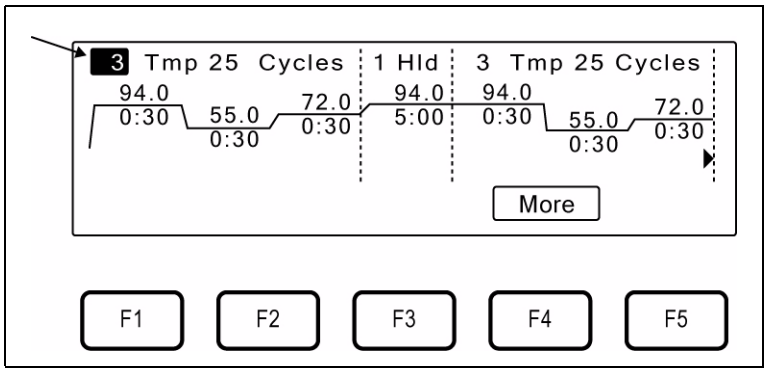


Figure B.10

Change the First Cycle Program to a Hold and Create the First 8 Holds

To change the first cycle program to a hold and set up the first 8 holds:

1. Press the up arrow to highlight the 3 of the first 3 **Temp 25 Cycles**.

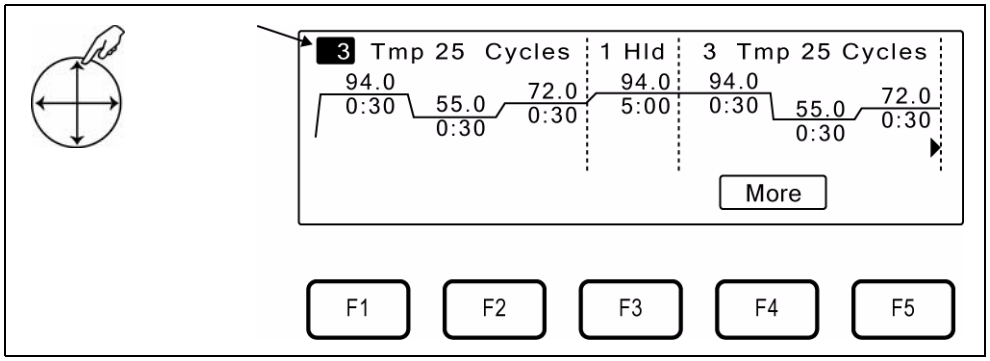


Figure B.11

2. Using the keypad, change the 3 to a 1.

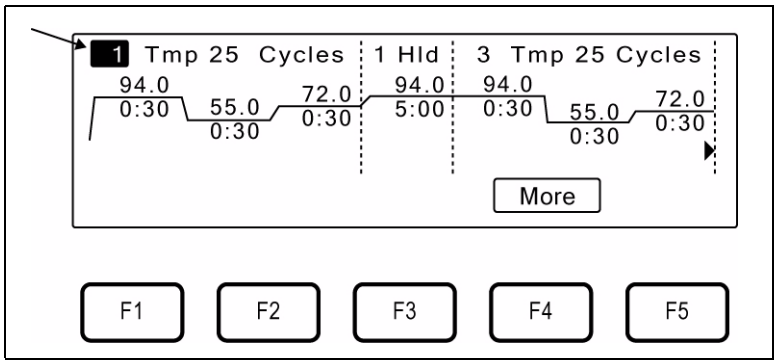


Figure B.12

3. Press the down arrow.

The wording in the display changes from **3 Temp 25 Cycles** to **1 Hld**.

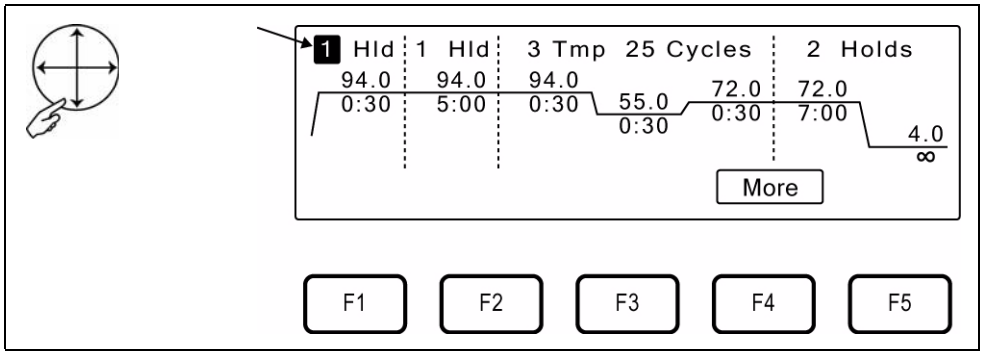


Figure B.13

4. Using the keypad, change the 1 to a 6.

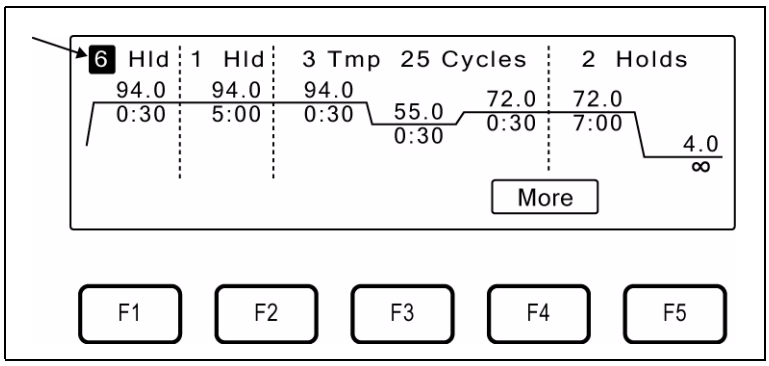


Figure B.14

5. Press the right arrow twice to move the highlight to the second hold program (immediately adjacent to the first 6-stage hold just created).

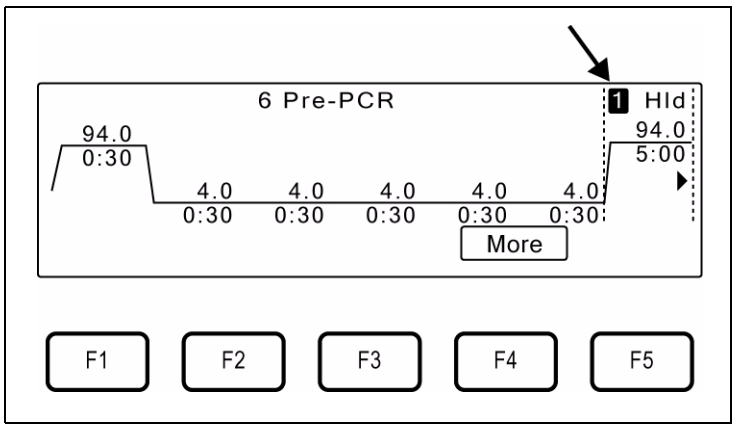


Figure B.15

6. Using the keypad, change the 1 to a 2.

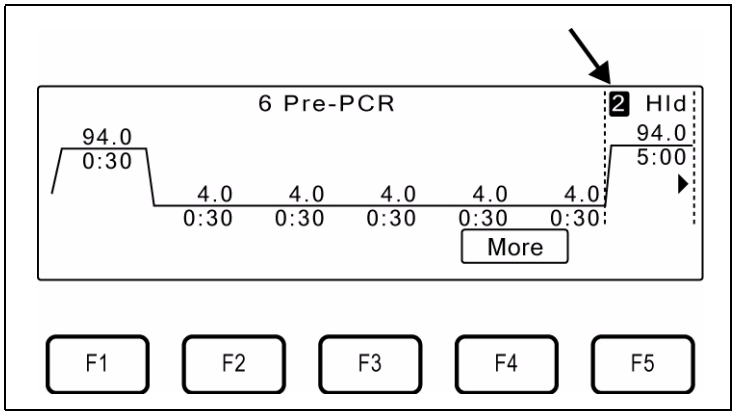


Figure B.16

Set the Times and Temperatures for the 6 Pre-PCR and 2 Hld Programs

1. Press the down arrow once, then the left arrow repeatedly to move from the 2 of 2 **Hld** back to the 94.0 of the 6 Pre-PCR program.

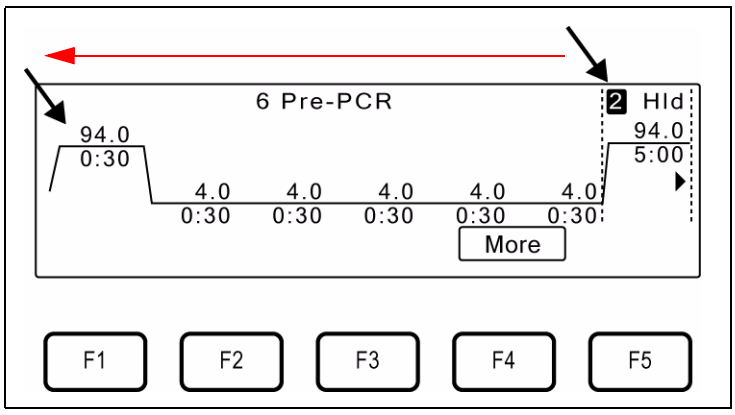


Figure B.17

2. Using the keypad, change 94.0 to 58.0.

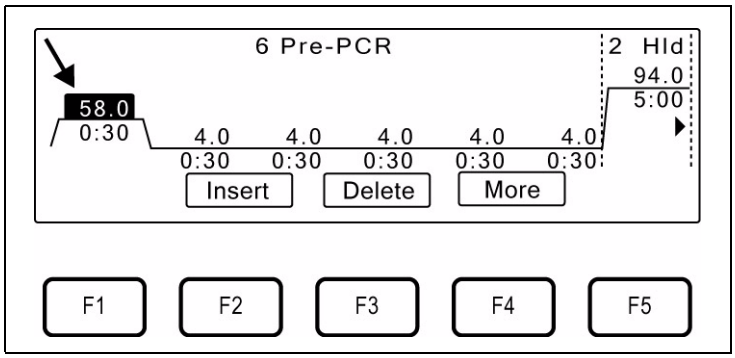


Figure B.18

3. Press the down arrow to highlight **0:30**; then use the keypad to change **0:30** to **2:00**.

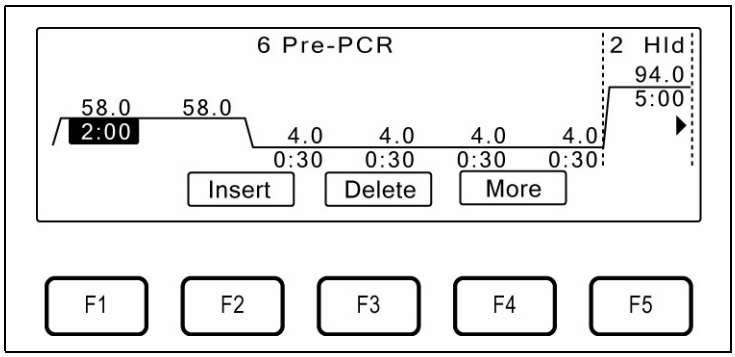
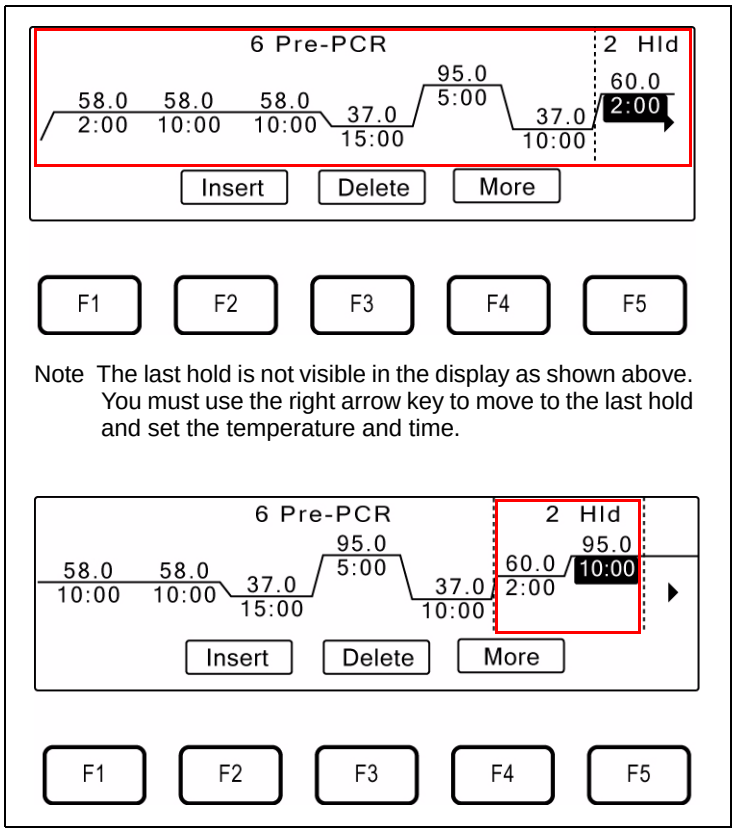


Figure B.19

4. Continue using the arrow keys and keypad to change each time and temperature in the 6 Pre-PCR and 2 Hld programs to those shown below.



Note The last hold is not visible in the display as shown above. You must use the right arrow key to move to the last hold and set the temperature and time.

Figure B.20

Set Up the Amplification Cycle

To set up the amplification cycle:

1. Continue using the arrow keys and keypad to set the temperature and duration of each PCR cycle as follows:

Table B.3

Temp of PCR Cycle	Temperature	Time	Cycles
Temp 1 (Denature)	95°C	20 seconds	Meg 3-5-10k: 22 cycles Meg 20k: 20 cycles
Temp 2 (Anneal)	64°C	45 seconds	
Temp 3 (Extend)	72°C	10 seconds	
Note: Because the products being amplified are <120 bases in length, long extension times are not required.			

Program the Last Two Holds

To program the last two holds:

1. Continue using the arrow keys and keypad to advance to the ninth hold and set it to 72°C for 10 seconds.
2. Advance to the last hold and set it to 4°C for Infinity.

Store the Program

To store the program:

1. Press **F2** to select **Store**.
2. If the User designation is:
   - Correct, proceed to the next step.
   - Incorrect, press F2 and select the correct user.
3. Press **F1** to select **Accept**.
4. Press **F3** to select **Method**.
5. Press **CE** on the keypad to clear.
6. Use the arrows to move to the letter **m**, then press **Enter** on the keypad.
7. Continue entering the program name in the same manner (meg 3-5-10k or meg 20k).
   - To select numbers (22 or 20), press the corresponding numbers on the keypad.
   - To include a space, move to and enter the blank after the letter z.
8. Press **F1** to select **Accept**.
9. Press **F1** again to select **Accept**.

SET THE RAMP SPEED AND VOLUME

IMPORTANT !

The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.

The ramp speed and volume for all of these programs are:

- Ramp speed
  - GeneAmp PCR System 9700 with a gold or silver block = Max
  - GeneAmp PCR System 9700 with an aluminum block = Std
- Volume: 67

Meg Hypcr Thermal Cycler Programs

ABOUT THE MEG HYPCR PROGRAMS

Each of the Meg Hypcr programs consist of three holds and 1 cycle. The only difference between these programs is the number of cycles. The version you will use is determined by the assay panel size.

- For 3K and 5K assay panels, use *Meg Hypcr 3-5k* (9 cycles)
- For 10K and 20K assay panels, use *Meg Hypcr 10-20k* (10 cycles)

Ramp speeds:

- GeneAmp PCR System 9700 Thermal Cycler with a gold or silver block: *Max*
- GeneAmp PCR System 9700 Thermal Cycler with an aluminum block: *Std*
- GeneAmp PCR System 9700 Thermal Cycler with a dual block: *96D*
- DNA Engine Peltier and Dyad: *Gradient*

Volume: 35 µL

IMPORTANT !

The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.

Table B.4
Stages of the Meg Hypcr Thermal Cycler Programs

Stage	Temperature	Time	Cycles
Denature	95°C	10 minutes	
Denature	95°C	20 seconds	3K/5K: 9 cycles 10K/20K: 10 cycles
Anneal	68°C	45 seconds	
Extend	68°C	10 seconds	
Finish	68°C	10 seconds	
Standby	4°C	Infinity	

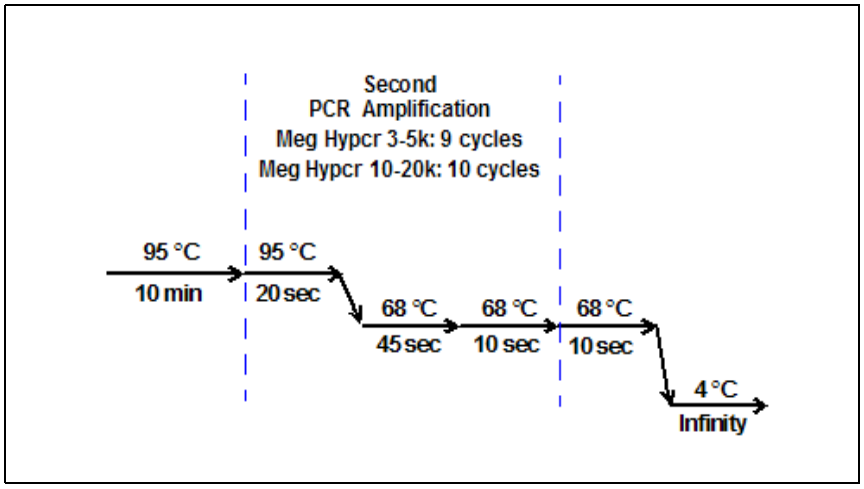


Figure B.21
Meg Hypcr Thermal Cycler Programs

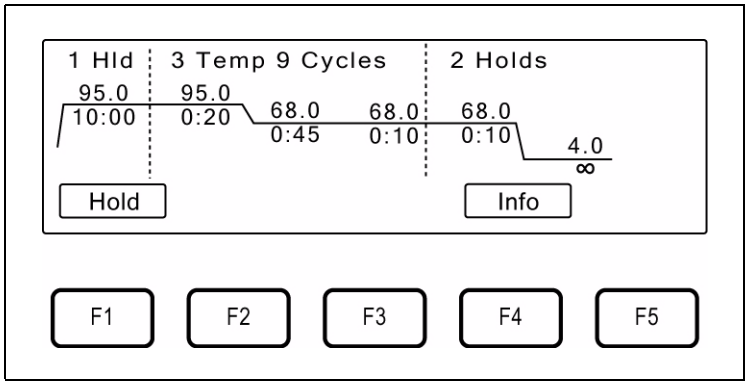


Figure B.22P
Thermal Cycler Display for the Meg Hypcr 3-5k Program

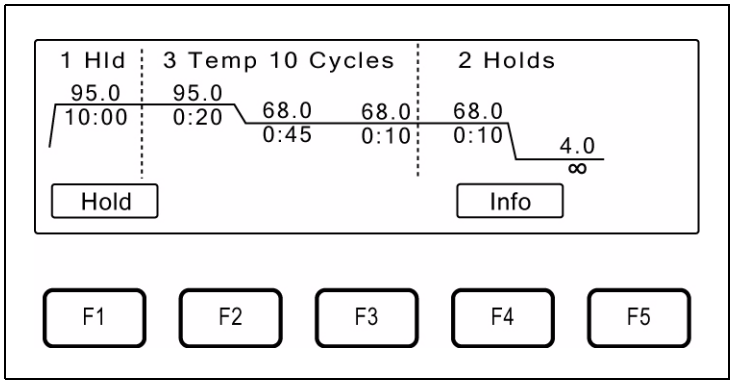


Figure B.23
Thermal Cycler Display for the Meg Hypcr 10-20k Program

Meg Hydigest-A Thermal Cycler Program

ABOUT THE MEG HYDIGEST-A PROGRAM

The *Meg Hydigest-a* program consists of three holds and no cycles.

Ramp speeds:

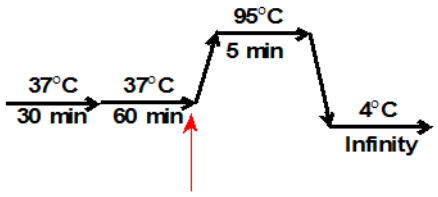
- GeneAmp PCR System 9700 Thermal Cycler with a gold or silver block: *Max*
- GeneAmp PCR System 9700 Thermal Cycler with an aluminum block: *Std*
- GeneAmp PCR System 9700 Thermal Cycler with a dual block: *96D*
- DNA Engine Peltier and Dyad: *Gradient*

Volume: 75 µL

IMPORTANT ! **The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.**

Table B.5
Stages of the Meg Hydigest-a Thermal Cycler Program

Stage	Temperature	Time
Digestion	37°C	30 minutes
		60 minutes
Heat Inactivation	95°C	5 minutes
Standby	4°C	Infinity



If running a second QC gel, remove 4.0 µL of each sample when timer reads 5:00 min remaining for the second 37°C hold.

Figure B.24
Meg Hydigest-a Thermal Cycler Program

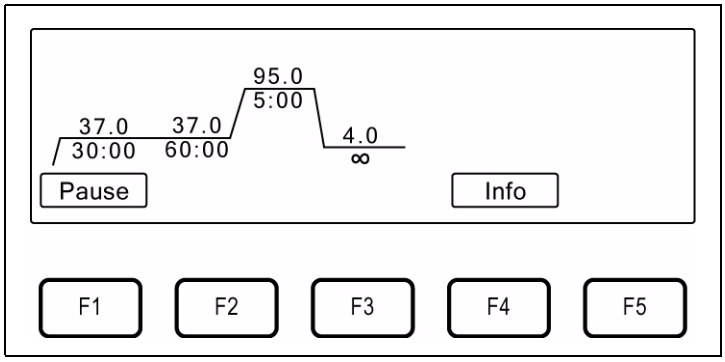


Figure B.25
Thermal Cycler Display for Meg Hydigest-a Program

Meg Denature Thermal Cycler Program

ABOUT THE MEG DENATURE PROGRAM

The *Meg Denature* program consists of two holds and no cycles.

Ramp speeds:

- GeneAmp PCR System 9700 Thermal Cycler with a gold or silver block: *Max*
- GeneAmp PCR System 9700 Thermal Cycler with an aluminum block: *Std*
- GeneAmp PCR System 9700 Thermal Cycler with a dual block: *96D*
- DNA Engine Peltier and Dyad: *Gradient*

Volume: 90 µL

IMPORTANT !

The ramp speed and volume must be set the first time you use the program. See *Setting the Ramp Speed and Volume for Each Program* on page 148.

Table B.6
Stages of the Meg Denature Thermal Cycler Program

Stage	Temperature	Time
Denature	95°C	6 minutes
Standby	4°C	Infinity

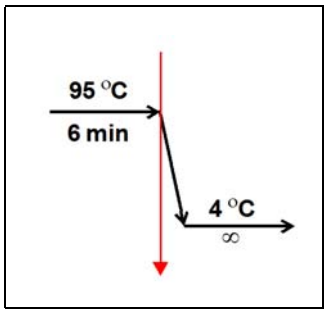


Figure B.26M
Meg Denature Thermal Cycler Program

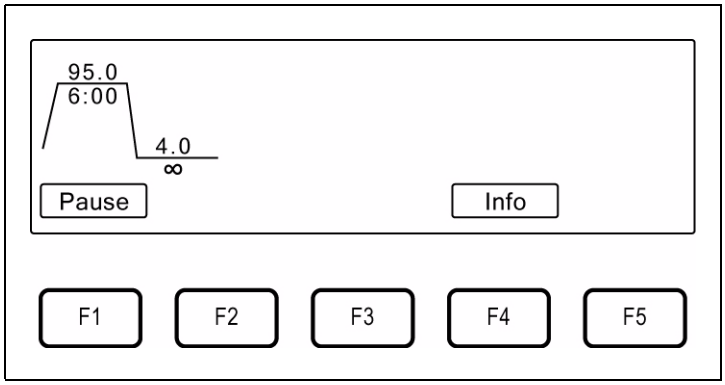


Figure B.27
Thermal Cycler Display for the Meg Denature Program

Appendix C

Guidelines for Increasing Sample Throughput

Guidelines for Increasing Sample Throughput

This appendix provides guidelines for increasing sample throughput for the MIP Assay Protocol from 48 to 96 samples per day based on a five day work week.

EQUIPMENT RECOMMENDATIONS

Increasing sample throughput based on the workflow recommended in Figure C.1 on page 171 requires the following additional equipment. Refer to Chapter 2, *Equipment, Supplies, Consumables* for part numbers.

- One additional thermal cycler in the Pre-Amp Lab (or an incubation block) GeneAmp® PCR System 9700 Thermal Cycler
- One additional GeneChip® Hybridization Oven 640 with 8 carriers
- One to two additional GeneChip® Fluidics Station 450

RESOURCE REQUIREMENTS

Increasing sample throughput based on the workflow recommended in Figure C.1 requires one additional FTE (for a total of 3 FTEs). FTEs must be available to process samples a minimum of 8 hours per day.

The process itself requires a minimum of 10 hours per day.

SUGGESTED WORKFLOW

The workflow shown in Figure C.1 on page 171 requires:

- That operators be proficient with running the MIP Assay Protocol.
- That every resource and all equipment is readily available and functional.
- A third thermal cycler or incubation block in the Pre-Amp Lab. This cycler is used to hold the second round of annealed samples at 58°C until the thermal cyclers in the Post-Amp Lab are available for use.
- Scanning 9 hours per day, assuming that new arrays are added to the Autoloader every 30 minutes. In addition, the scanner must be left to run overnight.

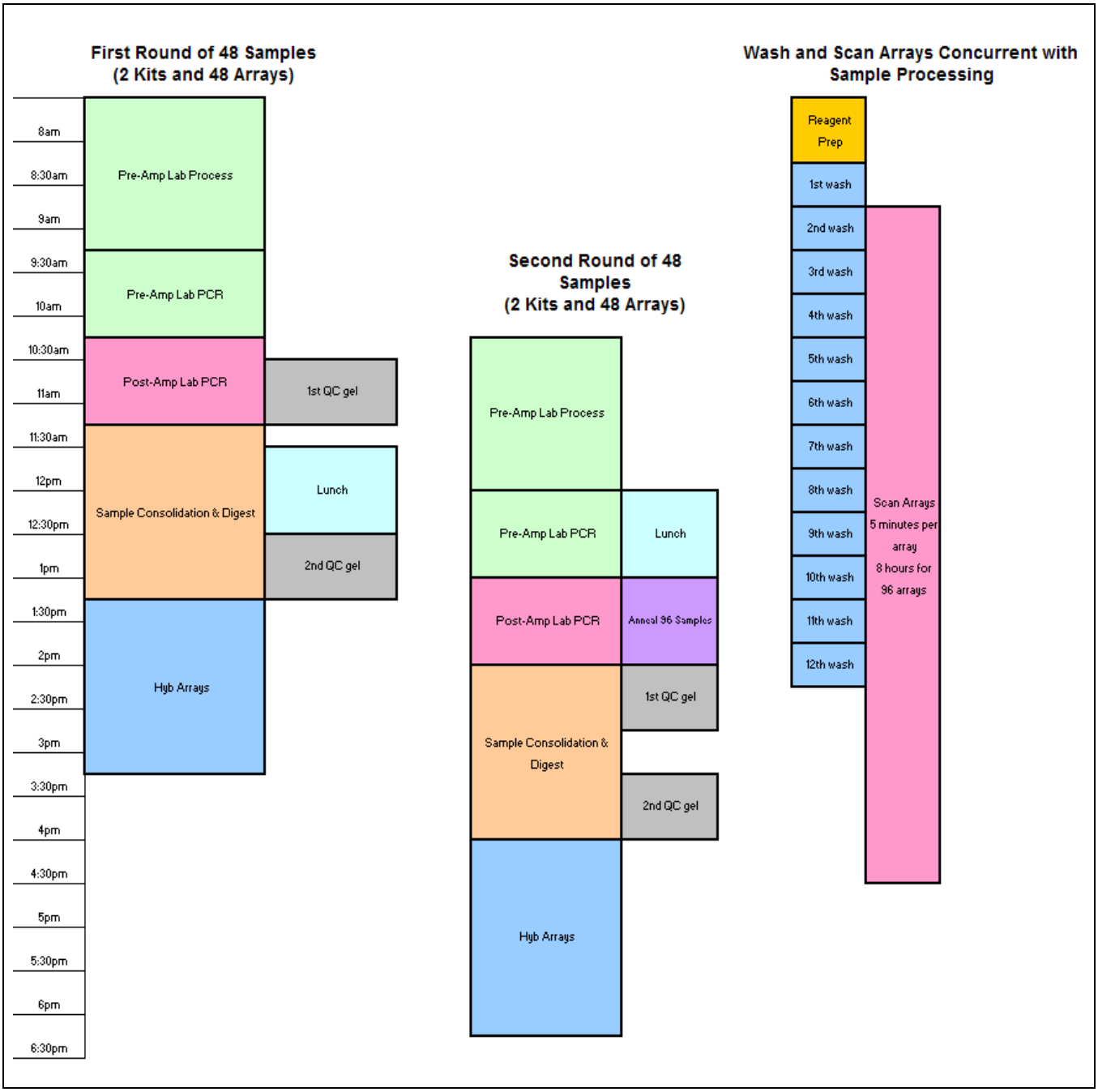


Figure C.1
Suggested Workflow for Processing 96 Samples Per Day

Appendix D

Changing Plate Barcode Restrictions

How to Change Plate Barcode Restrictions

This appendix describes how to change the default barcode restrictions required by Affymetrix GeneChip® Targeted Genotyping Analysis Software (GTGS). The default barcode designations correspond to the names of the various plates used for sample processing:

- Ann = Anneal Plate
- ASY = Assay Plate
- LBL = Label Plate
- Hyb = Hyb Plate

These designations help to ensure that the samples loaded onto each plate are properly tracked throughout the MIP Assay Protocol.

NOTE **The default barcode designations for plates help to ensure that the samples loaded onto each plate are properly tracked throughout the MIP Assay Protocol. Therefore we recommend that you do not change the default barcode designations.**

To change the default barcode properties, modify the AssayTracking.properties file by changing the designations highlighted below. The location of this file is:

C:\Program Files\Apache Group\Tomcat 4.1\webapps\geno\config\ Assay Tracking.properties

IMPORTANT **Modify only the text that is highlighted. Do not change the .***

```
# Defines plate tracking properties while performing assay

# Required barcode prefixes:

# Anneal plate:
ann-plate.pattern = ANN.*
ann-plate.errmsg = Barcode must start with ANN

# Assay plate:
asy-plate.pattern = ASY.*
asy-plate.errmsg = Barcode must start with ASY

# Label plate:
lbl-plate.pattern = LBL.*
lbl-plate.errmsg = Barcode must start with LBL

# Hybridization plate:
hyb-plate.pattern = HYB.*
hyb-plate.errmsg = Barcode must start with HYB

# Protocol:
protocol.pattern = PCL.*
protocol.errmsg = Barcode must start with PCL
```

```
# Valid Thermal Cyclers (leave blank for no checking)
# use comma delimited integers for specific thermal cyclers (e.g. 1, 2, 3)
ann-plate.thermal-cyclers =
asy-plate.thermal-cyclers =
lbl-plate.thermal-cyclers =
hyb-plate.thermal-cyclers =
```

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