applied biosystems

GeneChip™ Expression Analysis Technical Manual

For HT Array Plates Using the GeneChip™ Array Station

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Chapter 1 OVERVIEW

Introduction

Welcome to the GeneChip™ Array Station. This system uses robotic technology to automate many of the labor intensive tasks required when preparing a eukaryotic mRNA sample for gene expression analysis. The Array Station also automates the hybridization of a target to a GeneChip™ HT Array Plate, as well as the washing and staining of the HT Array Plate prior to scanning.

This manual describes the assay procedures recommended for eukaryotic target labeling for expression analysis and subsequent HT Array Plate hybridization and processing using the GeneChip™ Array Station. By following the protocols and using high-quality starting materials, a sufficient amount of biotin-labeled complementary RNA (cRNA) target can be obtained for hybridization to a HT Array Plate. The reagents and protocols have been developed and optimized specifically for use with the GeneChip™ Array Station.

The GeneChip™ One-Cycle Target Labeling Assay experimental outline is represented in Figure 1.1. Total RNA (1 µg to 2 µg) is first reverse transcribed using a T7-Oligo(dT) Promoter Primer in the first-strand cDNA synthesis reaction. Following RNase H-mediated second-strand cDNA synthesis, the double-stranded cDNA is purified and serves as a template in the subsequent *in vitro* transcription (IVT) reaction. The IVT reaction is carried out in the presence of T7 RNA Polymerase and a biotinylated nucleotide analog/ribonucleotide mix for cRNA amplification and biotin labeling. The biotinylated cRNA targets are then cleaned up, fragmented, and hybridized to GeneChip™ HT Array Plates.

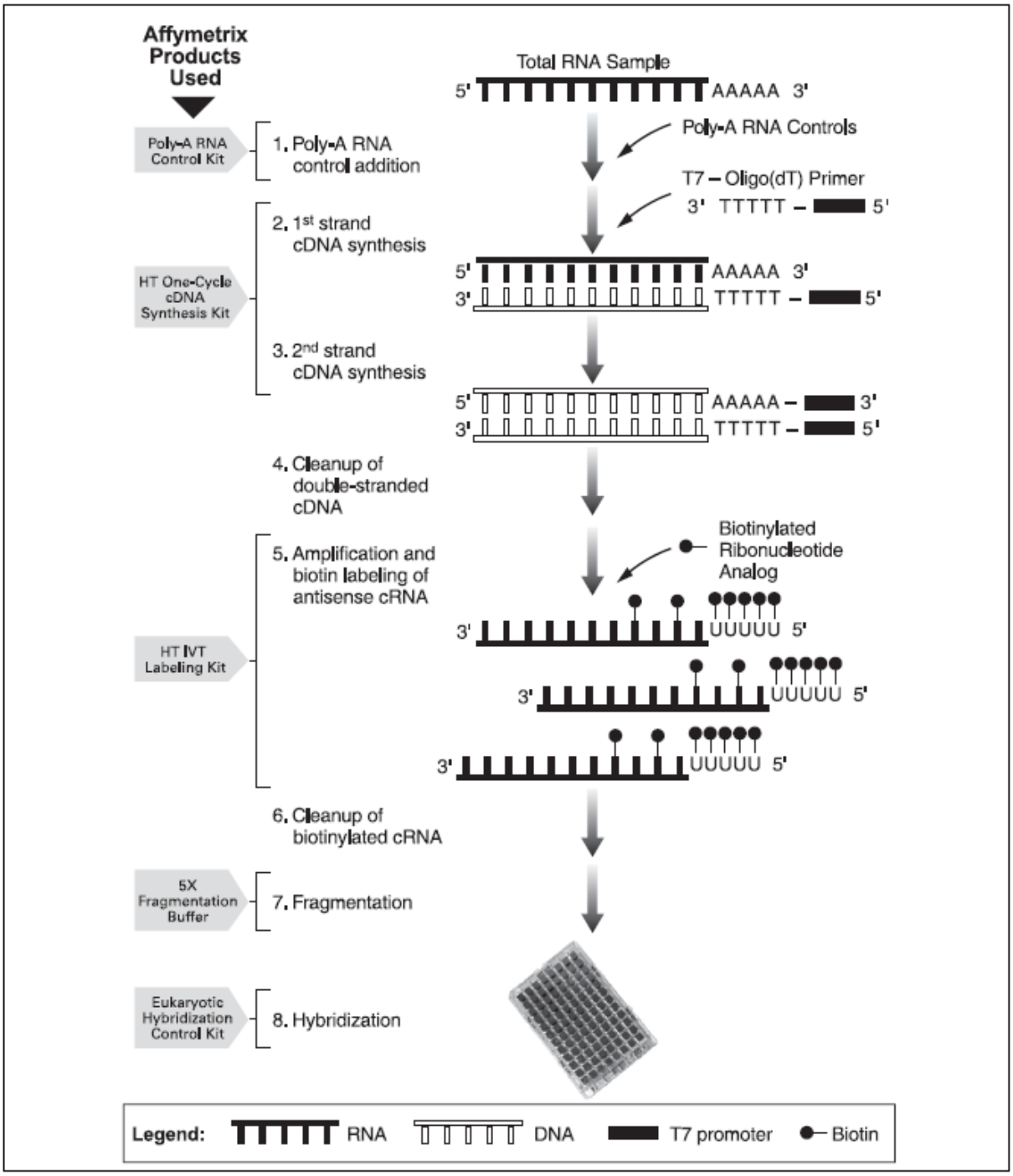


Figure 1.1 GeneChip™ Eukaryotic Labeling Assays for Expression Analysis Using GeneChip™ Array Station

Summary of the Array Station Run

Target preparation on the Array Station takes approximately 18.5 hours and requires three human interventions: initial sample preparation, change of deck layout, and cRNA quantitation. At the end of target preparation, the sample is ready to be denatured and hybridized onto a HT Array Plate. Hybridization takes 16 hours. Washing and staining on the Array Station takes 1.5 hours. Additional time is required for scanning and is dependent on the number of samples processed and the array type. The typical workflow and chapter references are provided below.

1. Isolate high quality total RNA (refer to Chapter 2).
2. Prepare reagents required for target preparation (refer to Chapter 3).
3. Perform Array Station system check (refer to Chapter 4).
4. Set-up first deck layout required for cDNA synthesis reaction through purification of cRNA (refer to Chapter 4).
5. Set-up second deck layout required for the quantitation and normalization steps and preparation of the hybridization-ready sample (refer to Chapter 4).
6. Denature sample and hybridize overnight onto GeneChip™ HT Array Plates
   - If using the GeneChip™ HT Hybridization, Wash and Stain Kit refer to Chapter 5.
   - If using self-prepared reagents refer to Chapter 6.
7. Wash and stain HT Array Plates using the Array Station
   - If using the GeneChip™ HT Hybridization, Wash and Stain Kit refer to Chapter 5.
   - If using self-prepared reagents refer to Chapter 6.
8. Scan HT Array Plates using the HT Scanner (refer to *GeneChip™ HT Scanner User's Guide*, P/N 701978).

Array Station Subsystems

The array station is composed of both mechanical and software subsystems. Refer to the *GeneChip™ Array Station User's Guide* (P/N 701859) for more information.

Typical Core System

Figure 1.2 illustrates the core mechanical subsystems of the Array Station including the Caliper Sciclone workstation, Bio-Rad DNA Engine™ Thermal Cycler, and Twister™ II microplate handler. For detailed information on the various mechanical components of the system, refer to the *GeneChip™ Array Station User's Guide* (P/N 701859).

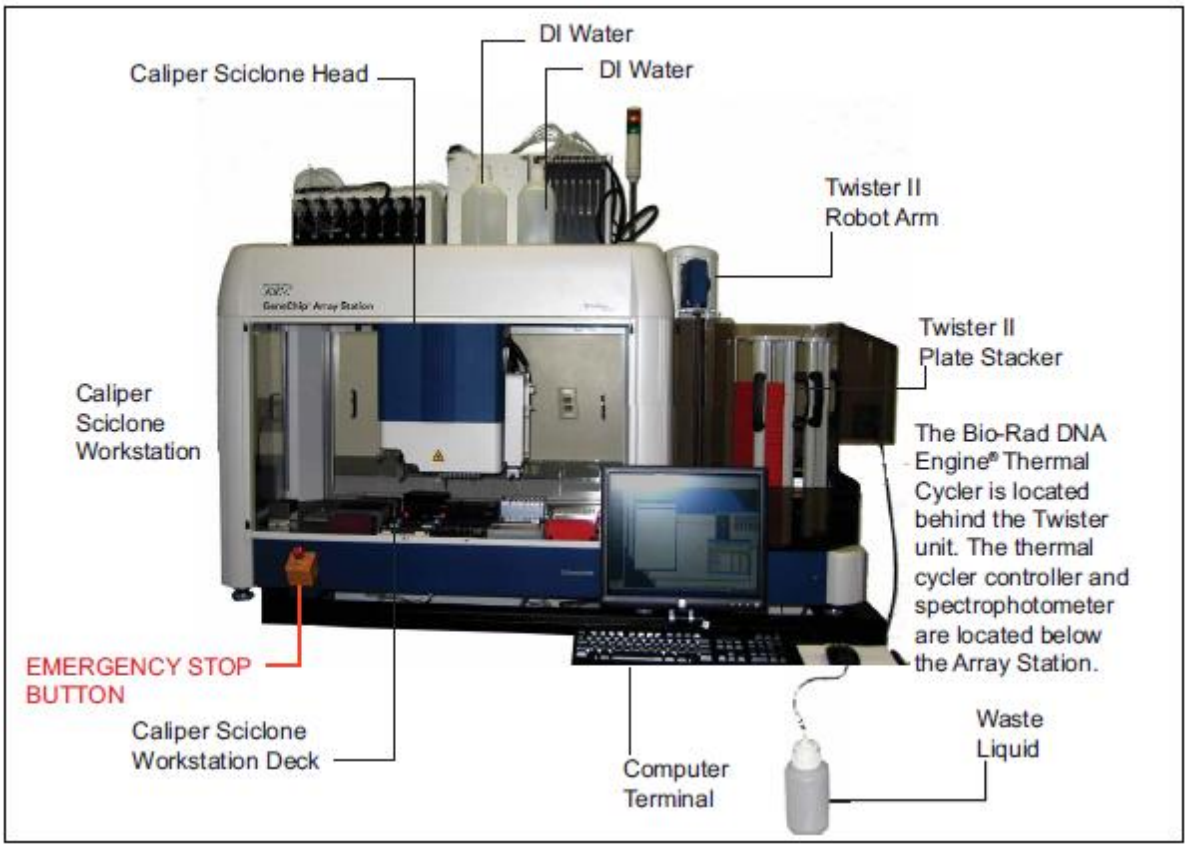


Figure 1.2 The GeneChip™ Array Station

The Sciclone 4.0 control software provides a graphical user interface (GUI) to run and track the various mechanical components that comprise the application. The Sciclone 4.0 software has integrated all instrument control programs (ICP's) into one GUI, thus enhancing software ease of use. See Figure 1.3 for a schematic of the software product configuration for the Array Station.

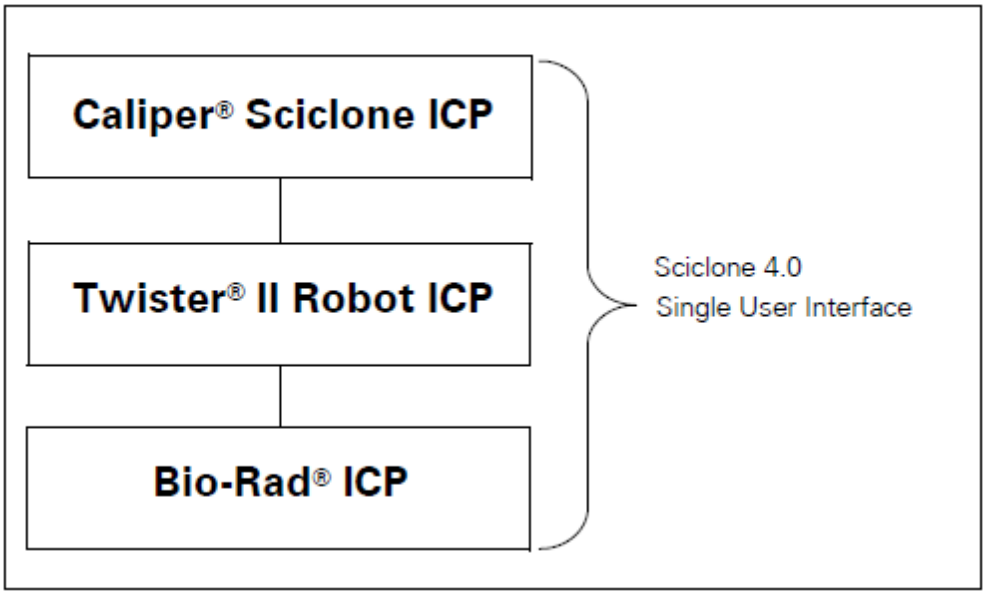


Figure 1.3 Sciclone 4.0 Software

User Documentation and Quick Reference Cards

The operation of the Array Station requires familiarity with the following user documentation.

The manuals that are relevant will depend on your system configuration. For the Array Station configuration, the following list presents the relevant guides:

1. *GeneChip™ Array Station Site Preparation Guide* (P/N 702020)
2. *GeneChip™ Array Station User's Guide* (P/N 701859)
3. *GeneChip™ Array Station Deck Layout Quick Reference Card* (P/N 702013)
4. *GeneChip™ Array Station Reagent Preparation Quick Reference Card* (P/N 702032)
5. *GeneChip™ Operating Software User's Guide* (P/N 701439): if you are using GeneChip™ analysis software, you should be familiar with GeneChip™ Operating System in order to interpret the assay results.
6. *Caliper Sciclone User's Manual* (from Caliper Life Sciences).
7. *Bio-Rad MJ Thermal Cycler ICP Guide* (from Bio-Rad™/MJ Research).

Regulatory Compliance

Please refer to the *GeneChip™Array Station User's Guide* (P/N 701859) for regulatory compliance and safety information for the Array Station.

Chapter 2 RNA PREPARATION

Total RNA Isolation for One-Cycle Eukaryotic Target Labeling Assay

This chapter describes the general requirements for RNA isolation methods and poly-A control preparation for spiking into your RNA sample.

IMPORTANT: The quality of the RNA is essential to the overall success of the analysis. Since the most appropriate protocol for the isolation of RNA can be source dependent, we recommend using a protocol that has been established for the tissues or cells being used. In the absence of an established protocol, using one of the commercially available kits designed for RNA isolation is suggested.

When using a commercial kit, follow the manufacturer's instructions for RNA isolation.

Materials List

Total RNA Isolation Reagents

- TRIzol™ Reagent: Invitrogen Life Technologies, P/N 15596-018, or QIAzol™ Lysis Reagent: QIAGEN, P/N 79306
- RNeasy™Mini Kit: QIAGEN, P/N 74104

Miscellaneous Reagents

- 80% ethanol (stored at –20°C)
- Pellet Paint™: Novagen, P/N 69049-3 (optional)
- Glycogen: Ambion, P/N 9510 (optional)
- 3M Sodium Acetate (NaOAc): Sigma-Aldrich, P/N S7899

Isolation of RNA from Yeast

Total RNA

Quality total RNA has been isolated successfully from yeast cells using a hot phenol protocol described by Schmitt, *et al. Nucl Acids Res* **18**:3091-3092 (1990).

Isolation of RNA from Arabidopsis

Total RNA

TRIzol™ Reagent from Invitrogen Life Technologies has been used to isolate total RNA from Arabidopsis. Follow the instructions provided by the supplier and, when necessary, use the steps outlined specifically for samples with high starch and/or high lipid content. QIAzol™ Lysis Reagent from QIAGEN™ can also be used.

Isolation of RNA from Mammalian Cells or Tissues

Total RNA

High-quality total RNA has been successfully isolated from mammalian cells (such as cultured cells and lymphocytes) using the RNeasy™ Mini Kit from QIAGEN™.

If mammalian tissue is used as the source of RNA, it is recommended to isolate total RNA with a commercial reagent, such as TRIzol™ or QIAzol™ reagent.

IMPORTANT: If going directly from TRIzol- or QIAzol™-isolated total RNA to cDNA synthesis, it may be beneficial to perform a second cleanup on the total RNA before starting. After the ethanol precipitation step in the Lysis Reagent extraction procedure, perform a cleanup using the QIAGEN RNeasy Mini Kit. Much better yields of labeled cRNA are obtained from the in vitro transcription-labeling reaction when this second cleanup is performed.

Precipitation of RNA

Total RNA

It is not necessary to precipitate total RNA following isolation or cleanup with the RNeasy Mini Kit. Adjust elution volumes from the RNeasy column to prepare for cDNA synthesis based upon expected RNA yields from your experiment. Ethanol precipitation is required following TRIzol or QIAzol reagent isolation and hot phenol extraction methods; see methods in Chapter 2 for details.

Precipitation Procedure

1. Add 1/10 volume 3M NaOAc, pH 5.2, and 2.5 volumes ethanol.*
2. Mix and incubate at –20°C for at least 1 hour.
3. Centrifuge at ≥ 12,000 x g in a microcentrifuge for 20 minutes at 4°C.
4. Wash pellet twice with 80% ethanol.
5. Air dry pellet. Check for dryness before proceeding.
6. Resuspend pellet in DEPC-treated H2O. The appropriate volume for resuspension depends on the expected yield and the amount of RNA required for the cDNA synthesis. Please read ahead to the cDNA synthesis protocol in order to determine the appropriate resuspension volume at this step.

*Addition of Carrier to Ethanol Precipitations

Adding carrier material has been shown to improve the RNA yield of precipitation reactions.

- Pellet Paint™

Addition of 0.5 µL of Pellet Paint per tube to nucleic acid precipitations makes the nucleic acid pellet easier to visualize and helps reduce the chance of losing the pellet during washing steps. The pellet paint does not appear to affect the outcome of subsequent steps in this protocol; however, it can contribute to the absorbance at 260 nm when quantifying the total RNA.

- Glycogen

Addition of 0.5 to 1 µL of glycogen (5 mg/mL) to nucleic acid precipitations aids in visualization of the pellet and may increase recovery. The glycogen does not appear to affect the outcome of subsequent steps in this protocol.

Quantitation of RNA

Quantify RNA yield by spectrophotometric analysis using the convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA.

- The absorbance should be checked at 260 and 280 nm for determination of sample concentration and purity.
- The A260/A280 ratio should be close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).
- Integrity of total RNA samples can also be assessed qualitatively on an Agilent 2100 Bioanalyzer. Refer to Figure 2.1 for an example of good-quality total RNA sample.

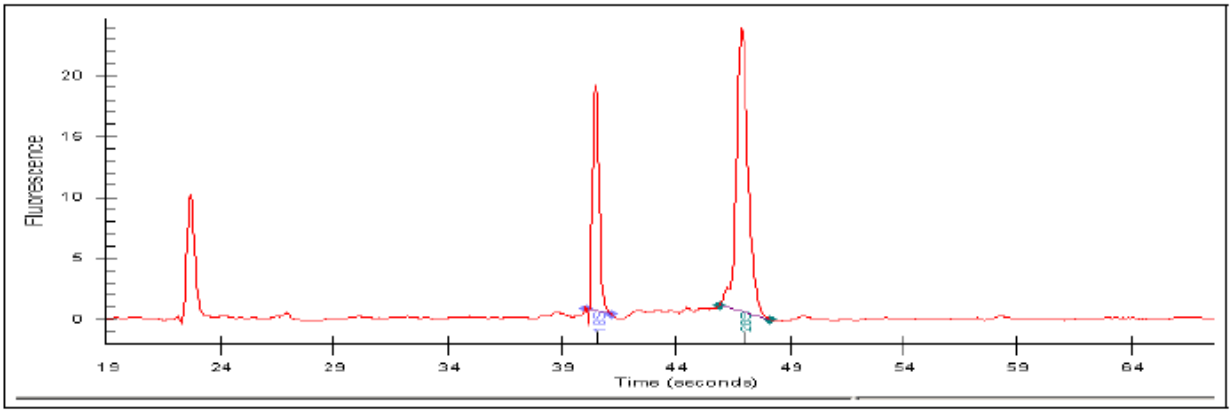


Figure 2.1 Electropherogram (from the Agilent 2100 Bioanalyzer) for HeLa Total RNA. For a high-quality total RNA sample, two well-defined peaks corresponding to the 18S and 28S ribosomal RNAs should be observed, similar to a denaturing agarose gel, with ratios approaching 2:1 for the 28S to 18S bands.

Preparation of Poly-A RNA Controls for One-Cycle cDNA Synthesis (Spike-in Controls)

Reagents and Equipment

- GeneChip™ Eukaryotic Poly-A RNA Control Kit: P/N 900433

Designed specifically to provide exogenous positive controls to monitor the entire eukaryotic target labeling process, a set of poly-A RNA controls is supplied in the GeneChip™ Eukaryotic Poly-A RNA Control Kit.

Each eukaryotic GeneChip™ probe array contains probe sets for several *B. subtilis* genes that are absent in eukaryotic samples (*lys*, *phe*, *thr*, and *dap*). These poly-A RNA controls are *in vitro* synthesized, and the polyadenylated transcripts for the *B. subtilis* genes are pre-mixed at staggered concentrations. The concentrated Poly-A Control Stock can be diluted with the Poly-A Control Dil Buffer and spiked directly into RNA samples to achieve the final dilutions (relative to estimated copy number of total mRNA population) summarized in Table 2.1.

Table 2.1 Final Dilutions of Poly-A RNA Controls in Samples

Poly-A RNA Spike	Final Dilution (estimated ratio of copy number)
lys	1:100,000
phe	1:50,000
thr	1:25,000
dap	1:6,667

The controls are then amplified and labeled together with the samples. Examining the hybridization intensities of these controls on GeneChip™ arrays helps to monitor the labeling process independently from the quality of the starting RNA samples. Anticipated relative signal strength follows the order of *lys* < *phe* < *thr* < *dap*.

The Poly-A RNA Control Stock and Poly-A Control Dil Buffer are provided with the kit to prepare the appropriate serial dilutions based on Table 2.2. This is a guideline when 1 or 2 µg of total RNA is used as starting material.

IMPORTANT: Use non-stick RNase-free microfuge tubes to prepare all of the dilutions.

Table 2.2 Serial Dilutions of Poly-A RNA Control Stock

Starting Amount		Serial Dilutions			Spike-in Volume
Total RNA mRNA Total RNA	mRNA	First	Second	Third	
1 µg		1:20	1:50	1:50	2 µL
2 µg		1:20	1:50	1:25	2 µL

TIP: Avoid pipetting solutions less than 2 µL in volume to maintain precision and consistency when preparing the dilutions.

For example, to prepare the Poly-A RNA dilutions for 1 µg of total RNA:

1. Add 2 µL of the Poly-A Control Stock to 38 µL of Poly-A Control Dil Buffer for the First Dilution (1:20).
2. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
3. Add 2 µL of the First Dilution to 98 µL of Poly-A Control Dil Buffer to prepare the Second Dilution (1:50).
4. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
5. Add 2 µL of the Second Dilution to 98 µL of Poly-A Control Dil Buffer to prepare the Third Dilution (1:50).
6. Mix thoroughly and spin down to collect the liquid at the bottom of the tube.
7. Add 2 µL of this Third Dilution to 1 µg of sample to total RNA directly. The final volume of the total RNA with the diluted poly-A controls should not exceed 5 µL.

NOTE: The first dilution of the Poly-A RNA controls can be stored up to six weeks in a frost-free freezer at –20°C and freeze-thawed up to eight times.

Preparation of Total RNA Plates for Processing on the Array Station

The Array Station Target Preparation protocol starts with 5 µL of material in a Bio-Rad 96-Well Hard-Shell PCR Plate. This plate can either be prepared offline (manually) or on the Array Station, as described below.

User-prepared Plate Preparation

Preparing Samples with PolyA Controls

Pipet 3 µL of the total RNA sample (1 to 2 µg) and 2 µL of the appropriate PolyA spike control solution into the 96-well plate. Samples should be placed into the plate in a column-wise fashion starting from the left side of the plate. For example, if preparing 24 samples, pipet the samples into the sample wells for columns 1, 2, and 3.

Optional – Running without PolyA Controls

We highly recommend that you utilize PolyA controls as described above. However, if you choose not to utilize these controls, the total RNA sample in the Bio-Rad 96-Well Hard-Shell PCR Plate must be adjusted to a final volume of 5 µL.

Once the Bio-Rad 96-Well Hard-Shell PCR Plate has been prepared with 5 µL of the material as described above, select the **Manual Sample Transfer** option when starting on the Array Station. Please refer to Chapter 4 for detailed information.

Automated Plate Preparation

If there is at least 20 µL of purified total RNA (0.2 to 0.4 µg/µL) in a Greiner-U-Bottom plate, the Array Station may be used to transfer 5 µL of this material to the Bio-Rad 96-Well Hard-Shell PCR Plate. Please refer to the instructions in Appendix B for an explanation of how to have the Array Station transfer 5 µL of sample to the starting plate at the beginning of a run.

Chapter 3 Reagent Preparation

Introduction

This chapter describes the reagent preparation for the One-Cycle Eukaryotic Target Labeling Assay. This involves manually preparing and loading strip tubes with reagent master mixes as well as filling the water and ethanol reservoirs. The initial reagent setup must be performed prior to starting the Target Preparation Protocol. Figure 3.1 illustrates the order of reagents loaded into the cold reagent block.

IMPORTANT: Before loading the strip tubes, please remove all bubbles from the bottom of the tubes by gently pipetting the solutions up and down.

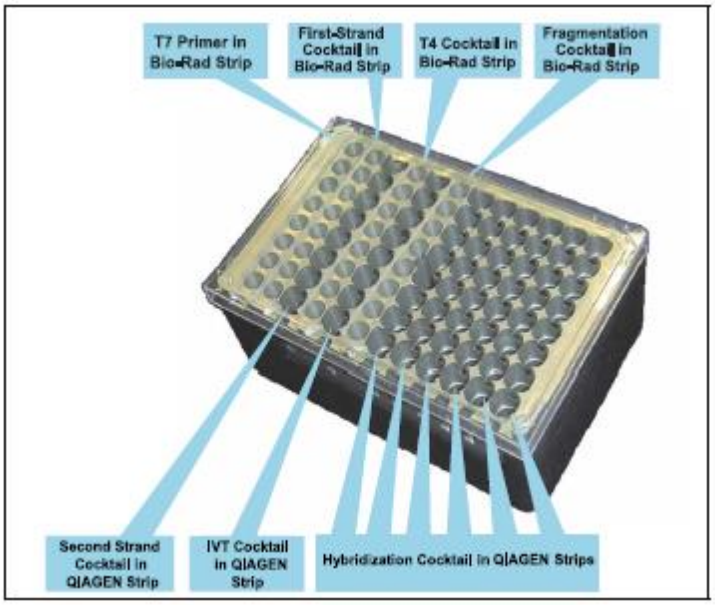


Figure 3.1 Cold Reagent Block Description

In the following sections, the components for 24-, 48-, and 96-sample master mixes are provided. Refer to Appendix A for detailed information on other reaction configurations.

Reagents and Materials Required

The following reagents and materials have been tested and evaluated. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

IMPORTANT: Do not store enzymes in a frost-free freezer.

Reagents and Equipment

- GeneChip™ HT One-Cycle cDNA Synthesis Kit, P/N 900687[1]
- GeneChip™ HT IVT Labeling Kit: P/N 900688
- GeneChip™ Eukaryotic Hybridization Control Kit, P/N 900454
- (30 reactions) or P/N 900457 (150 reactions), contains Control cRNA and Control Oligo B2
- Control Oligo B2 (3 nM), P/N 900301 (can be ordered separately)
- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen Life Technologies, P/N 15561-020
- EDTA Disodium Salt, 0.5M solution (100 mL): Sigma-Aldrich, P/N E7889
- Herring Sperm DNA: Promega Corporation, P/N D1811
- MES Hydrate SigmaUltra: Sigma-Aldrich, P/N M5287
- MES Sodium Salt: Sigma-Aldrich, P/N M5057
- 5M NaCl, RNase-free, DNase-free: Ambion, P/N 9760G
- Surfact-Amps 20 (Tween-20), 10%: Pierce Chemical, P/N 28320
- TMAC (5M), Sigma-Aldrich: P/N T3411
- Ethanol, 100%: Various Suppliers
- RNAClean™, Agencourt: P/N 000494
- Nuclease-free Water: Ambion, P/N 9932
- BD Falcon™ Test Tube, 5 mL: VWR International, P/N 60819-728
- BD Falcon™ Test Tube, 14 mL: VWR International, P/N 60819-761
- Elution Strip Tubes, 0.85 mL: QIAGEN, P/N 19588
- Low-Profile 0.2 mL PCR 8-Tube Strips: Bio-Rad, P/N TLS-0801
- Polypropylene Centrifuge Tubes with Caps, 50 mL: VWR International, P/N 20171-028
- RNase-Free 1.5 mL microfuge tube: Ambion, P/N 12400

[1] Users who do not purchase this Kit may be required to obtain a license under U.S. Patent Nos. 5,716,785, 5,891,636, 6,291,170, and 5,545,522 or to purchase another licensed kit.

Before You Begin

NOTE: Determine the number of reactions to run for target preparation and then prepare the reagents according to the number of reactions indicated in the tables provided in the following steps.

IMPORTANT: The tables in this guide are suited for RNA samples loaded in the total RNA plate columnwise.

IMPORTANT: The volume per strip tube for each reagent is adjusted to sufficiently accommodate the requirements of the GeneChip™ Array Station.

IMPORTANT: Assemble the cold reagent block to the Peltier on the GeneChip™ Array Station deck before preparing the reagent master mixes (refer to Figure 4.22 and Figure 4.23). Ensure that the cold reagent block is chilled to 4°C prior to loading the reagent strip tubes containing the reagent master mixes.

IMPORTANT: Reagent master mixes should not be vortexed. Gently pipet the solution to ensure reagents are uniformly mixed. The amount added to the wells of the strip tubes is slightly less than the total volume of prepared reagent. This is needed to compensate for technical differences that may result from pipette and operator variation, and the properties of the reagents (i.e., viscosity)..

Procedure 1: T7 Primer Master Mix

NOTE: Refer to Table 3.1 for the T7 Primer Master Mix composition.

1. Obtain a RNase-free 1.5 mL microfuge tube and label as "T7 Primer."
2. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "T7."
3. Add the components listed in Table 3.1 to the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 1 of the cold reagent block.

Table 3.1 T7 Primer Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
T7-Oligo(dT) Primer, 50 µM	1.0 µL	32.0 µL	57.0 µL	114.0 µL
Nuclease-free Water	4.0 µL	127.9 µL	228.0 µL	456.0 µL
Total Volume	5.0 µL	159.9 µL	285.0 µL	570.0 µL
Volume per Strip Tube Well		18.5 µL	34.0 µL	69.3 µL

Procedure 2: First-Strand cDNA Synthesis Master Mix

NOTE: Refer to Table 3.2 for the First-Strand cDNA Synthesis Cocktail composition.

1. Obtain a RNase-free 1.5 mL microfuge tube and label as "1st Strand."
2. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "1st."
3. Add the components listed in Table 3.2 to the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 2 of the cold reagent block.

Table 3.2 First-Strand cDNA Synthesis Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X 1st Strand Reaction Mix	4.0 µL	114.0 µL	228.0 µL	456.0 µL
DTT, 0.1 M	2.0 µL	57.0 µL	114.0 µL	228.0 µL
dNTP Mix, 10 mM	1.0 µL	28.5 µL	57.0 µL	114.0 µL
SuperScript™ II	1.0 µL	28.5 µL	57.0 µL	114.0 µL
Nuclease-free Water	2.0 µL	57.0 µL	114.0 µL	228.0 µL
Total Volume	10.0 µL	285.0 µL	570.0 µL	1,140.0 µL
Volume per Strip Tube Well		33.5 µL	69.3 µL	140.5 µL

Procedure 3: Second-Strand cDNA Synthesis Master Mix

NOTE: Refer to Table 3.3 for the Second-Strand cDNA Synthesis composition.

1. Obtain a 5 mL BD Falcon Test Tube with a clip-on cap for large volumes or a 1.5 mL microfuge tube for smaller volumes (for 32 reactions or less) and label as "2nd Strand."
2. Obtain an 0.85 mL QIAGEN Elution Strip Tube and label as "2nd."
3. Add the components listed in Table 3.3 to the BD Falcon Test Tube or the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the QIAGEN Elution Strip Tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 3 of the cold reagent block.

Table 3.3 Second-Strand cDNA Synthesis

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X 2nd Strand Reaction Mix	30.0 µL	810.0 µL	1,620.0 µL	3,240.0 µL
dNTP Mix, 10 mM	3.0 µL	81.0 µL	162.0 µL	324.0 µL
E. coli DNA Ligase, 10 unit/µL	1.0 µL	27.0 µL	54.0 µL	108.0 µL
E. coli DNA Polymerase I, 10 unit/µL	4.0 µL	108.0 µL	216.0 µL	432.0 µL
RNase H, 2 unit/µL	1.0 µL	27.0 µL	54.0 µL	108.0 µL
Total Volume	39.0 µL	1,053.0 µL	2,106.0 µL	4,212.0 µL
Volume per Strip Tube Well		129.0 µL	260.0 µL	522.5 µL

Procedure 4: T4 DNA Polymerase Master Mix

NOTE: Refer to Table 3.4 for the T4 DNA Polymerase Cocktail composition.

IMPORTANT: The T4 DNA polymerase buffer supplied in the GeneChip™ HT One-Cycle cDNA Synthesis Kit is 5X. First dilute 5X T4 DNA Polymerase Buffer to 1X buffer.

1. Dilute the 5X T4 DNA Polymerase Buffer to a 1X concentration with RNase-free water.
2. Obtain an RNase-free 1.5 mL microfuge tube and label as "T4 DNA Pol."
3. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "T4."
4. Add the components listed in Table 3.4 to the microfuge tube and mix well.
5. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the PCR strip tube.
6. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
7. Load the strip tube to column 4 of the cold reagent block.

Table 3.4 T4 DNA Polymerase Cocktail for Cold Reagent Block

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
T4 DNA Polymerase	2.0 µL	69.3 µL	118.0 µL	236.0 µL
1X T4 DNA Polymerase Buffer	2.0 µL	69.3 µL	118.0 µL	236.0 µL
Total Volume	4.0 µL	138.6 µL	236.0 µL	472.0 µL
Volume per Strip Tube Well		16.0 µL	27.8 µL	57.0 µL

Procedure 5: IVT Master Mix

NOTE: Refer to Table 3.5 for the IVT Cocktail Master Mix composition.

1. Obtain a 5 mL BD Falcon Test Tube with a clip-on cap for large volumes or a 1.5 mL microfuge tube for smaller volumes (for 32 reactions or less) and label as "IVT."
2. Obtain a 0.85 mL QIAGEN Elution Strip Tube and label as "IVT."
3. Add the components listed in Table 3.5 to the BD Falcon Test Tube or the microfuge tube and mix well.
4. Aliquot the appropriate volumes of the master mix as indicated in "Volume per Strip Tube Well" into each well of the QIAGEN Elution Strip Tube.
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tube to column 5 of the cold reagent block.

Table 3.5 IVT Cocktail Master Mix for Cold Reagent Block

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
10X IVT Buffer	6.0 µL	168.0 µL	336.0 µL	672.0 µL
IVT Labeling NTP Mix	18.0 µL	504.0 µL	1,008.0 µL	2,016.0 µL
IVT Labeling Enzyme Mix	6.0 µL	168.0 µL	336.0 µL	672.0 µL
T7 RNA Polymerase	1.0 µL	28.0 µL	56.0 µL	112.0 µL
Nuclease-free Water	7.0 µL	196.0 µL	392.0 µL	784.0 µL
Total Volume	38.0 µL	1,064.0 µL	2,128.0 µL	4,256.0 µL
Volume per Strip Tube Well		131.0 µL	264.0 µL	529.0 µL

Procedure 6: Fragmentation Buffer

NOTE: Refer to Table 3.6 for the Fragmentation Cocktail volumes required.

1. Obtain a Bio-Rad Low-Profile PCR Tube Strip and label as "Frag."
2. Aliquot the volume listed in Table 3.6 into each well of the strip tube.
3. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
4. Load the strip tube to column 6 of the cold reagent block.

Table 3.6 Fragmentation Cocktail for Cold Reagent Block

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
5X Fragmentation Buffer (Volume per Strip Tube Well)	7.5 µL	26.7 µL	53.4 µL	106.9 µL

Procedure 7: Hybridization Master Mix

The Array Station allows the choice of two different volumes of Hybridization Cocktails to be prepared for the HT Array Plates, 100 µL or 200 µL. The 100 µL Hybridization Cocktail is prepared when the samples are to be analyzed on one HT Array Plate. The 200 µL Hybridization Cocktail is prepared when samples are to be analyzed on two HT Array Plates.

NOTE: The Hybridization Cocktail for the HT Array Plates is a TMAC-based buffer. It differs from the Hybridization Cocktail prepared for cartridge expression arrays.

Please note that different tables are provided for those using the GeneChip™ HT Hybridization Wash and Stain Kit and for those using self-prepared reagents. Please refer to the appropriate tables for your use.

Reagent Preparation

12X MES Stock Buffer

(1.22M MES, 0.89M [Na+])

For 1,000 mL:

64.61 g of MES Hydrate

193.3 g of MES Sodium Salt 800 mL of Nuclease-free Water

Mix and adjust volume to 1,000 mL.

The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter.

IMPORTANT: Do not autoclave. Store at 2°C to 8°C, and shield from light. Discard solution if yellow.

1.23X Hybridization Buffer

(Final 1.0X concentration is 100 mM MES, 2.5M TMAC, 20 mM EDTA, 0.01% Tween-20)

For 50 mL:

mL of 12X MES Stock Buffer

30.73 mL of 5M TMAC

mL of 0.5M EDTA

0.06 mL of 10% Tween-20

11.71 mL of Nuclease-free Water Filter through a 0.2 µm filter

Store at 2°C to 8°C, and shield from light

IMPORTANT: Do not autoclave. Store at 2°C to 8°C, and shield from light. Discard solution if yellow.

NOTE: The recipes in Table 3.7 through Table 3.10 take into account that it is necessary to make extra hybridization cocktail due to a small loss of volume (10 to 20 µL) during each hybridization.

NOTE: Refer to Table 3.7 (or Table 3.9) for 100 µL Hybridization Cocktail Master Mix composition. The 100 µL volume is for Hybridization Cocktails that will be hybridized to one HT

Array Plate.

Refer to Table 3.8 (or Table 3.10) for 200 µL Hybridization Cocktail Master Mix composition. The 200 µL volume is for Hybridization Cocktails that will be hybridized to two HT Array Plates.

Procedure

1. Obtain a 14 mL BD Falcon Test Tube, or a 50 mL centrifuge tube for larger volumes, and label as "Hyb Mix."
2. Obtain one to six 0.85 mL QIAGEN Elution Strip Tubes and label as "Hyb."
3. For hybridizing to one HT Array Plate, follow Step 3A or Step 3B below. For hybridizing to two HT Array Plates, follow Step 3C or 3D, below.
   a. If using the GeneChip™ HT Hybridization Wash and Stain Kit, refer to Table 3.7 for 100 µL Hybridization Cocktail Master Mix composition. Combine the components as indicated in a 14 mL BD Falcon test tube. Go to Step 4 below.
   b. If using self-prepared reagents, refer to Table 3.9 for 100 µL Hybridization Cocktail Master Mix composition. Combine the components as indicated in a 14 mL BD Falcon test tube. Go to Step 4 below.
   c. If using the GeneChip™ HT Hybridization Wash and Stain Kit, refer to Table 3.8 for 200 µL Hybridization Cocktail Master Mix composition, combine the components as indicated in a 14 mL BD Falcon test tube, or a 50 mL centrifuge tube, depending on the total volume. Go to Step 4 below.
   d. If using self-prepared reagents, refer to Table 3.10 for 200 µL Hybridization Cocktail Master Mix composition, combine the components as indicated in a 14 mL BD Falcon test tube, or a 50 mL centrifuge tube, depending on the total volume.
4. Aliquot the appropriate volumes of the master mix as indicated into each well of the QIAGEN Elution Strip Tube(s).
   a. If using the HT Hyb, Wash and Stain kit, please refer to Table 3.7 or Table 3.8.
   b. If using self-prepared reagents, please refer to Table 3.9 or Table 3.10
5. Avoid air bubbles at the bottom of the strip tubes. If necessary, remove air bubbles by briefly pipetting the reaction mix out of the strip well and slowly pipetting it back.
6. Load the strip tubes to columns 7-12 of the cold reagent block. The number of strip tubes will vary depending on the number of reactions.

Hybridization Cocktail Master Mix for Cold Reagent Block using the GeneChip™ HT Hyb, Wash and Stain Kit

IMPORTANT: It is imperative that frozen stocks of 20X GeneChip™ Eukaryotic Hybridization Controls are heated to 65 °C for 5 minutes to completely resuspend the cRNA before aliquotting.

Table 3.7 100 µL Hyb Cocktail Master Mix (using the GeneChip™ HT Hyb, Wash and Stain Kit) - 1 HT Array Plate

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
20X Hybridization Control Stock	5.0 µL	139.1 µL	272.5 µL	545.0 µL
3 nM B2 Oligo	1.65 µL	45.9 µL	89.9 µL	179.9 µL
HT 1.2X Hybridization Mix*	83.35 µL	2,318.6 µL	4,542.6 µL	9,085.2 µL
Total Volume	90 µL	2,503.6 µL	4,905.0 µL	9,810.1 µL
# of QIAGEN Strips Used		1	2	3
Volume per Well in 1st Strip		306.0 µL	408.0 µL	408.0 µL
Volume per Well in 2nd Strip		204.0 µL		408.0 µL
Volume per Well in 3rd Strip		408.0 µL		

* The 1.2X Hybridization Mix is a component of the HT Hybridization, Wash, and Stain Kit containing BSA, HS DNA and 1.23X hybridization buffer.

Table 3.8 200 µL Hyb Cocktail Master Mix (using the GeneChip™ HT Hyb, Wash and Stain Kit) - 2 HT Array Plates

Volume per	Rxn	Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
20X Hybridization Control Stock	10.0 µL	272.5 µL	545.0 µL	1,090.0 µL
3 nM B2 Oligo	3.3 µL	89.9 µL	179.9 µL	359.7 µL
HT 1.2X Hybridization Mix*	166.7 µL	4,542.6 µL	9,085.2 µL	18,170.3 µL
Total Volume	180.0 µL	4,905.0 µL	9,810.1 µL	19,620.0 µL
# of QIAGEN Strips Used		2	3	6
Volume per Well in 1st Strip		408.0 µL	408.0 µL	408.0 µL
Volume per Well in 2nd Strip		204.0 µL	408.0 µL	408.0 µL
Volume per Well in 3rd Strip		408.0 µL		408.0 µL
Volume per Well in 4th Strip		408.0 µL		
Volume per Well in 5th Strip		408.0 µL		
Volume per Well in 6th Strip		408.0 µL		

* The 1.2X Hybridization Mix is a component of the HT Hybridization, Wash, and Stain Kit containing BSA, HS DNA and 1.23X hybridization buffer.

Hybridization Cocktail Master Mix for Cold Reagent Block Using Self-prepared Reagents

IMPORTANT: It is imperative that frozen stocks of 20X GeneChip™ Eukaryotic Hybridization Controls are heated to 65°C for 5 minutes to completely resuspend the cRNA before aliquotting.

Table 3.9 100 µL Hybridization Cocktail Master Mix for Cold Reagent Block - 1 HT Array Plate

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
20X Hybridization Control Stock	5.0 µL	139.1 µL	272.5 µL	545.0 µL
3 nM B2 Oligo	1.65 µL	45.9 µL	89.9 µL	179.9 µL
HS DNA (10 mg/mL)	1.0 µL	27.8 µL	54.5 µL	109.0 µL
Acetylated BSA (50 mg/mL)	1.0 µL	27.8 µL	54.5 µL	109.0 µL
1.23X Hybridization Buffer*	81.35 µL	2,263.0 µL	4,433.6 µL	8,867.2 µL
Total Volume	90 µL	2,503.6 µL	4,905.0 µL	9,810.1 µL
# of QIAGEN Strips Used		1	2	3
Volume per Well in 1st Strip		306.0 µL	408.0 µL	408.0 µL
Volume per Well in 2nd Strip		204.0 µL		408.0 µL
Volume per Well in 3rd Strip		408.0 µL		

* The 1.23x Hybridization Buffer is made from a recipe found in 1.23X Hybridization Buffer, and is not a component of the HT Hybridization, Wash, and Stain Kit.

IMPORTANT: It is imperative that frozen stocks of 20X GeneChip™ Eukaryotic Hybridization Controls are heated to 65 °C for 5 minutes to completely resuspend the cRNA before aliquotting.

Table 3.10 200 µL Hybridization Cocktail Master Mix for Cold Reagent Block - 2 HT Array Plates

Volume per Rxn		Adjusted Volumes: 24 Rxns	48 Rxns	96 Rxns
20X Hybridization Control Stock	10.0 µL	272.5 µL	545.0 µL	1,090.0 µL
3 nM B2 Oligo	3.3 µL	89.9 µL	179.9 µL	359.7 µL
HS DNA (10 mg/mL)	2.0 µL	54.5 µL	109.0 µL	218.0 µL
Acetylated BSA (50 mg/mL)	2.0 µL	54.5 µL	109.0 µL	218.0 µL
1.23X Hybridization Buffer*	162.7 µL	4,433.6 µL	8,867.2 µL	17,734.3 µL
Total Volume	180.0 µL	4,905.0 µL	9,810.1 µL	19,620.0 µL
# of QIAGEN Strips Used		2	3	6
Volume per Well in 1st Strip		408.0 µL	408.0 µL	408.0 µL
Volume per Well in 2nd Strip		204.0 µL	408.0 µL	408.0 µL
Volume per Well in 3rd Strip		408.0 µL		408.0 µL
Volume per Well in 4th Strip		408.0 µL		
Volume per Well in 5th Strip		408.0 µL		
Volume per Well in 6th Strip		408.0 µL		

* The 1.23x Hybridization Buffer is made from a recipie found in 1.23X Hybridization Buffer, and is not a component of the HT Hybridization, Wash, and Stain Kit.

Additional Reagent Preparation Steps Required

Set up 75% EtOH Reservoir

Materials Needed

- Ethanol, 100%
- Nuclease-free Water
- E&K Scientific 96 Well Reservoir, High Profile 300 mL
- Phenix Universal Lid for 96-well plates

Procedure

1. Prepare 250 mL 75% EtOH and pour into reagent reservoir.
2. Cover the reservoir with a lid.

Set up H_2O Reservoir

Materials Needed

- E&K Scientific 96 Well Reservoir, High Profile 300 mL
- Phenix Universal Lid for 96-well plates
- Nuclease-free Water

Procedure

1. Pour 250 mL Nuclease-free water into the reagent reservoir.
2. Cover the reservoir with a lid.

cDNA and cRNA Purification Preparation

Materials Needed

- Agencourt RNAClean™
- ABGene 1.2 mL Square Well Storage Plate, Low Profile
- Phenix Universal Lid for 96-well plates

Procedure

1. Inspect the bottle of RNAClean solution to verify that it is a brownish slurry in color and that it has been refrigerated.
2. Thoroughly shake the bottle of RNAClean solution before aliquoting 450 µL into each well of a 1.2 mL low profile reservoir.
3. Cover the reservoir with a lid.

Chapter 4 Array Station Setup and Target Preparation

Introduction

This chapter describes a typical Array Station run for GeneChip™ HT Array Plates. A schematic of the automated target preparation protocol is provided at the beginning to outline the steps in the procedure (Figure 4.1 to Figure 4.13).

This chapter also describes the procedures for using the GeneChip™ Array Station Software, setting up the Array Station deck, and performing quantitation and normalization of cRNA.

Automated Target Preparation Schematic

The following outlines a complete sample preparation protocol.

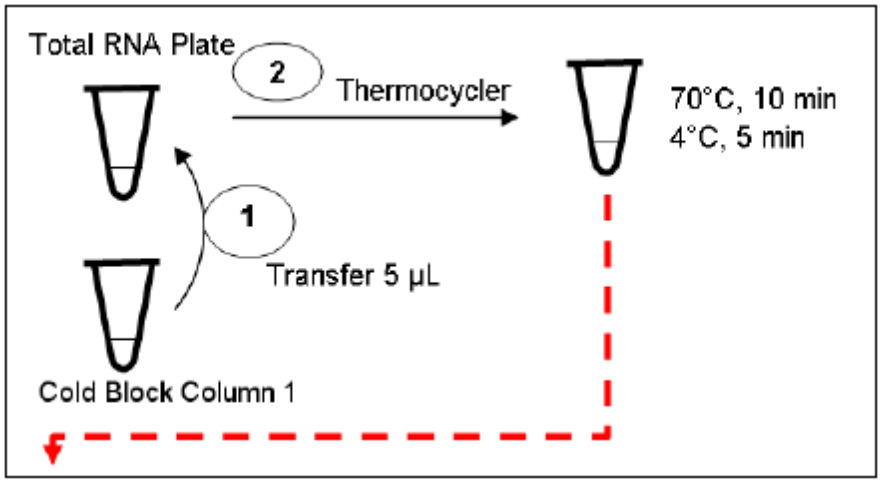


Figure 4.1 Step 1 & 2: Primer Anneal

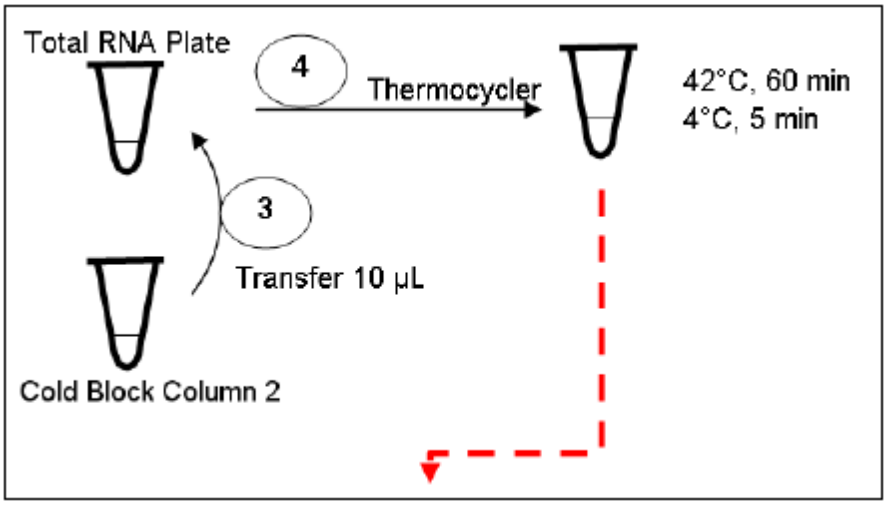


Figure 4.2 Step 3 & 4: First Strand cDNA Synthesis

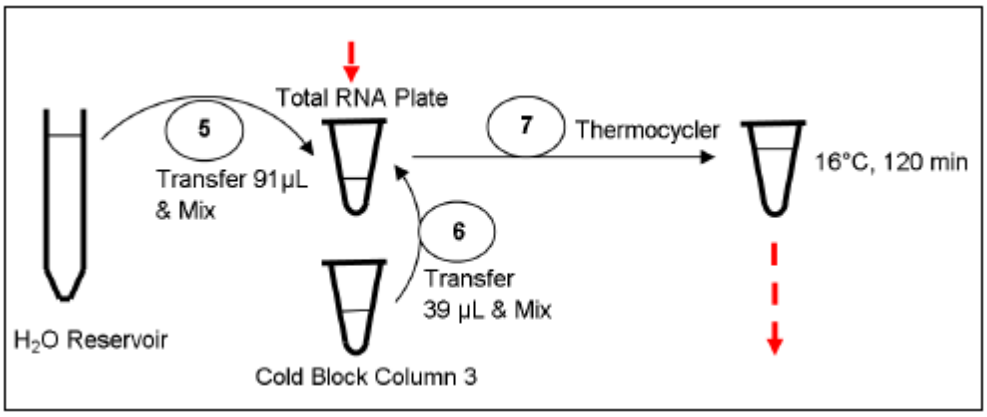


Figure 4.3 Steps 5, 6, and 7: Second Strand cDNA Synthesis

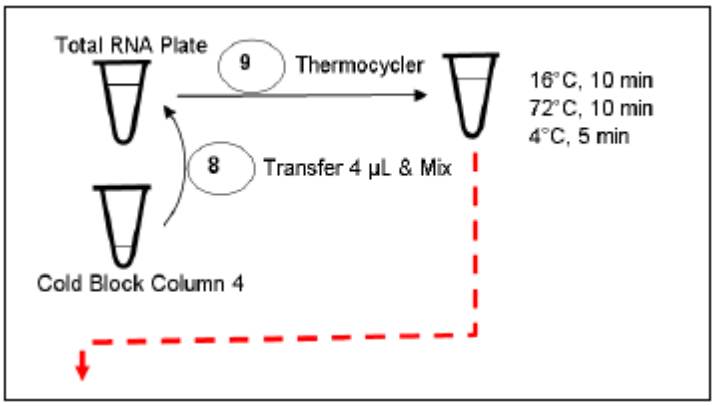


Figure 4.4 Step 8 & 9: T4 Polymerase Reaction

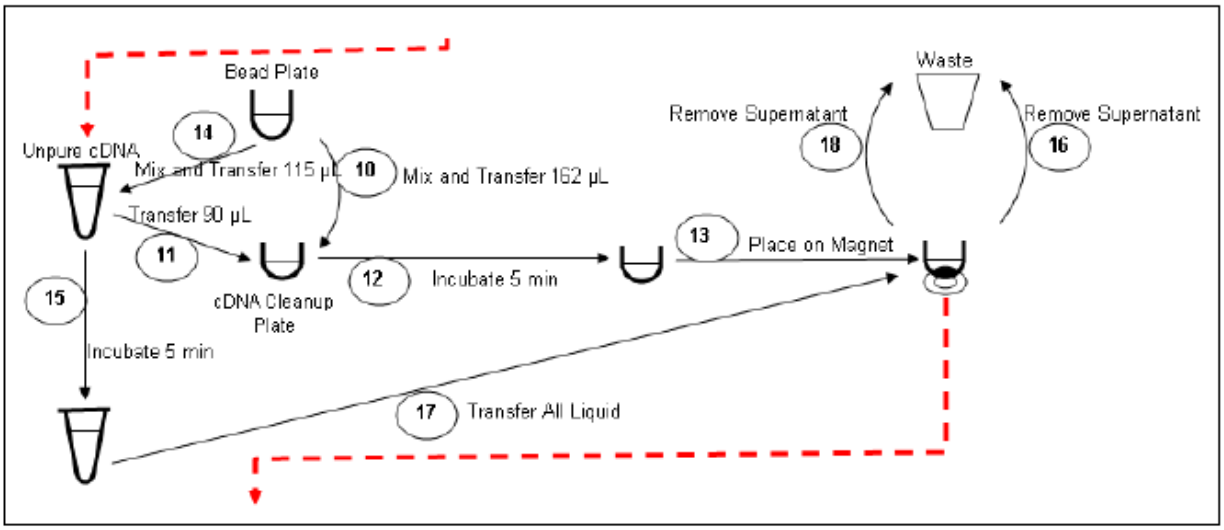


Figure 4.5 Steps 10 to 18: cDNA Capture

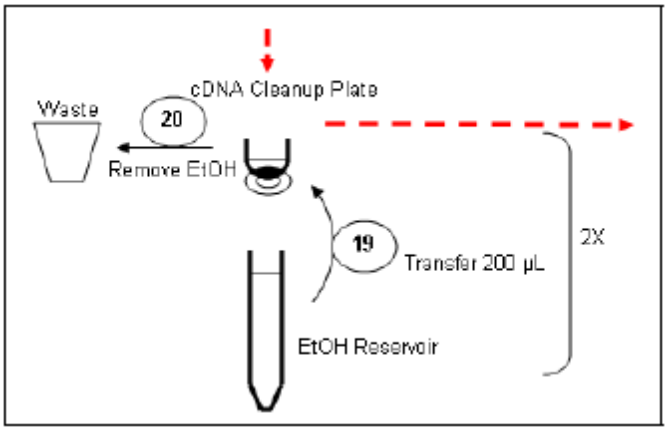


Figure 4.6 Step 19 & 20: cDNA Wash

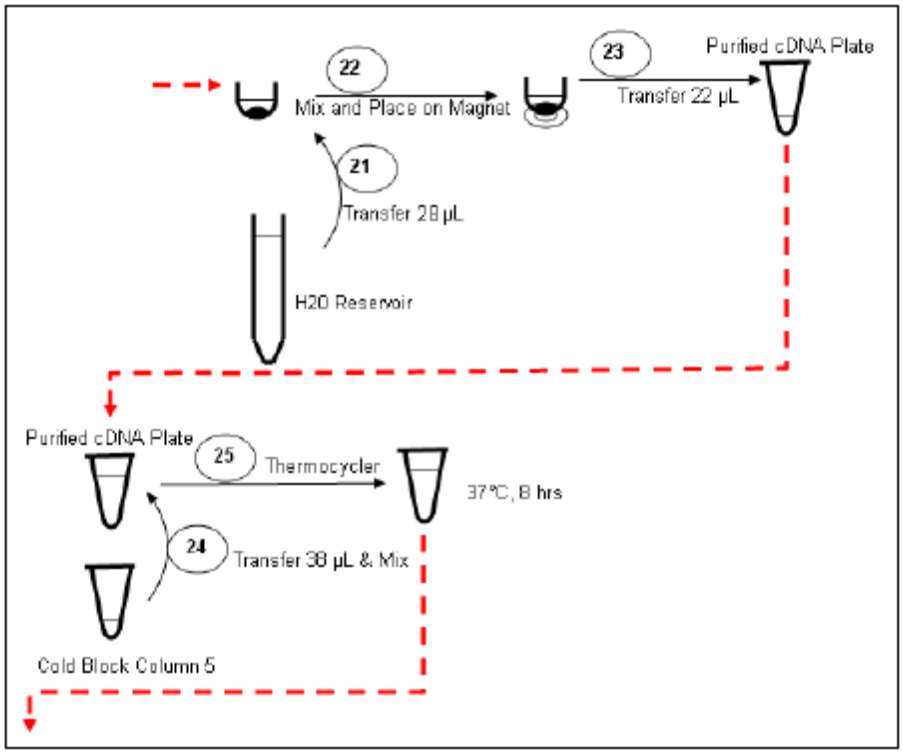


Figure 4.7 Steps 21 to 25: cDNA Elution and IVT Setup

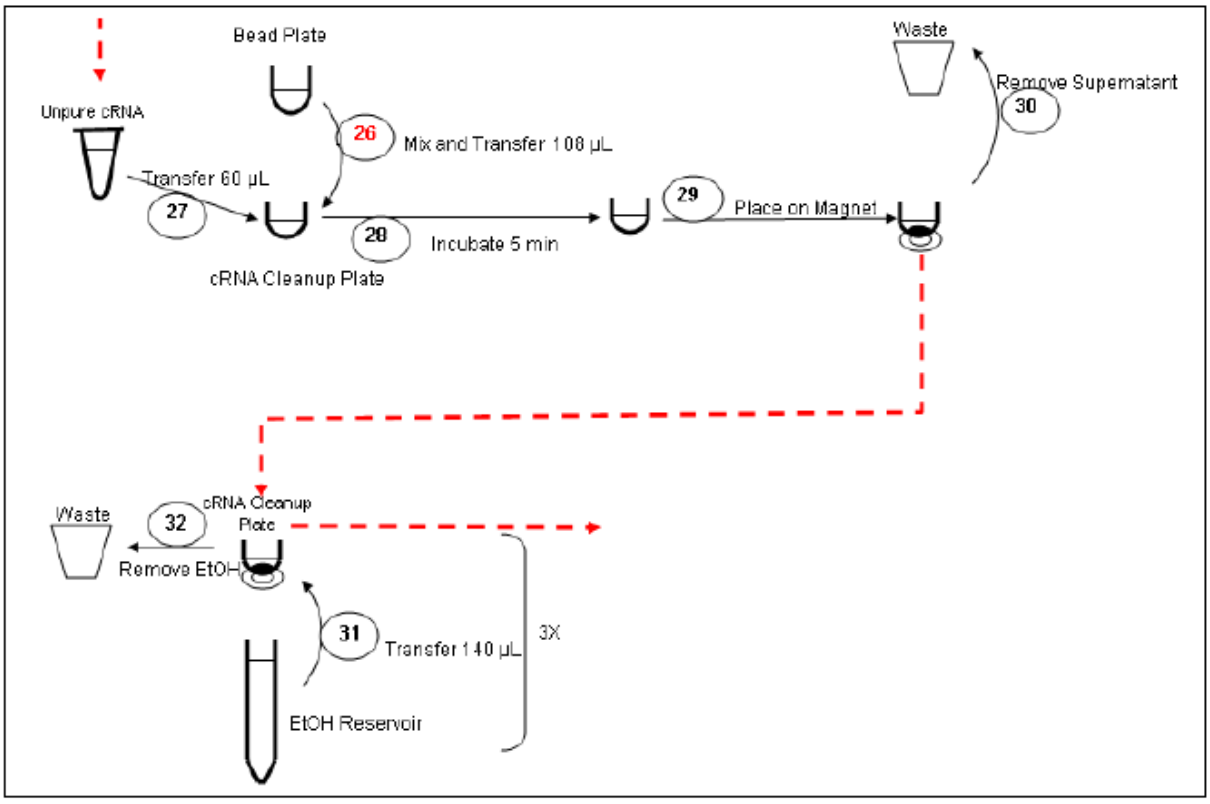


Figure 4.8 Steps 26 to 32: IVT Capture and Wash

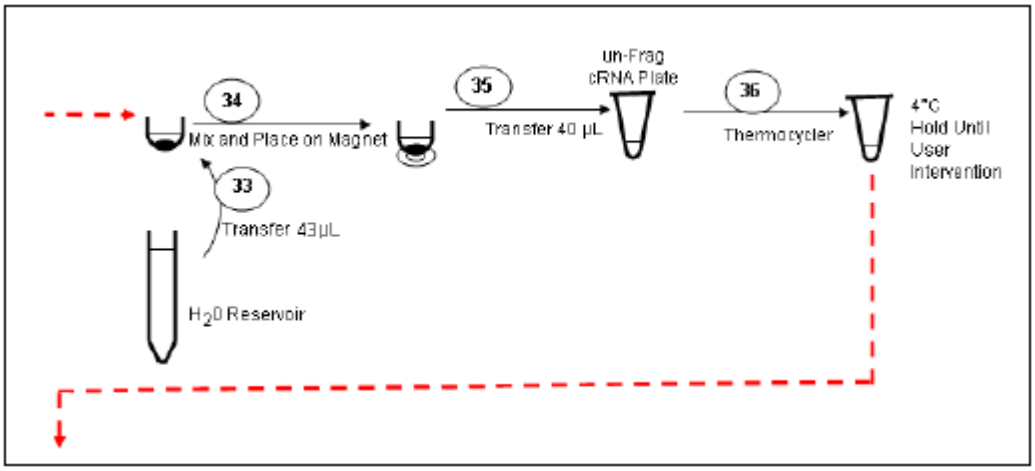


Figure 4.9 Steps 33 to 36: IVT Elution

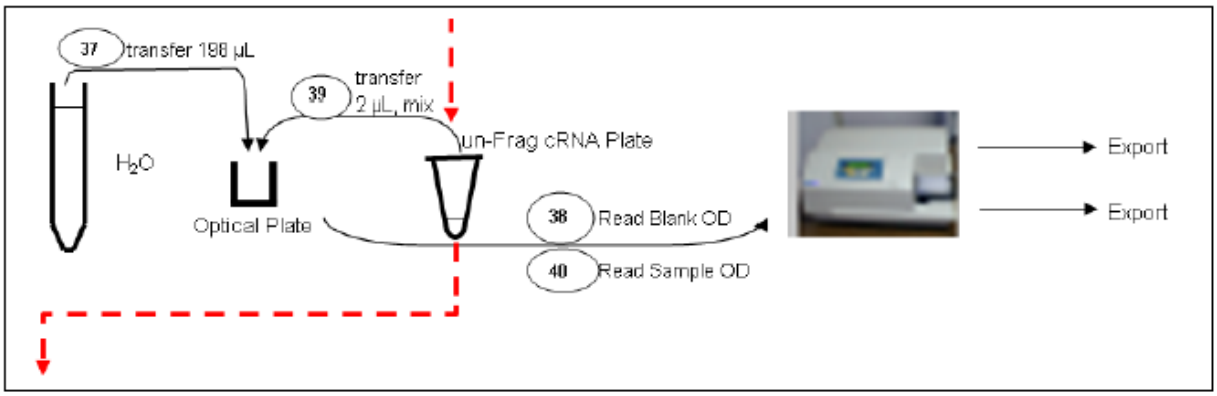


Figure 4.10 Steps 37 to 40: First Quantitation

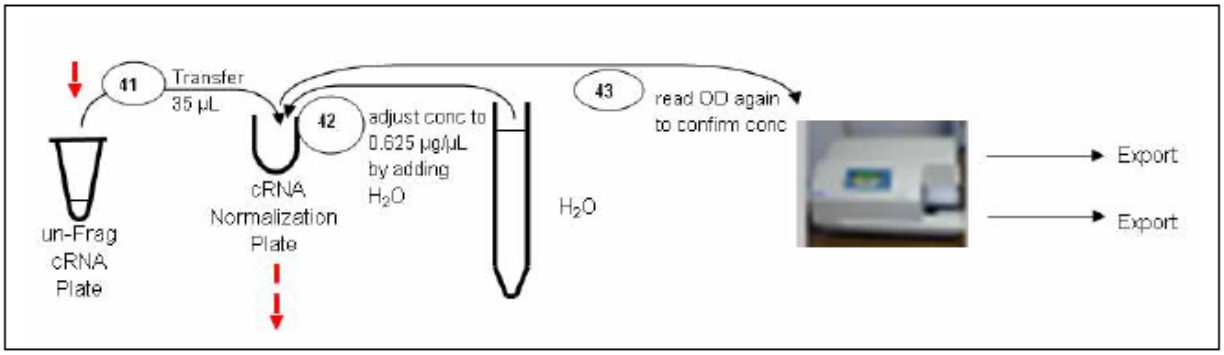


Figure 4.11 Steps 41 & 42 & 43: Normalization and Second Quantitation (Refer to Figure 4.11 for details of Step 43)

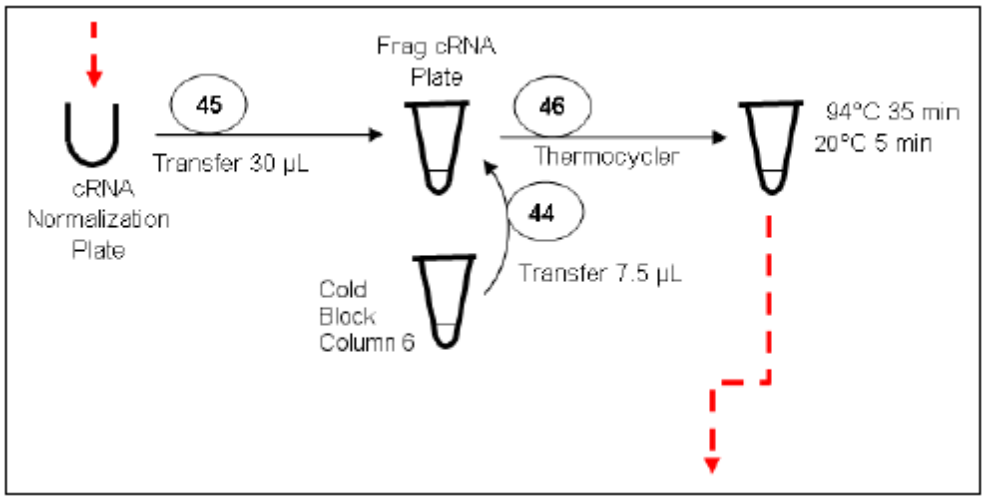


Figure 4.12 Steps 44 & 45 & 46: Fragmentation

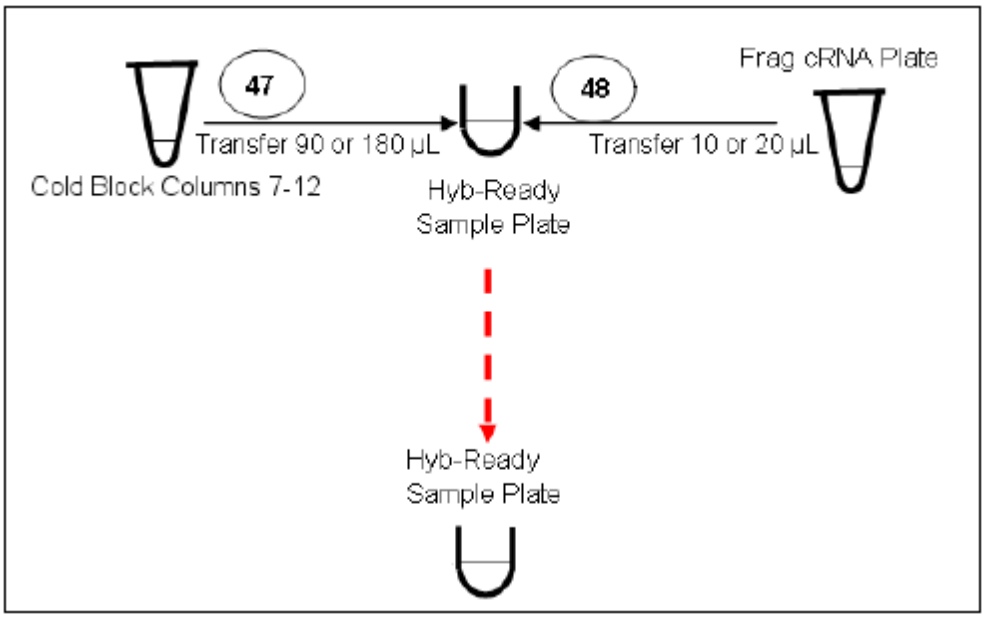


Figure 4.13 Steps 47 & 48: Hyb-sample Mix Transfer

Check List Before a Run

Before beginning a sample preparation run, you must make the following checks of the system.

1. Ensure that the water supply connections and waste water drainage are properly installed. In addition, the bottles (illustrated in Figure 4.14) must be filled with distilled or de-ionized water and the liquid waste container (illustrated in Figure 4.15) must be empty.

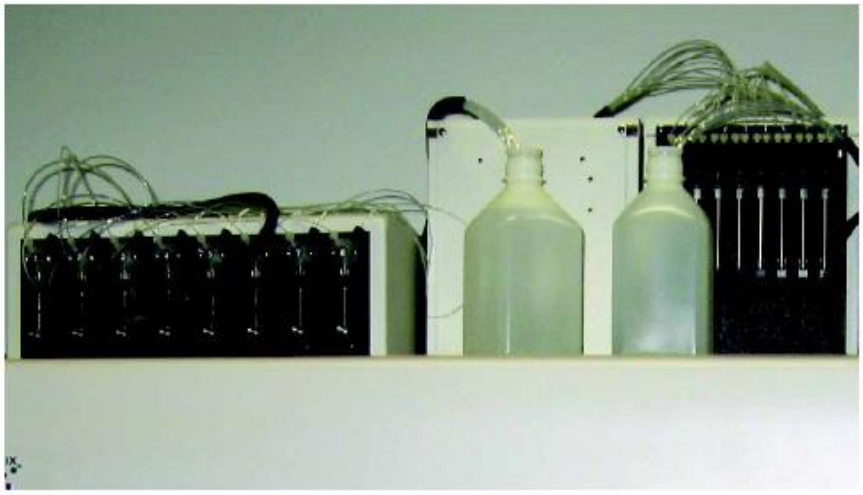


Figure 4.14 Tubing lines for water supply

Figure 4.15 Liquid waste drainage

2. Ensure that all of the pipette tip boxes are oriented correctly, fit snugly into the holders, and the holders fit into the Twister II Rack.

   Figure 4.16 to Figure 4.20 illustrate how to load the pipette tips into the Twister II Rack.

CAUTION: Tip Loading Requirement — The number of tip boxes needed for each run may vary depending on the deck layout used and the number of samples processed. Refer to Table 4.1 to determine the number of tip boxes to load.

CAUTION: Clean out empty tip boxes in Twister IITM Rack 2.

CAUTION: Do not remove tip boxes while run is in progress.

CAUTION: Tip boxes should not extend above the top of the Rack.

Table 4.1 Pipette Tip Usage

Number of Rxns	8	16	24	32	40	48	56	64	72	80	88	96
Number of Tip Boxes Needed	2	4	6	7	9	11	13	14	16	18	20	21

3. Controlling static electrical interference: Static attraction can cause pipette tips to cling to the heads and to each other. To control static attraction, use non-sterile, RNase/DNase-free tips and use a static gun to remove static discharge. See Figure 4.16 and Figure 4.17.

4. Another method used to control static electricity is the use of metal spacers. Spacers should be placed in between each and on top of the last RNase/DNase-free tip rack after the use of the static gun. See Figure 4.18.

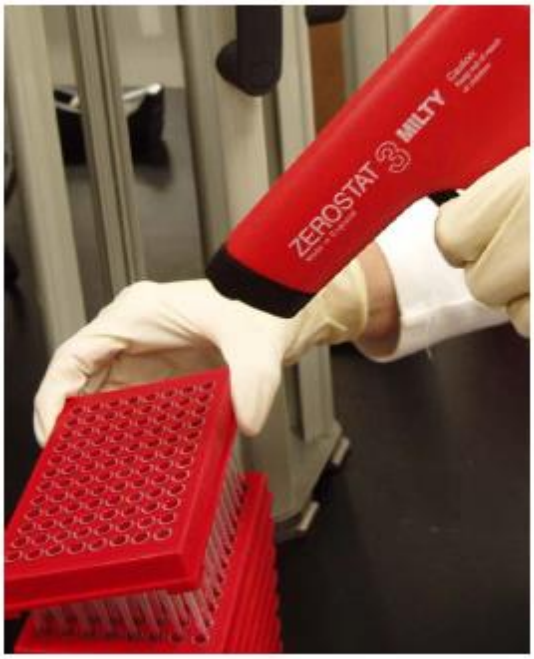


Figure 4.16 Removing static discharge from tip boxes with a static gun

Figure 4.17 Removing static discharge from tips with a static gun

Figure 4.18 Tips with anti-static metal spacers

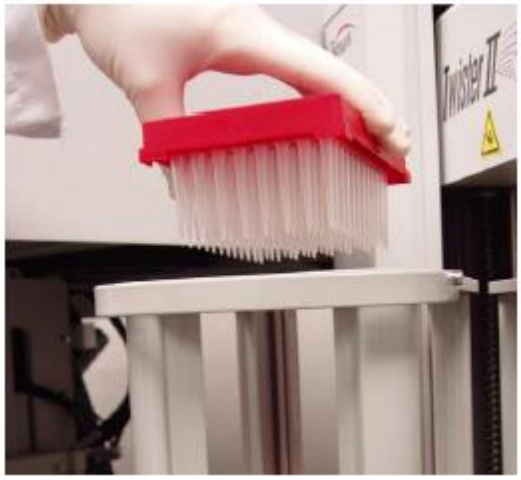


Figure 4.19 Loading the pipette tips into the holder and into the rack

Figure 4.20 Loading the pipette boxes into the rack

Clean the Bio-Rad 96-Well Hard-Shell PCR Plate Lids

NOTE: The disposable pad under the arched lids should be cleaned before every run.

Material required

- Ambion DNAZap™
- Ambion RNaseZap™ Wipes™

Procedure

1. Rinse the pad with DI water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI water.
6. Dry the pad with pressurized clean air or nitrogen.

NOTE: The disposable pad under the arched lids should be replaced every 15 runs. Refer to the *GeneChip™ Array Station User's Guide* (P/N 701859) for further details.

Beginning a Run — First Layout

This section shows you how to set up the deck and use the software to begin a sample preparation run for the first deck layout. The first layout protocol completes the following steps.

1. Primer anneal
2. First strand cDNA synthesis
3. Second strand cDNA synthesis
4. T4 polymerase synthesis
5. cDNA purification, wash and cDNA elution
6. IVT reaction
7. cRNA Cleanup and Elution
8. Pause for deck change to the second deck layout

Procedure

1. Set up the deck with the appropriate consumables. Refer to Figure 4.21.

NOTE: The deck layout shown in Figure 4.21 is for runs starting at the beginning of the target prep process. Custom target prep runs can also be started at various other places in the protocol. Please refer to layouts in Appendix C for runs starting at other steps in the process.

2. Assemble the Peltier adaptor and prechilled cold reagent block. Ensure that the unit is securely tightened. Refer to Figure 4.22 and Figure 4.23.
3. Turn on the Watlow Temperature Controller to 4°C. Refer Figure 4.24.

IMPORTANT: The prechilled cold reagent block and Peltier adaptor are assembled prior to the addition of the reagent strip tubes. It is important that the adaptor be at room temperature when joining with the cold reagent block as it is difficult to fit the cold reagent block flush against the adaptor if the adaptor is cold. Once the chilled cold reagent block is assembled into the adapter securely, adjust the control unit to 4°C for the remainder of the run.

4. Load the reagent strip tubes onto the assembled cold reagent block and adaptor. Refer to Figure 4.25.
5. Ensure that the tabs on the strip tubes are correctly seated in order to place the lid on the cold reagent block.

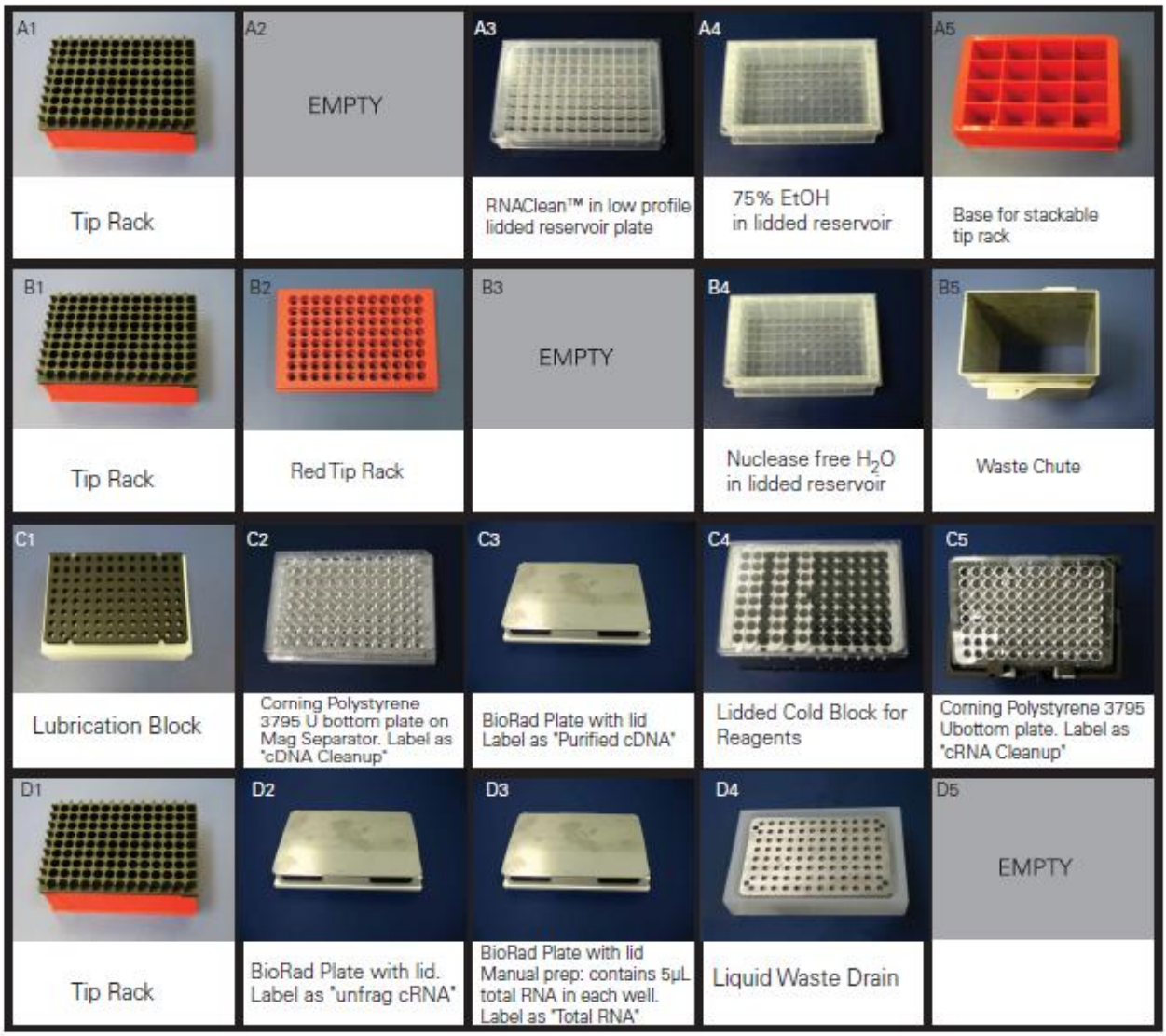


Figure 4.21 First Deck Layout. Refer to Table 4.1 to determine the number of tip boxes to load.

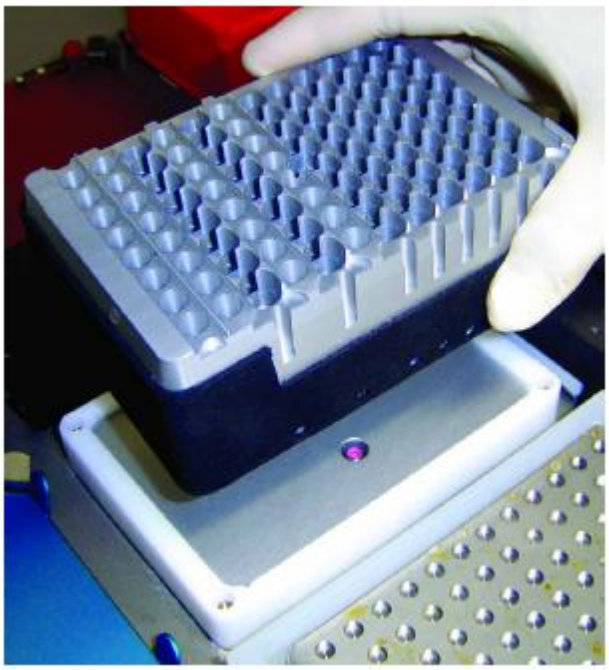


Figure 4.22 Loading the cold reagent block on to the deck fixture

Figure 4.23 Securing the cold reagent block. Do not fully tighten.

Figure 4.24 Setting the Watlow Temperature Controller for the Peltier at 4°C

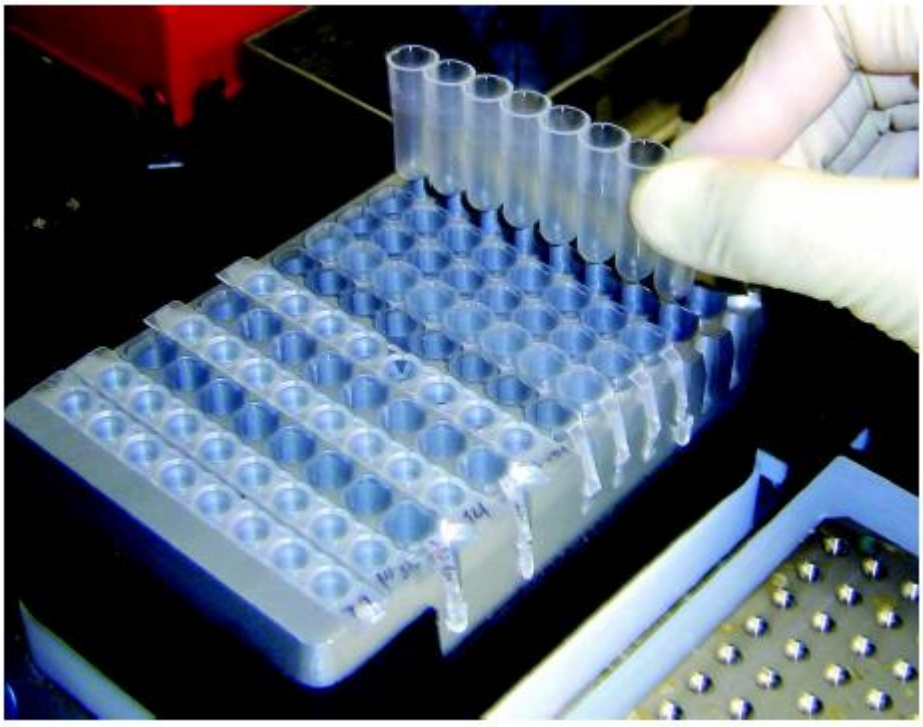


Figure 4.25 Loading the strips into the cold reagent block

Running Gene Expression Target Preparation (TP) Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc. and that the cold reagent block is loaded with the correct reagents.
2. Open the GeneChip™ Array Station Software by double-clicking the desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**.
3. A Login window appears (Figure 4.26). Enter your **User Name** and **Password** and click **OK**. All runs should be performed in Operator Mode. See Appendix D, *User Level Configuration* for a description of operational modes.

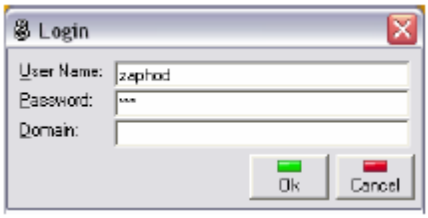


Figure 4.26 Sciclone Workstation Software Login Window

4. Once you have logged in, the main Operator window appears, as shown in Figure 4.27. This window is referred to as the Runtime Window.

Figure 4.27 Runtime window

5. To load the Target Preparation protocol, select **File → Open** and you will see the dialog box shown in Figure 4.28.

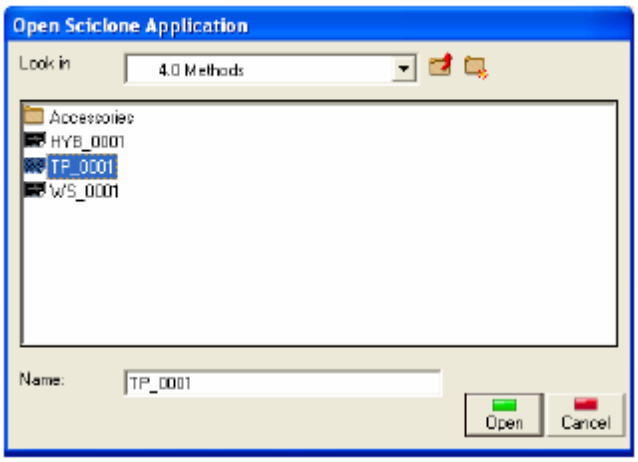


Figure 4.28 Open Sciclone Application window

6. Select the protocol **TP_0001** and click **Open**. When the Target Preparation Protocol has completed loading, the window will appear as shown in Figure 4.29.

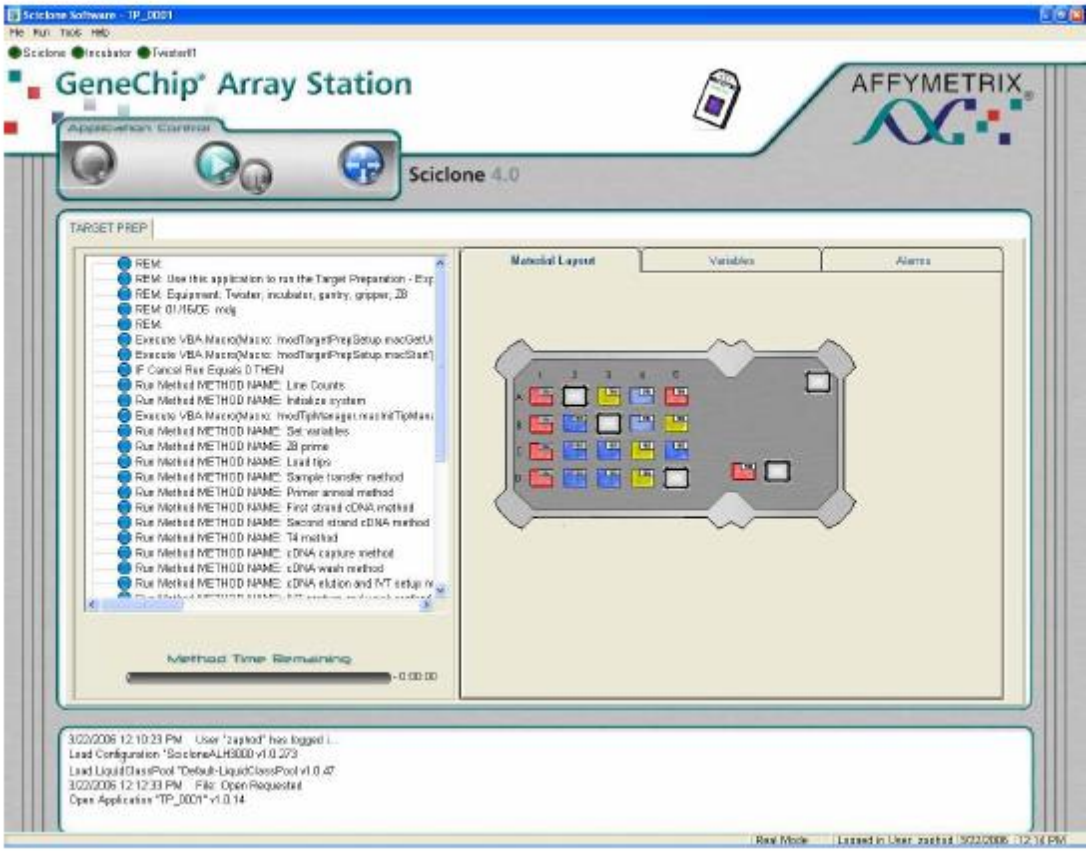


Figure 4.29 Target Preparation Protocol Loaded

7. To begin a run, click the green arrow button in the Application Control console of the Runtime window.

   Prior to the start of the run, you will be prompted to select various options. The windows and selections you will see are shown in the series of figures that follow.

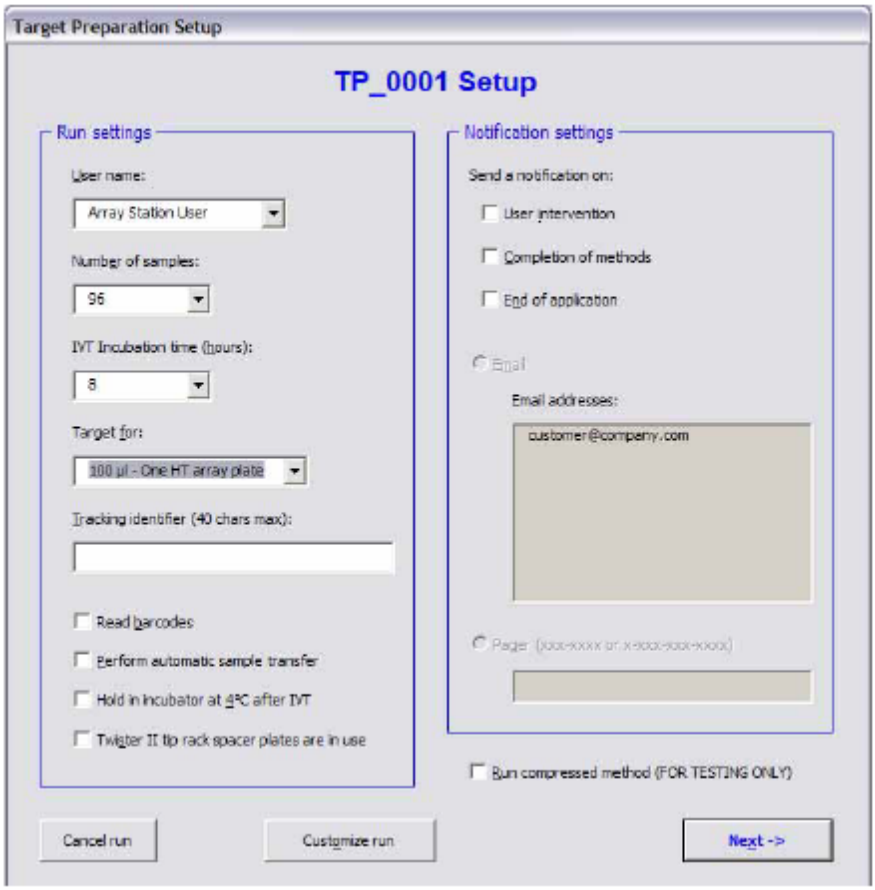


Figure 4.30 Target Preparation Setup window

8. For the Target Preparation Setup window you will need to select the following options:

a. **User name**: Select the appropriate user name from the drop down menu. Please contact support personnel for assistance with customizing this window.

b. **Number of samples**: Select either 24 or 96. Be sure your samples are laid out in the plate in column wise fashion starting column 1.

c. **Incubation time**: Select 8 hours from the drop down menu.

NOTE: Target Prep currently is only validated for an 8 hour IVT. If you choose an incubation time other than 8 hours, ensure you have consulted support.

d. **Target for**: Select the desired final volume of hyb-ready sample needed for further processing on HT Array plates. 100 µL will provide sufficient material for hybridization to one HT Array plate. 200 µL is required if running samples on two HT Array plates.

e. **Tracking identifier**: This is an optional field that can be utilized for your individual runs. You can fill in up to 40 characters of text here. The information you enter will be recorded in the Target Preparation Summary Report for your run (see Figure 4.44).

f. **Read barcodes**: Check this box if you want the system to automatically read and track the barcodes of various plates used during the run. You must supply the barcodes (code 128c format) and affix them to any or all of the following plates for tracking total RNA, unfragmented cRNA, normalized cRNA, fragmented cRNA, and hyb-ready sample. Barcodes should be affixed to the right side of the plate (see Figure 4.31). Barcodes recorded during the run will be reported in the Target Preparation Summary Report for your run (see Figure 4.44).

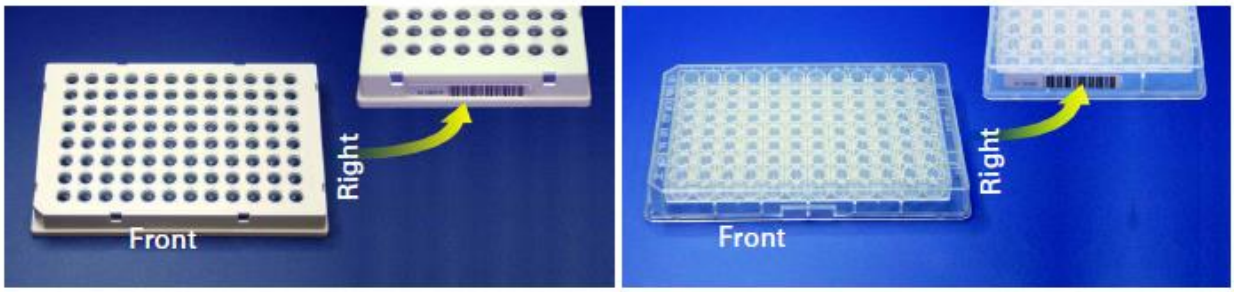


Figure 4.31 Barcode Positioning on Plate

g. **Perform automatic sample transfer**: Check this box to have the Array Station transfer 5 µL of starting total RNA into the Bio-Rad 96 Well Hard Shell PCR plate. To use automated sample transfer, a minimum of 20 µL of your sample (0.2 to 0.4 µg/µL) must be in a Greiner U-bottom plate. If you select Automated Sample Transfer, you will be prompted when to place and remove plates as needed. Please refer to Appendix B and Chapter 2 for more information on using the automated sample transfer option.

NOTE: If you select the automatic sample transfer option you must use the initial deck layout shown in Figure B.1 of Appendix B. Do not use the layout shown in Figure 4.21.

h. **Hold in** incubator **at 4°C after IVT**: If you select this option, the plate will be held in the incubator at 4°C after the IVT step until you prompt the Array Station to resume the process. Once prompted, the process will proceed to the IVT cleanup steps.

i. **Twister II tip rack spacer plates are in use**: Select this option if you are using the tip rack spacer plates to help control static. We strongly suggest that you use the tip rack spacer plates. A spacer plate should be placed between each rack of tips as well as on the top rack of tips before loading tips into the Twister II Rack 1.

j. **Notification Settings**: Select each of the steps at which you would like to receive a notification. Notification can be received via email or pager. Ensure that the email address or pager information in the selection is correct and active. Contact support personnel for assistance with configuring email or pager notification.

Run compressed method (FOR TESTING ONLY):

Do not select this option unless you are performing a blank test run.

NOTE: Please see Appendix C for a description of how to use the "Customize Run" option.

9. When you have completed the options on this setup window, click **Next**.

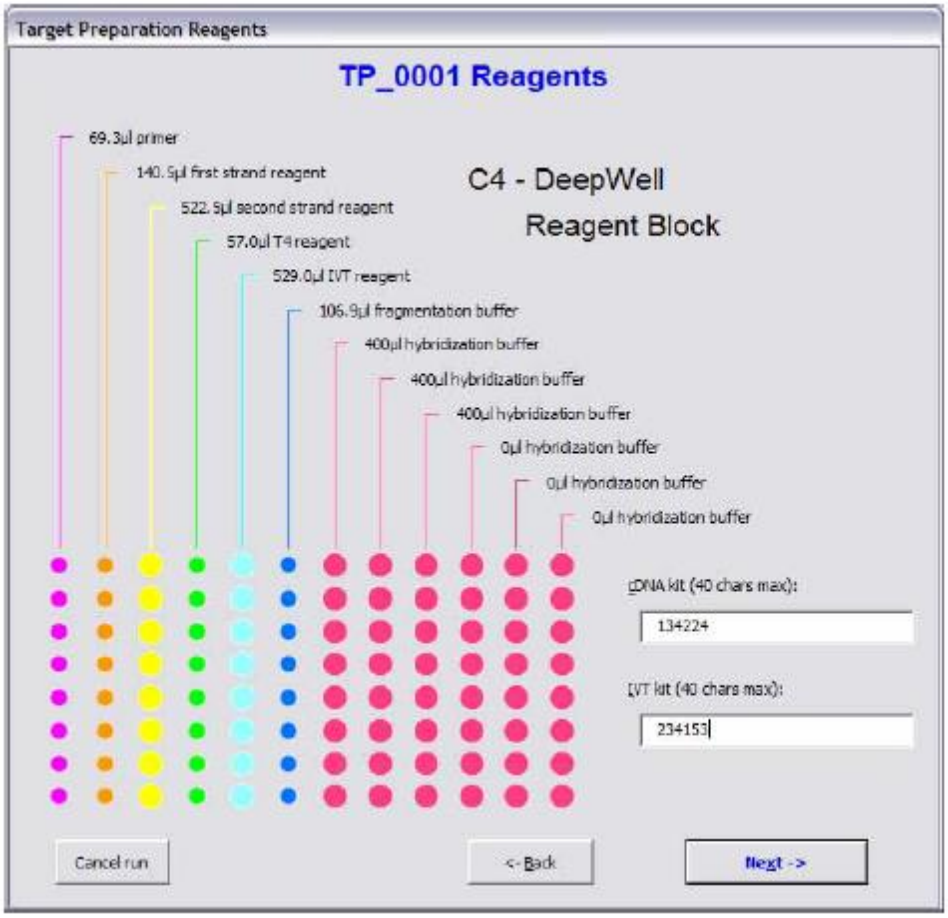


Figure 4.32 Target Preparation Reagents window

10. The Target Preparation Reagents window graphically displays the correct setup for the Reagent Block according to your selections. In the example shown, the user has selected 96 samples and a final hybridization volume of 100 µL. The boxes labeled **cDNA kit** and **IVT kit** provide an optional way to track lot numbers of your reagent kits. If you place the lot numbers in the boxes at this time, they will be captured in the Target Preparation Summary Report at the end of the run (see Figure 4.44 for an example of this report). After verifying you have correctly set up the Reagent Block, click **Next**.

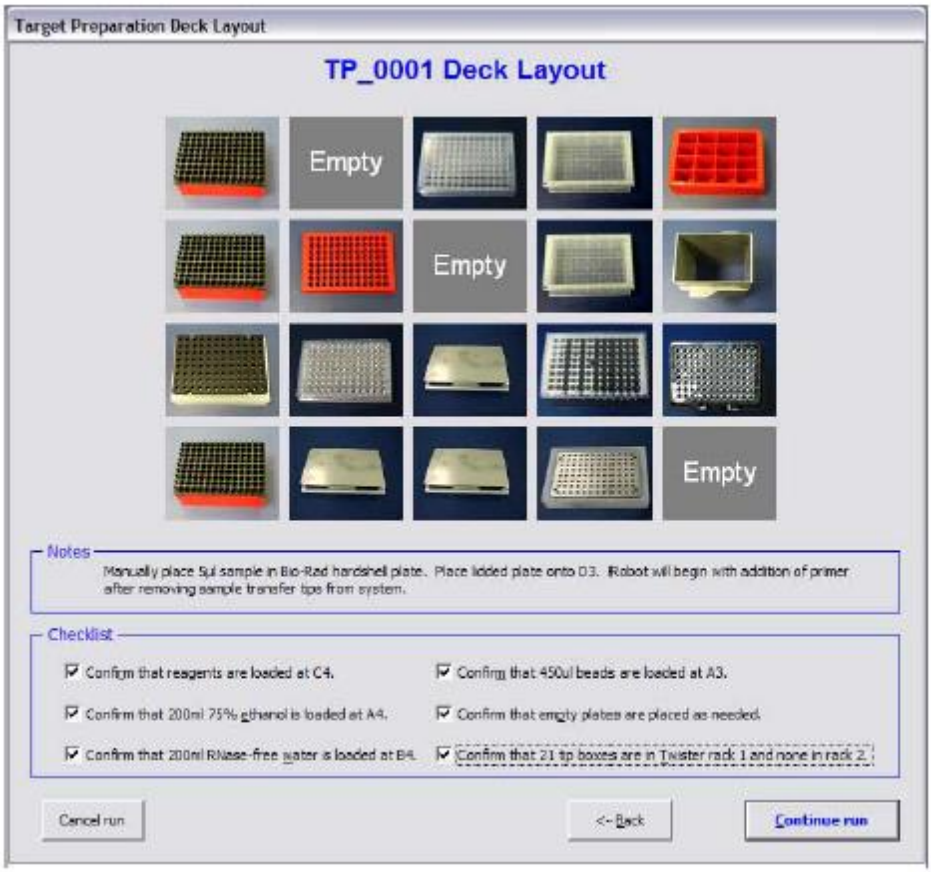


Figure 4.33 Target Preparation Deck Layout window

11. This TP_0001 Deck Layout window (Figure 4.33) shows a sample window for the **target preparation** 96 samples. In this window the user has NOT selected the **Automatic sample transfer** option. Be sure the deck layout shown in the Target Preparation Deck Layout window matches the layout appropriate for your starting selection. Refer to Appendix C for a listing and illustration of the deck layouts used for custom starting options. Be sure you have performed all steps in the **Checklist** and check each box to continue. Click **Continue run** to proceed. The Array Station proceeds to run your selected methods.

12. If you have selected the option to **Hold in the incubator at 4°C after IVT**, you will see the following message after completion of the IVT method:

Figure 4.34 User Message window if holding at 4°C after IVT.

13. To proceed to the cRNA cleanup, you must click **OK**. Do not make any changes to the deck. The Array Station will continue with the cRNA cleanup method.

Second Layout (User Intervention)

This section shows you how to begin a sample preparation run for the second deck layout.

The second layout protocol completes the following steps:

1. FirstS quantitation
2. Normalization
3. Second quantitation
4. Fragmentation
5. Preparation of hybridization-ready sample.

Procedure

CAUTION:. Do not click OK, as illustrated in the User Message in Figure 4.35, until the deck layout is changed. See Figure 4.36 for an illustration of the deck layout.

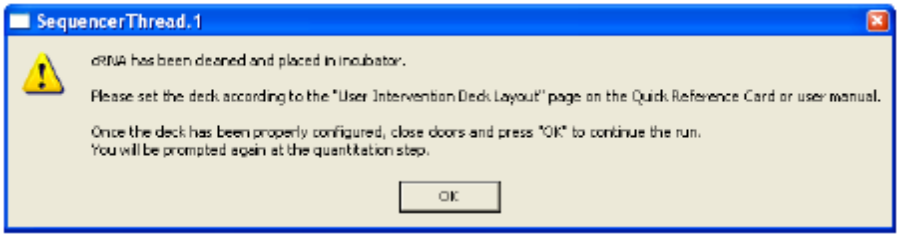


Figure 4.35 User Message window indicating a deck layout change for First quantitation.

NOTE: Your samples are being held at 4°C in the incubator. Do not disturb them. The robot will bring them back on the deck after you have completed the deck layout change.

1. Change the deck layout as shown in Figure 4.36.

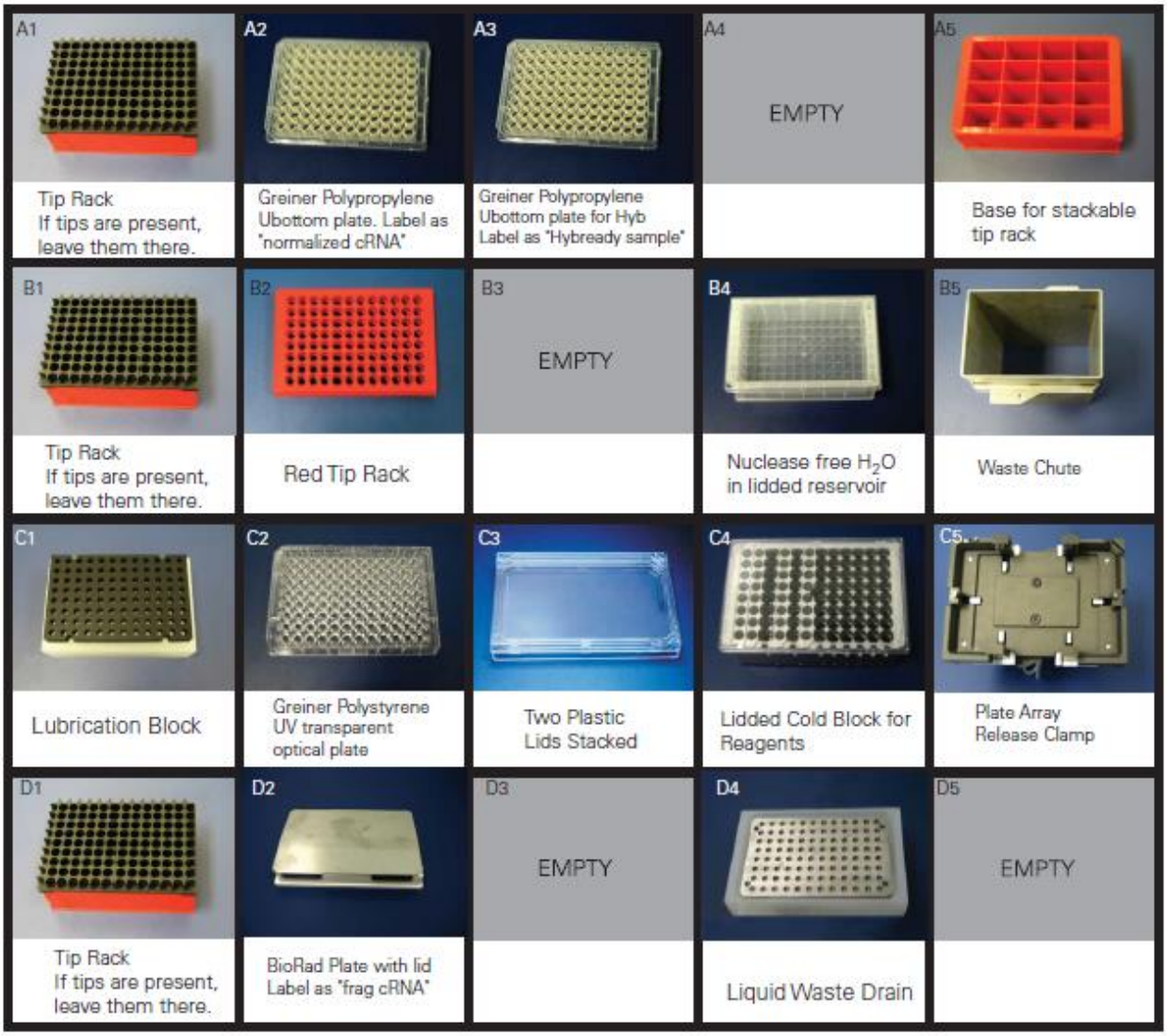


Figure 4.36 Second layout. This requires user intervention to set up.

2. Follow the prompts on the workstation to complete this section of the protocol.

NOTE: After the completion of this section, the samples are ready for hybridization to the cartridge arrays.

cRNA Quantitation and Normalization

This section of the protocol details the user intervention steps necessary for calculating the yield of cRNA generated from the *in vitro* transcription reaction. The accurate calculation of the yield is necessary so that the correct amount of cRNA is added to the fragmentation reaction. Too much or too little cRNA added to the fragmentation reaction can result in incomplete or over fragmented cRNA and cause hybridization effects. This cRNA yield can be used as a check point to ensure that all the proceeding steps have been successfully completed and sufficient cRNA yield has been generated.

The GeneChip™ Array Station uses spectrophotometric analysis to determine the cRNA yield. The convention that 1 absorbance unit at 260 nm equals 40 µg/mL RNA is used.

- The absorbance at 260 nm and 280 nm is checked to determine sample concentration and purity.
- The A_{260}/A_{280} ratio is maintained close to 2.0 for pure RNA (ratios between 1.9 and 2.1 are acceptable).

NOTE: The following method assumes the use of the recommended spectrophotometer as part of the Array Station system. If a different spectrophotometer is used, refer to instructions in that user guide.

cRNA First Quantitation

1. Turn on the spectrophotometer. Before proceeding, ensure that the LCD screen on the spectrophotometer indicates that the UV lamp has warmed up successfully. Confirm that you have selected the proper wavelengths (260 and 280).
2. Start the SoftMax™ Pro software. Either click the SoftMax Pro icon on the desktop or click **Start → All Programs → SoftMax Pro → SoftMax Pro**.

NOTE: The robot will remove the reaction plate from the thermal cycler and put 198 µL of water into the optical plate.

CAUTION:. When the User Message appears, as illustrated in Figure 4.37, do not click "OK" until Step 7.

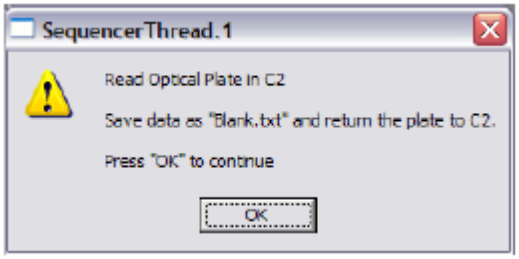


Figure 4.37 User Message window indicating to read Blank plate.

3. Remove the optical plate from position C2 on the deck and place the plate on the spectrophotometer tray.
4. Press the **Read** button in the SoftMax Pro software and make sure that the **Replace** option is selected.

   The spectrophotometer will read the plate as a blank.
5. Using the SoftMax Pro software, export the readings as a file called "Blank.txt" to the folder c:\Affymetrix\Reader Data. Also save the blank reading on floppy disc as a backup.

IMPORTANT: Use the export function to save the file in text format. If you are using other software, export or save data as a text file and ensure that the file is reformatted to the appropriate format.

6. Go to c:\Affymetrix\Reader Data to check that the software has updated "Blank.txt" with the correct date and time.
7. Return the plate on the deck to position C2 and click **OK**.

NOTE: The Array Station will now add 2 µL to the 198 µL of the water in the optical plate and will mix the sample with pipette mixes.

CAUTION:. When the User Message appears, as illustrated in Figure 4.38, do not click "OK" until Step 2 of *Normalization*.

Figure 4.38 User Message window indicating reading optical plate and placing a clean optical plate.

8. Remove the optical plate and place in the spectrophotometer.
9. Open the SoftMax Pro software.
10. Press the **Read** button in the SoftMax Pro software and ensure that you have selected the **Replace** option.

    The spectrophotometer will now read the plate.
11. When the spectrophotometer completes the optical read, export the data as "sample.txt" to the folder c:\Affymetrix\Reader Data. Save the sample data on a floppy disc as a backup.
12. Go to c:\Affymetrix\Reader Data to check that the software has updated "Sample.txt" with the correct date and time.
13. Discard optical plate.

Normalization

1. Place a clean optical plate on the Array Station deck at position C2.
2. Click **OK** in the window shown in Figure 4.38.

   The Array Station will calculate the concentrations and yields of your samples. A graphical output will be produced to show you the yields. The Figure 4.39 shows an example.

   If any wells fail to generate an amount of sample that can be correctly normalized, a red "X" will mark that well.

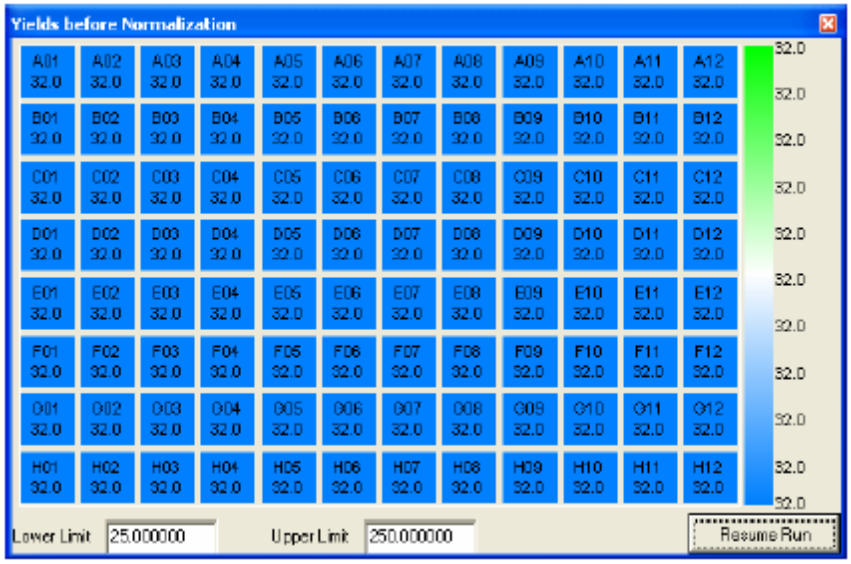


Figure 4.39 Graphic displaying cRNA yields (in µg)

NOTE: Samples which are outside the range of normalization (25 to 250 µg) will be carried through the remainder of the procedure but will not be properly normalized. Hybridization results from improperly normalized samples may not be valid.

3. Click **Resume Run** and the robot will add the correct amount of water to each well to make the dilution of cRNA ready for fragmentation. This process takes approximately 30 minutes to complete for a full 96 sample plate.

NOTE: The blank.txt and sample.txt files you saved for the First Quantitation above, will be renamed with the following convention:

Sample_TP_0001_Pre-Norm_3_28_2006_5_44_59_PM.txt Where

Sample = sample or blank

TP_0001 = method used

Pre-Norm or Post-Norm (dependent on when it is used)

3_28_2006 = date stamp of when the TP_0001 *starts*

5_44_59_PM = time stamp of when the TP_0001 *starts*

These renamed files can still be found in the folder c:\Affymetrix\Reader Data after the run. In addition, all processed OD readings will be automatically saved to a Data Report at the end of the run.

Following normalization, the Array Station will prepare a blank plate so that the normalized samples can be read to verify normalization. The blank plate will contain 198 µL of water in each well.

CAUTION:. When the User Message appears, as illustrated in Figure 4.40, do not click "OK" until Step 6 of *Second Quantitation*.

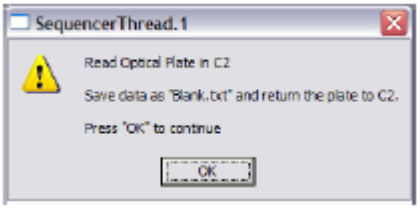


Figure 4.40 User Message window indicating read Blank plate.

Second Quantitation

1. Remove the optical plate from position C2 and place the optical plate on the spectrophotometer tray.
2. Start the SoftMax Pro software. Either click the Softmax Pro icon on the desktop or click **Start → All Programs → SoftMax Pro → SoftMax Pro**.
3. Press the **Read** button in the SoftMax Pro software and ensure that you have selected the **Replace** option.

   The spectrophotometer will now read the plate.
4. When the spectrophotometer completes the optical read, export the data in the appropriate format as "Blank.txt" to the folder c:\Affymetrix\Reader Data. Save the blank reading on a floppy disc as a backup.
5. Go to c:\Affymetrix\Reader Data, to ensure the "Blank.txt" file is updated with the correct date and time.
6. Replace the optical plate back on the deck in position C2 and click **OK**.

NOTE: The Array Station will now add 2 µL to the 198 µL of the water and will mix the sample with pipette mixes.

CAUTION:. When the User Message appears, as illustrated in Figure 4.41, do not click "OK" until Step 12.

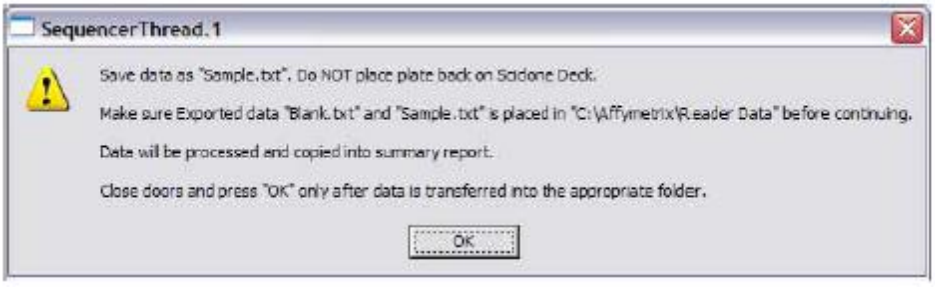


Figure 4.41 User Message window indicating the transfer of Blank.txt and Sample.txt.

7. Remove the optical plate from position C2 on the Array Station and place in the spectrophotometer.
8. Press the **Read** button in the SoftMax Pro software make sure that the **Replace** option is selected.

   The spectrophotometer will now read the plate.
9. When the spectrophotometer completes the optical read, export the file in the appropriate format as "Sample.txt" to the folder c:\Affymetrix\Reader Data. Save the data on a floppy disc as a backup.
10. Go to c:\Affymetrix\Reader Data, ensure the software has updated "Sample.txt" with the correct date and time.
11. Exit the SoftMax Pro program.
12. Discard the optical plate and click **OK**.

    The Array Station calculates the concentrations and yields of your samples. A graphical output is produced to show you the yields. Figure 4.42 shows an example.

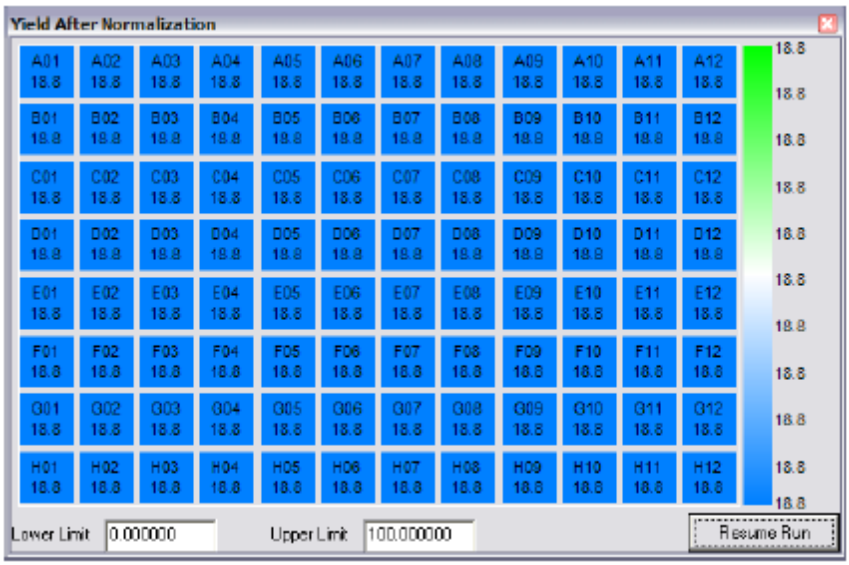


Figure 4.42 Graphic displaying cRNA yields (in µg)

13. Click **Resume Run** and the sample preparation method continues with the fragmentation step.

    After the second quantitation, the samples should be at a concentration of 0.625 µg/µL. If the dilution of the sample is below 0.625 µg/µL, the sample will not be normalized, but will continue through the method.

Fragmentation and Hybridization Cocktail

After the second quantitation and cRNA yield calculation, the Array Station will continue to the fragmentation step and make the hybridization-ready sample without user intervention. These two processes take approximately two hours for a full 96 sample plate. After the run has completed, a message appears as illustrated in Figure 4.43.

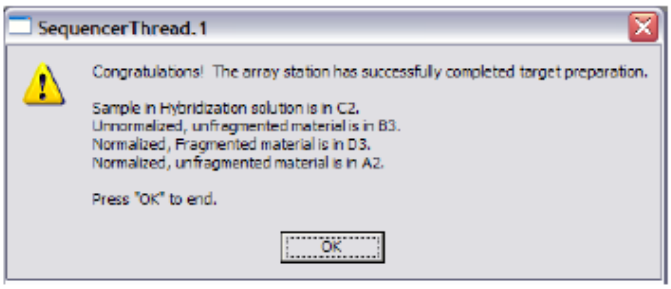


Figure 4.43 User Message indicating the completion of target preparation.

The hybridization-ready samples can now be hybridized to a HT Array Plate or stored.

For short-term storage:

1. Cover the hybridization-ready sample plate with an adhesive plate sealer.
2. Store the sealed plate at –20°C.

For long-term storage:

1. Cover the hybridization-ready sample plate with an adhesive plate sealer.
3. Store the sealed plate at –80°C.

Target Preparation Final Reports

After completion of the target preparation, three report files are saved automatically.

1. **Main Summary Report**: This report is found in the folder c:\Affymetrix\Reports\Summary. This is a user readable report that captures user information, start and stop times, set up information, lot numbers (if input), barcodes (if used), wells flagged as outside the pre-normalization range limits, and reports of all errors encountered during run. An example of a Summary Report is shown in Figure 4.44.

   The summary report will be named with the following convention (example):
   SummaryReport_TP_0001_3_21_2006_6_14_52_PM.rtf
   SummaryReport: type of report
   TP_0001: type of run
   3_21_2006: date of run
   6_14_52_PM: time run started

2. Yield **Data Summary**: The Yield Data Summary is found in the folder c:\Affymetrix\Reports\Data. This report captures the background subtracted A260 and A280 readings, A_{260}/A_{280} ratio, concentrations, yields, and volumes for both the Pre and Post Normalization process.

   The naming convention for the Data Summary report is (example):
   ODs_TP_0001_3_21_2006_6_14_52_PM.txt
   ODs: indicates yield data report
   TP_0001: type of run
   3_21_2006: date run started
   6_14_52_PM: time run started

3. Data **Summary**: The Data Summary report is found in the folder c:\Affymetrix\Reports\Data. It contains the same information as the Main Summary report. However, this report is in a tab delimited text file useful for data upload to many LIMS systems.

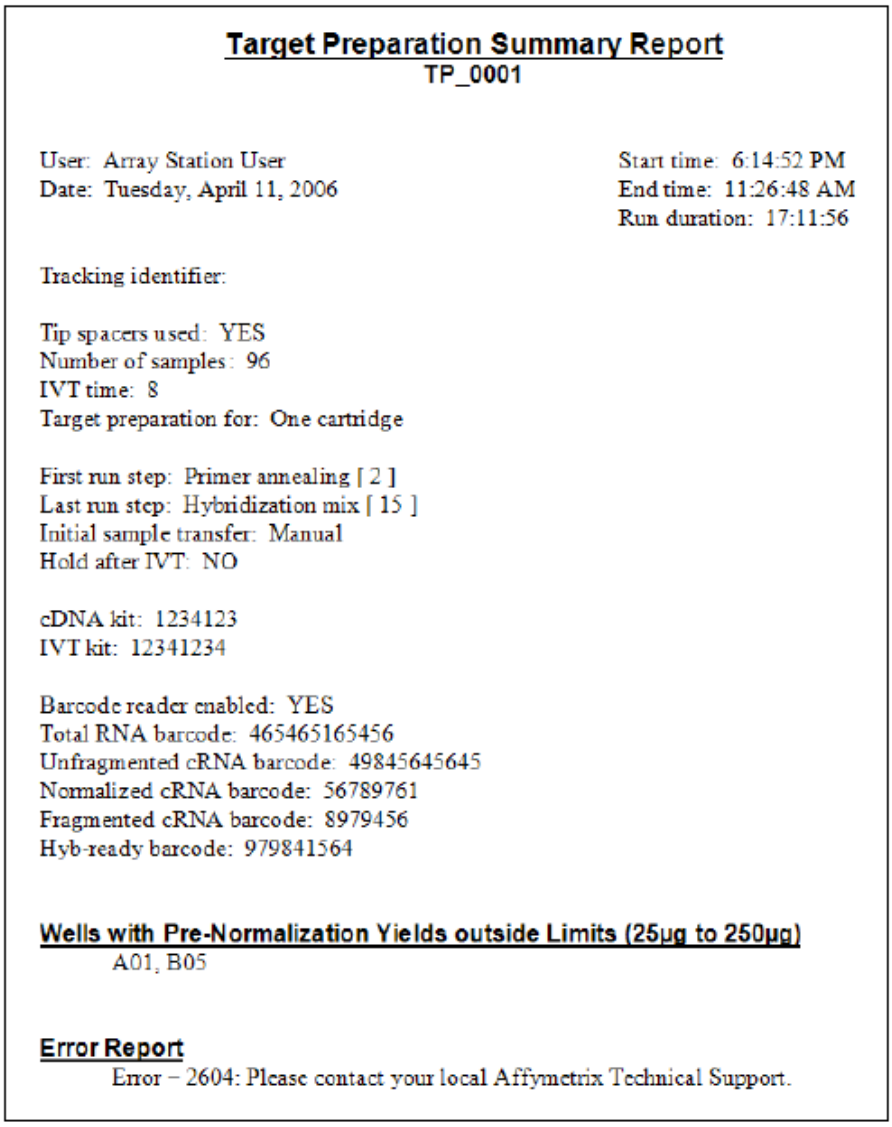
   The naming convention for the Data Summary report is (example):
   SummaryReport_TP_0001_3_21_2006_6_14_52_PM.txt

   SummaryReport: type of report

   TP_0001: type of run

   3_21_2006: date run started

   6_14_52_PM: time run started



Target Preparation Summary Report
TP_0001

User: Array Station User
Date: Tuesday, April 11, 2006

Start time: 6:14:52 PM
End time: 11:26:48 AM
Run duration: 17:11:56

Tracking identifier:

Tip spacers used: YES
Number of samples: 96
IVT time: 8
Target preparation for: One cartridge

First run step: Primer annealing [2]
Last run step: Hybridization mix [15]
Initial sample transfer: Manual
Hold after IVT: NO

cDNA kit: 1234123
IVT kit: 12341234

Barcode reader enabled: YES
Total RNA barcode: 465465165456
Unfragmented cRNA barcode: 49845645645
Normalized cRNA barcode: 56789761
Fragmented cRNA barcode: 8979456
Hyb-ready barcode: 979841564

Wells with Pre-Normalization Yields outside Limits (25µg to 250µg)
A01, B05

Error Report
Error – 2604: Please contact your local Affymetrix Technical Support.

Figure 4.44 Example of Final Summary Report for a Target Preparation Run

GeneChip™ Array Station Clean Up

After completion of the target preparation, follow the steps below to clean up the Array Station.

1. Cover the unfragmented, normalized, and fragmented RNA with an adhesive plate sealer. Use the plate roller to securely cover the sealer on the plate to prevent evaporation.
2. Store the sealed plates at –20°C.
3. Dispose of the EtOH, water, pipette tips, and waste water appropriately.
4. Wipe up any spills that may have occurred.
5. Close the GeneChip™ Array Station Software.
6. Check and refill Z8 water reservoir level.

Chapter 5 Hybridization, Wash and Stain Kit Protocol

Introduction

This chapter is for users of the GeneChip™ HT Hybridization, Wash, and Stain Kit (P/N 901219).

IMPORTANT: For customers preparing their own hybridization, wash, and stain reagents, refer to Chapter 6.

In order to distinguish kit reagents from self-prepared reagents a naming and formatting convention is utilized in this chapter. All GeneChip™ HT Hybridization, Wash, and Stain Kit reagents are preceded with the letters "HT" and are formatted with an italic font. For example:

Pour 20 mL of the *HT Pre-Hybridization Mix* into the VWR Omnitray.

This chapter is divided into three main sections:

- *Section 1: Hybridization Setup*
- *Section 2: Hybridization*
- *Section 3: Wash & Stain*

Figure 5.1 Components of the GeneChip™ HT Hybridization Wash and Stain Kit

Reagents and Materials Master List for Hybridization, Wash and Stain

The following is a comprehensive list of the reagents and materials required for this entire chapter. Each subsection in this chapter will define the specific reagents and materials used in that section.

All reagents and materials listed have been tested and evaluated. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- GeneChip™ HT Hybridization, Wash, and Stain Kit (P/N 901219). Kit includes:
- Box 1 of 2:
  - *HT Pre-Hybridization Mix* (sufficient for 1 x 96 HT array plate)
  - *HT 1.2X Hybridization Mix* (sufficient for 1 x 96 HT array plate)
  - *HT Stain Cocktail 1 & 3* (sufficient for 1 x 96 HT array plate)
  - *HT Stain Cocktail 2* (sufficient for 1 x 96 HT array plate)
  - *HT Array Holding Buffer* (sufficient for 1 HT array plate run)
- Box 2 of 2:
  - *HT Wash Buffer A* (sufficient for 1 HT array plate run)
  - *HT Wash Buffer B* (sufficient for 1 HT array plate run)

IMPORTANT: For users of GeneChip™ HT 24-array plate products, additional HT Wash Buffers A and B (P/N 901220) and HT Array Holding Buffer.

(P/N 901218) will need to be purchased for each run of additional plates on GCAS. For more information, please see Table 5.1.

- GeneChip™ HT Wash Buffer A and HT Wash Buffer B (P/N 901220)[1]
- GeneChip™ *HT Array Holding Buffer* (P/N 901218)[1]
- De-ionized water
- DNAZap: Ambion™, 9890
- RNaseZap: Ambion™, 9786
- HT Hybridization Tray[2] (qty. 1): P/N 202111 (may be ordered separately)
- HT Wash Tray[2] (qty. 5), P/N 900752(may be ordered separately)
- HT Stain Tray[2] (qty. 4): P/N 900745 (may be ordered separately)
- HT Scan Tray[2] (qty. 1), P/N 900746 (may be ordered separately)
- 96-Well Hard-Shell PCR Plate (qty. 2): Bio-Rad, P/N HSP-9601

[1] Available for purchase separately. For users of GeneChip™ HT 24-array plate products, additional HT Wash Buffers A and B and HT Array Holding Buffer need to be purchased for each run of additional plates on GCAS.

[2] HT Hybridization, Wash, Stain and Scan Trays are packaged with the HT Array Plates.

- Omnitray (qty. 1): VWR, P/N 4660-638 - or - Greiner Round Bottom Clear Polypropylene Plate (qty. 1): E&K Scientific Products, P/N 20261 (for Hyb Setup)
- Omnitray (qty. 2): VWR International 4660-638 (for Wash & Stain)
- Round Bottom Clear Polypropylene Plate(qty. 1): E&K Scientific Products, 20261
- Quarter Reservoir Modules (qty. 2): Beckman Coulter, 372790
- Quarter Reservoir Frame (qty. 1): Beckman Coulter, 372795
- Stacker Tips 200 µL non-sterile (qty. 3): Caliper LifeSciences, P/N 78642
- Stacker Tips 200 µL non-sterile (1 box): Caliper LifeSciences, 78641
- BD Falcon™ Test Tube, 14 mL (qty. 2): VWR International 60819-761
- BD Falcon™ Test Tube, 5 mL (or equivalent) (qty. 1): VWR International 60819-728
- Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL (qty. 2): VWR International, 20171-028
- RNase-Free 1.5 mL Microfuge tube (qty. 1): Ambion, 12400
- Aluminum Foil Lids: Beckman Coulter, 538619

Table 5.1 Hybridization, Wash, and Stain Reagent Purchasing Scenarios for GCAS Plates

Run Size	Reagents Required	P/N
1 x HT 96-Array Plate	GeneChip™ HT Hybridization, Wash, and Stain Kit	901219
4 x 24-Array Plate	1 x GeneChip™ HT Hybridization, Wash, and Stain Kit 3 x GeneChip™ HT Wash Buffer A and HT Wash Buffer B 3 x GeneChip™ HT Array Holding Buffer	901219 901220 901218

Section 1: Hybridization Setup

Introduction

This section describes the GeneChip™ Array Station Hybridization Setup protocol for HT Array Plates using GeneChip™ HT Hybridization, Wash, and Stain Kit reagents. A schematic of the automated Hybridization Setup run is provided to outline the steps in the procedure (Figure 5.2).

Additionally, this section provides details on how to set up reagents on the Array Station Deck, and how to use the GeneChip™ Array Station Software to hybridize a HT Array Plate.

IMPORTANT: This chapter is for customers who have purchased the preformulated and validated GeneChip™ HT Hybridization, Wash, and Stain Kit. A protocol is also provided for users wishing to prepare their own hybridization, wash, and stain reagents. Please refer to Chapter 6 for information on this protocol.

Reagents and Materials for Hybridization Setup

The following reagents and materials required in the Hybridization Setup protocol have been tested and evaluated. Information and part numbers listed below are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- From the GeneChip™ HT Hybridization, Wash, and Stain Kit, P/N 901219:
  - HT Pre-hybridization Mix – one 20 mL bottle
- HT Hybridization Tray[1] (qty. 1): P/N 202111 (may be ordered separately)
- HT Stain Tray[1] (qty. 1): P/N 900745 (may be ordered separately)
- 96-Well Hard-Shell PCR Plate (qty. 1): Bio-Rad, P/N HSP-9601
- Omnitray (qty. 1): VWR, P/N 4660-638 - or - Greiner Round Bottom Clear Polypropylene Plate (qty. 1): E&K Scientific Products, P/N 20261
- Stacker Tips 200 µL non-sterile (qty. 3): Caliper LifeSciences, P/N 78642
- DNAZap: Ambion, 9890
- RNaseZap: Ambion, 9786
- De-ionized water

1 Hybridization and Stain Trays are packaged with the HT Array Plates.

Automated Hybridization Setup Schematic

Figure 5.2 summarizes the steps of the Hybridization Setup protocol.

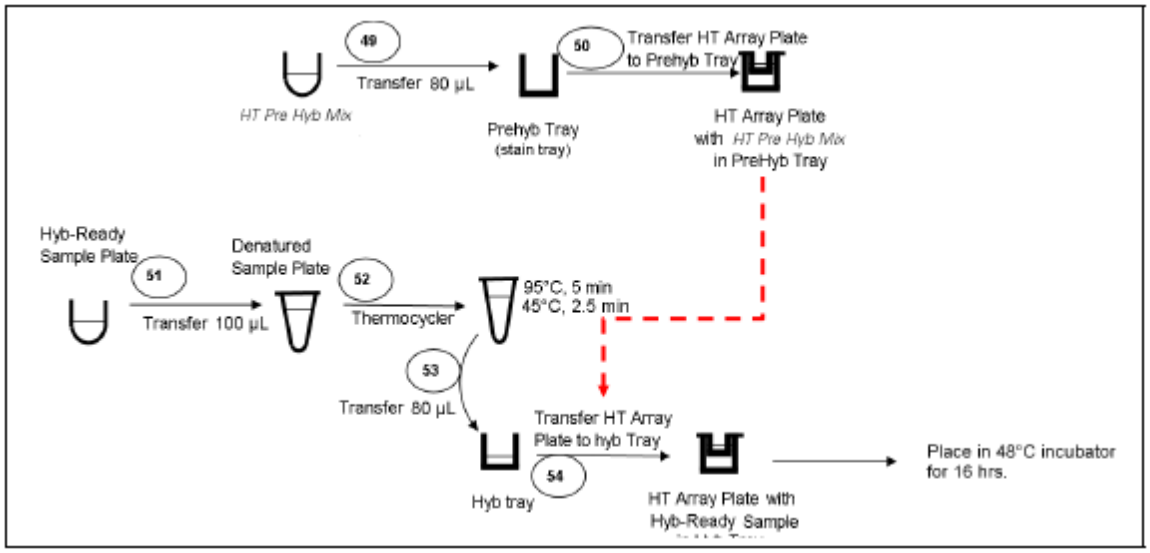


Figure 5.2 Steps 49 to 54: Hybridization Setup and Hybridization

HT Pre-Hybridization Mix for HT Array Plates

NOTE: If processing a 96 array plate, a Greiner Polypropylene U-bottom Plate or a VWR Omnitray can be used in position B3 (see Figure 5.3) to hold the *HT Pre-Hybridization Mix*. However, if processing a 24-array plate, a Greiner Polypropylene U-bottom Plate must be used.

If using a Greiner Polypropylene U-bottom Plate at position B3:

1. Aliquot 100 µL of the *HT Pre-Hybridization Mix* into each well of the Greiner Polypropylene U-bottom Plate.

NOTE: If processsing a 24-array plate, add *HT Pre-Hybridization Mix* only into columns 5, 7, & 9 of the U-bottom plate.

If using a VWR Omnitray at position B3:

1. Pour 20 mL of the *HT Pre-Hybridization Mix* into the VWR Omnitray.

WARNING: Use good lab practices when handling TMAC-containing solutions. Please consult the appropriate MSDS for reagent storage and handling requirements.

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.

Wipe the pad with RNaseZap.

Rinse the pad with DI Water.

Wipe the pad with DNAZap.

Thoroughly rinse the pad with DI Water.

Dry the pad with pressurized clean air or nitrogen.

Beginning a Run – Deck Layout for Hybridization Setup

This section describes how to assemble the deck and use the GeneChip™ Array Station Software to begin a Hybridization Setup run. The Hybridization Setup protocol will perform the following methods:

- Pre-hybridize the HT Array Plates
- Denature the sample for hybridization
- Assemble the HT Array Plate and the Hybridization Tray containing the hybridization-ready sample

Procedure - Assembling the Deck

1. Setup the deck with the appropriate consumables. Refer to Figure 5.3.

Load at least four tip boxes into Rack 1.

Remove the cold reagent block.

Place the hybridization-ready samples on the deck at location C2.

NOTE: If using a HT 24-Array Plate, it is NOT necessary to re-array the samples into columns 5, 7 and 9. Samples should be in columns 1, 2, and 3 of the Greiner plate. The Array Station will transfer samples to columns 5, 7, and 9 during the protocol.

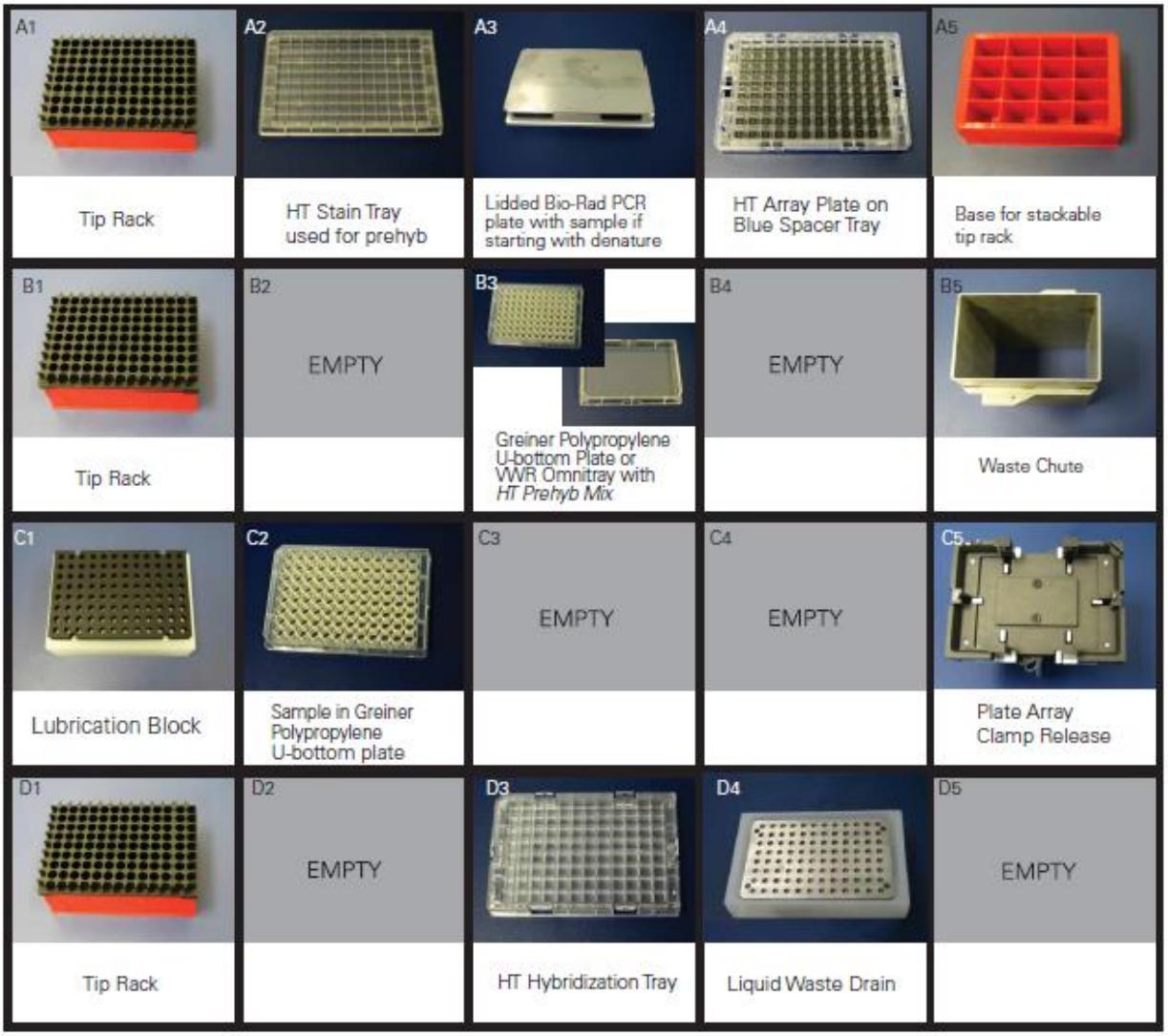


Figure 5.3 The deck layout for the Hybridization Setup protocol.

Load four tip boxes into Rack 1.

Empty Rack 2, leaving the tip rack base there.

NOTE: If you are using recovered samples and your samples are already in the Bio-Rad 96-Well plate, you will need to leave position C2 empty. Your samples will be in the Bio-Rad plate placed at A3.

Running the Hybridization Setup Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.
2. Open **the** software by double clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The **login** window appears (Figure 5.4).

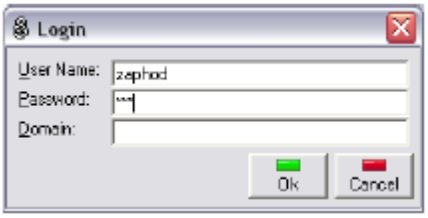


Figure 5.4 Sciclone Workstation Software Login window

3. Enter your **User Name** and **Password** and click **OK**.

   The Operator Runtime interface window appears (Figure 5.5).

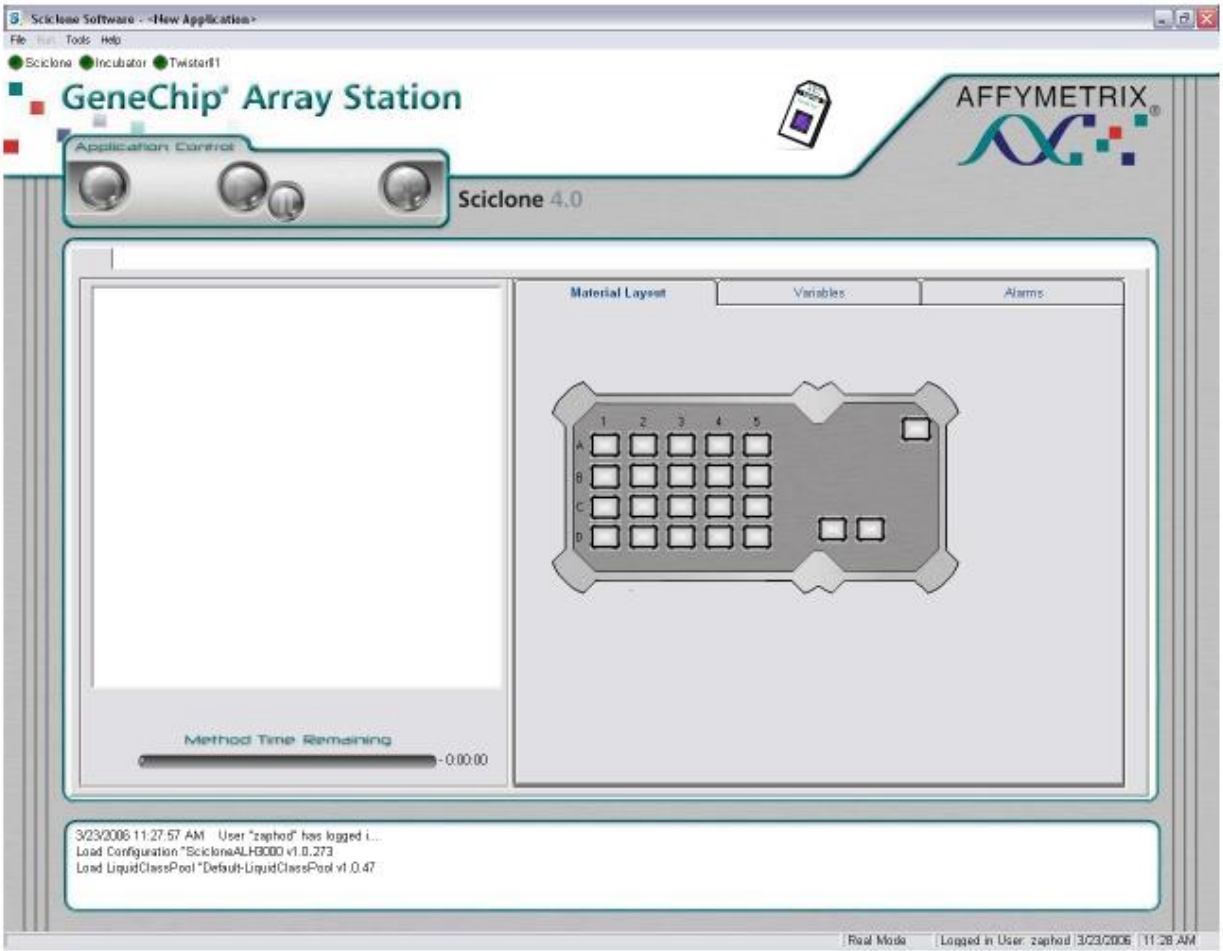


Figure 5.5 Sciclone Workstation Software Operator window

4. Select **File → Open** to access the load application window (Figure 5.6).

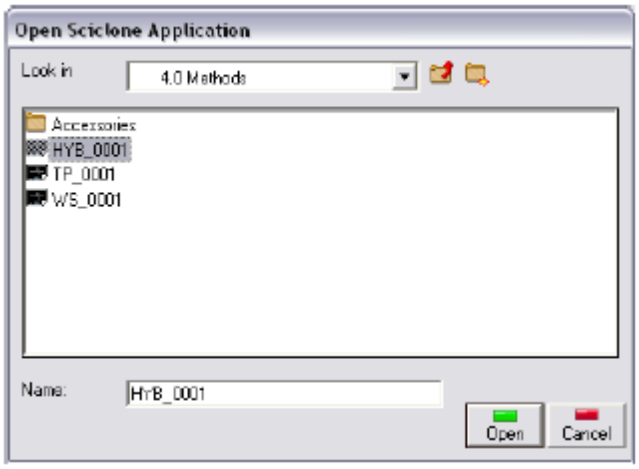


Figure 5.6 Open Sciclone Application window

5. Select **HYB_0001** and click **Open**.

   The hybridization setup application is loaded into Sciclone Workstation Software.

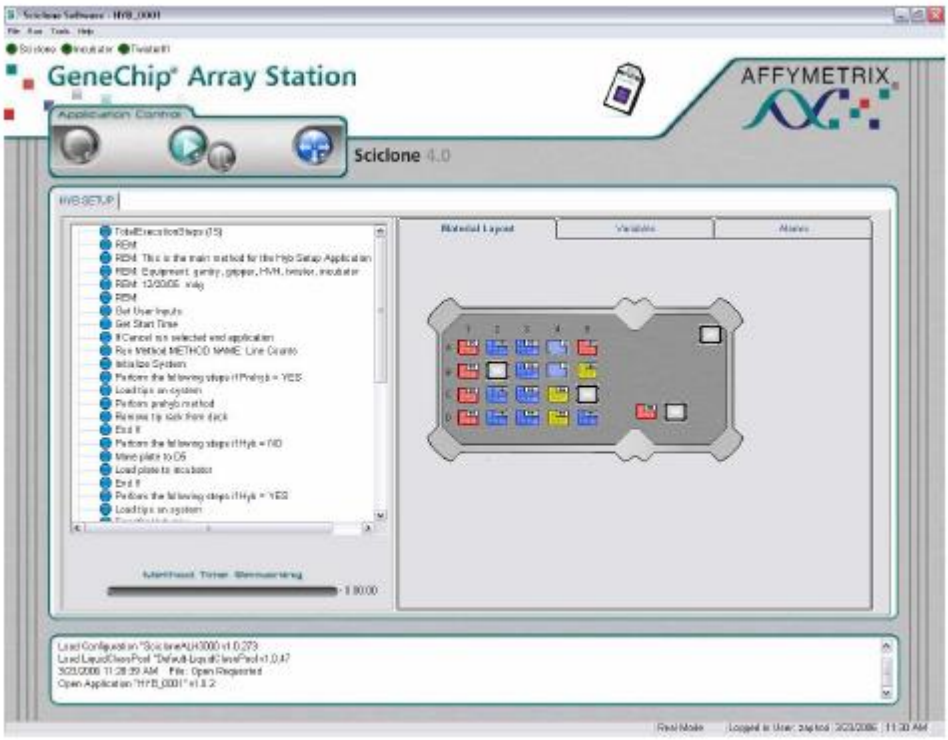


Figure 5.7 Hybridization Setup Protocol Loaded

6. Click the green **Start** arrow to begin the application.

   The application will prompt you to select several options through the interactive window shown in Figure 5.8.

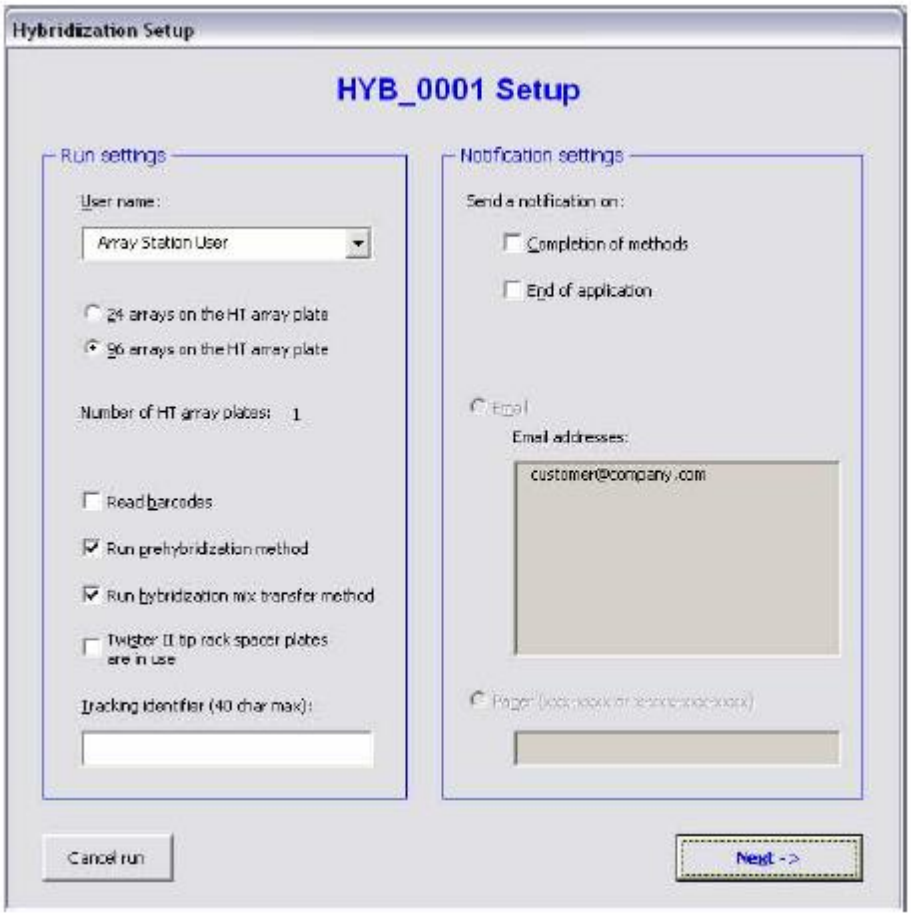


Figure 5.8 Hybridization Setup window

7. Select the protocol parameters.
    a. Select your **User name** from the drop-down menu.
    b. Select either 24 or 96 well HT array plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.
    c. If you wish the protocol to read and store the sample plate barcode and the HT array plate barcode, check the Read barcodes option. Barcodes will be reported in the user summary at the end of the run.
    d. Select Run prehybridization method.
    e. Selection of the Run hybridization mix transfer method depends on if your samples are in the Greiner plate or are recovered samples in the Bio-Rad plate.
        1) If **Run hybridization mix transfer method** is selected, the Array Station will transfer the hyb-ready sample from a Greiner U-bottom Plate at C2 to a Bio-Rad Plate at A3. If you are running a HT 24-Array Plate, your samples should be in columns 1, 2, and 3 of the Greiner plate. The Array Station will automatically transfer these samples to columns 5,7, and 9 of the hybridization tray to match the HT 24-Array Plate.
        2) If **Run hybridization mix transfer method** is NOT selected, it is required that 100 µL of Hyb-Ready sample is placed in a Bio-Rad Plate at position A3. If you are running a HT 24-Array Plate, your samples should be in columns 1, 2, and 3 of the Bio-Rad plate. The Array Station will automatically transfer these samples to columns 5, 7, and 9 of the hybridization tray to match the HT 24-Array Plate.

NOTE: In most cases you will select and run the hybridization mix transfer method. However, if you are re-hybridizing samples recovered from a previous hybridization, your samples will already be in a Bio-Rad Plate. You should select NOT to run the hybridization mix transfer if your samples are in the Bio-Rad Plate.

f. If you are using spacer plates for tip static control (recommended), check the box **Twister tip rack spacer plates are in use**. If this box is checked, ensure you have placed spacer plates between each nested tip rack and on the top rack.

g. Enter a **Tracking identifier** (limited to 40 characters) if desired. The **Tracking identifier** will be recorded in the Hybridization Setup Summary Report at the end of the run (refer to Figure 5.12 for a description of the Summary Report).

h. Select the desired notification settings. Be sure the email address or pager information is properly configured.

8. After selecting the settings parameters, click **Next**.

You will see one of the following windows, depending on whether you have selected

Run hybridization mix transfer method

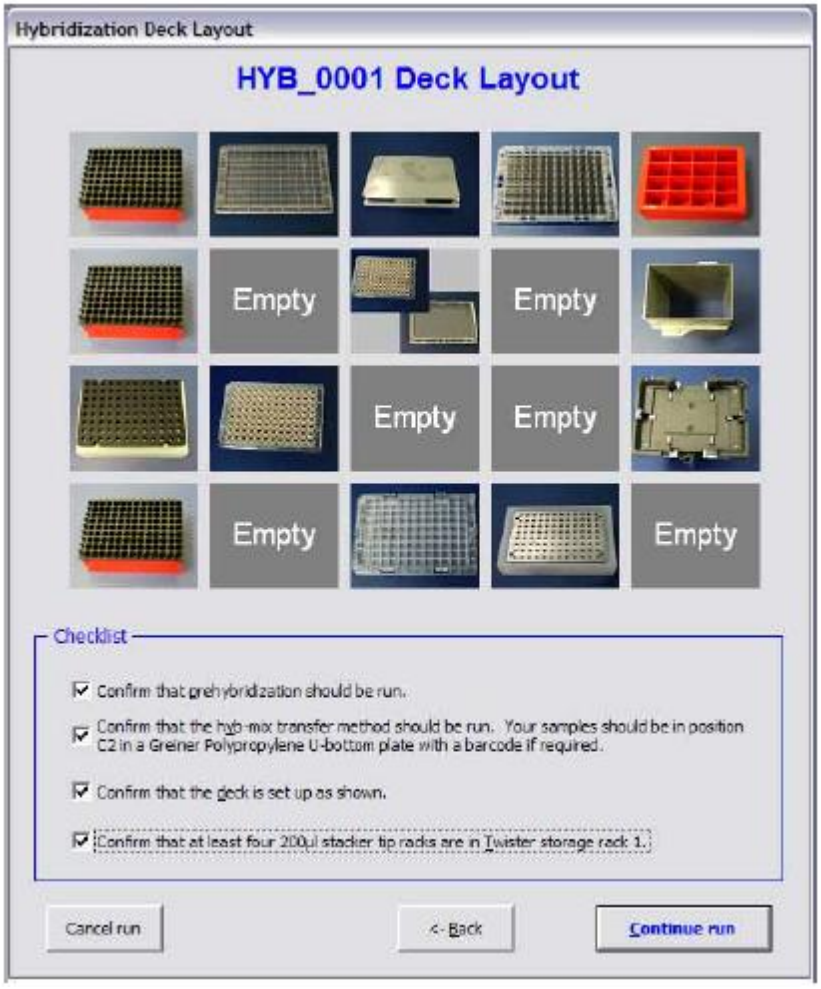


Figure 5.9 Hybridization Deck Layout when Run hybridization mix transfer method is selected

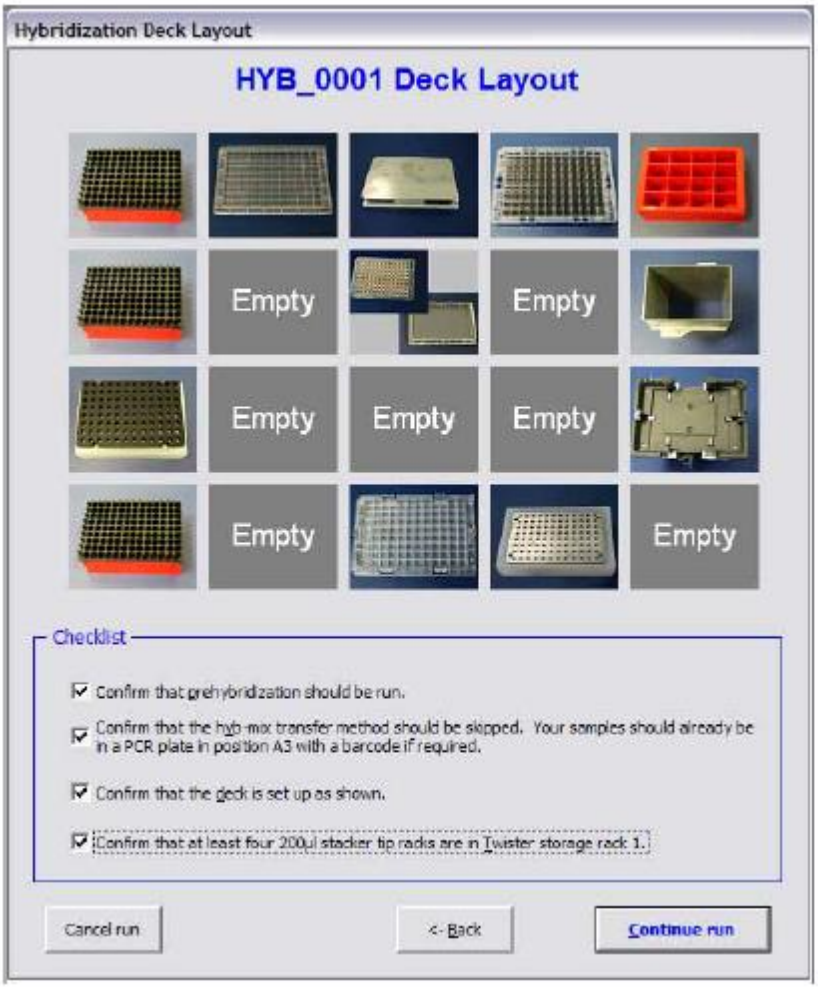


Figure 5.10 Hybridization Deck Layout when Run hybridization mix transfer method is NOT selected

9. Verify that you have configured the deck properly and that you have completed and checked the items on the checklist. Click **Continue** and the Hybridization Setup protocol continues until the HT Array Plate/Hybridization Tray Sandwich is assembled.
10. At the end of the protocol, The End message appears (Figure 5.11). Click **OK**.

Figure 5.11 Run Complete message

You must place the HT Array Plate hybridization sandwich in an incubator equilibrated to 48°C. Please refer to *Section 2: Hybridization*.

11. At the conclusion of each run, two summary reports are automatically saved by the program.

    A user readable summary of the run is saved to the folder C:\Affymetrix\Reports\Summary. This file has the

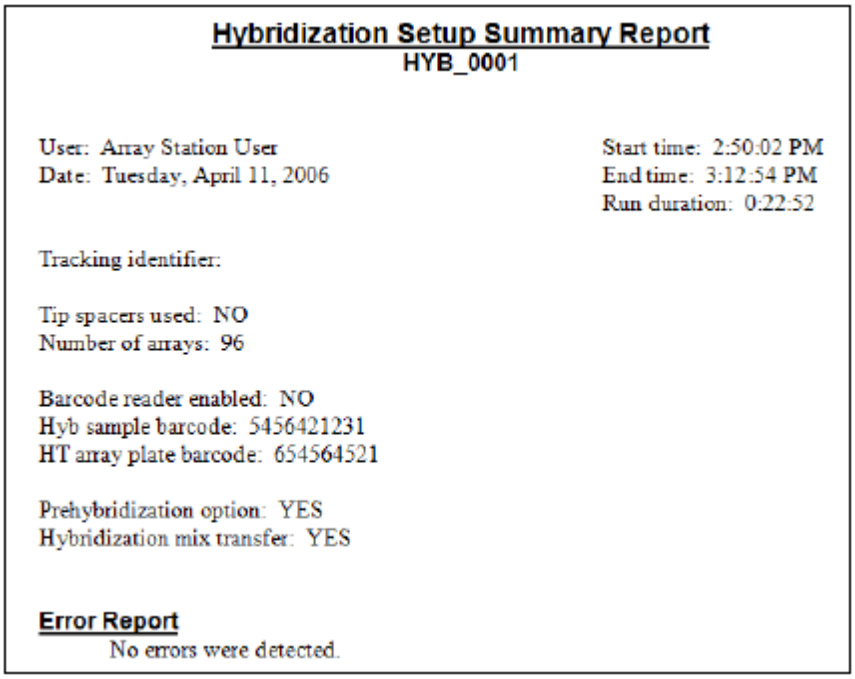
following naming convention:

SummaryReport_HYB_0001_3_23_2006_10_03_36_AM.rtf

HYB_0001 = method used

3_23_2006 = date stamp of when the HYB_0001 starts

10_03_36_AM = time stamp of when the HYB_0001 starts Figure 5.12 shows an example of a Hybridization Setup Summary Report.



Hybridization Setup Summary Report
HYB_0001

User: Array Station User
Date: Tuesday, April 11, 2006

Start time: 2:50:02 PM
End time: 3:12:54 PM
Run duration: 0:22:52

Tracking identifier:

Tip spacers used: NO
Number of arrays: 96

Barcode reader enabled: NO
Hyb sample barcode: 5456421231
HT array plate barcode: 654564521

Prehybridization option: YES
Hybridization mix transfer: YES

Error Report
No errors were detected.

Figure 5.12 Hybridization Setup Summary Report Example

12. A tab-delimited text report useful for transferring data to many LIMS systems is also saved. This report is saved in the folder C:\Affymetrix\Reports\Data. An example if this report is shown in Figure 5.13.

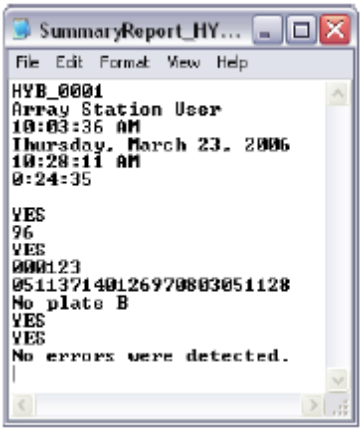


Figure 5.13 LIMS Summary Report Example

13. The HT Array Plate/Hybridization Tray Sandwich is ready to be placed into a 48°C incubator for 16 hours.

CAUTION:. Take care in transferring the Hyb Sandwich to the hybridization oven. The sandwich should remain close to level to prevent sample contamination.

Section 2: Hybridization

This section describes how to incubate a HT Array Plate/Hybridization Tray Sandwich for hybridization.

Overnight Hybridization

1. Remove the HT Array Plate/Hybridization Tray Sandwich from the Array Station, taking care to keep the sandwich level, and place in the hybridization oven.
2. Incubate the HT Array Plate/Hybridization Tray Sandwich for 16 hours at 48°C.

IMPORTANT: The hybridization oven should be monitored with a digital thermometer (such as VWR, P/N 23226-656) to ensure that the temperature is stabilized at 48°C.

IMPORTANT: The HT Array Plate/Hybridization Tray Sandwich must remain level during hybridization. A bubble level can be placed on top of the sandwich to ensure that all four corners are level.

Section 3: Wash & Stain

Introduction

This section describes the GeneChip™ Array Station Wash and Stain protocol for HT Array Plates. A schematic of the automated Wash and Stain is provided to outline the steps of the procedure (Figure 5.21 to Figure 5.27). Also provided in this section are details on how to set up the Array Station deck and use the Sciclone 4.x software to perform the Wash and Stain protocol.

For convenience, prepackaged and ready-to-use components in the GeneChip™ HT Hybridization, Wash, and Stain Kit replace the previous reagent preparation protocols. *HT Wash Buffer A* is used for Low Stringency Wash, and *HT Wash Buffer B* is used for High Stringency Wash. These reagents have the same formulations as the previous low and high stringency wash buffers except for the addition of preservative. Pre-formulated *HT Stain Cocktails 1 & 3* and *HT Stain Cocktail 2* replace the previous stain cocktails of the same name.

The volumes contained in the *HT Stain Cocktail* bottles are sufficient to process 96 HT arrays and must be aliquoted appropriately when running either 24 or 96 HT array plates. *HT Wash Buffer A and B* are sufficient to process any single plate of either 24 or 96 HT arrays. For users processing additional 24 HT array plates, one additional *HT Wash Buffer A and B* (P/N 901220) and *HT Array Holding Buffer* (P/N 901218) must be purchased separately for each additional plate to be processed.

Reagents and Materials for Wash and Stain

The following reagents and materials required in the Wash and Stain protocol have been tested and evaluated. Information and part numbers listed below are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- From the GeneChip™ HT Hybridization, Wash, and Stain Kit (P/N 901219):
  - HT Wash Buffer A – one 2-L bottle
  - HT Wash Buffer B – one 150-mL bottle
  - HT Stain Cocktail 1 & 3 – one 22-mL bottle
  - HT Stain Cocktail 2 – one 13-mL bottle
  - HT Array Holding Buffer – one 20 mL bottle
- Quarter reservoir modules (qty. 2), Beckman Coulter P/N 372790
- Quarter reservoir frame, Beckman Coulter P/N 372795
- HT Wash Tray[1] (qty. 5), P/N 900752
- HT Stain Tray[1] (qty. 3), P/N 900745
- HT Scan Tray[1] (qty. 1), P/N 900746
- 96-Well Hard-Shell PCR Plate (qty 1): Bio-Rad, HSP-9601
- Omnitray (qty. 2): VWR International 4660-638
- Round Bottom Clear Polypropylene Plate(qty. 1): E&K Scientific Products, 20261
- Quarter Reservoir Modules (qty. 2): Beckman Coulter, 372790
- Quarter Reservoir Frame (qty. 1): Beckman Coulter, 372795

[1] HT Stain, Wash, and Scan Trays are packaged with the HT Array Plates.

- BD Falcon™ Test Tube, 14 mL (qty. 2): VWR International 60819-761
- BD Falcon™ Test Tube, 5 mL (or equivalent) (qty. 1): VWR International 60819-728
- Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL (qty. 2): VWR International, 20171-028
- RNase-Free 1.5 mL Microfuge tube (qty. 1): Ambion, 12400
- Stacker Tips 200 µL non-sterile (1 box): Caliper LifeSciences, 78641
- Aluminum Foil Lids: Beckman Coulter, 538619
- DNAZap: Ambion, 9890
- RNaseZap: Ambion, 9786
- De-ionized water

Wash and Stain Preparation

Prior to running the Wash and Stain protocol, deck preparation is required. This involves manually filling the appropriate consumable. The reagent setup must be performed prior to starting the Wash and Stain protocol.

New with this version of the manual is an automated Stain Dispensing Setup Protocol. Alternatively, you may perform this function manually.

- If you plan to use the automated Stain Dispensing Setup Protocol, please go to *Stain Dispensing Setup Protocol.*
- If you plan to perform the stain dispensing manually, please go to *Manual Stain Dispensing Setup*.

Stain Dispensing Setup Protocol

This version of the manual features a new Stain Dispensing Setup Protocol provided to facilitate efficiency of time, accuracy, and ease of use. This new step is an alternative option to manual dispensing and automates the aliquoting of stain reagents prior to the Wash and Stain protocol (WS_0001).

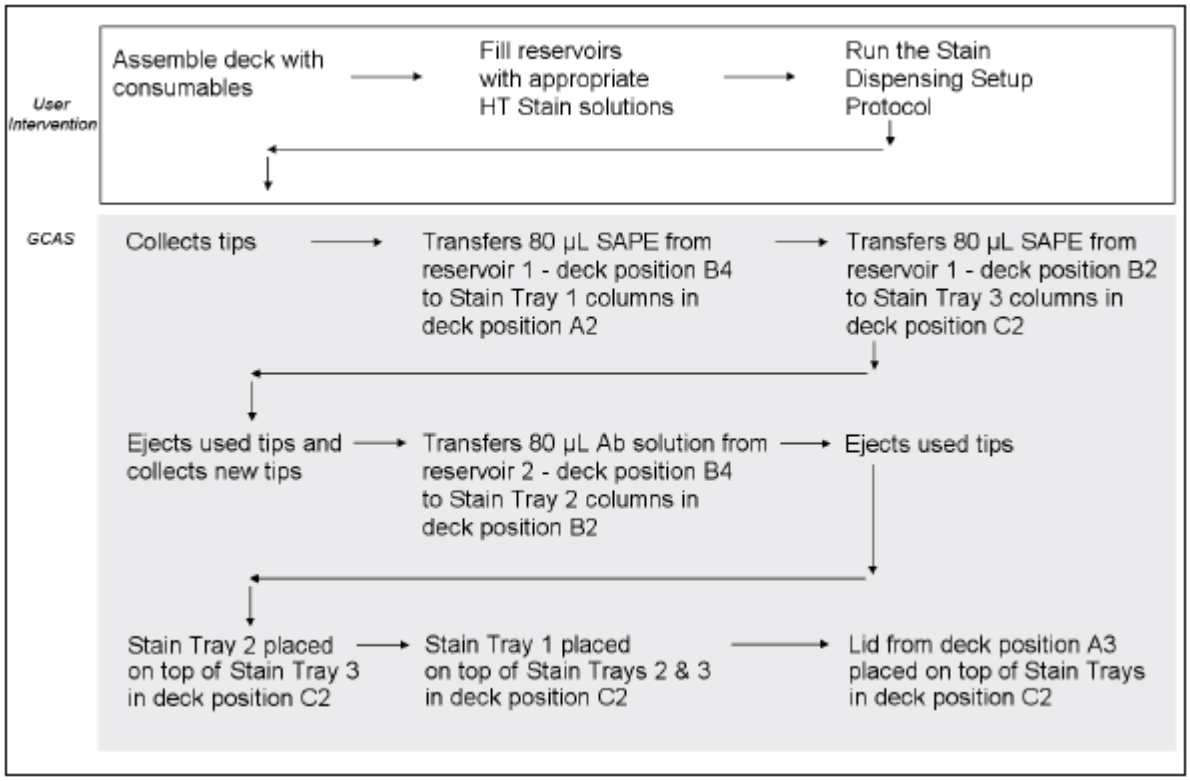


Figure 5.14 Stain Dispensing Setup Schematic

Procedure - Assembling the Deck for the Stain Dispensing Setup Protocol

1. Set up deck with appropriate consumables. Refer to Figure 5.15.

NOTE: The red tip rack at position A3 is covered with the flat lid.

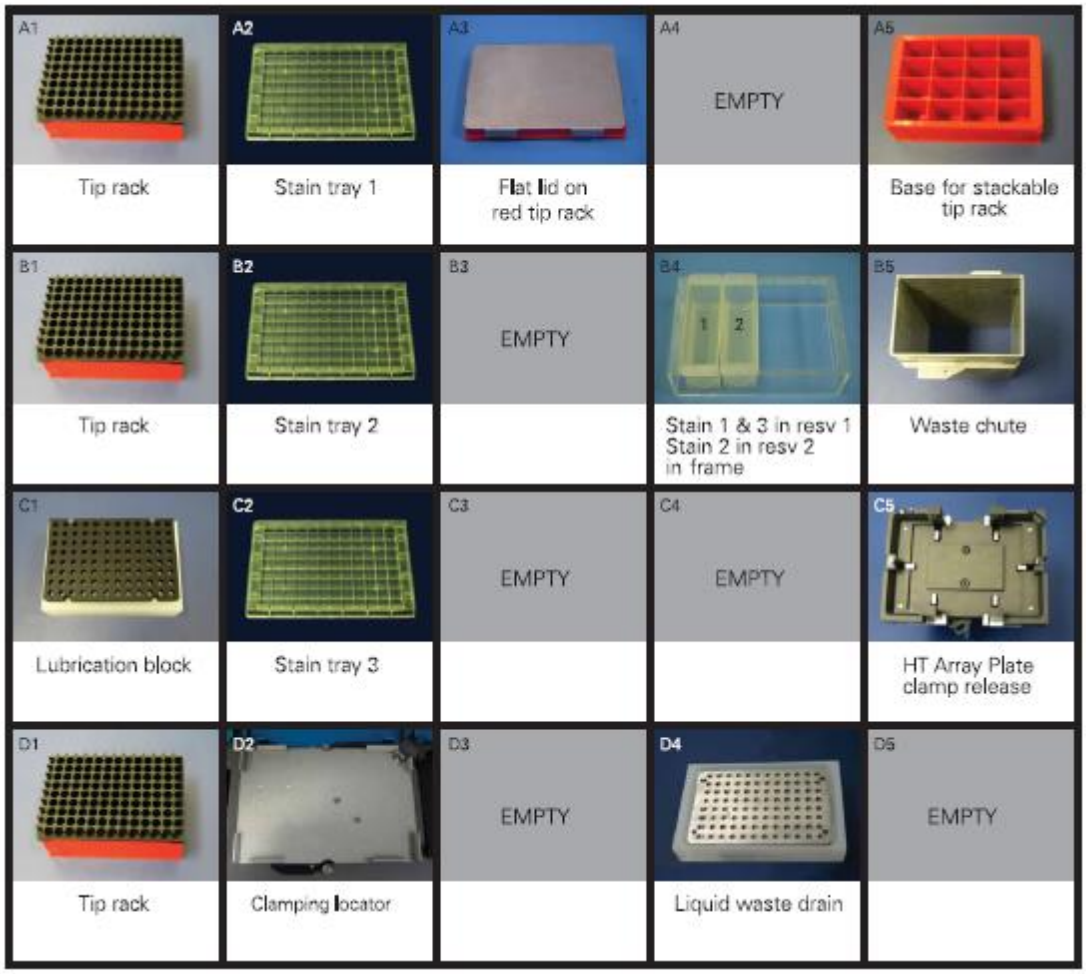


Figure 5.15 The deck layout for Stain Dispensing Setup

2. Fill the quarter reservoirs at position B4 as follows:
   a. If processing a 24-array plate, aliquot 5 mL HT Stain Cocktail 1 & 3 (SAPE) into the first quarter reagent reservoir at B4. Aliquot 3 mL for HT Stain Cocktail 2 (Ab) into the second quarter reservoir at B4.
   b. If processing a 96-array plate, pour the entire contents of HT Stain Cocktail 1 & 3 bottles (SAPE) into the first quarter reagent reservoir at B4. Pour the entire contents of the HT Stain Cocktail 2 bottle (Ab) into the second quarter reservoir at B4.
3. Load at least one tip box in Rack 1.
4. Place the buffer lines in correct liquid carboys.
   - The bulk dispenser buffer line should be placed in the carboy containing *HT Wash Buffer A*.
   - The Z8 buffer line should be placed in DI water.

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI Water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI Water.
6. Dry the pad with pressurized clean air or nitrogen.

Running the Stain Dispensing Setup Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.
2. Open the software by double-clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The login window appears **(**Figure 5.16**)**.

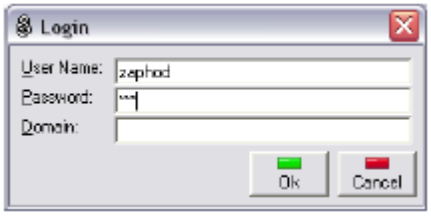


Figure 5.16 Sciclone Workstation Software Login window

3. Enter your **User name** and **Password** and click **OK**. The Operator Runtime interface appears **(**Figure 5.17**)**.

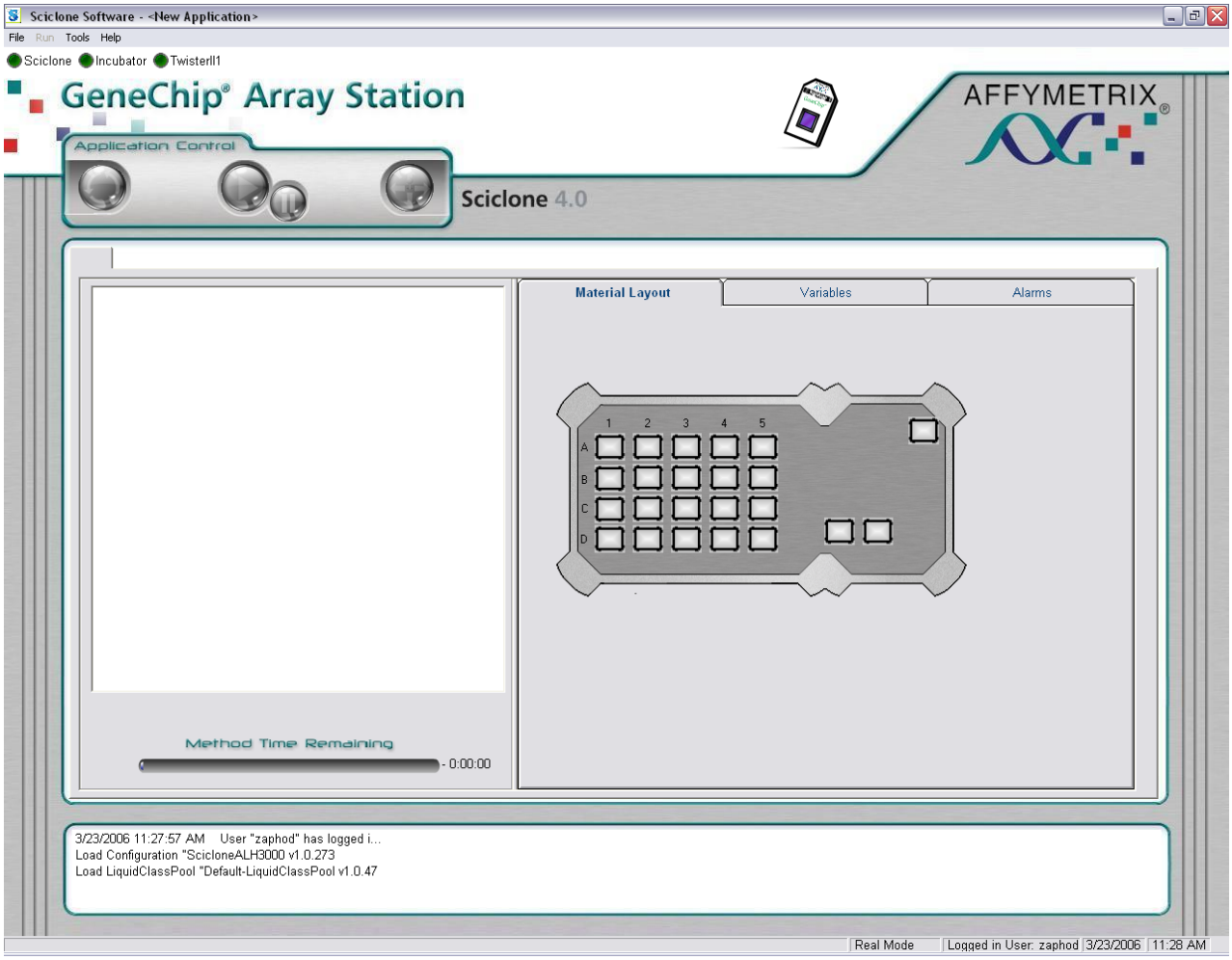


Figure 5.17 Sciclone Workstation Software Operator window

4. Select **File → Open** to access the load application window.
5. Select **Setup_0001** and click **Open**.

   The Stain Dispensing Setup application is loaded into Sciclone Workstation software (Figure 5.18).

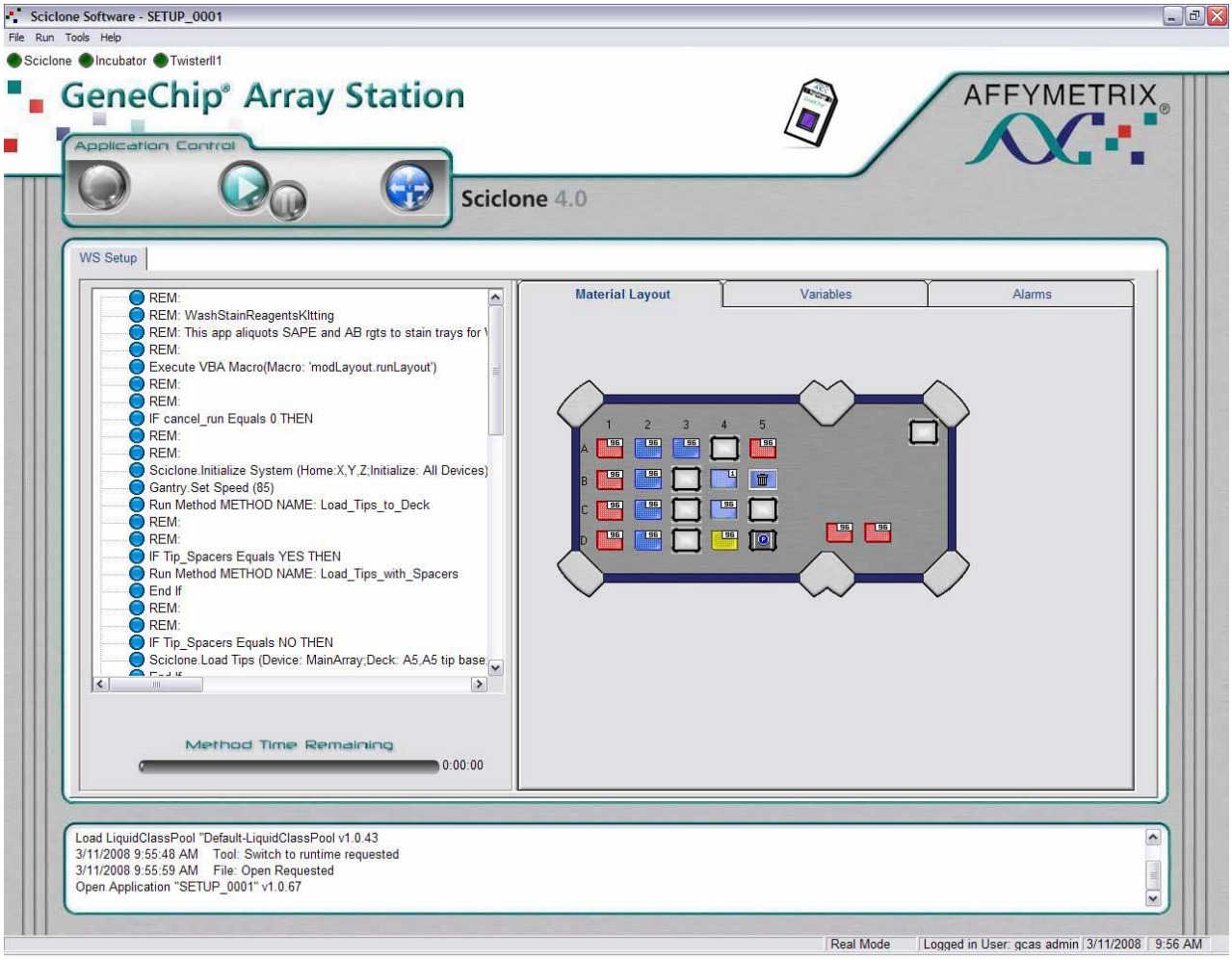


Figure 5.18 Wash and Stain Protocol Loaded

6. Click the green **Start** arrow to begin the application.

   The Application will prompt you to select several options through the interactive window shown in Figure 5.19

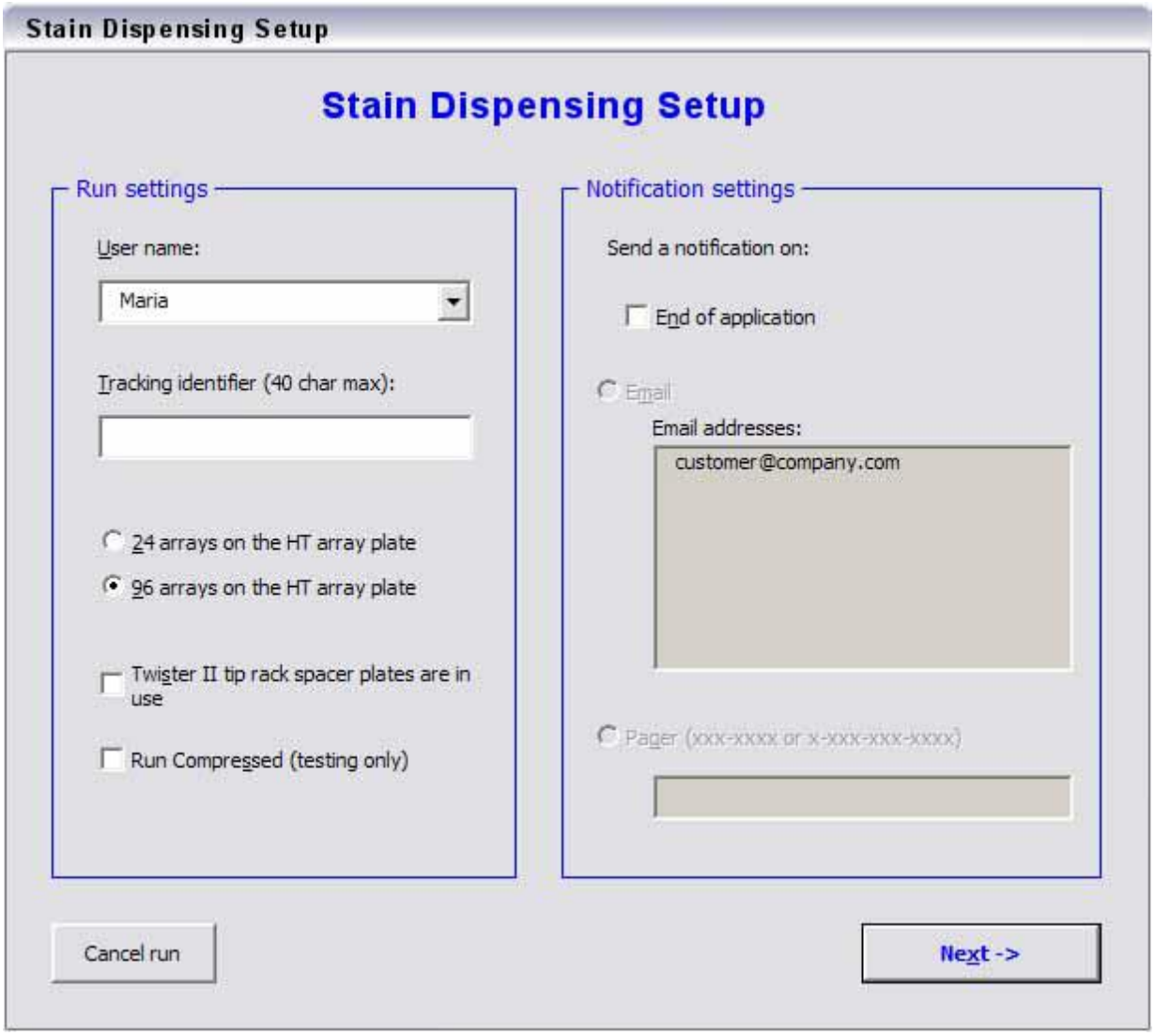


Figure 5.19 Stain Dispensing Setup window

7. Select the protocol parameters: Select either a HT 24- or 96-Array Plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.
8. Click Next.

   The Stain Dispensing Setup deck layout window appears (Figure 5.20).

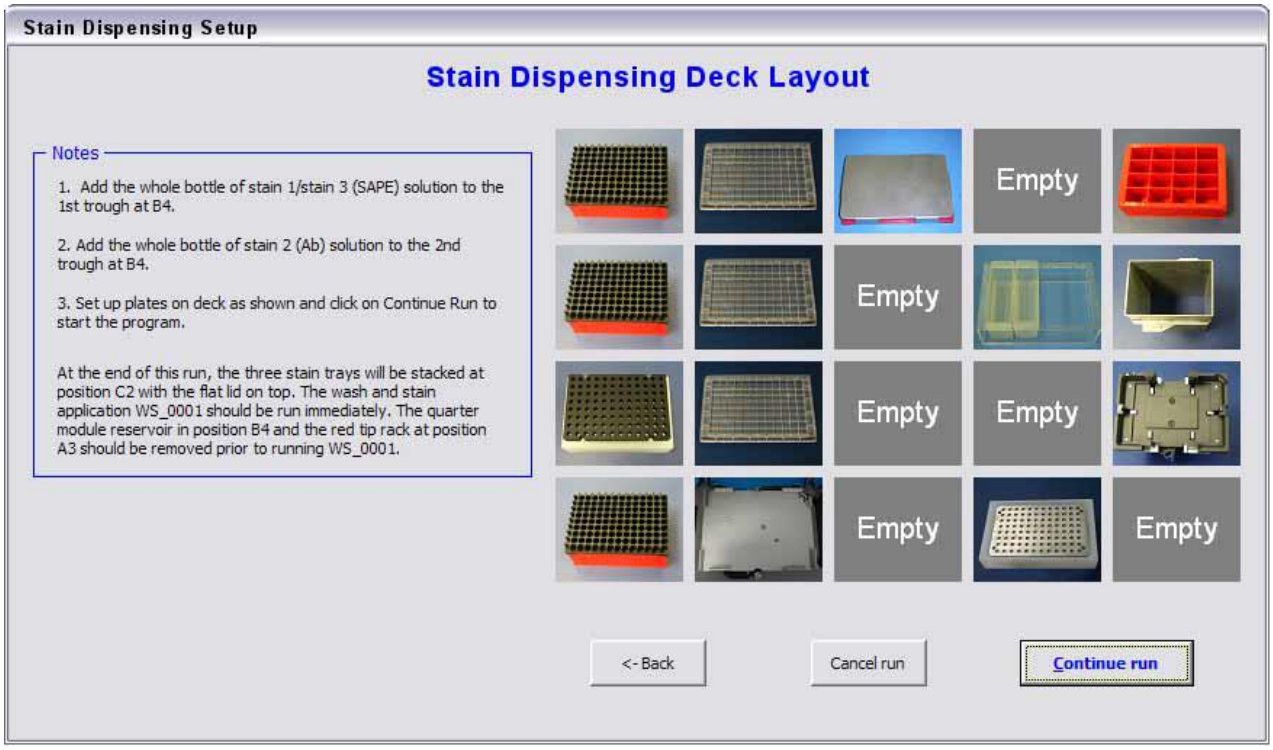


Figure 5.20 Stain Dispensing Setup Deck Layout

9. Verify that you have set the deck up properly and that you have completed all items in the Notes section.
10. Click Continue run and the Array Station will perform the Stain Dispensing Setup protocol.
11. When completed, remove the red tip rack at A3 and the reagent reservoirs at B4 before continuing on to the WS_0001 application (see *Beginning a Run – Wash and Stain Deck Layout*).

Manual Stain Dispensing Setup

HT Stain Cocktail 1 & 3

1. Obtain two HT Stain trays and label one “Stain 1” and the other “Stain 3.”

Aliquot 80 µL of *HT Stain Cocktail 1 & 3* into the appropriate wells of both labeled HT Stain Trays.

NOTE: If using a HT 24-Array Plate, the stain mixture should be aliquoted into columns 5, 7, and 9 of the HT Stain Trays.

HT Stain Cocktail 2

1. Obtain one HT Stain tray and label it “Stain 2.”
2. Aliquot 80 µL of *HT Stain Cocktail 2* into the appropriate wells of the labeled HT Stain Tray.

NOTE: If using a HT 24-Array Plate, the stain mixture should be aliquotted into columns 5, 7, and 9 of the HT Stain Trays.

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI Water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI Water.
6. Dry the pad with pressurized clean air or nitrogen.

Automated Wash and Stain Protocol

Automated Wash and Stain Schematic

Figure 5.21 through Figure 5.27 summarize the steps in the Wash and Stain protocol.

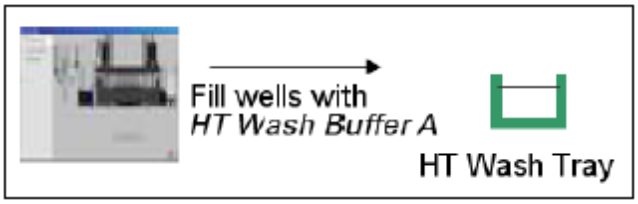


Figure 5.21 Step 1: Fill HT Wash Trays with *HT Wash Buffer A*

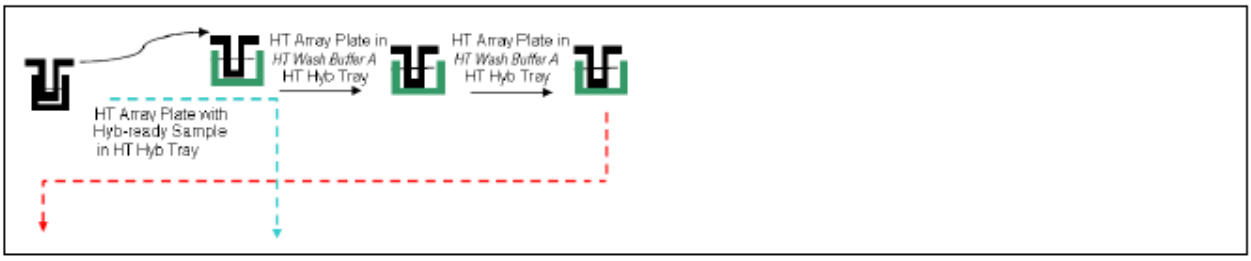


Figure 5.22 Step 2: Low Stringency Wash (LSW) using *HT Wash Buffer A*

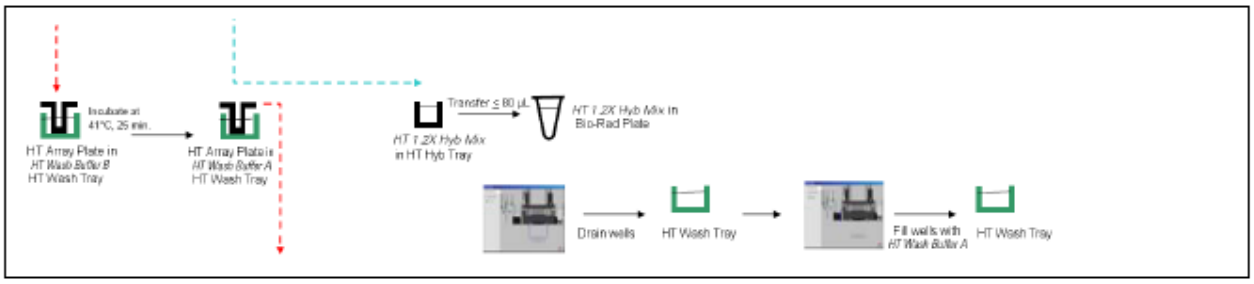


Figure 5.23 Step 3: High Stringency Wash (HSW) using *HT Wash Buffer B;* Transfer of Hybridization Cocktail; Drain/Refill HT Wash Trays

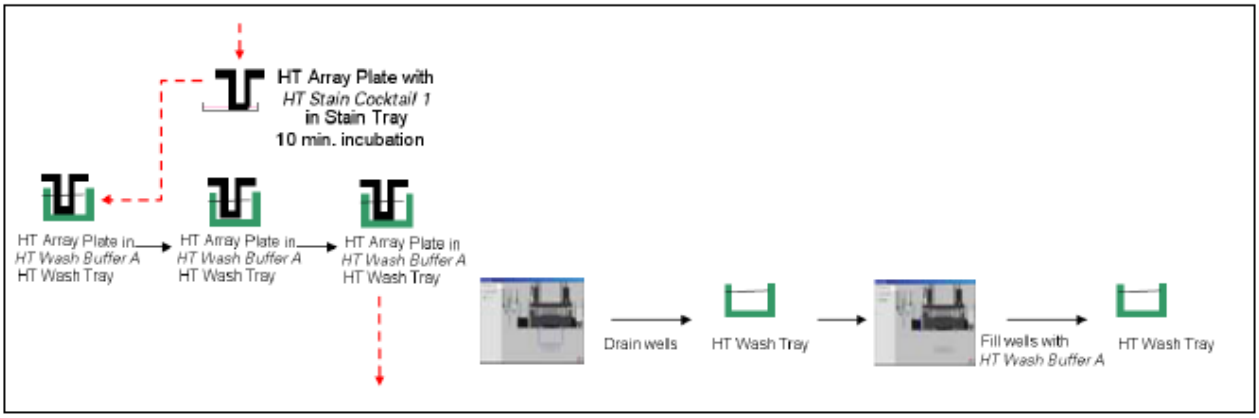


Figure 5.24 Step 4: First Stain (using *HT Stain Cocktail 1 & 3*); Low Stringency Wash; Drain/Refill HT Wash Trays

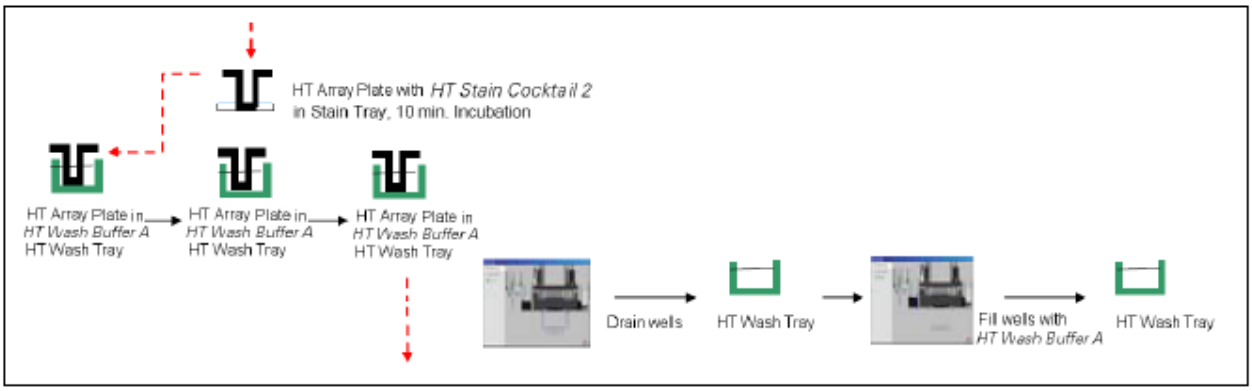


Figure 5.25 Step 5: Second Stain (using *HT Stain Cocktail 2*); Low Stringency Wash; Drain/Refill HT Wash Tray

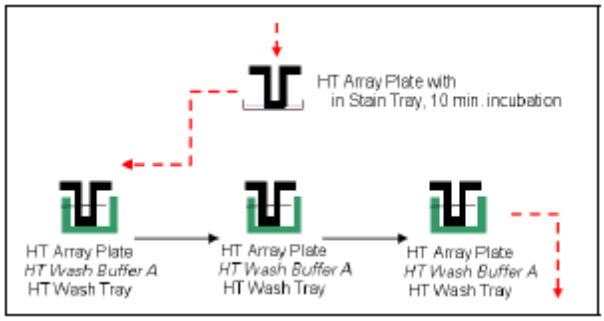


Figure 5.26 Step 6: Third Stain (using *HT Stain Cocktail 1 & 3*); Low Stringency Wash

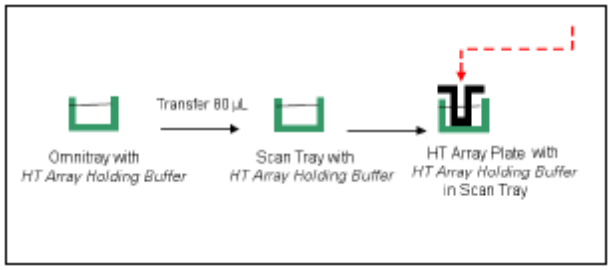


Figure 5.27 Step 7: Filling HT Scan Tray with *HT Array Holding Buffer* and Insertion of the HT Array Plate

Beginning a Run – Wash and Stain Deck Layout

This section describes how to assemble the deck and use the Sciclone Workstation Software to begin a Wash and Stain run. The Wash and Stain protocol will perform the following methods on the GeneChip™ Array Station:

- Wash the hybridized HT Array Plate in *HT Wash Buffer A and B*
- Stain the hybridized HT Array Plate
- Assemble the hybridized HT Array Plate for scanning

Wash and Stain Deck Layout Procedure

1. Set up deck with appropriate consumables. Refer to Figure 5.28.

NOTE: The HT Stain Trays containing Stain 1, Stain 2, and Stain 3 are stacked and covered with a flat lid. The three trays should be stacked in ascending order, with Stain 1 on top, and Stain 3 on bottom. Stain 1 is covered with the flat lid.

2. Load at least three tip boxes in Rack 1.
3. Assemble the Peltier adaptor and Hybridization Block Fixture. Refer to Figure 5.29 and Figure 5.29.
4. Set the temperature of the Watlow Temperature Controller to 48°C. Refer to Figure 5.30.

***TIP: HT Wash Buffer B* must be between 41 °C and 42 °C for the Wash and Stain protocol. During the Wash and Stain deck setup, *HT Wash Buffer B* is placed in a HT Wash Tray and placed on the Peltier adaptor to reach a temperature between 41 °C and 42 °C. The temperature adjustment between 41 °C and 42 °C takes a long time. To decrease this time requirement, place 100 mL of *HT Wash Buffer B* in a 48 °C incubator the night before so it is prewarmed.**

5. Place the buffer lines in correct liquid carboys.

- The bulk dispenser buffer line should be placed in the carboy containing HT Wash Buffer A bottle.
- The Z8 buffer line should be placed in DI water.

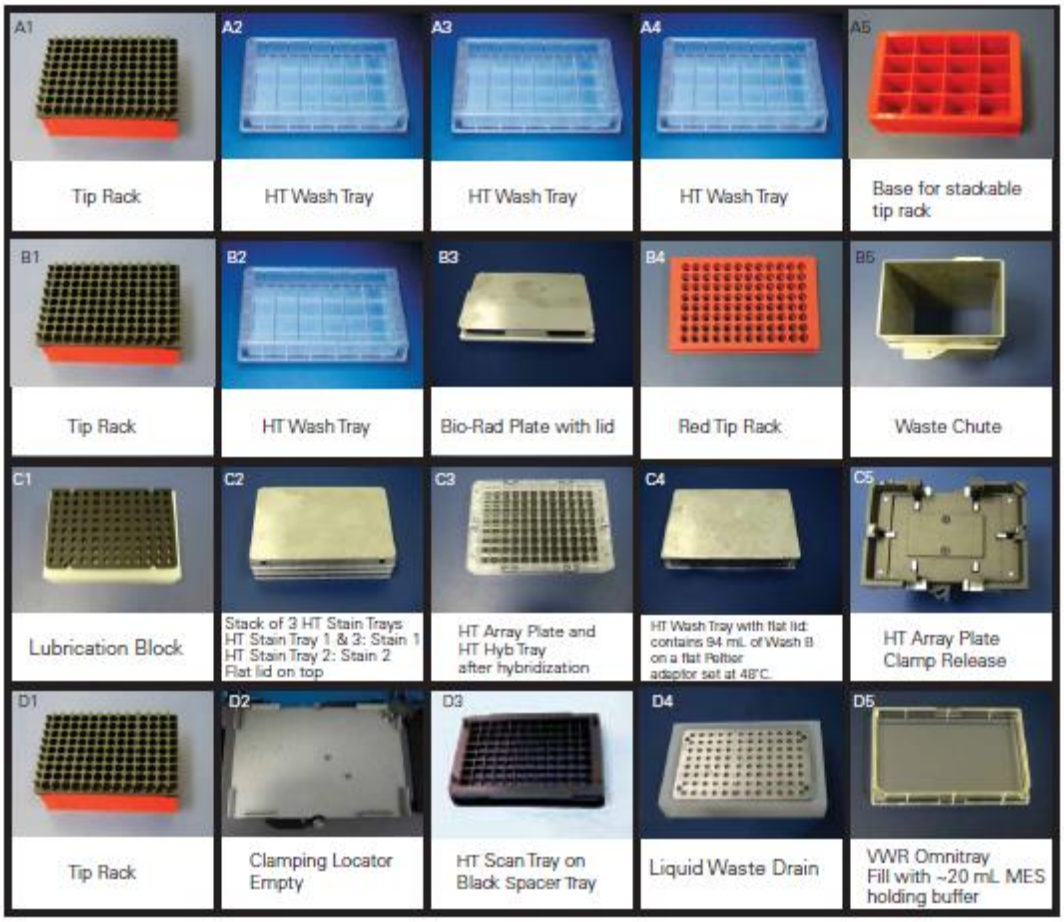


Figure 5.28 The deck layout for the wash and stain protocol—load at least three tip racks in rack 1.

NOTE: The red tip rack used in B4 should be used only once to prevent the High Stringency Wash lid from adhering to it.

Reagent Setup for Wash and Stain Protocol

The Wash and Stain protocol requires reagent setup. This involves loading consumables on to the GCAS deck and filling the appropriate consumables with reagents. The reagent setup must be performed prior to starting the Wash and Stain protocol.

1. ***HT Wash Buffer A:*** Transfer Bulk Dispenser Buffer Line to container containing 2 Liters of *HT Wash Buffer A.*
2. ***HT Wash Buffer B:*** Dispense 94 mL of *HT Wash Buffer B* into one HT Wash Tray.
3. **HT Array Holding Buffer:** Pour 20 mL of HT Array Holding Buffer into a VWR Omnitray.

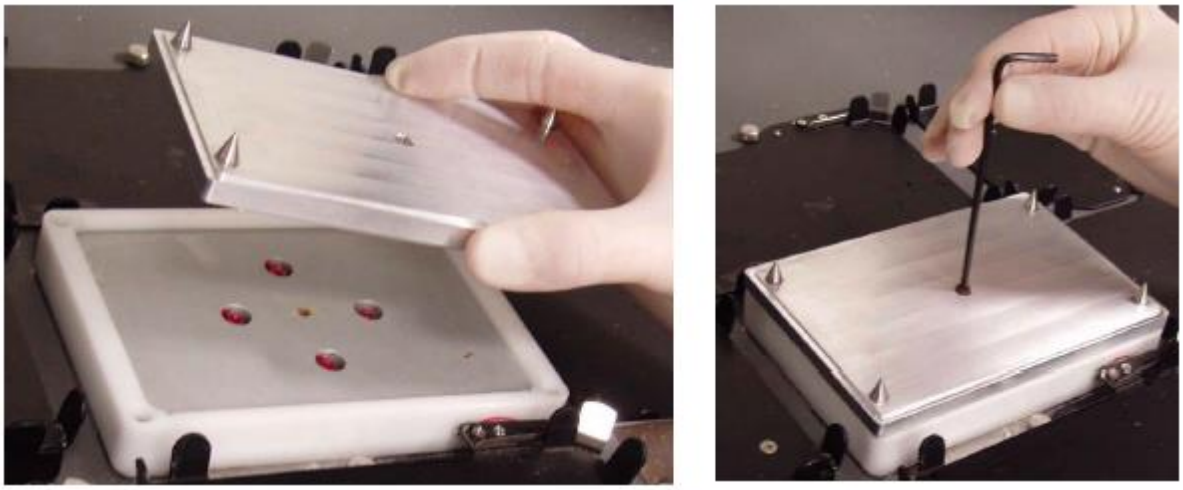


Figure 5.29 Installing (left) and Securing (right) the hybridization block fixture

Figure 5.30 Setting the 48°C temperature on the Watlow Temperature Controller

Running the Wash and Stain Protocol

Running the Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.
2. Remove the HT Array Plate/Hybridization Tray Sandwich from the incubator and place on the deck at location C3.
3. Open the software by double-clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The login window appears **(**Figure 5.16).

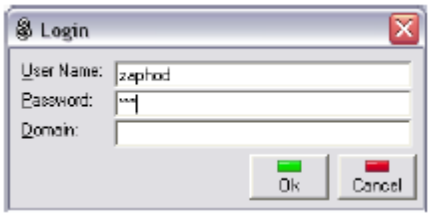


Figure 5.31 Sciclone Workstation Software Login window

4. Enter your **User name** and **Password** and click **OK**. The Operator Runtime interface appears **(**Figure 5.17).

Figure 5.32 Sciclone Workstation Software Operator window

5. Select **File → Open** to access the load application window (Figure 5.33).

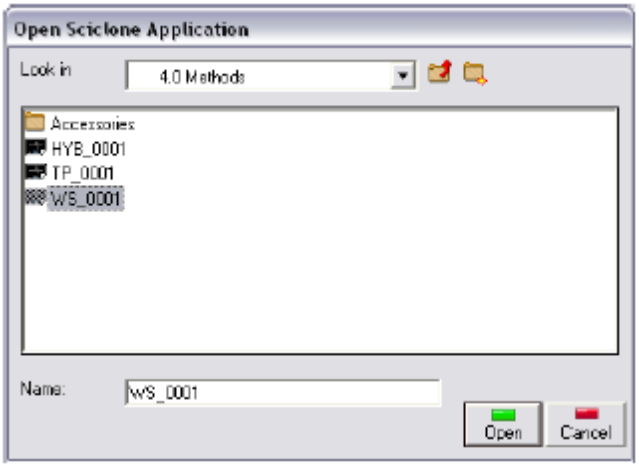


Figure 5.33 Open Sciclone Application window

6. Select **WS_0001** and click **Open**.

   The Wash Stain Protocol is loaded into Sciclone Workstation software (Figure 5.18).

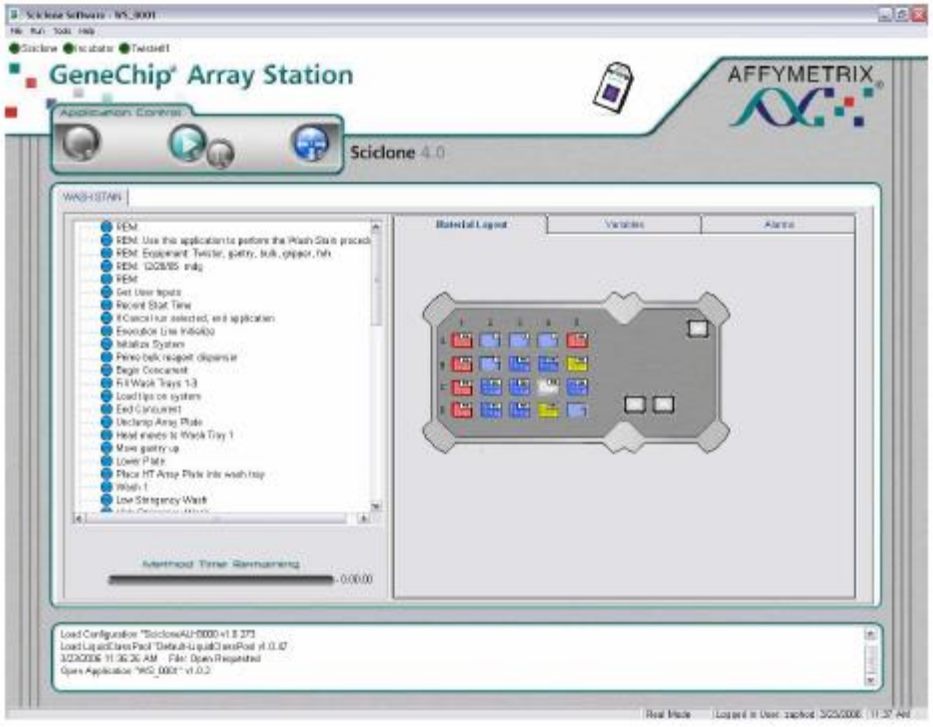


Figure 5.34 Wash and Stain Protocol Loaded

7. Click the green **Start** arrow to begin the application.
8. The Application will prompt you to select several options through the interactive window shown in Figure 5.35.

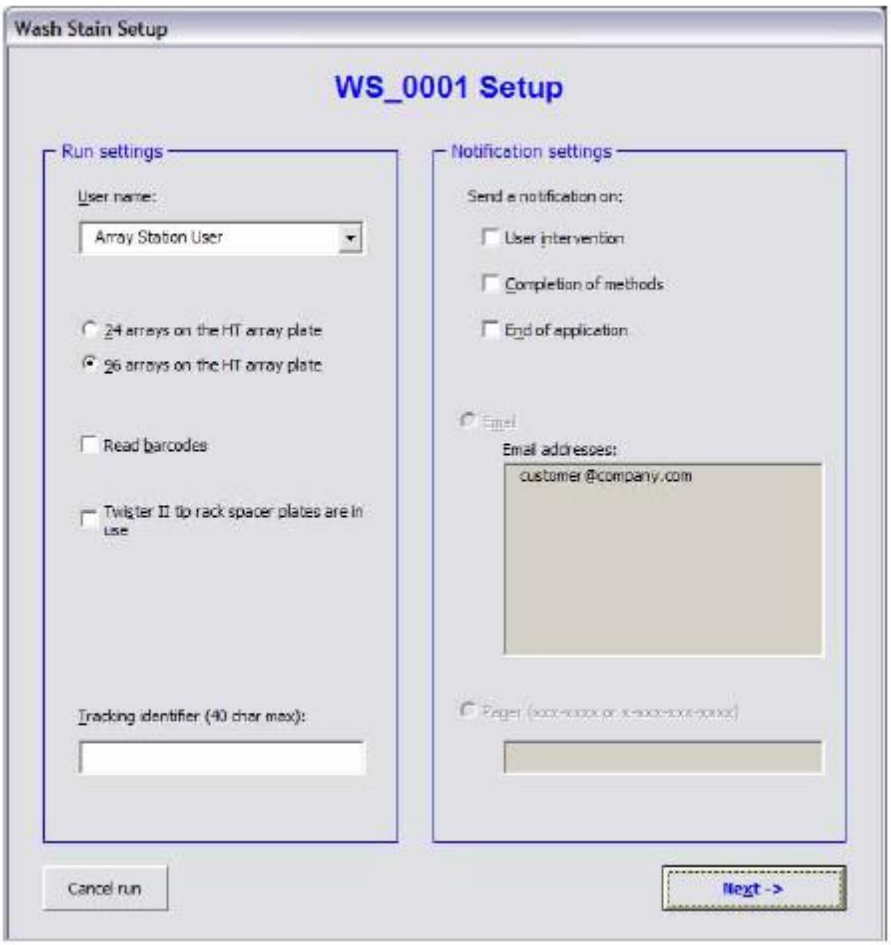


Figure 5.35 Wash and Stain Setup window

9. Select the protocol parameters:
    a. Select your User name from the drop down menu.
    b. Select either a HT 24- or 96-Array Plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.
    c. If you wish the protocol to read and store the HT array plate barcode and the recovered sample plate barcode, check the **Read barcodes** option. Barcodes will be reported in the Wash/ Stain Summary Report at the end of the run.
    d. If you are using spacer plates for tip static control (recommended), check the box **Twister tip rack spacer plates are in use**. If this box is checked, ensure you have placed spacer plates between each nested tip rack and on the top tip rack.
    e. Enter a Tracking identifier (limited to 40 characters) if desired. The Tracking identifier will be recorded in the Summary report at the end of the run.
    f. Select the desired notification settings. Be sure the email address or pager information is properly configured.
10. After selecting the settings parameters, click **Next**. The Wash Stain Deck Layout window appears (Figure 5.20).

Figure 5.36 Wash/Stain Deck Layout Setup window

11. Verify that you have setup the deck properly and that you have completed all items in the **Notes** section. Verify and check the boxes that you have completed all items in the **Checklist** section.
12. Click **Continue run** and the Array Station will perform the Wash/Stain protocol.

    The Wash/Stain protocol continues until the HT Array Plate is stained and ready to be scanned. When the protocol has completed, the following message appears (Figure 5.37).

Figure 5.37 Ready to Scan window

13. Remove the stained HT Array Plate for scanning. Take care not to disturb or disrupt the array from the scan tray. Refer to the HT Scanner User's Guide (P/N 701978) for instructions on scanning the HT Array Plate.
14. Remove the sample recovery plate. Recovered hyb sample can be sealed and stored at –80°C for further use.
15. Change the Bulk Dispenser tubing back to the water bottle, Click **OK**. The Array Station will proceed to drain the wash trays and clean up the deck.

    At the end of the protocol, The End message appears (Figure 5.38). Click **OK**.

Figure 5.38 Run Complete window

At the conclusion of each run, two summary reports are automatically saved by the program:

a. A user readable summary of the run is saved to the folder C:\Affymetrix\Reports\Summary. This file has the following naming convention:

SummaryReport_WS_0001_4_4_2006_9_56_08_AM.rtf

WS_0001 = method used

4_4_2006 = date stamp of when the HYB_0001 starts

9_56_08_AM = time stamp of when the HYB_0001 starts

Figure 5.39 shows an example of a Summary Report.

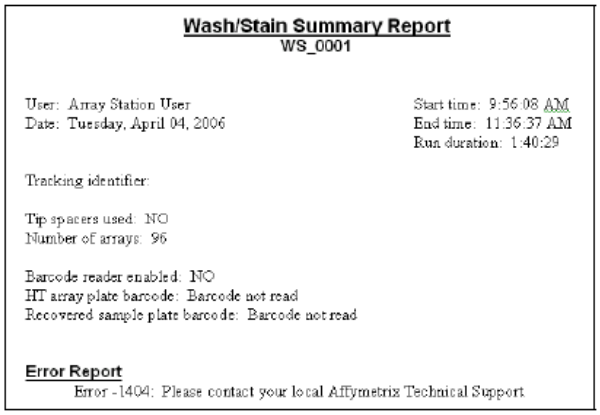


Figure 5.39 Wash/Stain Summary Report Example

b. A report useful for transferring data to many LIMS systems is also saved. This report is saved in the folder C:\Affymetrix\Reports\Data. An example if this report is shown in Figure 5.40.

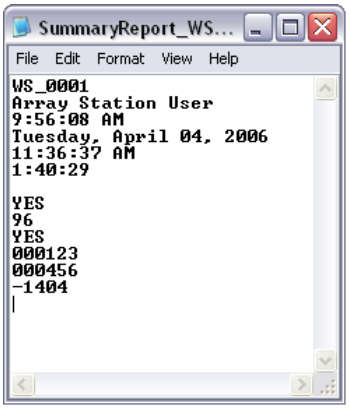


Figure 5.40 LIMS-friendly Report Example

Chapter 6 Hybridization, Wash and Stain Protocol with User-prepared Reagents

Introduction

This chapter is for customers preparing their own hybridization, wash, and stain reagents.

IMPORTANT: For customers using the GeneChip™ HT Hybridization, Wash, and Stain Kit (P/N 901219) please refer to Chapter 5.

This chapter is divided into three main sections:

- Section 1: Hybridization Setup
- Section 2: Hybridization
- Section 3: Wash & Stain

Reagents and Materials Master List for Hybridization, Wash and Stain

The following is a comprehensive list of the reagents and materials required for this entire chapter. Each subsection in this chapter will define the specific reagents and materials used in that section.

All reagents and materials listed have been tested and evaluated. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen™ Life Technologies, P/N 15561-020
- Herring Sperm DNA (HS DNA) solution (10 mg/mL): Promega Corporation, P/N D1811
- Nuclease-free Water: Ambion™, P/N 9932
- Distilled Water: Invitrogen™ Life Technologies, P/N 15230-147
- EDTA Disodium Salt, 0.5M solution (100 mL): Sigma-Aldrich™, P/N E7889
- Surfact-Amps™ 20 (Tween-20), 10%: Pierce™ Chemical, P/N 28320
- Tetramethyl ammonium chloride (TMAC), 5M solution (500 mL): Sigma-Aldrich™, P/N T-3411
- 12X MES – see recipe Chapter 3
- Nuclease-free Water: Ambion™, P/N 9932
- 20X SSPE (3M NaCl, 0.2M NaH_2PO_4, 0.02M EDTA): Cambrex, P/N 51214
- 5M NaCl, RNase-free, DNase-free: Ambion™, P/N 9760G
- Anti-streptavidin antibody (goat), biotinylated: Vector Laboratories, P/N BA-0500
- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen™ Life Technologies, P/N 15561-020
- Distilled Water: Invitrogen™ Life Technologies, P/N 15230-147
- Goat IgG, Reagent Grade: Sigma-Aldrich™, P/N I 5256
- PBS, pH 7.2: Invitrogen™ Life Technologies, P/N 20012-027
- R-Phycoerythrin Streptavidin: Molecular Probes, P/N S-866
- Surfact-Amps™ 20 (Tween-20), 10%: Pierce™ Chemical, P/N 28320

- De-ionized water
- DNAZap: Ambion™, 9890
- RNaseZap: Ambion™, 9786
- HT Hybridization Tray[1] (qty. 1), P/N 202111 (may be ordered separately)
- HT Wash Tray[1] (qty. 5),P/N 900752 (may be ordered separately)
- HT Stain Tray[1] (qty. 4): P/N 900745 (may be ordered separately)
- HT Scan Tray[1] (qty. 1), P/N 900746 (may be ordered separately)
- 96-Well Hard-Shell PCR Plate (qty. 2): Bio-Rad, P/N HSP-9601
- Omnitray (qty. 1): VWR, P/N 4660-638 - or - Greiner Round Bottom Clear
- Polypropylene Plate (qty. 1): E&K Scientific Products, P/N 20261 (for Hyb Setup)
- Omnitray (qty. 2): VWR International 4660-638 (for Wash & Stain)
- Round Bottom Clear Polypropylene Plate(qty. 1): E&K Scientific Products, 20261
- Quarter Reservoir Modules (qty. 2): Beckman Coulter™, 372790
- Quarter Reservoir Frame (qty. 1): Beckman Coulter™, 372795
- Stacker Tips 200 µL non-sterile (qty. 3): Caliper LifeSciences, P/N 78642
- Stacker Tips 200 µL non-sterile (1 box): Caliper LifeSciences, 78641
- BD Falcon™ Test Tube, 14 mL (qty. 2): VWR™ International 60819-761
- BD Falcon™ Test Tube, 5 mL (or equivalent) (qty. 1): VWR™ International 60819-728
- Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL (qty. 2): VWR™ International, 20171-028
- RNase-Free 1.5 mL Microfuge tube (qty. 1): Ambion™, 12400
- Aluminum Foil Lids: Beckman Coulter™, 538619

[1]*HT Hybridization, Wash, Stain and Scan Trays are packaged with the HT Array Plates.*

Section 1: Hybridization Setup

Introduction

This section describes the Array Station Hybridization Setup protocol for HT Array Plates. A schematic of the automated Hybridization Setup run is provided to outline the steps in the procedure (Figure 6.1).

This chapter also provides details on how to prepare reagents, how to set up the Array Station Deck, and how to use the GeneChip™ Array Station Software to pre-hybridize a HT Array Plate. Information on how to hybridize a HT Array Plate is also provided.

Reagents and Materials for Hybridization Setup

The reagents and materials required in the Hybridization Setup protocol have been tested and evaluated. Information and part numbers listed are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen Life Technologies, P/N 15561-020
- Herring Sperm DNA (HS DNA) solution (10 mg/mL): Promega Corporation, P/N D1811
- Nuclease-free Water: Ambion, P/N 9932
- Distilled Water: Invitrogen Life Technologies, P/N 15230-147
- EDTA Disodium Salt, 0.5M solution (100 mL): Sigma-Aldrich, P/N E7889
- Surfact-Amps™ 20 (Tween-20), 10%: Pierce Chemical, P/N 28320
- Tetramethyl ammonium chloride (TMAC), 5M solution (500 mL): Sigma-Aldrich, P/N T-3411
- 12X MES – see recipe Chapter 3
- HT Hybridization Tray[1] (qty. 1), P/N 202111 (may be ordered separately)
- HT Stain Tray[1] (qty. 1), P/N 900745 (may be ordered separately)
- 96-Well Hard-Shell PCR Plate (qty. 1): Bio-Rad, P/N HSP-9601
- Omnitray (qty. 1): VWR, P/N 4660-638 - or - Greiner Round Bottom Clear Polypropylene Plate (qty. 1): E&K Scientific Products, P/N 20261
- Stacker Tips 200 µL non-sterile (qty. 3): Caliper LifeSciences, P/N 78642
- DNAZap: Ambion, 9890
- RNaseZap: Ambion, 9786
- De-ionized water

[1]*Hybridization and Stain Trays are packaged with the HT Array Plates.*

Reagent Preparation

The Hybridization Setup protocol requires reagent preparation. This involves manually preparing reagents and filling the appropriate consumable. The reagent setup must be performed prior to starting the Hybridization Setup protocol.

Automated Hybridization Setup Schematic

This section summarizes the Hybridization Setup protocol steps.

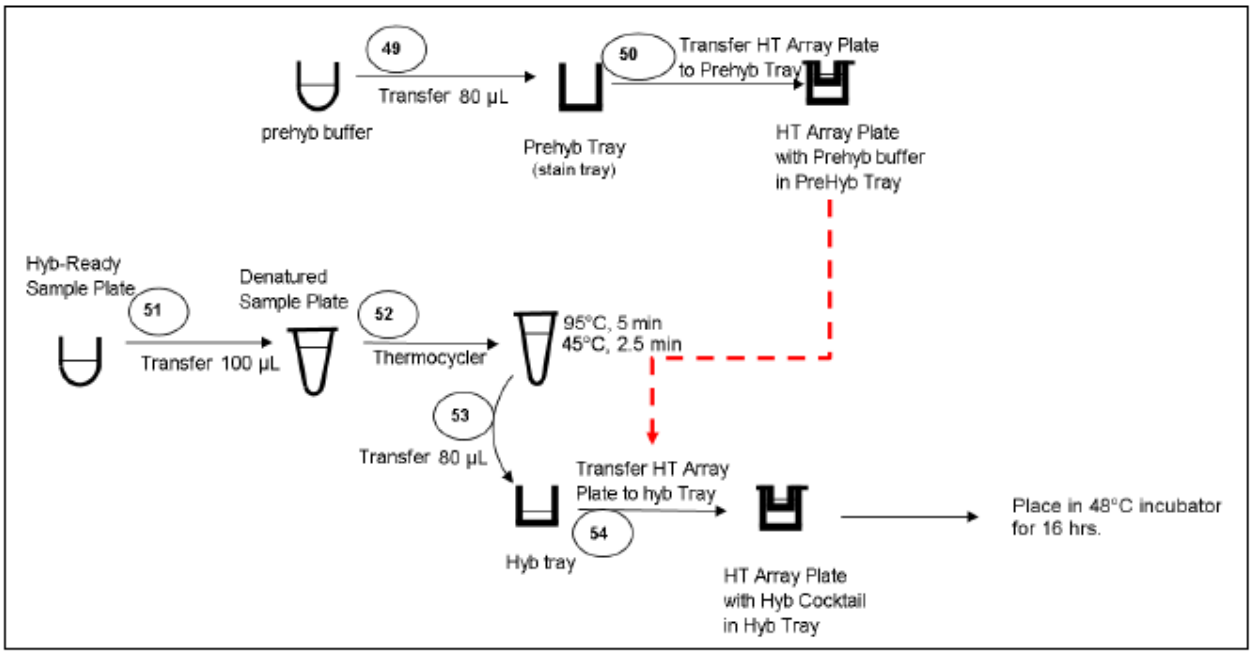


Figure 6.1 Steps 49 to 54: Hybridization Setup and Hybridization

Pre-Hybridization Buffer for HT Array Plates

NOTE: A Greiner Polypropylene U-bottom Plate or a VWR Omnitray can be used in position B3 to hold the Pre-Hybridization Buffer.

If using a Greiner Polypropylene U-bottom Plate at position B3:

1. Prepare the Pre-Hybridization Buffer for 24 or 96 wells as shown in Table 6.1.
2. Aliquot 100 µL of this buffer into each well of the Greiner Polypropylene U-bottom Plate.

If using a VWR Omnitray at position B3:

1. Prepare the Pre-Hybridization Buffer for the Omnitray as shown in Table 6.1.
2. Pour 20 mL into the VWR Omnitray.

WARNING: Use good lab practices when handling TMAC-containing solutions. Wear gloves, safety glasses, and lab coats when preparing the pre-hybridization buffers. Please consult the appropriate MSDS for reagent storage and handling requirements.

Table 6.1 Pre-Hybridization Buffer Recipe

	1 Well Volume	24 Wells Volume	96 Wells Volume	Omnitray Volume
HS DNA (10 mg/mL)	1.00 µL	27 µL	108 µL	200.0 µL
BSA (50 mg/mL)	1.00 µL	27 µL	108 µL	200.0 µL
1.23X Hybridization Buffer*	81.4 µL	2,197.8 µL	8,791.0 µL	16,280.0µL
Nuclease-free Water	16.6 µL	448.2 µL	1,793.0 µL	3,320.0 µL
Total Volume	100 µL	2,700 µL	10,800.0 µL	20,000.0 µL

* The 1.23X hybridization buffer is TMAC containing buffer. See Table 6.2 for recipe.

Table 6.2 1.23X Hybridization Buffer recipe.

50 mL		1 L	Final 1X Concentration
12X MES	5.04 mL	100.8 mL	100 mM
5M TMAC	30.73 mL	614.6 mL	2.5M
0.5M EDTA	2.46 mL	49.2 mL	20 mM
10% Tween-20	0.06 mL	1.2 mL	0.01%
Nuclease-free Water	11.71 mL	234.2 mL	
Total Volume	50 mL	1 L	

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.

2. Wipe the pad with RNaseZap.

3. Rinse the pad with DI Water.

4. Wipe the pad with DNAZap.

5. Thoroughly rinse the pad with DI Water.

6. Dry the pad with pressurized clean air or nitrogen.

Beginning a Run – Deck Layout for Hybridization Setup

This section describes how to assemble the deck and use the GeneChip™ Array Station Software to begin a Hybridization Setup run. The Hybridization Setup protocol will perform the following methods:

- Pre-hybridize the HT Array Plates
- Denature the sample for hybridization
- Assemble the HT Array Plate and the Hybridization Tray containing the hybridization-ready sample

Procedure - Assembling the Deck

1. Setup the deck with the appropriate consumables. Refer to Figure 6.2.

2. Load at least four tip boxes into Rack 1.

3. Remove the cold reagent block.

4. Place the hybridization-ready samples on the deck at location C2.

NOTE: If using a HT 24-Array Plate, it is NOT necessary to re-array the samples into columns 5, 7 and 9. Samples should be in columns 1, 2, and 3 of the Greiner plate. The Array Station will transfer samples to columns 5, 7, and 9 during the protocol.

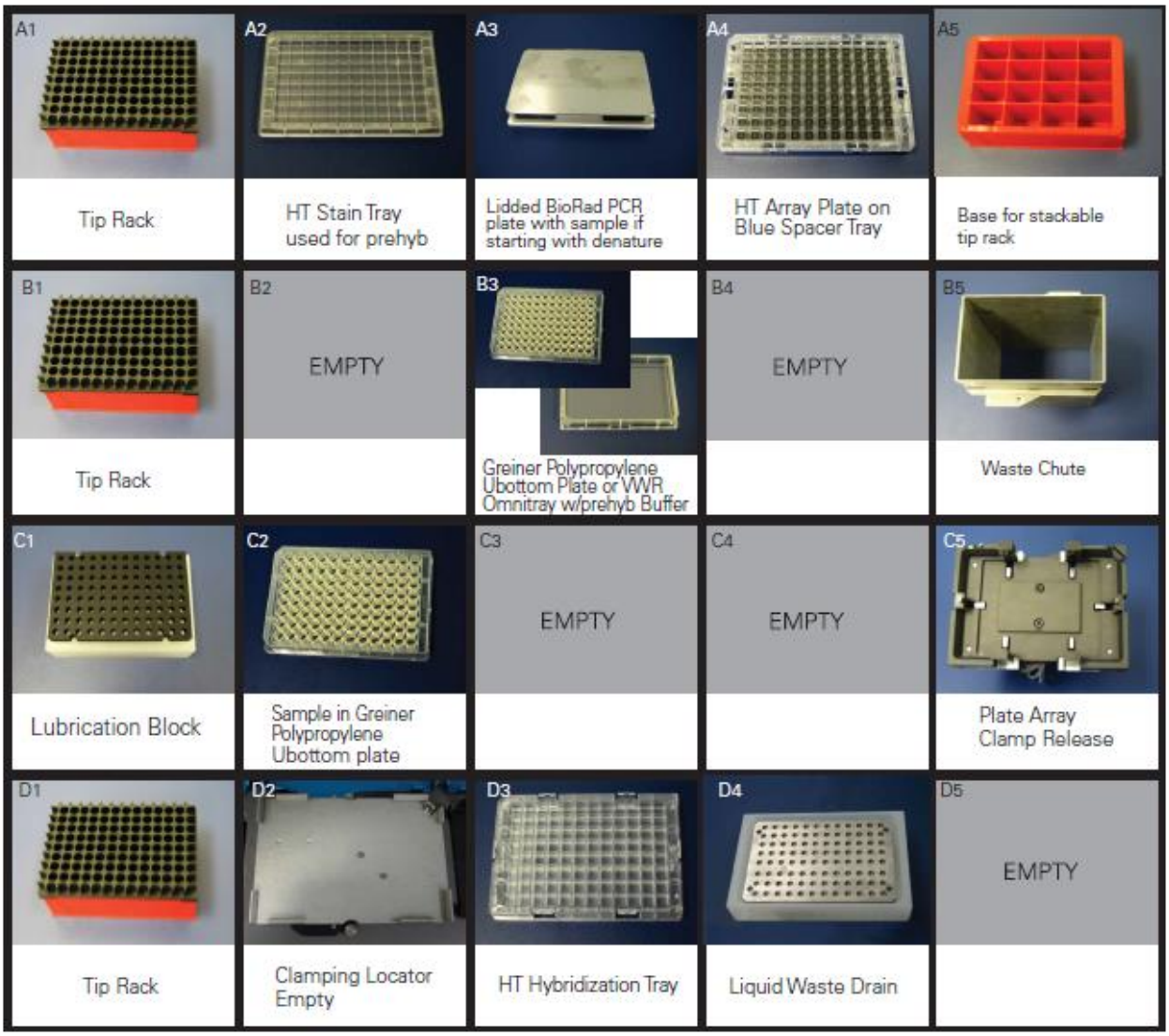


Figure 6.2 The deck layout for the Hybridization Setup protocol.

Load four tip boxes into Rack 1.

Empty Rack 2, leaving the tip rack base there.

NOTE: If you are using recovered samples and your samples are already in the Bio-Rad 96-Well plate, you will need to leave position C2 empty. Your samples will be in the Bio-Rad plate placed at A3.

Running the Hybridization Setup Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.
2. Open the software by double clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The **login** window appears (Figure 6.3).

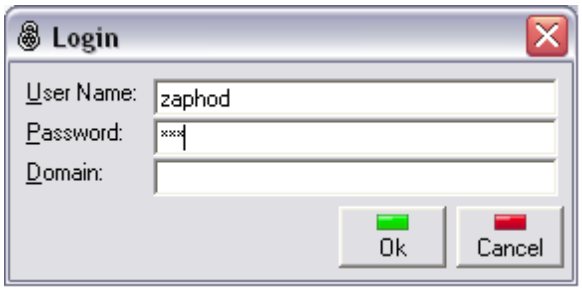


Figure 6.3 Sciclone Workstation Software Login window

3. Enter your **User Name** and **Password** and click **OK**.

The Operator Runtime interface window appears (Figure 6.4).

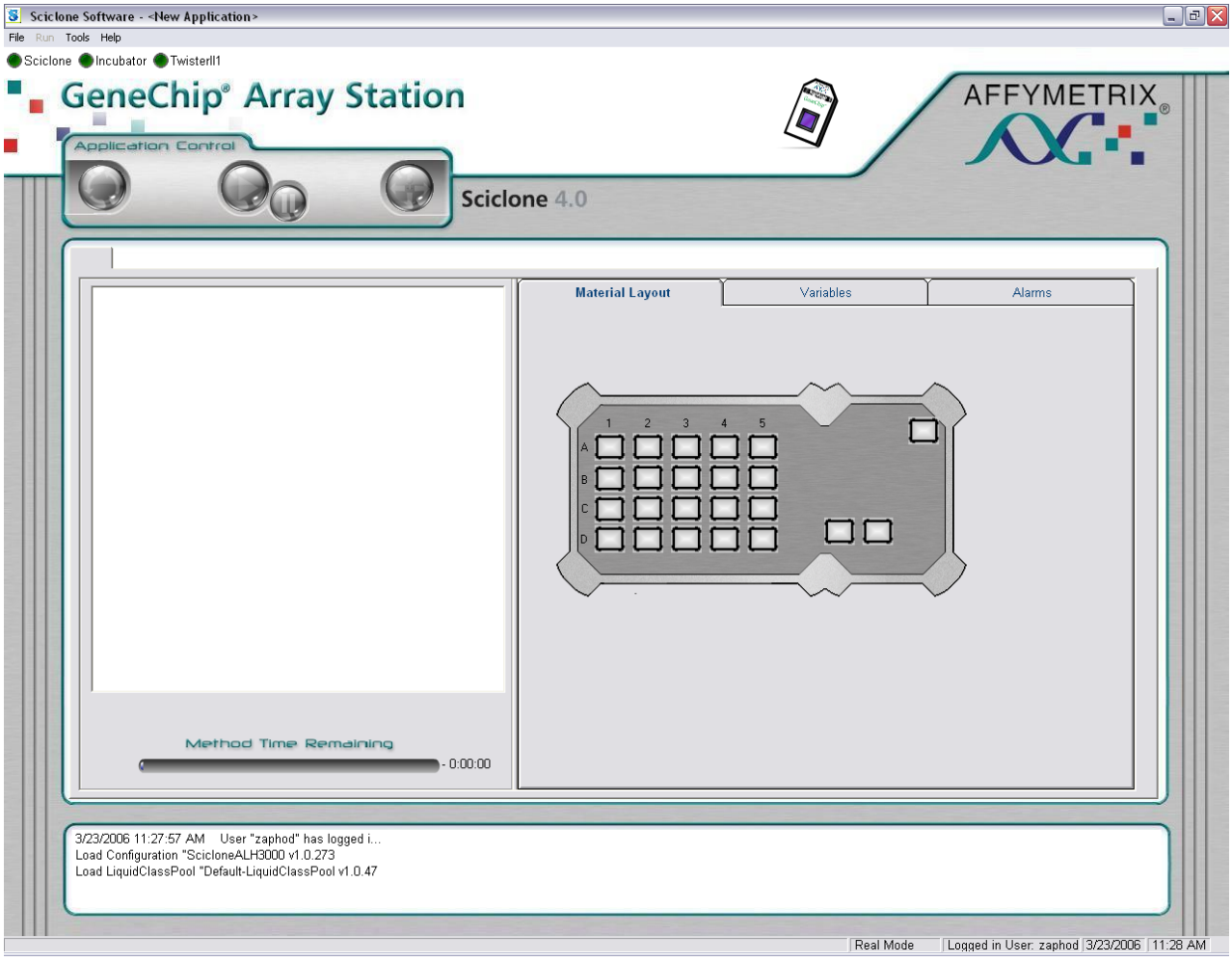


Figure 6.4 Sciclone Workstation Software Operator window

4. Select **File** → Open to access the load application window (Figure 6.5).

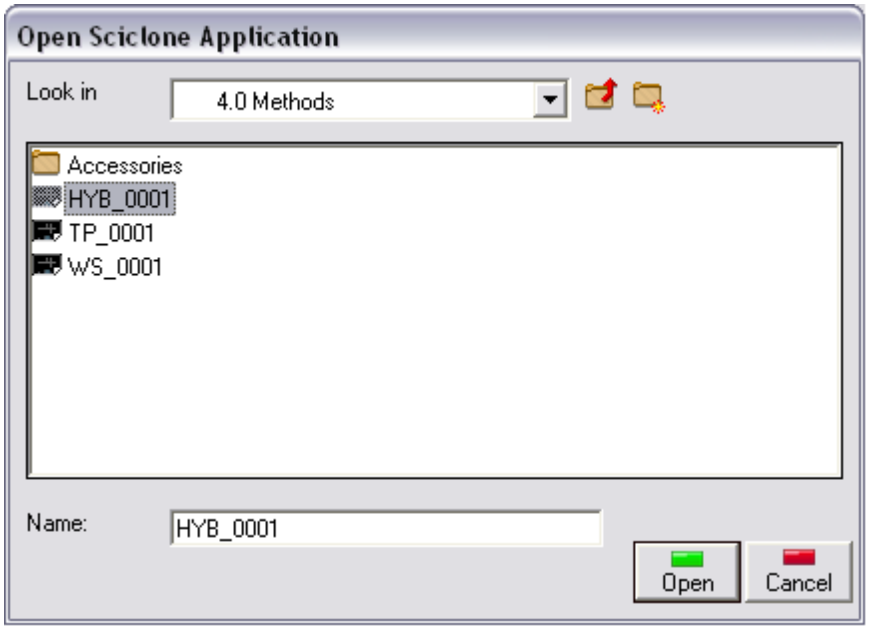


Figure 6.5 Open Sciclone Application window

5. Select **HYB_0001** and click **Open**.

The hybridization setup application is loaded into Sciclone Workstation Software.

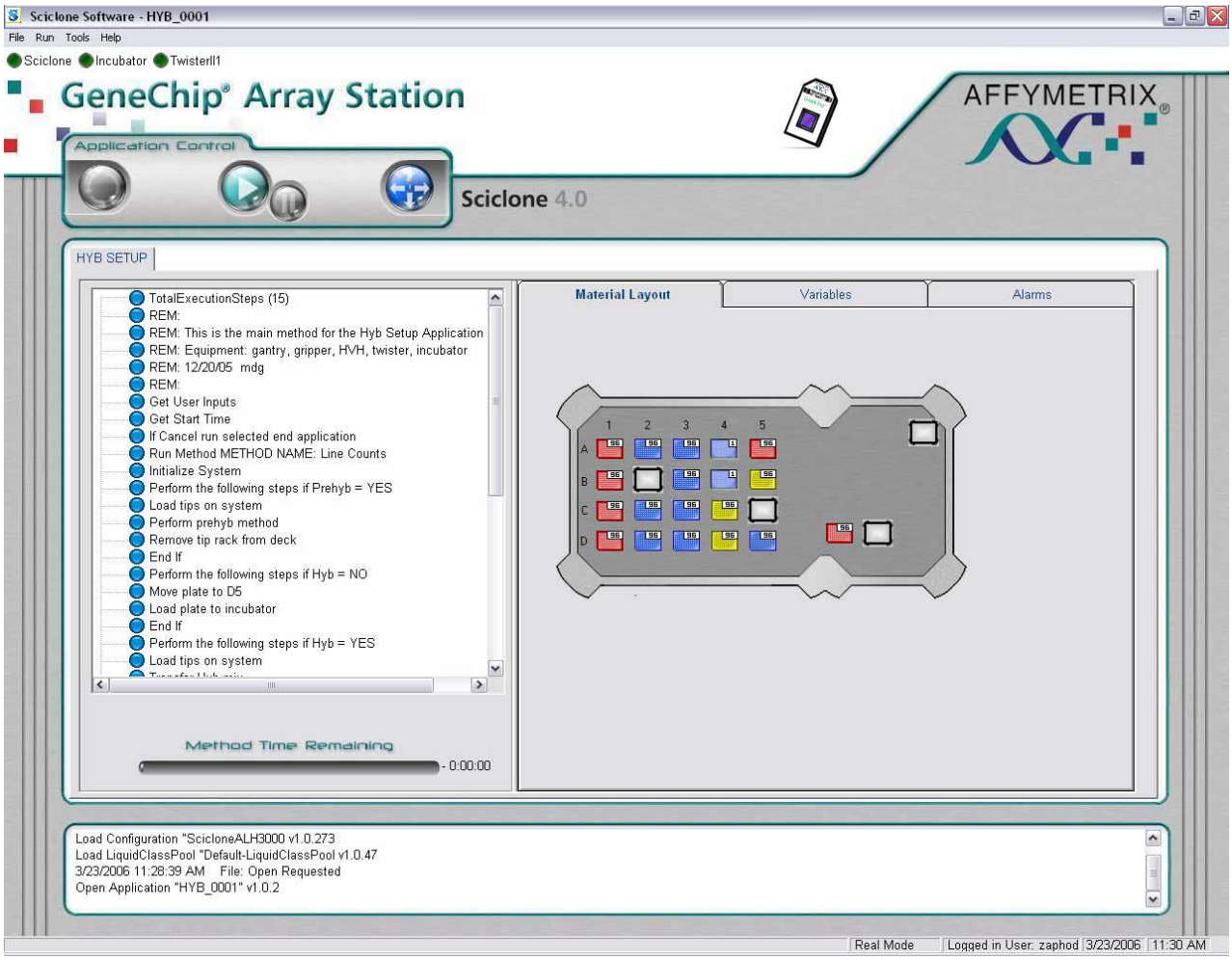


Figure 6.6 Hybridization Setup Protocol Loaded

6. Click the green **Start** arrow to begin the application.

The application will prompt you to select several options through the interactive window shown in Figure 6.7.

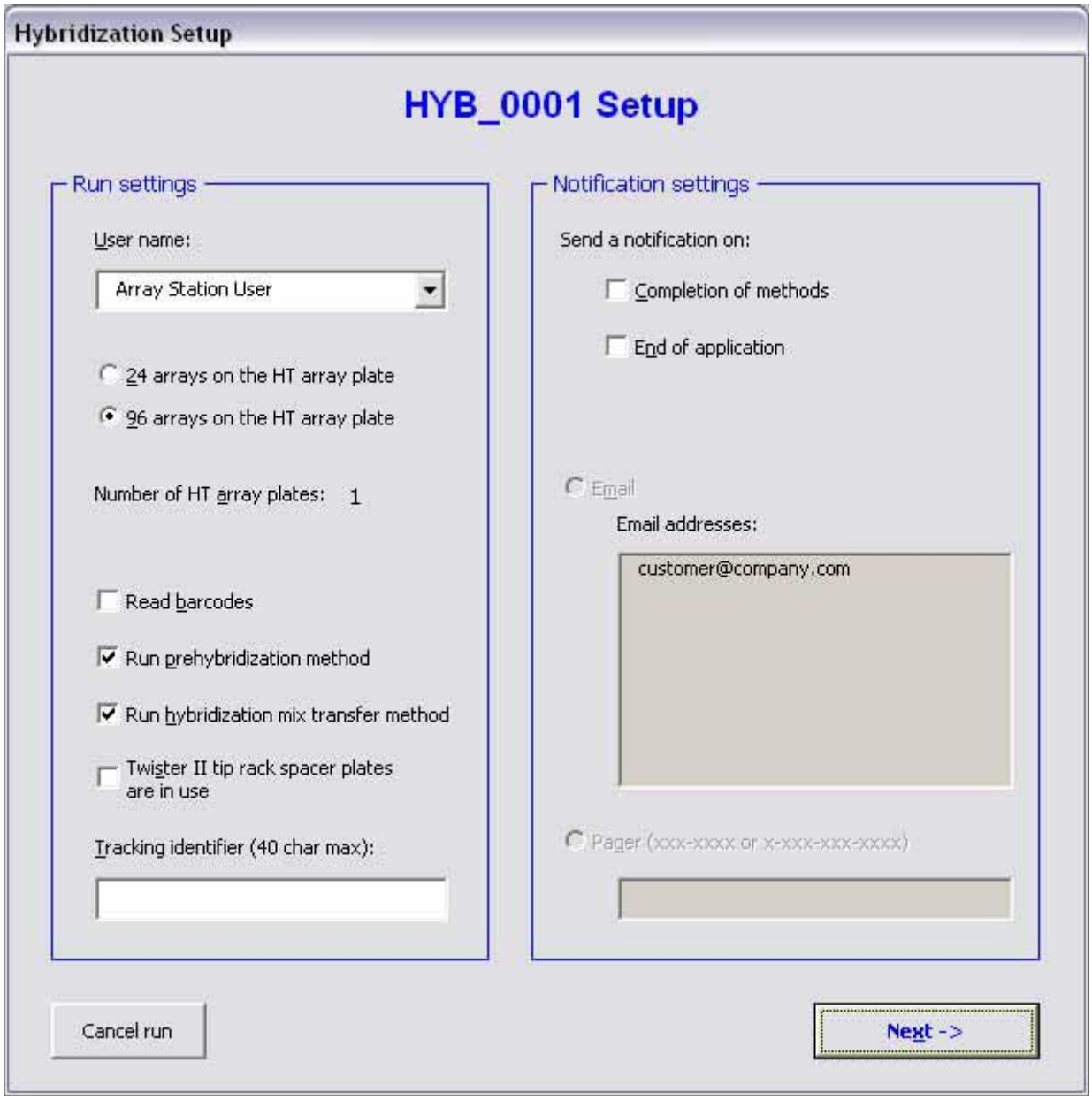


Figure 6.7 Hybridization Setup window

7. Select the protocol parameters.

- **a.** Select your **User name** from the drop down menu.
- **b.** Select either 24 or 96 well HT array plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.
- **c.** If you wish the protocol to read and store the sample plate barcode and the HT array plate barcode, check the Read barcodes option. Barcodes will be reported in the user summary at the end of the run.
- **d.** Select Run prehybridization method.

e. Selection of the Run hybridization mix transfer method depends on if your samples are in the Greiner plate or are recovered samples in the Bio-Rad plate.

1) If Run hybridization mix transfer method is selected, the Array Station will transfer the hyb-ready sample from a Greiner U-bottom Plate at C2 to a Bio-Rad Plate at A3. If you are running a HT 24-Array Plate, your samples should be in columns 1, 2, and 3 of the Greiner plate. The Array Station will automatically transfer these samples to columns 5,7, and 9 of the hybridization tray to match the HT 24-Array Plate.

2) If Run hybridization mix transfer method is NOT selected, it is required that 100 µL of Hyb-Ready sample is placed in a Bio-Rad Plate at position A3. If you are running a HT 24-Array Plate, your samples should be in columns 1, 2, and 3 of the Bio-Rad plate. The Array Station will automatically transfer these samples to columns 5, 7, and 9 of the hybridization tray to match the HT 24-Array Plate.

NOTE: In most cases you will select and run the hybridization mix transfer method. However, if you are re-hybridizing samples recovered from a previous hybridization, your samples will already be in a Bio-Rad Plate. You should select NOT to run the hybridization mix transfer if your samples are in the Bio-Rad Plate.

f. If you are using spacer plates for tip static control (recommended), check the box Twister tip rack spacer plates are in use. If this box is checked, ensure you have placed spacer plates between each nested tip rack and on the top rack.

g. Enter a Tracking identifier (limited to 40 characters) if desired. The Tracking identifier will be recorded in the Hybridization Setup Summary Report at the end of the run (refer to Figure 6.11 for a description of the Summary Report).

h. Select the desired notification settings. Be sure the email address or pager information is properly configured.

8. After selecting the settings parameters, click **Next**.

You will see one of the following windows, depending on whether you have selected **Run hybridization mix transfer method.**

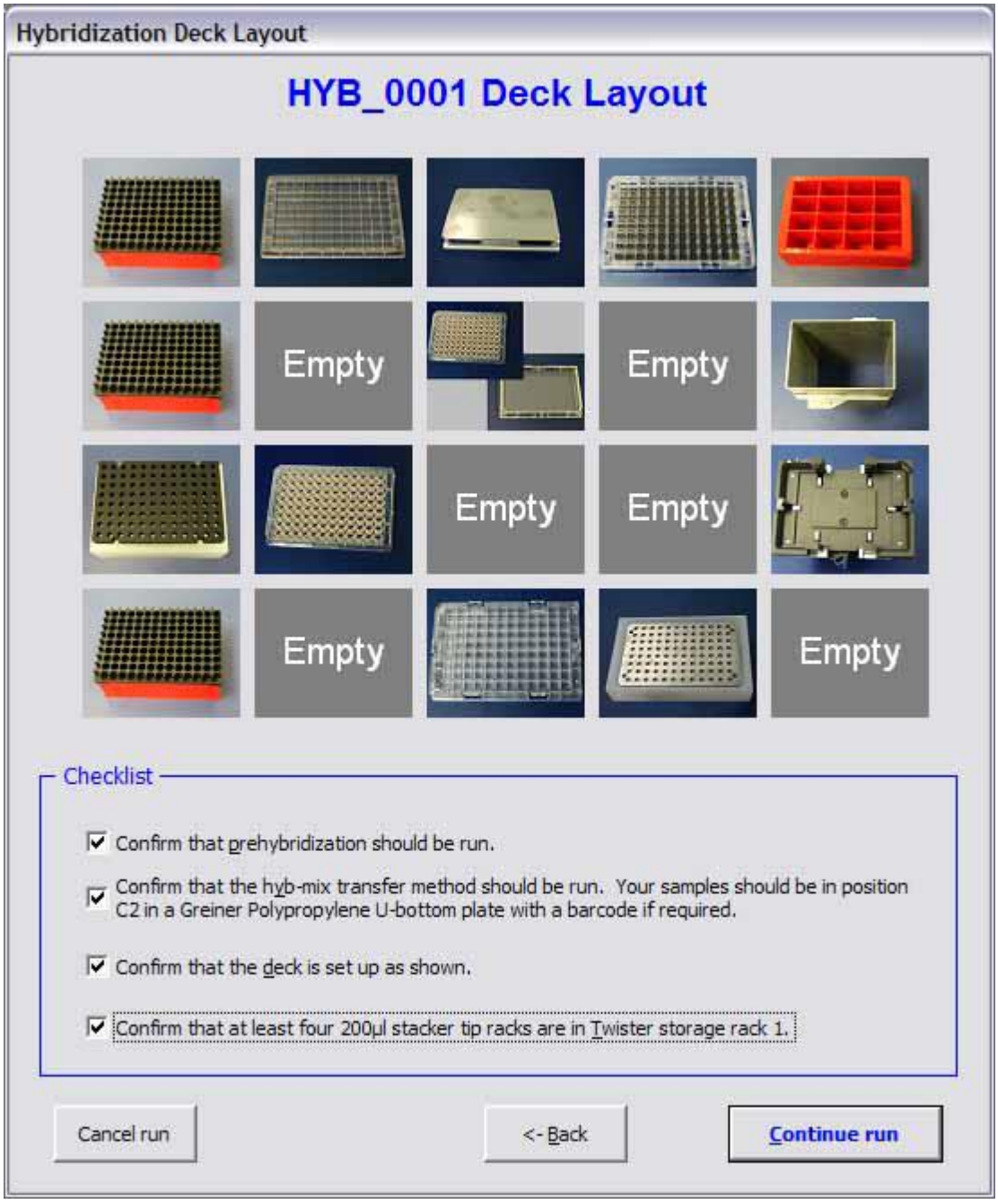


Figure 6.8 Hybridization Deck Layout when Run hybridization mix transfer method is selected

Figure 6.9 Hybridization Deck Layout when Run hybridization mix transfer method is NOT selected

9. Verify that you have configured the deck properly and that you have completed and checked the items on the checklist. Click **Continue** and the Hybridization Setup protocol continues until the HT Array Plate/Hybridization Tray Sandwich is assembled.

10. At the end of the protocol, The End message appears (Figure 6.10). Click **OK**.

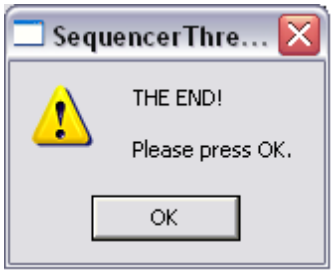


Figure 6.10 Run Complete message

You must place the HT Array Plate hybridization sandwich in an incubator equilibrated to 48°C.

11. At the conclusion of each run, two summary reports are automatically saved by the program.

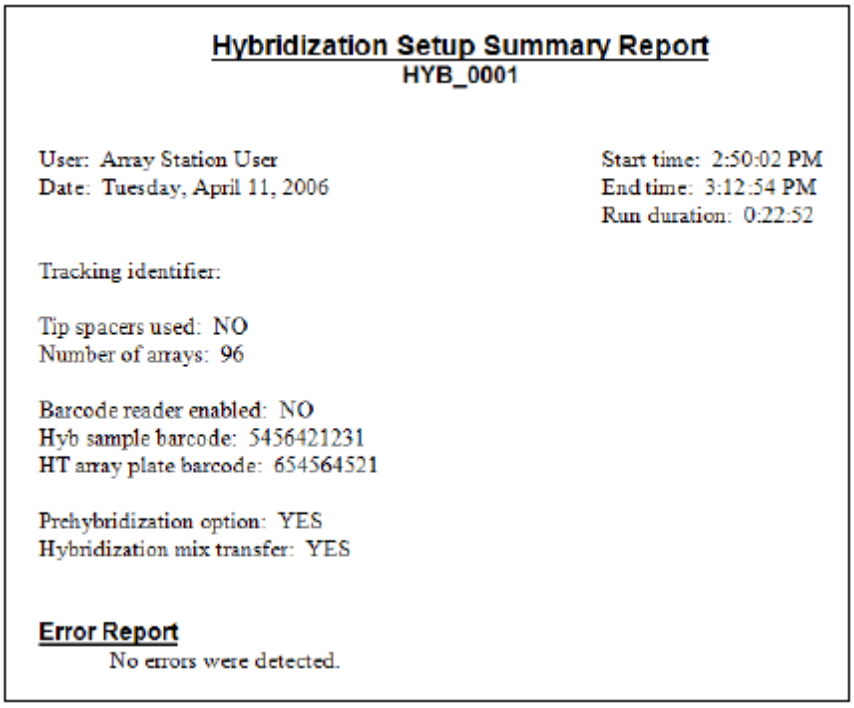
    a. A user readable summary of the run is saved to the folder C:\Affymetrix\Reports\Summary. This file has the following naming convention:

    SummaryReport_HYB_0001_3_23_2006_10_03_36_AM.rtf

    HYB_0001 = method used

    3_23_2006 = date stamp of when the HYB_0001 starts

    10_03_36_AM = time stamp of when the HYB_0001 starts Figure 6.11 shows an example of a Hybridization Setup Summary Report.



Hybridization Setup Summary Report
HYB_0001

User: Array Station User
Date: Tuesday, April 11, 2006

Start time: 2:50:02 PM
End time: 3:12:54 PM
Run duration: 0:22:52

Tracking identifier:

Tip spacers used: NO
Number of arrays: 96

Barcode reader enabled: NO
Hyb sample barcode: 5456421231
HT array plate barcode: 654564521

Prehybridization option: YES
Hybridization mix transfer: YES

Error Report
No errors were detected.

Figure 6.11 Hybridization Setup Summary Report Example

12. A tab-delimited text report useful for transferring data to many LIMS systems is also saved. This report is saved in the folder C:\Affymetrix\Reports\Data. An example if this report is shown in Figure 6.12.

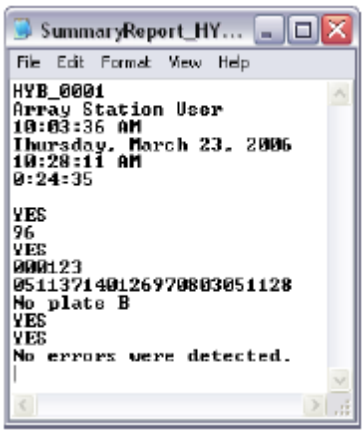


Figure 6.12 LIMS Summary Report Example

13. The HT Array Plate/Hybridization Tray Sandwich is ready to be placed into a 48°C incubator for 16 hours.

CAUTION: Take care in transferring the Hyb Sandwich to the hybridization oven. The sandwich should remain close to level to prevent sample contamination.

Section 2: Hybridization

This section describes how to incubate a HT Array Plate/Hybridization Tray Sandwich for hybridization.

Overnight Hybridization

1. Remove the HT Array Plate/Hybridization Tray Sandwich from the Array Station, taking care to keep the sandwich level, and place in the hybridization oven.
2. Incubate the HT Array Plate/Hybridization Tray Sandwich for 16 hours at 48°C.

IMPORTANT: The hybridization oven should be monitored with a digital thermometer (such as VWR, P/N 23226-656) to ensure that the temperature is stabilized at 48°C.

IMPORTANT: The HT Array Plate/Hybridization Tray Sandwich must remain level during hybridization. A bubble level can be placed on top of the sandwich to ensure that all four corners are level.

Section 3: Wash & Stain

Introduction

This section describes the Array Station Wash and Stain protocol for HT Array Plates. A schematic of the automated Wash and Stain is provided at the beginning of this chapter to outline the steps of the procedure (Figure 6.20 to Figure 6.25).

This chapter also provides detail on how to prepare reagents, how to set up the Array Station deck, and how to use the Sciclone 4.0 Software to perform a Wash and Stain protocol.

Reagents and Materials for Wash and Stain

The following reagents and materials required in the Wash and Stain protocol have been tested and evaluated. Information and part numbers listed below are based on U.S. catalog information. For supplier information, please refer to the Supplier Reference List in Appendix F of this manual.

- Nuclease-free Water: Ambion™, P/N 9932
- 20X SSPE (3M NaCl, 0.2M NaH_2PO_4, 0.02M EDTA): Cambrex, P/N 51214
- 5M NaCl, RNase-free, DNase-free: Ambion, P/N 9760G
- Anti-streptavidin antibody (goat), biotinylated: Vector NTI™ Laboratories, P/N BA-0500
- Bovine Serum Albumin (BSA) solution (50 mg/mL): Invitrogen™ Life Technologies™, P/N 15561-020
- Distilled Water: Invitrogen™ Life Technologies™, P/N 15230-147
- Goat IgG, Reagent Grade: Sigma-Aldrich™, P/N I 5256
- PBS, pH 7.2: Invitrogen™ Life Technologies™, P/N 20012-027
- R-Phycoerythrin Streptavidin: Molecular Probes™, P/N S-866
- Surfact-Amps™ 20 (Tween-20), 10%: Pierce™ Chemical, P/N 28320
- Quarter reservoir modules (qty. 2), Beckman Coulter™ P/N 372790
- Quarter reservoir frame, Beckman Coulter™ P/N 372795
- HT Wash Tray[1] (qty. 5), P/N 900752
- HT Stain Tray[1] (qty. 3), P/N 900745
- HT Scan Tray[1] (qty. 1), P/N 900746

[1]*HT Stain, Wash, and Scan Trays are packaged with the HT Array Plates.*

- 96-Well Hard-Shell PCR Plate (qty 1): Bio-Rad™, HSP-9601
- Omnitray (qty. 2): VWR™ International 4660-638
- Round Bottom Clear Polypropylene Plate(qty. 1): E&K Scientific Products, 20261
- Quarter Reservoir Modules (qty. 2): Beckman Coulter™, 372790
- Quarter Reservoir Frame (qty. 1): Beckman Coulter™, 372795
- BD Falcon™ Test Tube, 14 mL (qty. 2): VWR International 60819-761
- BD Falcon™ Test Tube, 5 mL (or equivalent) (qty. 1): VWR™ International 60819-728
- Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL (qty. 2): VWR™ International, 20171-028
- RNase-Free 1.5 mL Microfuge tube (qty. 1): Ambion™, 12400
- Stacker Tips 200 µL non-sterile (1 box): Caliper™ LifeSciences, 78641
- Aluminum Foil Lids: Beckman Coulter™, 538619
- DNAZap: Ambion™, 9890
- RNaseZap: Ambion™, 9786
- De-ionized water

Wash and Stain Preparation

The Wash and Stain protocol requires reagent preparation. This involves manually preparing and filling the appropriate consumable. The reagent setup must be performed prior to starting the Wash and Stain protocol.

New with this version of the manual is an automated Stain Dispensing Setup Protocol. Alternatively, instructions are provided for performing this function manually.

Reagent Preparation

Stock Buffers

12X MES Stock Buffer Recipe

(1.22M MES, 0.89M [Na+])

For 1,000 mL:

64.61 g of MES Hydrate

193.3 g of MES Sodium Salt 800 mL of Nuclease-free Water

Mix and adjust volume to 1,000 mL.

The pH should be between 6.5 and 6.7. Filter through a 0.2 µm filter.

IMPORTANT: Do not autoclave. Store at 2°C to 8°C, and shield from light. Discard solution if yellow.

2X MES Stain Buffer Recipe

(100 mM MES, 1.0M Na+, 0.05% Tween-20)

41.7 mL 12X MES Buffer

92.5 mL 5M NaCl

2.5 mL 10% Tween-20

113.3 mL Distilled Water

Stain Cocktails

Stain 1 and Stain 3 (SAPE)

1. In a 50 mL conical tube, add the components listed in the Stain 1 + Stain 3 column of Table 6.3, and mix well.

Table 6.3 Stain 1 and Stain 3 Recipe

	Stain 1 + Stain 3		
	1 Well	24 Wells	96 Wells
Nuclease-free Water	36.0 µL	1,944.0 µL	7,776.0 µL
2X MES Stain Buffer	40.0 µL	2,160 µL	8,640.0 µL
BSA (50 mg/mL)	3.2 µL	172.8 µL	691.2 µL
SAPE	0.8 µL	43.2 µL	172.8 µL
Total Volume	80.0 µL	4,320.0 µL	17,280.0 µL

Stain 2 (Ab)

1. In a 14 mL conical tube, add the components listed in the 24 wells or 96 wells column of Table 6.4 as appropriate, and mix well.

Table 6.4 Stain 2 Synthesis

	1 Well	24 Wells	96 Wells
Nuclease-free Water	35.52 µL	959.0 µL	3,836.16 µL
2X MES Stain Buffer	40.0 µL	1,080.0 µL	4,320.0 µL
BSA (50 mg/mL)	3.2 µL	86.4 µL	345.6 µL
10 mg/mL Goat IgG Stock*	0.8 µL	21.6 µL	86.4 µL
0.5 mg/mL Biotinylated Antibody†	0.48 µL	13 µL	51.84 µL
Total Volume	80.0 µL	2,160.0 µL	8,640.0 µL

* Goat IgG Stock should be made by resuspending 50 mg in 5 mL of 150 mM NaCl. Store at 4°C. Note: If a larger volume of the 10 mg/mL IgG stock is prepared, aliquot and store at –20°C until use. After the solution has been thawed it should be stored at 4°C. Avoid additional freezing and thawing.

† Stock solution of the biotinylated antibody should be made by resuspending 0.5 mg in 1 mL of nuclease-free water.

Wash Buffers

Low Stringency Wash Buffer

(6X SSPE, 0.01% Tween-20)

For 2,000 mL:

600.0 mL 20X SSPE

2.0 mL 10% Tween-20

1,398.0 mL Distilled Water

Adjust pH to 7.4.

Filter through a 0.2 µm filter. Store at 2°C to 8°C and shield from light.

NOTE: The Low Stringency Wash Buffer can be made in large volumes and placed in a 20 L carboy.

High Stringency Wash Buffer

(100 mM MES, 100 mM NaCl, 0.01% Tween-20)

For 1,000.0 mL:

83.3 mL 12X MES

5.2 mL 5M NaCl

1.0 mL 10% Tween-20

910.5 mL Distilled Water

Filter through a 0.2 µm filter. Store at 2°C to 8°C and shield from light.

MES Holding Buffer

MES Holding Buffer Recipe

(100 mM MES, 1M [Na+], 0.01% Tween-20)

For 1,000 mL:

83.3 mL 12X MES

185.2 mL 5M NaCl

1.0 mL 10% Tween-20

731.8 mL Distilled Water

Filter through a 0.2 µm filter. Store at 2°C to 8°C and shield from light.

Stain Dispensing

- If you plan to use the automated Stain Dispensing Setup Protocol, please go to *Stain Dispensing Setup Protocol.*
- If you plan to perform the stain dispensing manually, please go to *Manual Stain Dispensing Setup.*

Stain Dispensing Setup Protocol

This version of the manual features a new Stain Dispensing Setup Protocol provided to facilitate efficiency of time, accuracy, and ease of use. This new step is an alternative option to manual dispensing and automates the aliquoting of wash and stain reagents prior to the Wash and Stain protocol (WS_0001).

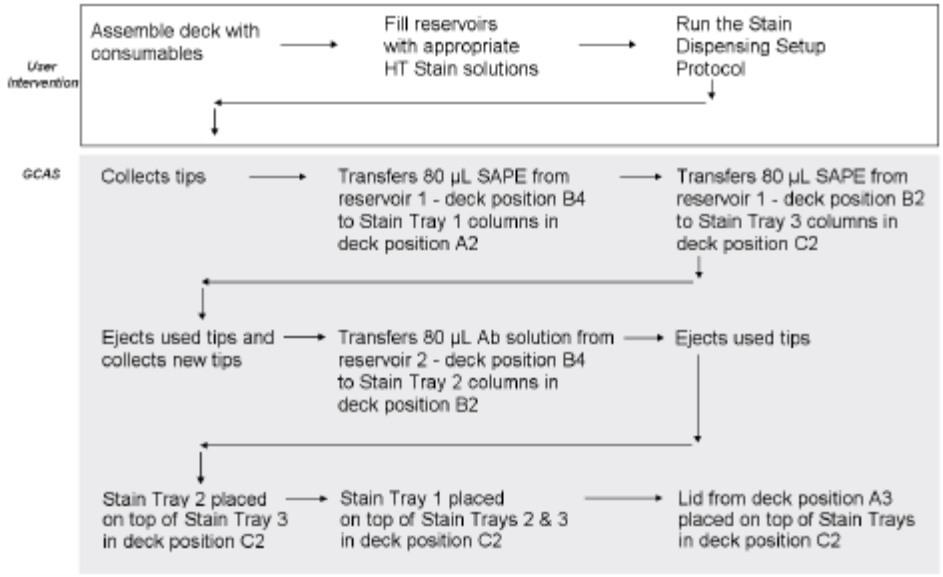


Figure 6.13 Stain Dispensing Setup Schematic

Procedure - Assembling the Deck for the Stain Dispensing Setup Protocol

1. Set up deck with appropriate consumables. Refer to Figure 6.27.

NOTE: The red tip rack at position A3 is covered with the flat lid.

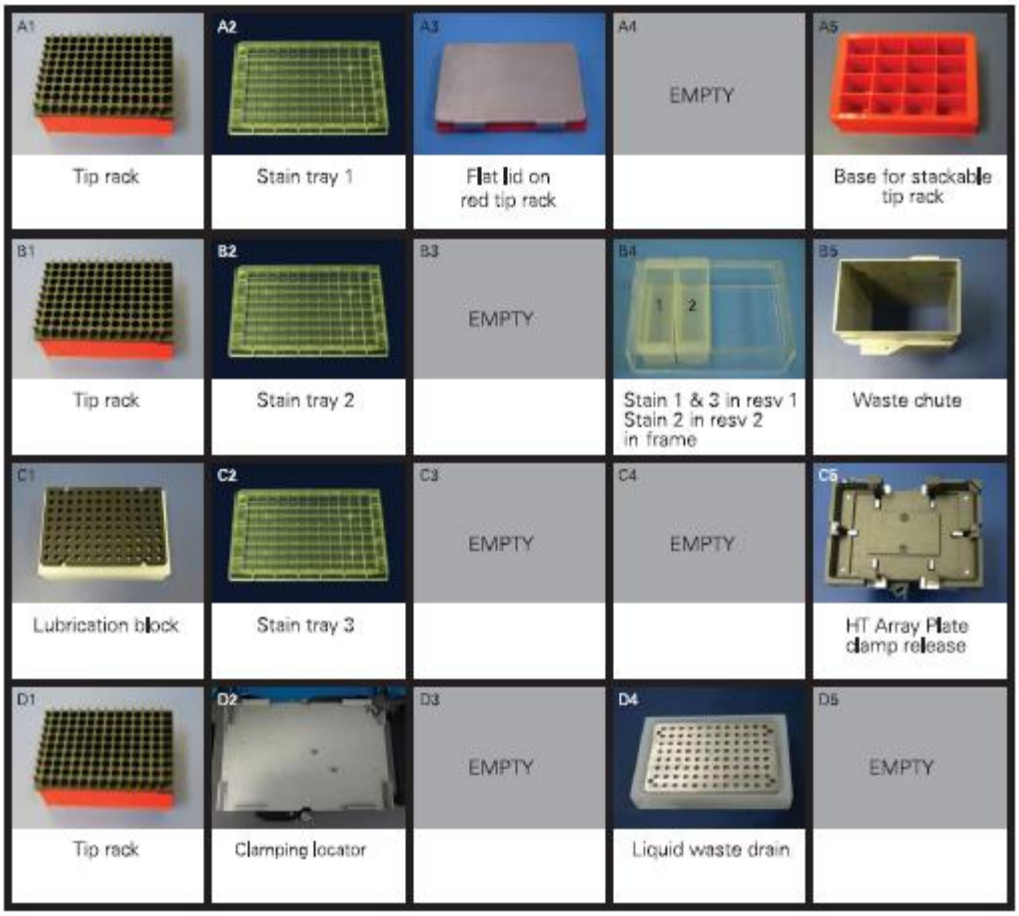


Figure 6.14 The deck layout for Stain Dispensing Setup

2. Fill the quarter reservoirs at position B4 as follows:

a. If processing a 24-array plate, aliquot 5 mL HT Stain Cocktail 1 & 3 (SAPE) into the first quarter reagent reservoir at B4. Aliquot 3 mL for HT Stain Cocktail 2 (Ab) into the second quarter reservoir at B4.

b. If processing a 96-array plate, pour the entire contents of HT Stain Cocktail 1 & 3 bottles (SAPE) into the first quarter reagent reservoir at B4. Pour the entire contents of the HT Stain Cocktail 2 bottle (Ab) into the second quarter reservoir at B4.

3. Load at least one tip box in Rack 1.

4. Place the buffer lines in correct liquid carboys.

- The bulk dispenser buffer line should be placed in the carboy containing Low Stringency Buffer.
- The Z8 buffer line should be placed in DI water.

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI Water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI Water.
6. Dry the pad with pressurized clean air or nitrogen.

Running the Stain Dispensing Setup Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.

2. Open the software by double-clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The login window appears **(**Figure 6.30).

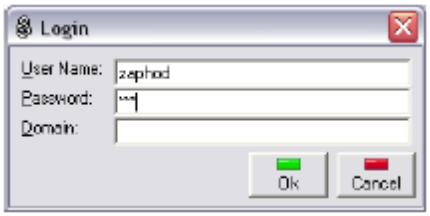


Figure 6.15 Sciclone Workstation Software Login window

3. Enter your **User name** and **Password** and click **OK**. The Operator Runtime interface appears **(**Figure 6.31).

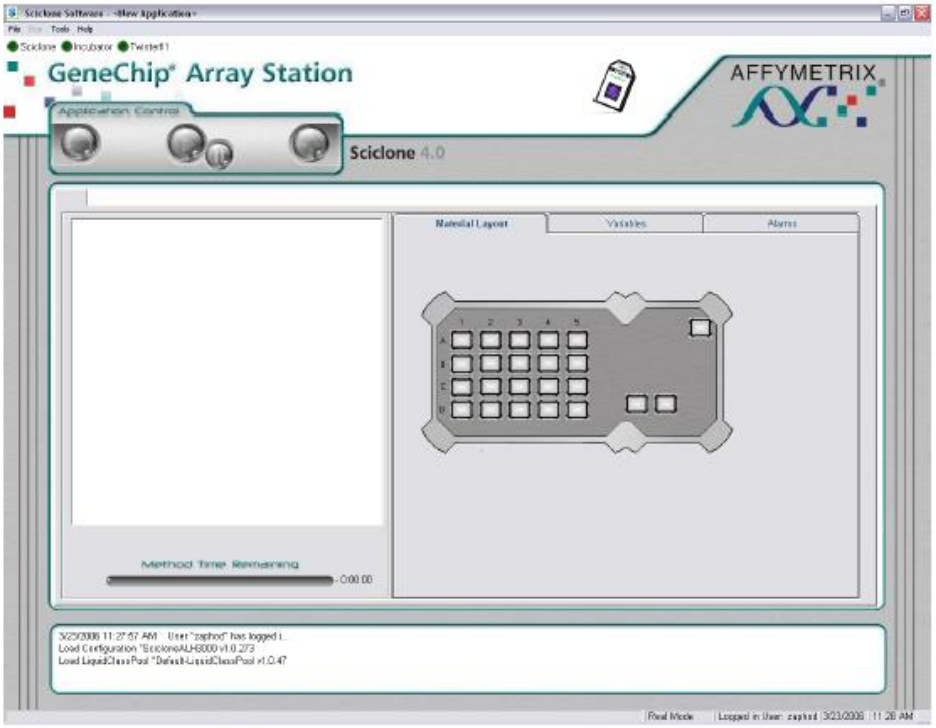


Figure 6.16 Sciclone Workstation Software Operator window

4. Select File → Open to access the load application window.
5. Select Setup_0001 and click Open.

The Stain Dispensing Setup application is loaded into Sciclone Workstation software (Figure 6.33).

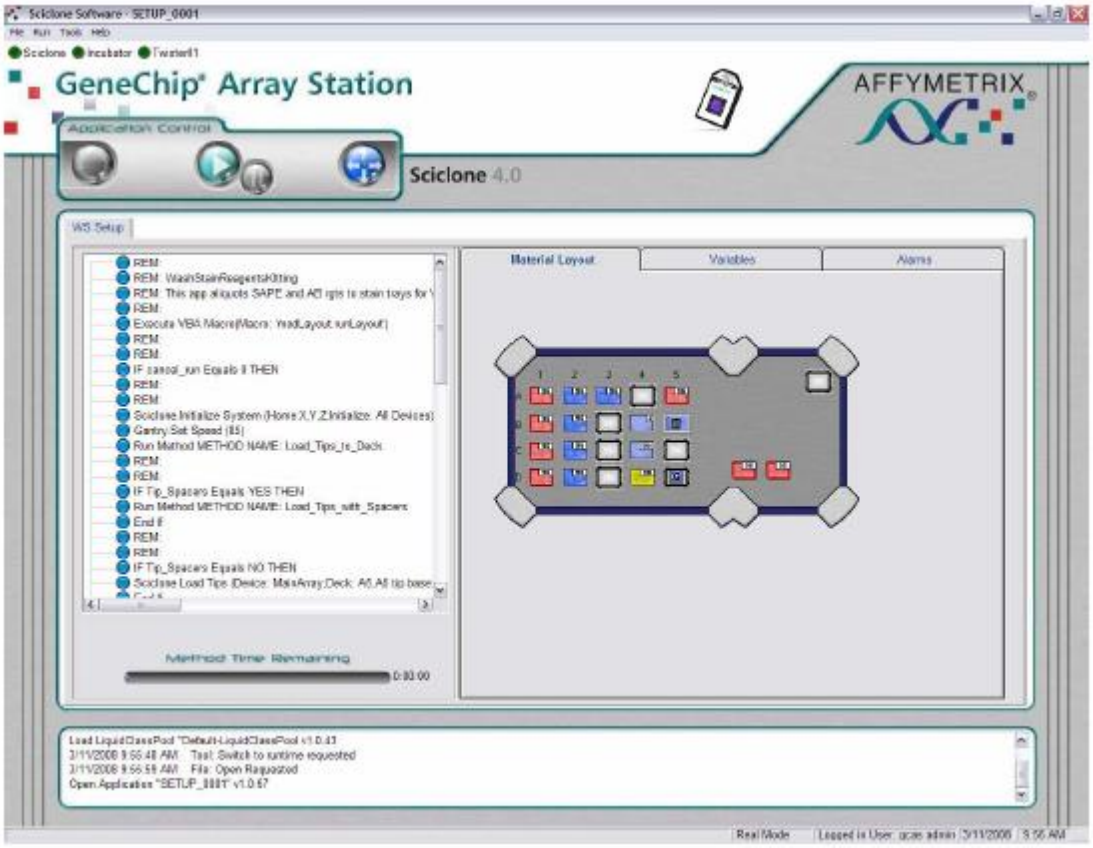


Figure 6.17 Wash and Stain Protocol Loaded

6. Click the green **Start** arrow to begin the application.

   The Application will prompt you to select several options through the interactive window shown in Figure 6.18

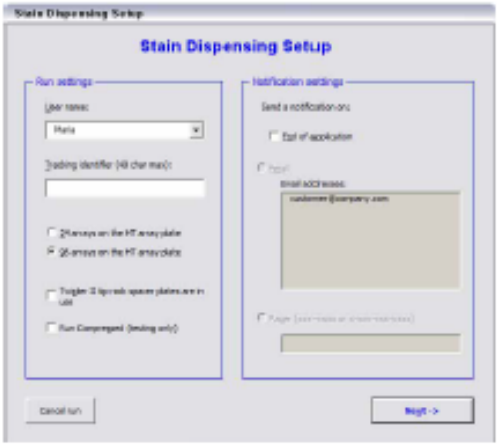


Figure 6.18 Stain Dispensing Setup window

7. Select the protocol parameters: Select either a HT 24- or 96-Array Plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.

8. Click **Next**.

The Stain Dispensing Setup deck layout window appears (Figure 6.35).

Figure 6.19 Stain Dispensing Setup Deck Layout

a. Verify that you have set the deck up properly and that you have completed all items in the Notes section.

c. Click Continue run and the Array Station will perform the Stain Dispensing Setup protocol.

d. When completed, remove the red tip rack at A3 and the reagent reservoirs at B4 before continuing on to the WS_0001 application (see *Beginning a Run – Wash and Stain Deck Layout*).

Manual Stain Dispensing Setup

Stain 1 and 3 (SAPE)

1. Obtain two HT Stain trays and label one “Stain 1” and the other “Stain 3.”
2. Aliquot 80 µL of the Stain 1 + Stain 3 mixture (Table 6.3) into the appropriate wells of each labeled HT Stain Tray.

NOTE: If using a HT 24-Array Plate, the stain mixture should be aliquoted into columns 5, 7, and 9 of the HT Stain Trays.

HT Stain Cocktail 2

1. Obtain one HT Stain tray and label it “Stain 2.”
2. Aliquot 80 µL of Stain 2 mixture (Table 6.4) into the appropriate wells of the labeled HT Stain Tray.

NOTE: If using a HT 24-Array Plate, the stain mixture should be aliquotted into columns 5, 7, and 9 of the HT Stain Trays.

Low Stringency Buffer

1. Prepare 2,000 mL of Low Stringency Wash Buffer (see *Wash Buffers*).
2. Connect Bulk Dispenser Buffer Line to container containing Low Stringency Wash Buffer.

High Stringency Buffer

1. Prepare 1 L of High Stringency Wash Buffer (see

2. *Wash* Buffers).
3. Pour 94 mL into one HT Wash Tray.

MES Holding Buffer

1. Prepare 1 L of MES Holding Buffer (see *Wash Buffers).*
2. Pour 20 mL into a VWR Omnitray.

Clean the Bio-Rad Plate Lids with DNAZap™ and RNaseZap™

The disposable pad under the lids should be cleaned before the run.

1. Rinse the pad with DI Water.
2. Wipe the pad with RNaseZap.
3. Rinse the pad with DI Water.
4. Wipe the pad with DNAZap.
5. Thoroughly rinse the pad with DI Water.
6. Dry the pad with pressurized clean air or nitrogen.

Automated Wash and Stain Schematic

This section summarizes the Wash and Stain protocol steps.

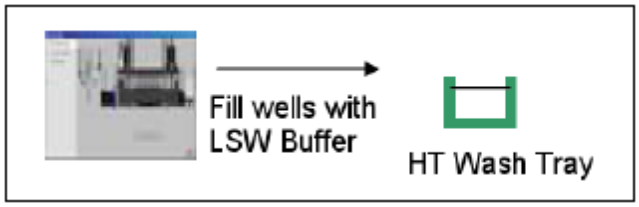


Figure 6.20 Step 1: Fill HT Wash Trays with Low Stringency Wash (LSW) Buffer

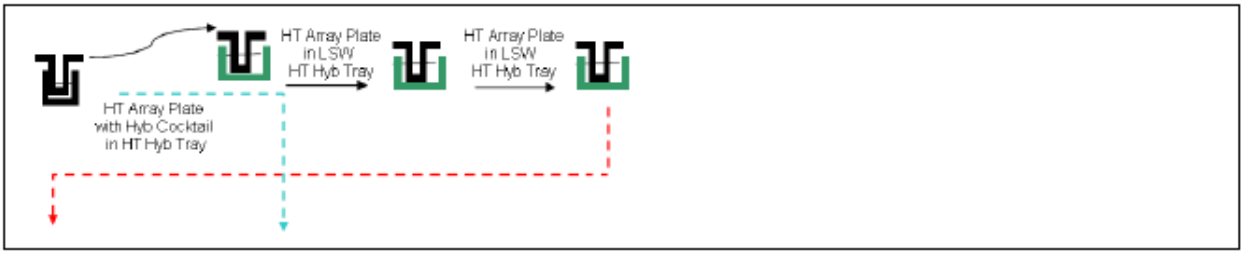


Figure 6.21 Step 2: Low Stringency Wash (LSW)

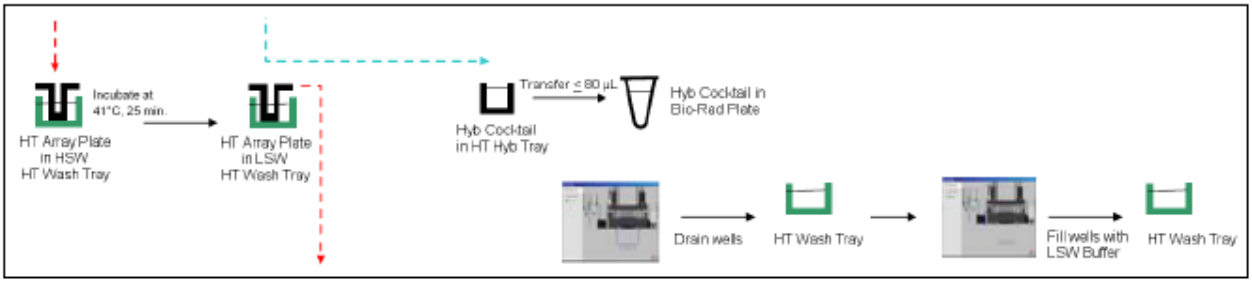


Figure 6.22 Step 3: High Stringency Wash (HSW); Transfer of Hybridization Cocktail; Drain/Refill HT Wash Trays

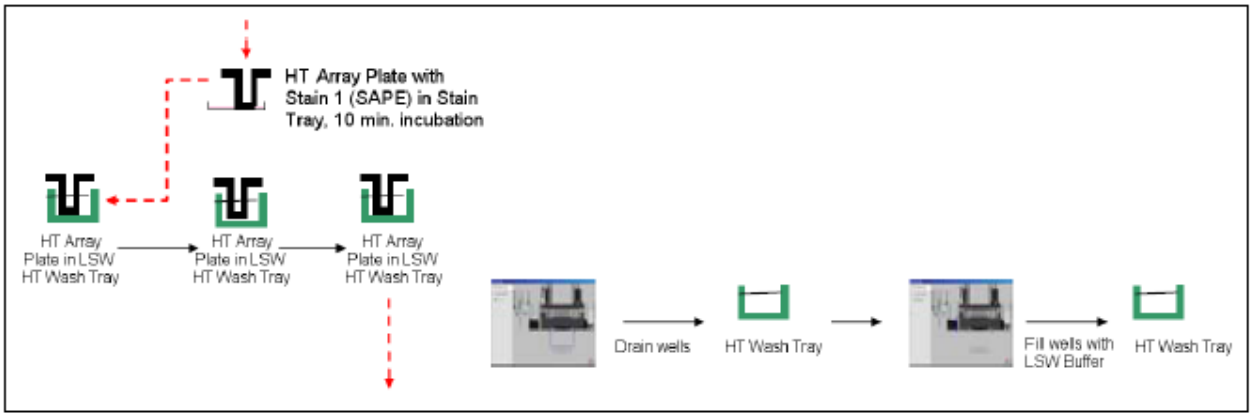


Figure 6.23 Step 4: First Stain (Phycoerythrin); Low Stringency Wash; Drain/Refill HT Wash Trays

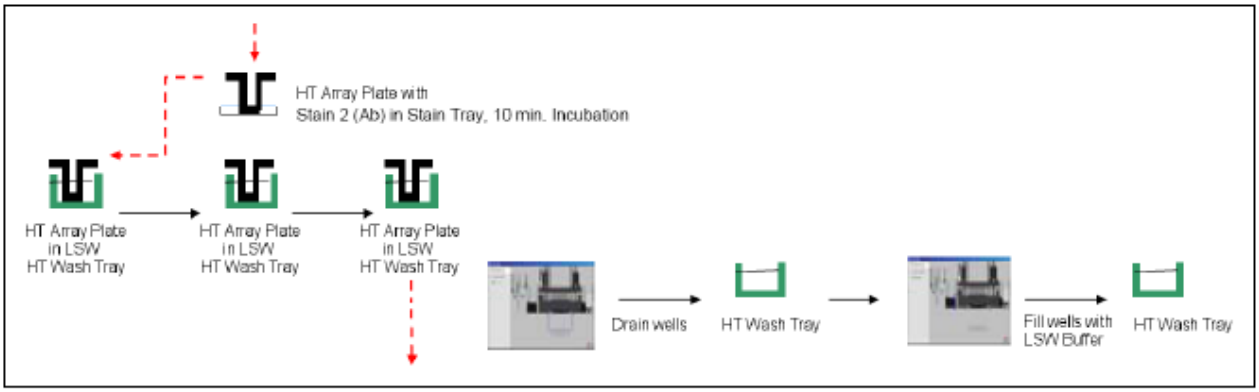


Figure 6.24 Step 5: Second Stain (Ab); Low Stringency Wash; Drain/Refill HT Wash Tray

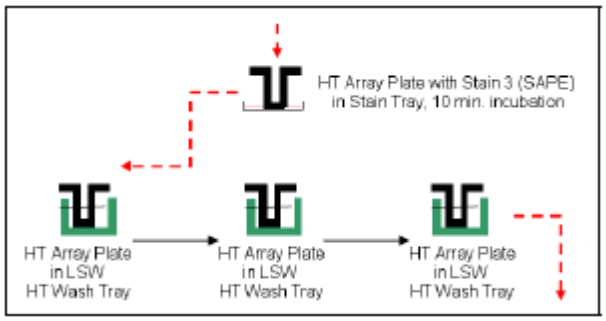


Figure 6.25 Step 6: Third Stain (Streptavidin-Phycoerythrin); Low Stringency Wash

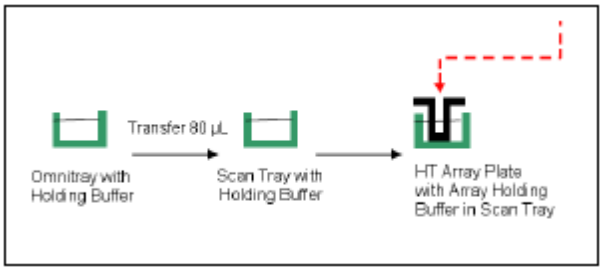


Figure 6.26 Step 7: Filling HT Scan Tray with Holding Buffer and Insertion of the HT Array Plate

Beginning a Run – Wash and Stain Deck Layout

This section describes how to assemble the deck and use the Sciclone Workstation Software to begin a Wash and Stain run. The Wash and Stain protocol will perform the following methods on the GeneChip™ Array Station:

- Wash the hybridized HT Array Plate in Low Stringency and High Stringency Buffer
- Stain the hybridized HT Array Plate
- Assemble the hybridized HT Array Plate for scanning

Procedure

1. Set up deck with appropriate consumables. Refer to Figure 6.27.

NOTE: The HT Stain Trays containing Stain 1, Stain 2, and Stain 3 are stacked and covered with a flat lid. The three trays should be stacked in ascending order, with Stain 1 on top, and Stain 3 on bottom. Stain 1 is covered with the flat lid.

2. Load at least three tip boxes in Rack 1.
3. Assemble the Peltier adaptor and Hybridization Block Fixture. Refer to Figure 6.28 and Figure 6.28.
4. Set the temperature of the Watlow Temperature Controller to 48°C. Refer to Figure 6.29.

TIP: The High Stringency Buffer must be between 41°C and 42°C for the Wash and Stain protocol. During the Wash and Stain deck setup, the High Stringency Buffer is placed in a HT Wash Tray and placed on the Peltier adaptor to reach a temperature between 41°C and 42°C. The temperature adjustment between 41°C and 42°C takes a long time. To decrease this time requirement, place 100 mL of the High Stringency Buffer in a 37°C incubator the night before so it is prewarmed.

5. Place the buffer lines in correct liquid carboys. The bulk dispenser buffer line should be placed in the carboy containing Low Stringency Wash Buffer. The Z8 buffer line should be placed in DI water.

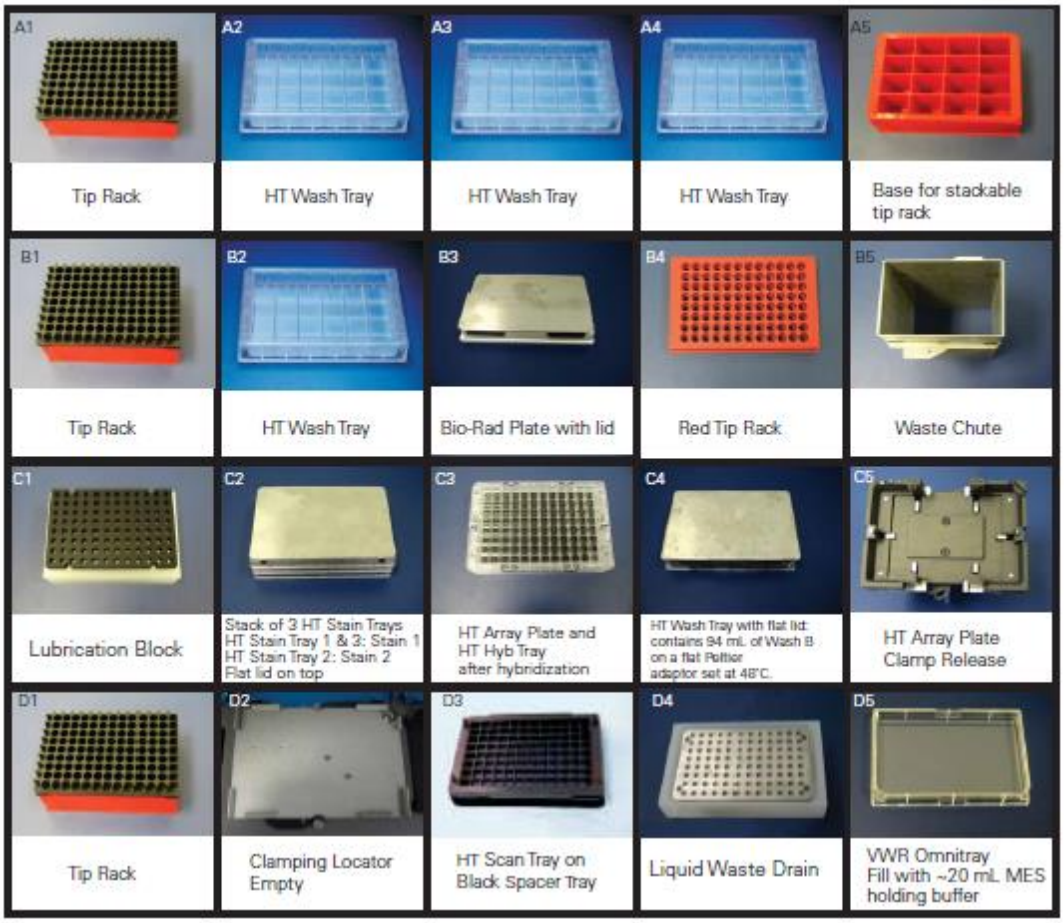


Figure 6.27 The deck layout for the wash and stain protocol—load at least three tip racks in rack 1.

NOTE: The red tip rack used in B4 should be used only once to prevent the High Stringency Wash lid from adhering to it.

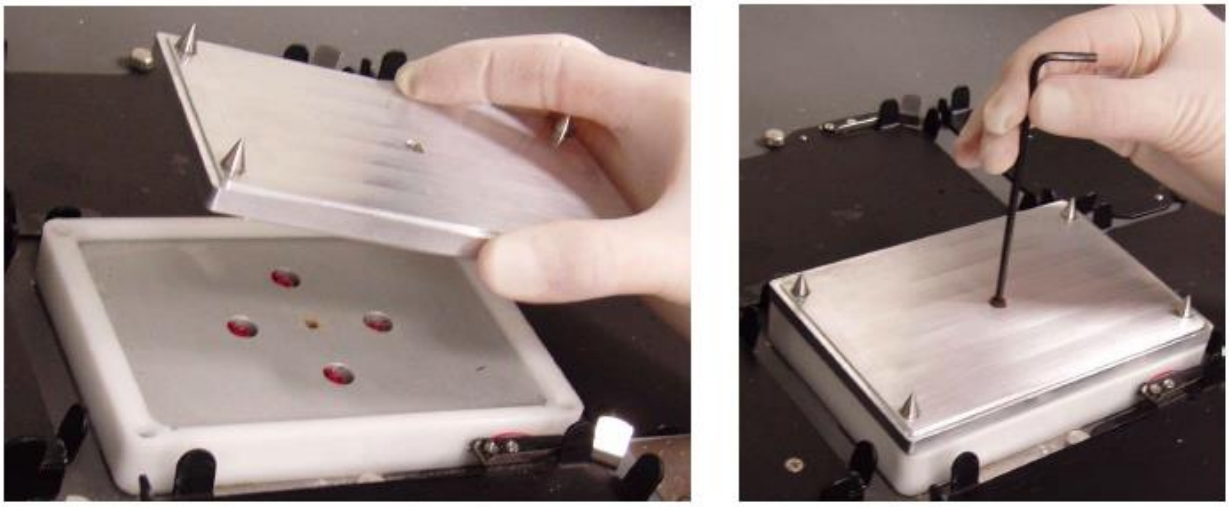


Figure 6.28 Installing the hybridization block fixture (left) and Securing the hybridization block fixture (right)

Figure 6.29 Setting the 48°C temperature on the Watlow Temperature Controller

Running the Wash and Stain Protocol

1. Check to confirm that the deck and racks are correctly populated with well plates, tips, etc.
2. Remove the HT Array Plate/Hybridization Tray Sandwich from the incubator and place on the deck at location C3.
3. Open the software by double-clicking the GeneChip™ Array Station desktop icon, or click **Start → Programs → Caliper Life Sciences → Instruments → Sciclone → Sciclone Workstation Software**. The login window appears **(**Figure 6.30).

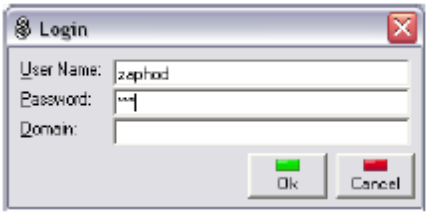


Figure 6.30 Sciclone Workstation Software Login window

4. Enter your **User name** and **Password** and click **OK**. The Operator Runtime interface appears **(**Figure 6.31).

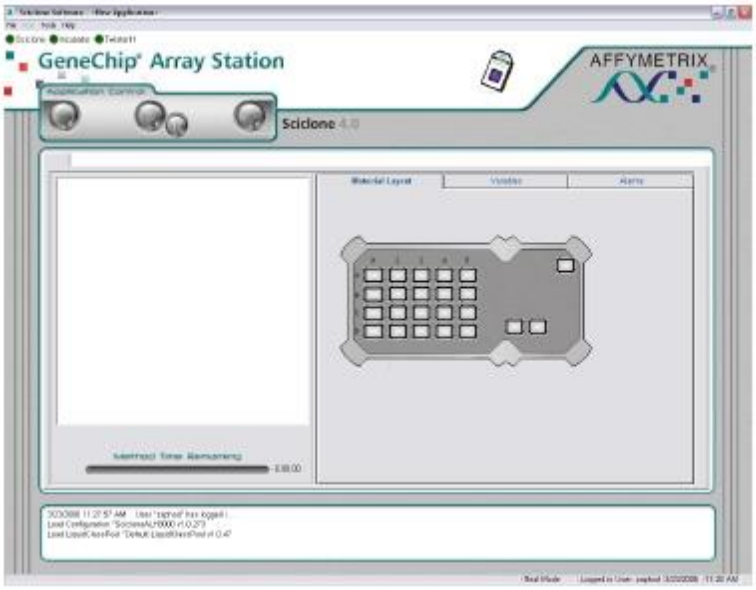


Figure 6.31 Sciclone Workstation Software Operator window

5. Select **File → Open** to access the load application window (Figure 6.32).

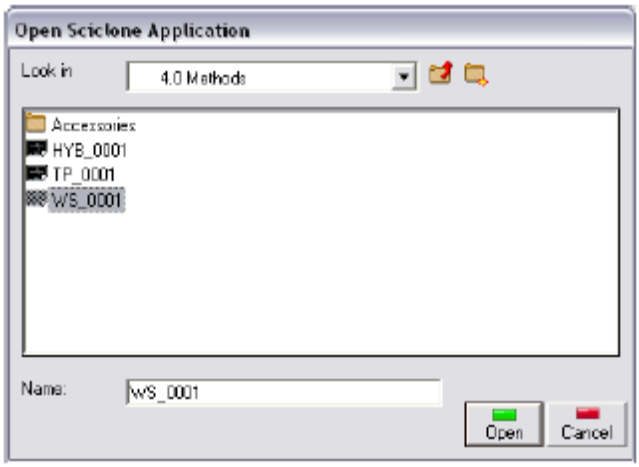


Figure 6.32 Open Sciclone Application window

6. Select **WS_0001** and click **Open**.

   The Wash Stain Protocol is loaded into Sciclone™ Workstation software (Figure 6.33).

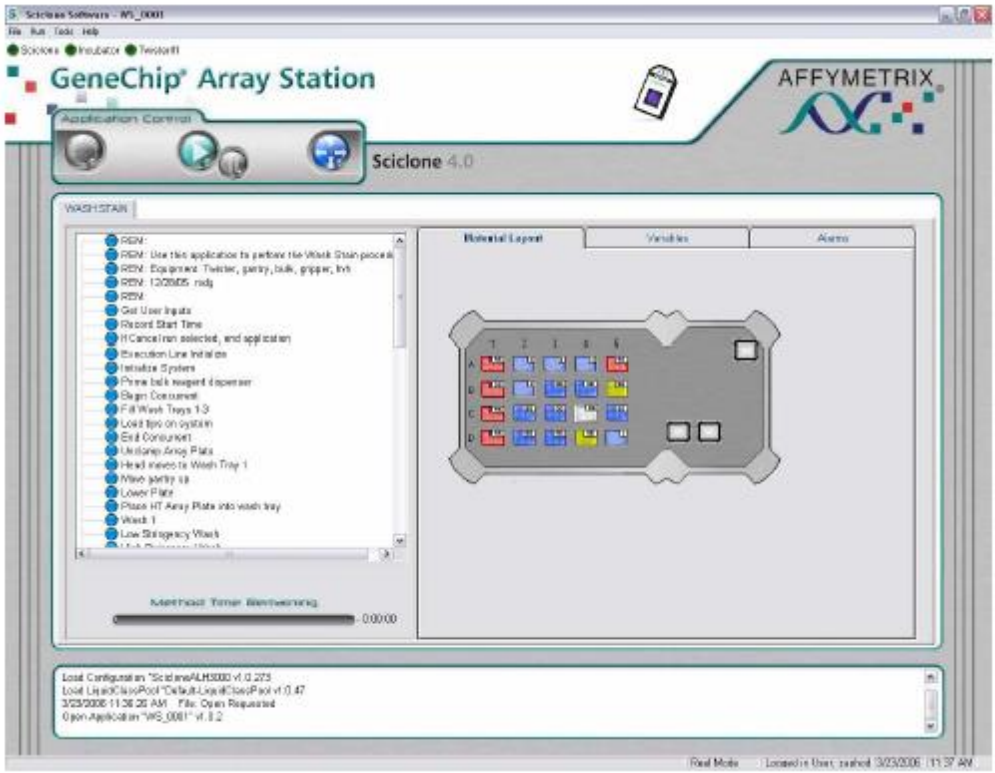


Figure 6.33 Wash and Stain Protocol Loaded

7. Click the green **Start** arrow to begin the application.

The Application prompts you to select several options through the interactive window shown in Figure 6.34.

Figure 6.34 Wash and Stain Setup window

8. Select the protocol parameters:

a. Select your **User name** from the drop down menu.

b. Select either a HT 24- or 96-Array Plate. It is important that you select the proper plate type to ensure samples are transferred to the correct wells.

c. If you wish the protocol to read and store the HT array plate barcode and the recovered sample plate barcode, check the Read barcodes option. Barcodes will be reported in the Wash/ Stain Summary Report at the end of the run.

d. If you are using spacer plates for tip static control (recommended), check the box Twister tip rack spacer plates are in use. If this box is checked, ensure you have placed spacer plates between each nested tip rack and on the top tip rack.

e. Enter a Tracking identifier (limited to 40 characters) if desired. The Tracking identifier will be recorded in the Summary report at the end of the run.

f. Select the desired notification settings. Be sure the email address or pager information is properly configured.

9. After selecting the settings parameters, click **Next**. The Wash Stain Deck Layout window appears (Figure 6.35).

Figure 6.35 Wash/Stain Deck Layout Setup window

10. Verify that you have set the deck up properly and that you have completed all items in the Notes section. Verify and check the boxes that you have completed all items in the Checklist section.

11. Click **Continue run** and the Array Station will perform the Wash/Stain protocol.

The Wash/Stain protocol continues until the HT Array Plate is stained and ready to be scanned. When the protocol has completed, the following message appears (Figure 6.36).

Figure 6.36 Ready to Scan window

12. Remove the stained HT Array Plate for scanning. Take care not to disturb or disrupt the array from the scan tray. Refer to the HT Scanner User's Guide (P/N 701978) for instructions on scanning the HT Array Plate.

13. Remove the sample recovery plate. Recovered hyb sample can be sealed and stored at –80°C for further use.

14. Change the Bulk Dispenser tubing back to the water bottle, Click **OK**. The Array Station will proceed to drain the wash trays and clean up the deck.

15. At the end of the protocol, The End message appears (Figure 6.37). Click **OK**.

Figure 6.37 Run Complete window

16. At the conclusion of each run, two summary reports are automatically saved by the program:

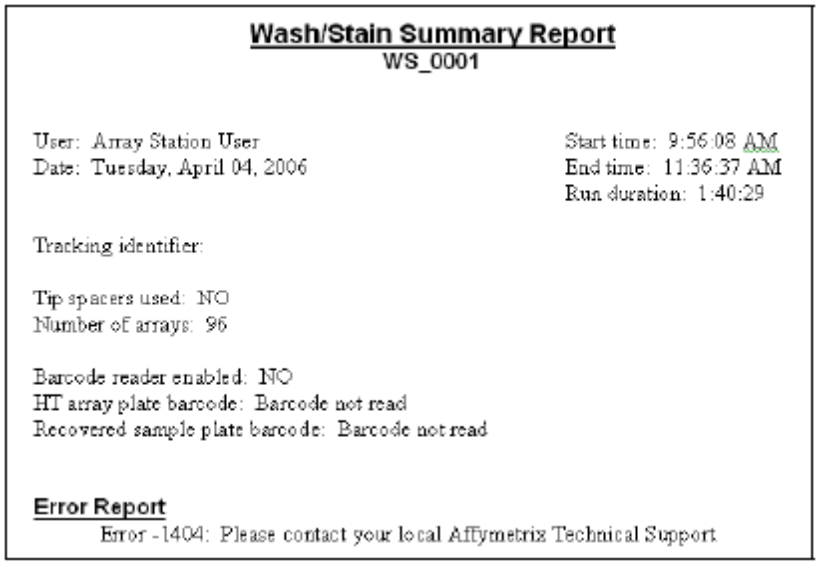
a. A user readable summary of the run is saved to the folder C:\Affymetrix\Reports\Summary. This file has the following naming convention: SummaryReport_WS_0001_4_4_2006_9_56_08_AM.rtf

WS_0001 = method used

4_4_2006 = date stamp of when the HYB_0001 starts

9_56_08_AM = time stamp of when the HYB_0001 starts

Figure 6.38 shows an example of a Summary Report.



Wash/Stain Summary Report
WS_0001

User: Array Station User
Date: Tuesday, April 04, 2006

Start time: 9:56:08 AM
End time: 11:36:37 AM
Run duration: 1:40:29

Tracking identifier:

Tip spacers used: NO
Number of arrays: 96

Barcode reader enabled: NO
HT array plate barcode: Barcode not read
Recovered sample plate barcode: Barcode not read

Error Report
Error -1404: Please contact your local Affymetrix Technical Support

Figure 6.38 Wash/Stain Summary Report Example

b. A report useful for transferring data to many LIMS systems is also saved. This report is saved in the folder C:\Affymetrix\Reports\Data. An example if this report is shown in Figure 6.39.

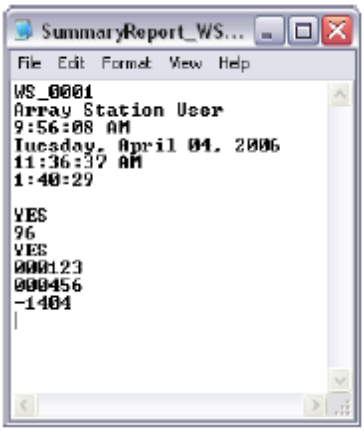


Figure 6.39 LIMS-friendly Report Example

Appendix A Master Mix Spreadsheet

Master Mix Volume Spreadsheets

This appendix contains master mix formulation tables for all of the reagents placed in the cold reagent block for the Target Preparation protocol. These master mix formulation tables are calculated for use on the GeneChip™ Array Station.

The GeneChip™ Array Station is designed to facilitate higher-throughput target preparation for the GeneChip™ Expression Assay. Reagent volumes provided in the GeneChip™ HT One-Cycle cDNA Synthesis Kit (P/N 900687) and the GeneChip™ HT IVT Labeling Kit (P/N 900688) are sufficient for four 24-sample master mixes, with overage to accommodate for pipetting error. Occasionally, smaller sample master mixes may be useful. Please note in the tables that the "Volume per Strip Tube Well" values for each reagent is adjusted to sufficiently accommodate the requirements of the GeneChip™ Array Station.

Table A.1 T7 Primer Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
T7-Oligo(dT) Primer, 50 µM	1.0	12.0	23.2	32.0	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
Nuclease-free Water	4.0	48.0	92.8	127.9	152.0	190.0	228.0	266.0	304.0	342.0	380.0	418.0	456.0
Total Volume	5.0	60.0	116.0	159.9	190.0	237.5	285.0	332.5	380.0	427.5	475.0	522.5	570.0
Vol per Strip Tube Well		7.0	13.5	18.5	22.0	28.0	34.0	40.0	45.5	51.4	57.4	63.3	69.3

Table A.2 First-Strand cDNA Synthesis Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X 1st Strand Rxn Mix	4.0	46.4	82.8	114.0	152.0	190.0	228.0	266.0	304.0	342.0	380.0	418.0	456.0
DTT, 0.1 M	2.0	23.2	41.4	57.0	76.0	95.0	114.0	133.0	152.0	171.0	190.0	209.0	228.0
dNTP Mix, 10 mM	1.0	11.6	20.7	28.5	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
SuperScript™ II	1.0	11.6	20.7	28.5	38.0	47.5	57.0	66.5	76.0	85.5	95.0	104.5	114.0
Nuclease-free Water	2.0	23.2	41.4	57.0	76.0	95.0	114.0	133.0	152.0	171.0	190.0	209.0	228.0
Total Volume	10.0	116.0	207.0	285.0	380.0	475.0	570.0	665.0	760.0	855.0	950.0	1,045.0	1,140.0
Vol per Strip Tube Well		14.0	24.0	33.5	45.5	57.4	69.3	81.1	93.0	104.9	116.8	128.6	140.5

Table A.3 Second-Strand cDNA Synthesis Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X 2nd Strand Rxn Mix	30.0	270.0	540.0	810.0	1,080.0	1,350.0	1,620.0	1,890.0	2,160.0	2,430.0	2,700.0	2,970.0	3,240.0
dNTP Mix, 10 mM	3.0	27.0	54.0	81.0	108.0	135.0	162.0	189.0	216.0	243.0	270.0	297.0	324.0
DNA Ligase, 10 unit/µL	1.0	9.0	18.0	27.0	36.0	45.0	54.0	63.0	72.0	81.0	90.0	99.0	108.0
DNA Polymerase I, 10 unit/µL	4.0	36.0	72.0	108.0	144.0	180.0	216.0	252.0	288.0	324.0	360.0	396.0	432.0
RNase H, 2 unit/µL	1.0	9.0	18.0	27.0	36.0	45.0	54.0	63.0	72.0	81.0	90.0	99.0	108.0
Total Volume	39.0	351.0	702.0	1,053.0	1,404.0	1,755.0	2,106.0	2,457.0	2,808.0	3159.0	3,510.0	3,861.0	4,212.0
Vol per Strip Tube Well		41.0	85.0	129.0	172.0	216.0	260.0	303.0	347.0	391.0	435.0	479.0	522.5

Table A.4 T4 DNA Polymerase Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
T4 DNA Polymerase	2.0	30.7	49.7	69.3	81.1	98.3	118.0	137.7	157.3	177.0	196.7	216.3	236.0
1X T4 DNA Polymerase Buffer	2.0	30.7	49.7	69.3	81.1	98.3	118.0	137.7	157.3	177.0	196.7	216.3	236.0
Total Volume	4.0	61.4	99.4	138.6	162.2	196.6	236.0	275.4	314.6	354.0	393.4	432.6	472.0
Vol per Strip Tube Well		7.0	11.0	16.0	18.5	23.0	27.8	33.0	38.0	42.3	47.3	52.1	57.0

Table A.5 IVT Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
10X IVT Buffer	6.0	56.0	112.0	168.0	224.0	280.0	336.0	392.0	448.0	504.0	560.0	616.0	672.0
IVT Labeling NTP Mix	18.0	168.0	336.0	504.0	672.0	840.0	1,008.0	1,176.0	1,344.0	1,512.0	1,680.0	1,848.0	2,016.0
IVT Labeling Enzyme Mix	6.0	56.0	112.0	168.0	224.0	280.0	336.0	392.0	448.0	504.0	560.0	616.0	672.0
T7 RNA Polymerase	1.0	9.3	18.7	28.0	37.3	46.7	56.0	65.3	74.7	84.0	93.3	102.7	112.0
Nuclease-free Water	7.0	65.3	130.7	196.0	261.3	326.7	392.0	457.3	522.7	588.0	653.3	718.7	784.0
Total Volume	38.0	354.6	709.4	1,064.0	1,418.6	1,773.4	2,128.0	2,482.6	2,837.4	3,192.0	3,546.6	3,901.4	4,256.0
Vol per Strip Tube Well		43.0	87.0	131.0	174.0	219.0	264.0	308.0	352.0	396.0	440.0	484.0	529.0

Table A.6 Fragmentation Cocktail for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
5X Fragmentation Buffer (Vol per Strip Tube Well)	7.5	11.4	17.8	26.7	35.6	44.5	53.4	62.3	71.3	80.2	89.1	98.0	106.9

Table A.7 1 HT Array Plate
100 µL Hyb Cocktail Master Mix (using the GeneChip™ HT Hyb, Wash and Stain Kit)

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
20X Hybridization Control Stock	5.0	51.1	93.7	139.1	184.5	232.8	272.5	317.9	363.3	408.8	454.2	499.6	545.0
3nM B2 Oligo	1.65	16.9	30.9	45.9	60.9	76.8	89.9	104.9	119.9	134.9	149.9	164.9	179.9
HT 1.2X Hybridization Mix*	83.35	851.7	1,561.4	2,318.6	3,075.7	3,880.2	4,542.6	5,299.7	6,056.8	6,814	7,570.9	8,328	9,085.2
Total	90.0	919.7	1,686.0	2,503.6	3,321.1	4,189.8	4,905.0	5,722.5	6,540.0	7,357.7	8,175.0	8,992.5	9,810.1
# of QIAGEN Strips Used		1	1	1	1	2	2	2	2	3	3	3	3
Volume per Well 1st Strip		114.0	204.0	306.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0
Volume per Well 2nd Strip						114.0	204.0	306.0	400.0	400.0	400.0	400.0	400.0
Volume per Well 3rd Strip										114.0	204.0	306.0	400.0

* The 1.2X Hybridization Mix is a component of the HT Hybridization, Wash, and Stain Kit containing BSA, HS DNA and 1.23X hybridization buffer.

Table A.8 2 HT Array Plates

200 µL Hyb Cocktail Master Mix (using the GeneChip™ HT Hyb, Wash and Stain Kit)

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
20X Hybridization Control Stock	10.0	90.8	181.7	272.5	363.3	454.2	545.0	635.8	726.7	817.5	908.3	999.2	1,090.0
3nM B2 Oligo	3.3	30.0	60.0	89.9	119.9	149.9	179.9	209.8	239.8	269.8	299.8	329.7	359.7
HT 1.2X Hybridization Mix*	166.7	1,514.3	3,028.3	4,542.6	6,056.8	7,570.9	9,085.2	10,599.4	12,113.5	13,627.7	15,142	16,656	18,170.3
Total	180.0	1,635.1	3,270.0	4,905.0	6,540.0	8,175.0	9,810.1	11,445.0	13,080.0	14,715.0	16,350.1	17,984.9	19,620.0
# of QIAGEN Strips Used		1	1	2	2	3	3	4	4	5	5	6	6
Volume per Well 1st Strip		204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 2nd Strip				204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 3rd Strip						204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 4th Strip								204.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 5th Strip										204.0	408.0	408.0	408.0
Volume per Well 6th Strip												204.0	408.0

*** The 1.2X Hybridization Mix is a component of the HT Hybridization, Wash, and Stain Kit containing BSA, HS DNA and 1.23X hybridization buffer.**

Table A.9 1 HT Array Plates

100 µL Hybridization Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
20X Hybridization Control Stock	5.0	51.1	93.7	139.1	184.5	232.8	272.5	317.9	363.3	408.8	454.2	499.6	545.0
3nM B2 Oligo	1.65	16.9	30.9	45.9	60.9	76.8	89.9	104.9	119.9	134.9	149.9	164.9	179.9
HS DNA (10 mg/mL)	1.0	10.2	18.7	27.8	36.9	46.6	54.5	63.6	72.7	81.8	90.8	99.9	109.0
Acetylated BSA (50 mg/mL)	1.0	10.2	18.7	27.8	36.9	46.6	54.5	63.6	72.7	81.8	90.8	99.9	109.0
1.23x Hybridization Buffer*	81.35	831.3	1,524.0	2,263.0	3,001.9	3,787.0	4,433.6	5,172.5	5,911.4	6,650.4	7,389.3	8,128.2	8,867.2
Total	90.0	919.7	1,686.0	2,503.6	3,321.1	4,189.8	4,905.0	5,722.5	6,540.0	7,357.7	8,175.0	8,992.5	9,810.1
# of QIAGEN Strips Used		1	1	1	1	2	2	2	2	3	3	3	3
Volume per Well 1st Strip		114.0	204.0	306.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0	400.0
Volume per Well 2nd Strip						114.0	204.0	306.0	400.0	400.0	400.0	400.0	400.0
Volume per Well 3rd Strip										114.0	204.0	306.0	400.0

* The 1.23x Hybridization Buffer is made from a recipe found in *1.23X Hybridization Buffer*, and is not a component of the HT Hybridization, Wash, and Stain Kit.

Table A.10 2 HT Array Plates

200 µL Hybridization Cocktail Master Mix for Cold Reagent Block

	Volume per Rxn	Adjusted Volumes (µL): 8 Rxns	16 Rxns	24 Rxns	32 Rxns	40 Rxns	48 Rxns	56 Rxns	64 Rxns	72 Rxns	80 Rxns	88 Rxns	96 Rxns
20X Hybridization Control Stock	10.0	90.8	181.7	272.5	363.3	454.2	545.0	635.8	726.7	817.5	908.3	999.2	1,090.0
3nM B2 Oligo	3.3	30.0	60.0	89.9	119.9	149.9	179.9	209.8	239.8	269.8	299.8	329.7	359.7
HS DNA (10 mg/mL)	2.0	18.2	36.3	54.5	72.7	90.8	109.0	127.2	145.3	163.5	181.7	199.8	218.0
Acetylated BSA (50 mg/mL)	2.0	18.2	36.3	54.5	72.7	90.8	109.0	127.2	145.3	163.5	181.7	199.8	218.0
1.23x Hybridization Buffer*	162.7	1,477.9	2,955.7	4,433.6	5,911.4	7,389.3	8,867.2	10,345.0	11,822.9	13,300.7	14,778.6	16,256.4	17,734.3
Total	180.0	1,635.1	3,270.0	4,905.0	6,540.0	8,175.0	9,810.1	11,445.0	13,080.0	14,715.0	16,350.1	17,984.9	19,620.0
# of QIAGEN Strips Used		1	1	2	2	3	3	4	4	5	5	6	6
Volume per Well 1st Strip		204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 2nd Strip				204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 3rd Strip						204.0	408.0	408.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 4th Strip								204.0	408.0	408.0	408.0	408.0	408.0
Volume per Well 5th Strip										204.0	408.0	408.0	408.0
Volume per Well 6th Strip												204.0	408.0

* The 1.23x Hybridization Buffer is made from a recipie found in *1.23X Hybridization Buffer*, and is not a component of the HT Hybridization, Wash, and Stain Kit.

Appendix B Automated Sample Transfer

Loading Sample Plate and Initial Deck Layout for Automated Sample Transfer

The Automated Sample Transfer function uses the Array Station to transfer 5 µL of total RNA from a Greiner U-bottom plate to a lidded Bio-Rad 96-Well Hard-Shell PCR Plate. To use this option, you must have at least 20 µL of your sample at a concentration of 0.2 to 0.4 µg/µL in a Greiner U-bottom plate. The samples should be placed in the wells in a column wise fashion in the same wells to be utilized for the Array Station Target Preparation run.

Procedure

1. Set up the deck with the appropriate consumables, as illustrated in Figure B.1.

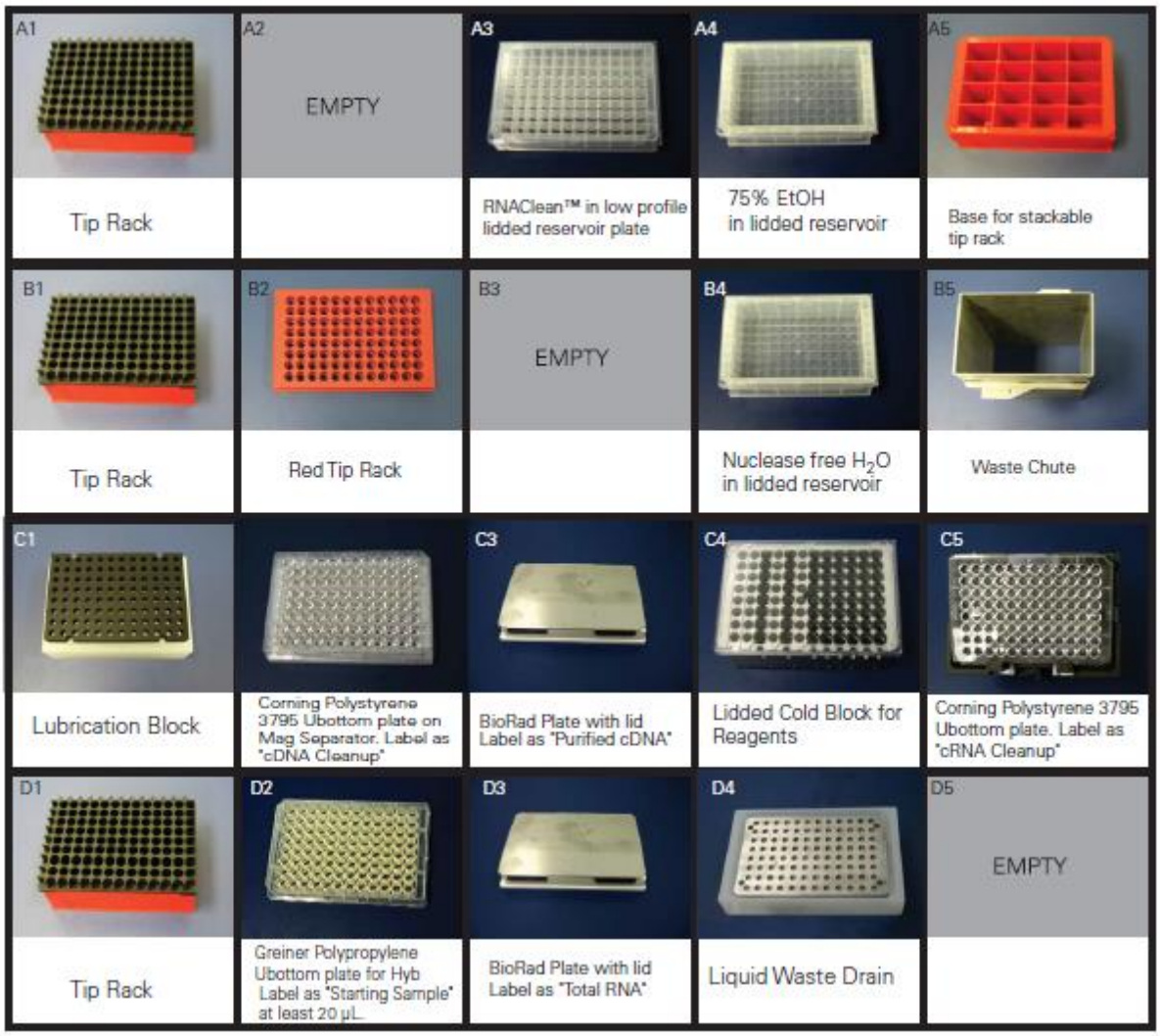


Figure B.1 Auto Starting Deck Layout

2. Start the software and load the TP_0001 application. Start the run by clicking the green arrow on the Application Control Console. The first window you see is shown in Figure B.2.

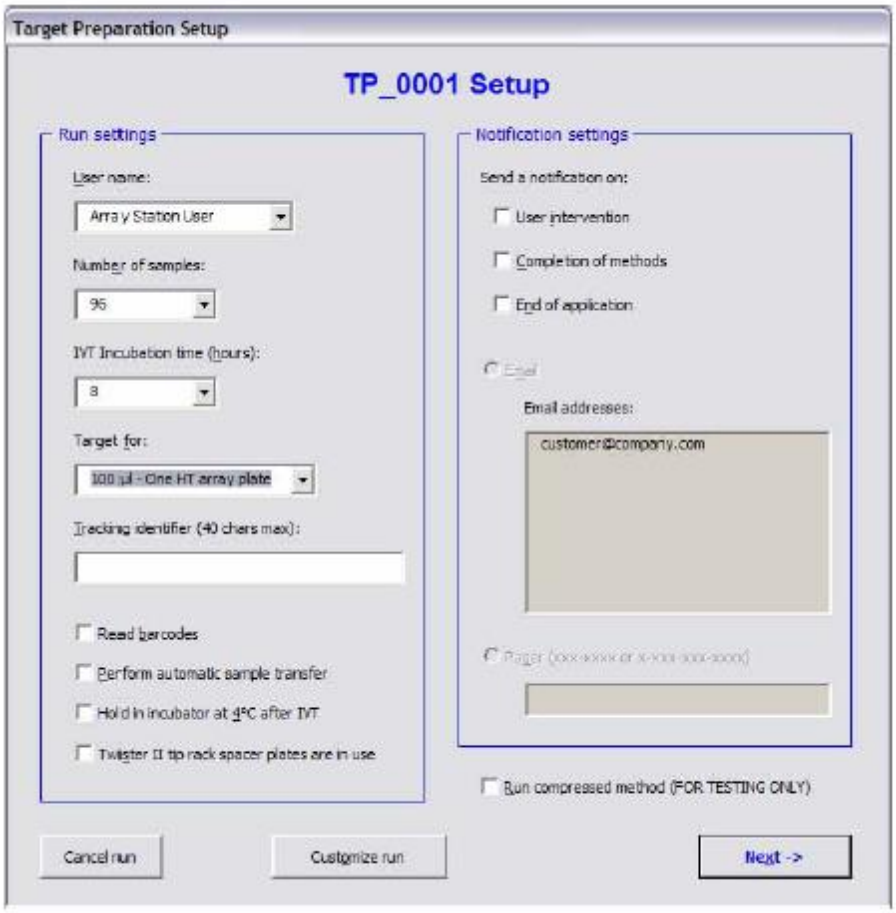


Figure B.2 Target Preparation Setup window

a. From the drop-down option boxes, select **User Name**, **Number of Samples** by 8's (by column starting with position A1), **IVT Incubation Time**, and either 100 µL one HT Array plate, or 200 µL - two HT Array plates.

b. In the Tracking Identifier box, you may put barcodes or other unique identification codes.

c. There are five options at the bottom of the Run Settings section in the TP-0001 Setup window:

- If using barcodes, select the barcode option. Please refer to Chapter 4, substep f for a description on how to use barcoded plates.
- Select the Perform automatic sample transfer option.
- Select the option, Hold in incubator at 4°C after IVT if you want the plate to be held at 4°C before proceeding to the cRNA cleanup steps. User intervention is required after IVT if you select this option. See Figure 4.34.
- Select the option, **Twister II tip rack spacer plates are in use** when using the spacer plates if there is a static electricity concern.
- The last option, Run compressed method (FOR TESTING ONLY) is for testing purposes only and should not be selected.

d. Notification **Settings**: During unattended operation, if problems arise the user notification feature allows an operator to be notified remotely by sending an email or dialing a phone number for pager/text purposes.

3. When the appropriate options are selected, click the **Next** button.

To cancel before continuing the run follow these steps:

1. **Click the "Cancel Run" button.**
2. **Two windows appear in succession.**
3. **Click "Yes" and "OK" sequentially to cancel the run.**

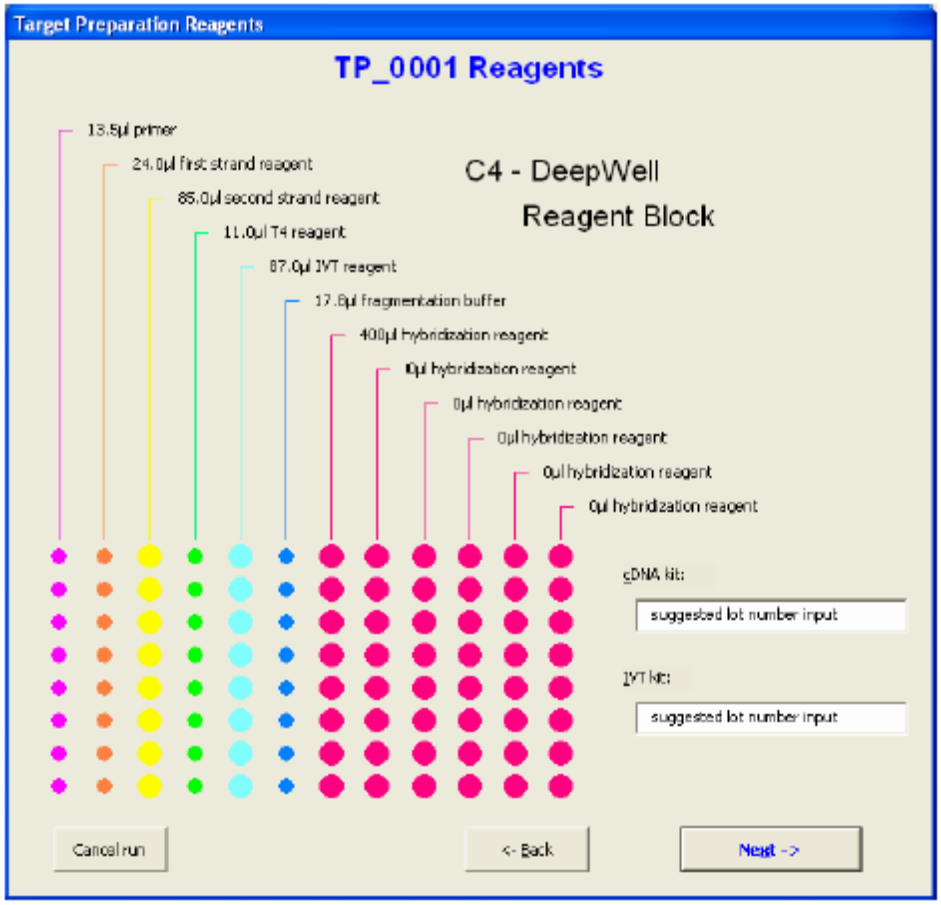


Figure B.3 TP_0001 Reagents window

4. Use the TP_0001 Reagents window to ensure all reagents are loaded. There are two user entry fields found in this window, one for the cDNA Kit and one for the IVT Kit. Lot numbers of the kits are tracked by typing them into the appropriate field.
5. After you have verified that your reagents are loaded and have typed in the lot numbers of the kits, click **Next**.

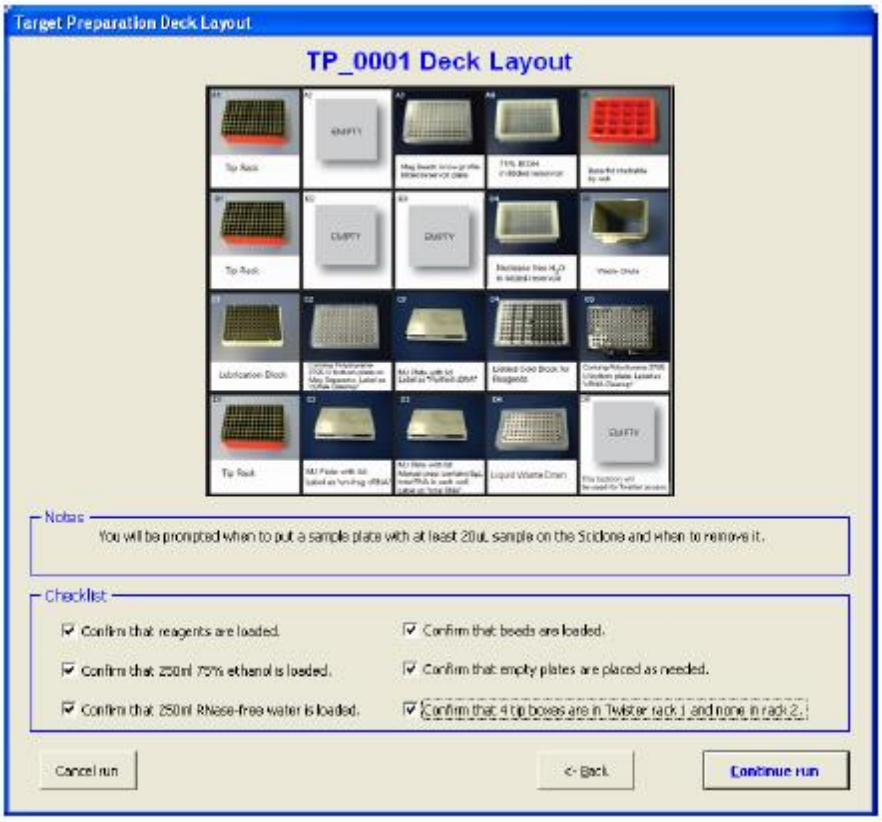


Figure B.4 TP_0001 Deck Layout Checklist window

To cancel before continuing the run follow these steps:

1. **Click the "Cancel Run" button.**
2. **Two windows appear in succession.**
3. **Click "Yes" and "OK" sequentially to cancel the run**

6. Use the six-option **Checklist** of this window to ensure all consumables are loaded. After selecting each option (all six boxes should be checked as in Figure B.4) the **Continue Run** button becomes enabled.
7. Click Continue Run.
8. After transfer is complete, Figure B.5 appears.

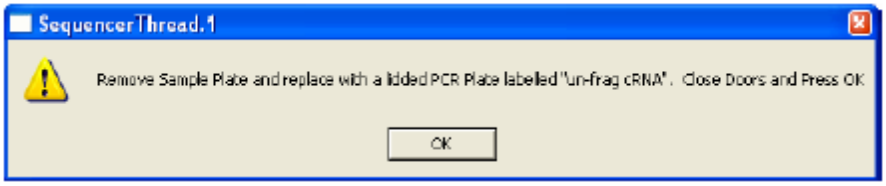


Figure B.5 User message to replace sample plate with lidded PCR plate.

9. Remove the Greiner plate from the D2 position and replace it with the "unfrag cRNA" plate (Bio-Rad 96-well Hard Shell PCR Plate). Close the doors to the Array Station and select **OK** to proceed.
10. Refer to Chapter 4, *Array Station Setup and Target Preparation* instructions for the remaining steps of the process.

Appendix C Array Station Customized Applications And Deck Layouts

Deck layouts for the Array Station Target Preparation Options

The Array Station Automated Target Preparation Application offers options for running all or portions of the One Cycle Eukaryotic Labeling Process.

You will need to configure the Deck Layout properly depending upon where in the process you elect to start. Deck layouts for all possible starting options are presented in this Appendix.

1. To run customized applications, click the **Customize run** button as shown in **Figure C.1.**

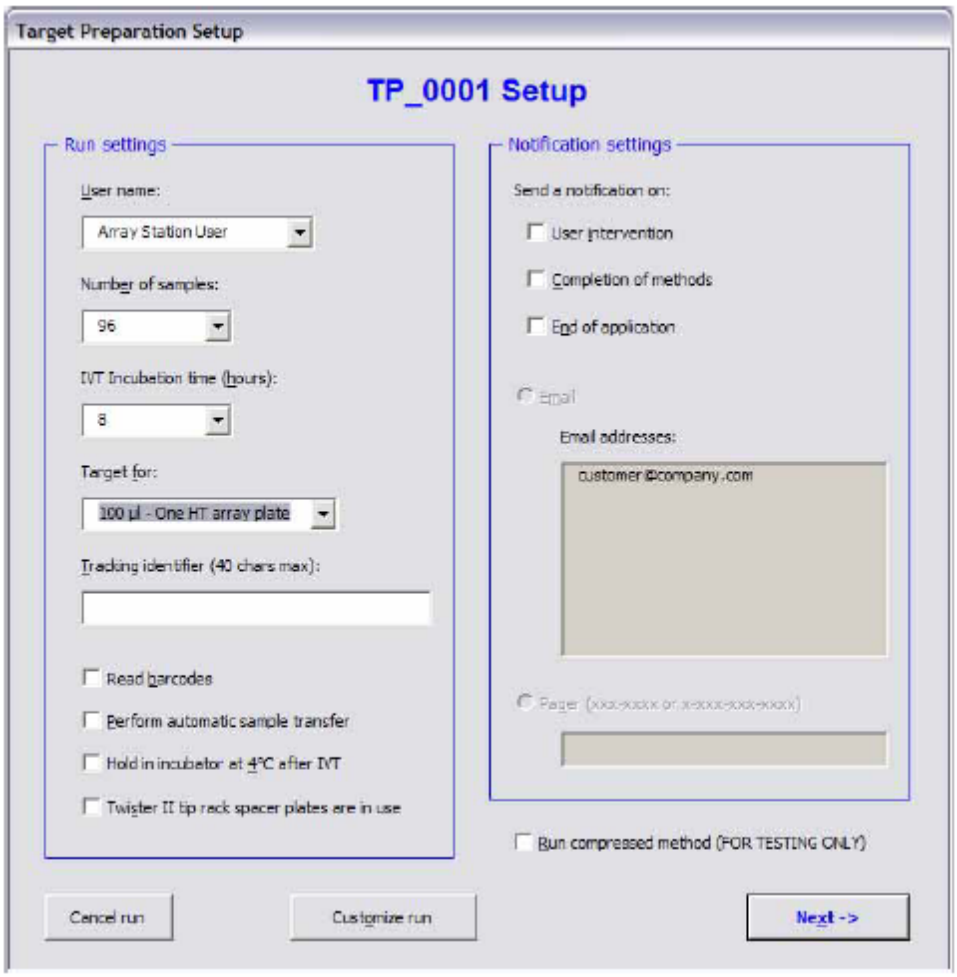


Figure C.1 Target Preparation Setup window

You are prompted for information on the Run Type as shown in Figure C.2.

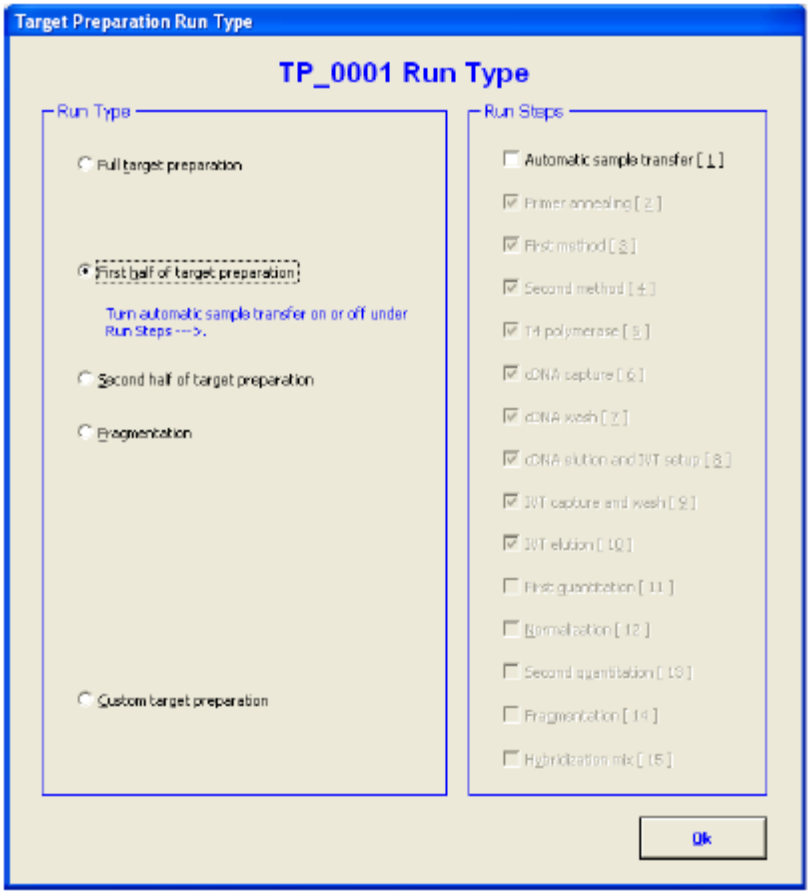


Figure C.2 Customized Run Setup window

2. For the **Run Type** window, you will need to select one of the following options:

   a. **Full target preparation**: When this option is selected, the protocol will run all methods associated with target preparation from Primer Anneal through Hybridization Mix preparation. Notice that when you select this option, all methods from **Primer annealing [2]** to **Hybridization mix [15]** will be selected for you in the **Run Steps**. In addition, if you want the Array Station to transfer your sample from a Greiner U-bottom to the Bio-Rad 96 Well plate, you must select the checkbox **Automatic Sample Transfer [1]**. If 5 µL of your sample is already in the Bio-Rad plate, leave this box unchecked. When running the **Full target preparation** selection, you will be prompted to change the deck, read plates and transfer plate reader data to the Array Station at indicated times.

   b. **First half of target preparation**: When this option is **selected**, only the methods from Primer anneal through IVT elution will execute. Notice that when you select this option, all methods from **Primer annealing [2]** to **IVT elution [10]** will be selected for you in the **Run Steps**. In addition, if you want the Array Station to transfer your sample from a Greiner U-bottom to the Bio-Rad 96 Well plate, you will need to select the checkbox **Automatic Sample Transfer [1]**. If 5 µL of your sample is already in the Bio-Rad plate, leave this box unchecked. At the end of this process, the cRNA will be cleaned and placed in the incubator at 4°C. This RNA should be removed and stored at –80°C until ready for further processing.

   c. **Second half of target preparation**: When this option is selected, only the methods First quantitation through Hybridization mix will execute. Notice that when you select **this** option, methods from **First Quantitation [11]** to **Hybridization mix [15]** will be selected for you in the **Run Steps**. This selection assumes you are starting with the purified cRNA obtained from the **First half of target preparation**. When running the **Second half of target preparation** selection, you will be prompted to read plates and transfer plate reader data to the Array Station at indicated times.

d. **Fragmentation**: **When** this option is selected, only the last two methods, Fragmentation and Hybridization mix will execute. Notice that when you select this option, the methods **Fragmentation [14]** and **Hybridization mix [15]** are selected for you in the **Run Steps**.

e. The option **Custom target preparation** enables you to select the steps you choose to run. If you select Custom target preparation, you gain the ability to manually select methods in the **Run Steps** window. You may choose the starting and ending methods by selecting the desired boxes. You must select contiguous methods. Please pay special attention that you set the deck up correctly for the starting method you choose. Please see Table C.1 for starting deck layouts for custom method selections.

NOTE: Starting at Normalization [12] is not supported. If you select normalization as your starting option and proceed, an error message will appear.

3 When you have selected the **Run Type** you wish to perform, click **OK** to continue. A window displaying the appropriate deck layout for your selection appears.

Table C.1 through Table C.3 lists the correct Deck Layouts for all possible starting selections. Figure C.3 through Figure C.17 illustrate the various deck layouts. Be sure to refer to the deck layout corresponding to your starting selection when configuring the deck for your run.

Table C.1 The Array Station Starting Deck Layouts for Major Run Types

Starting Selection	Deck Layout
Full Target Prep Starting Layout – Manual Transfer	Layout A
Full Target Prep Starting Layout – Automated Transfer	Layout B
First Half of Target Prep Starting Layout – Manual Transfer	Layout A
First Half of Target Prep Starting Layout – Automated Transfer	Layout B
Second Half of Target Prep Starting Layout	Layout C
Fragmentation Starting Layout	Layout D

Table C.2 The Array Station Starting Deck Layouts for Custom Selection of Run Steps

Starting Selection	Deck Layout
Primer Annealing	Layout A
First Method	Layout E
Second Method	Layout F
T4 Polymerase	Layout G
cDNA Capture	Layout H
cDNA Wash	Layout I
cDNA Elution and IVT Setup	Layout J
IVT Capture and Wash	Layout K
IVT Elution	Layout L
First Quantitation	Layout C
Normalization	Not Supported
Second Quantitation	Layout M
Fragmentation	Layout D
Hybridization Mix	Layout N

Table C.3 Deck Layout for User Intervention

Starting Selection	Deck Layout
Full Target Prep User Intervention Layout	Layout O

NOTE: Please be sure to place all plates on deck as indicated in layouts. Even if the process for which that plate is used is complete, the robot expects the plate to be in that position. Serious errors can occur if plates are missing.

NOTE: When starting at any selection (except Full Target Prep User Intervention) tip boxes on the Deck at A1, B1 and D1 should be empty of any tips.

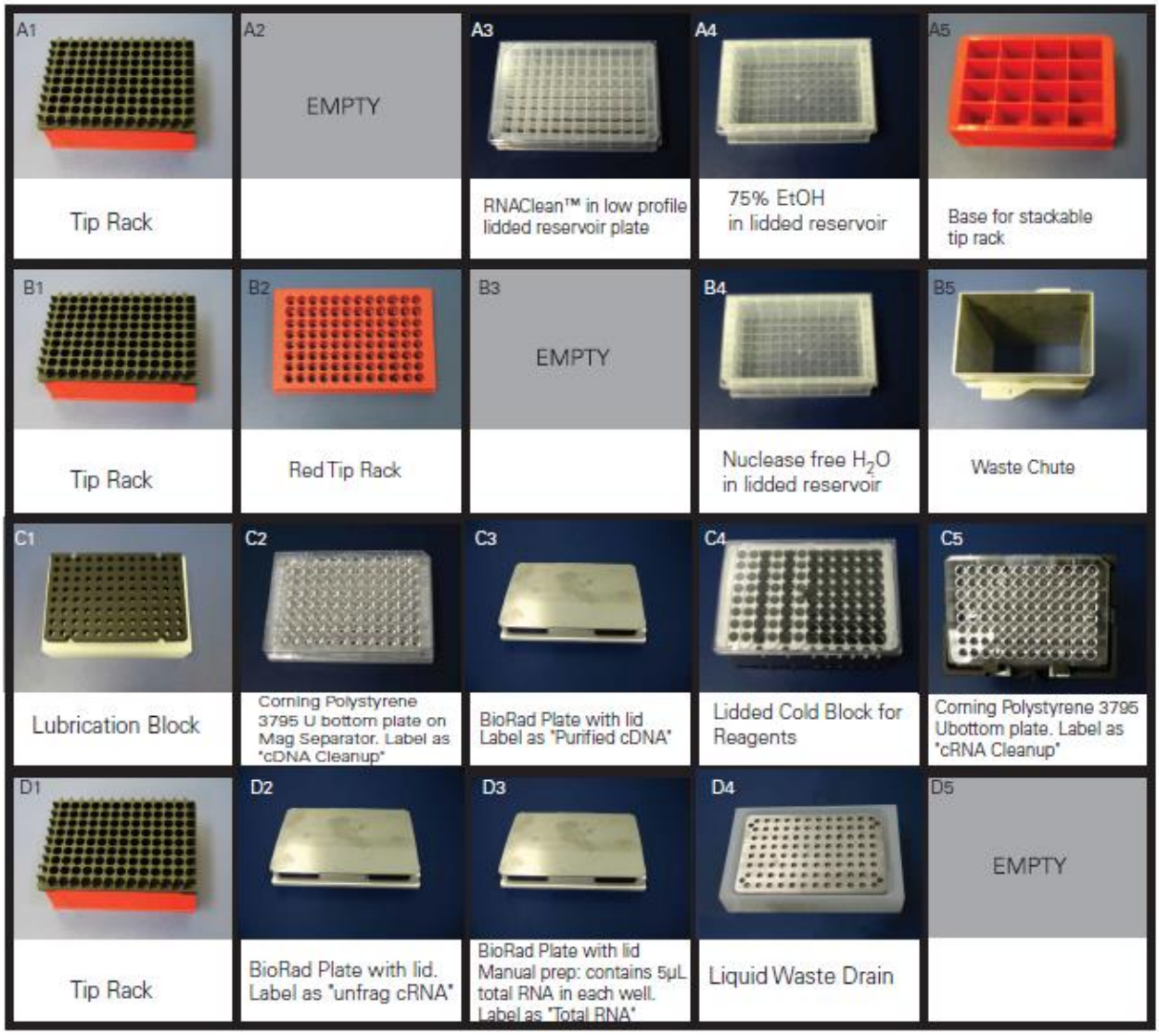


Figure C.3 Array Station Deck Layout Configuration A

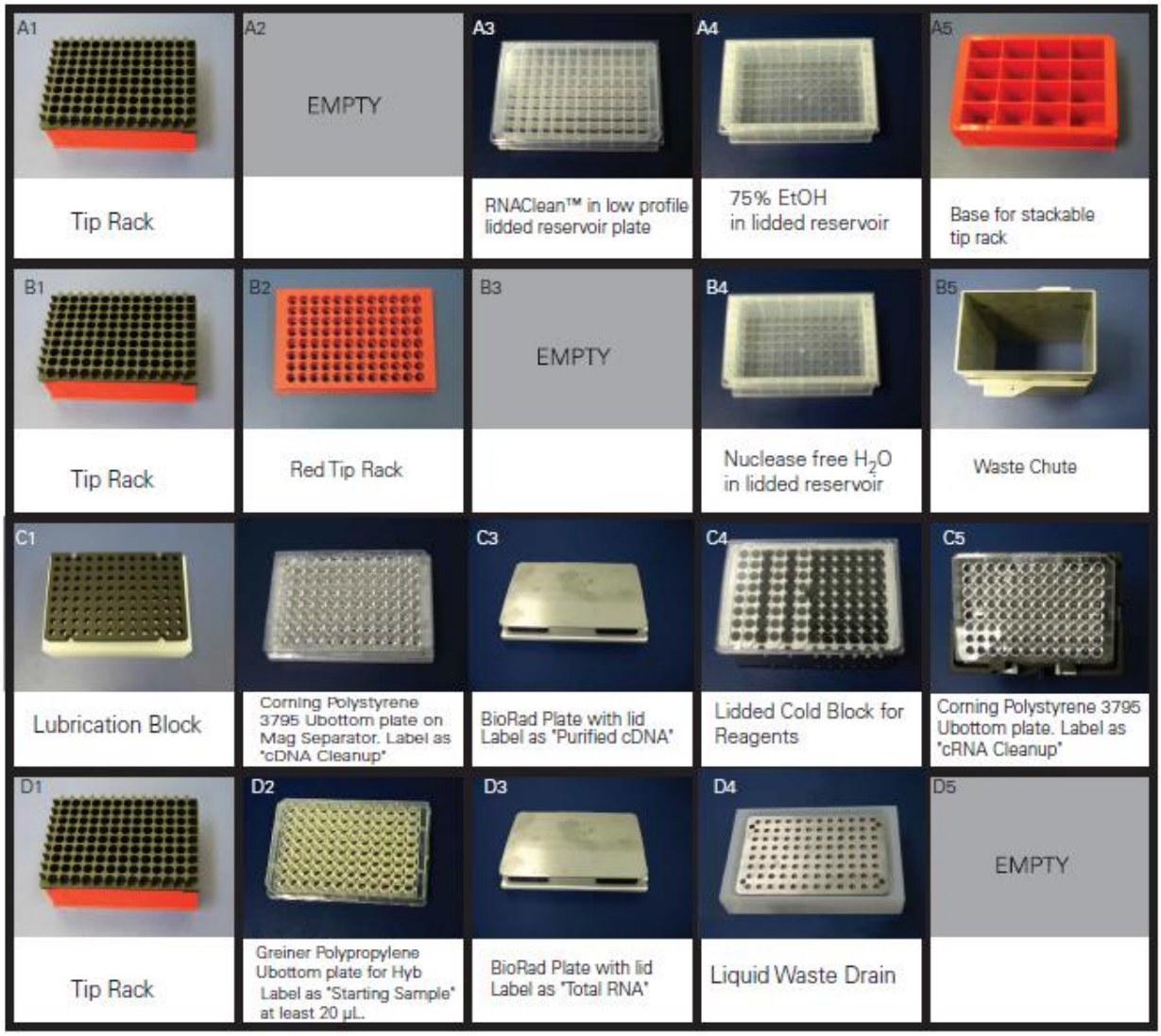


Figure C.4 Array Station Deck Layout Configuration B

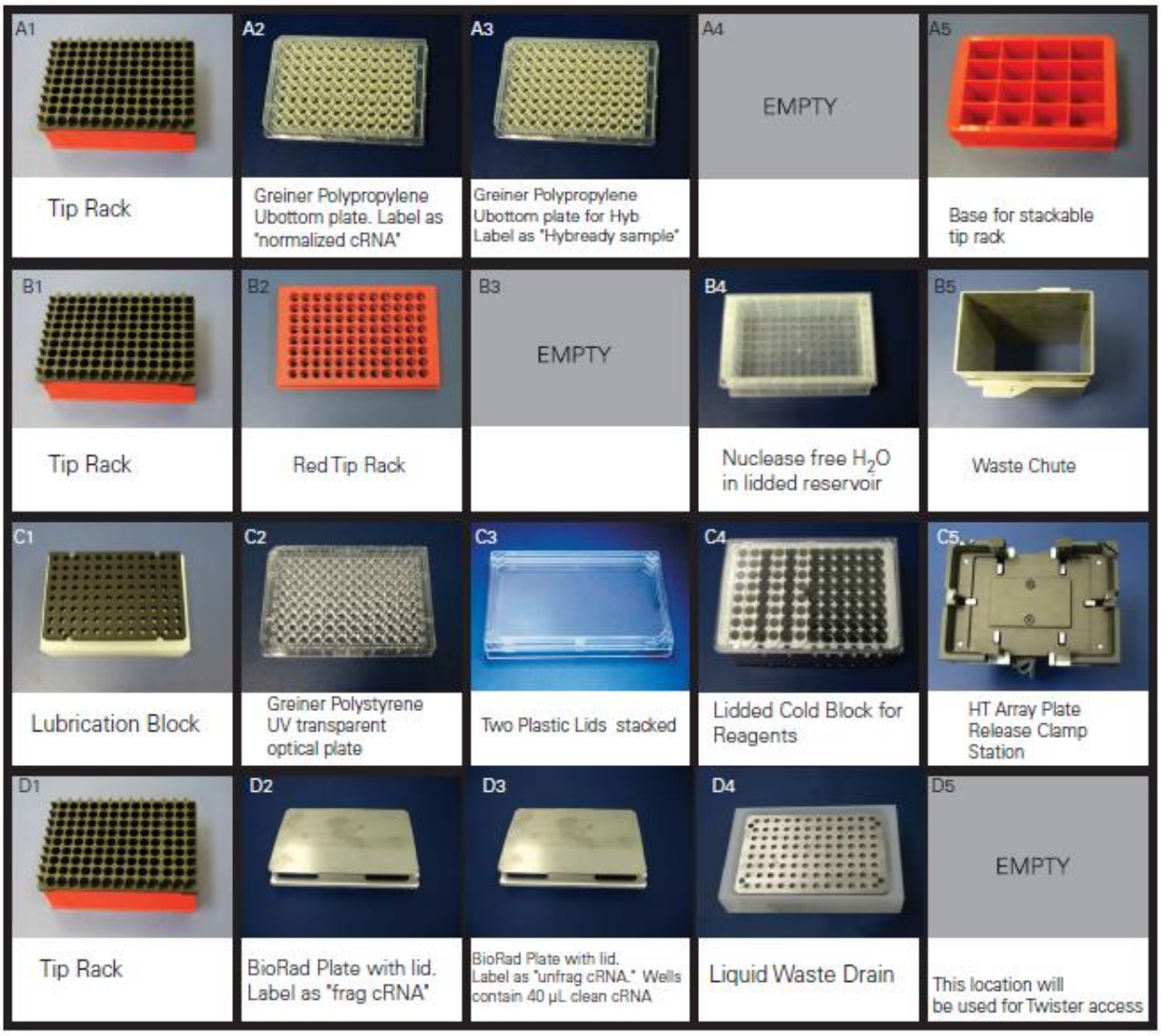


Figure C.5 Array Station Deck Layout Configuration C

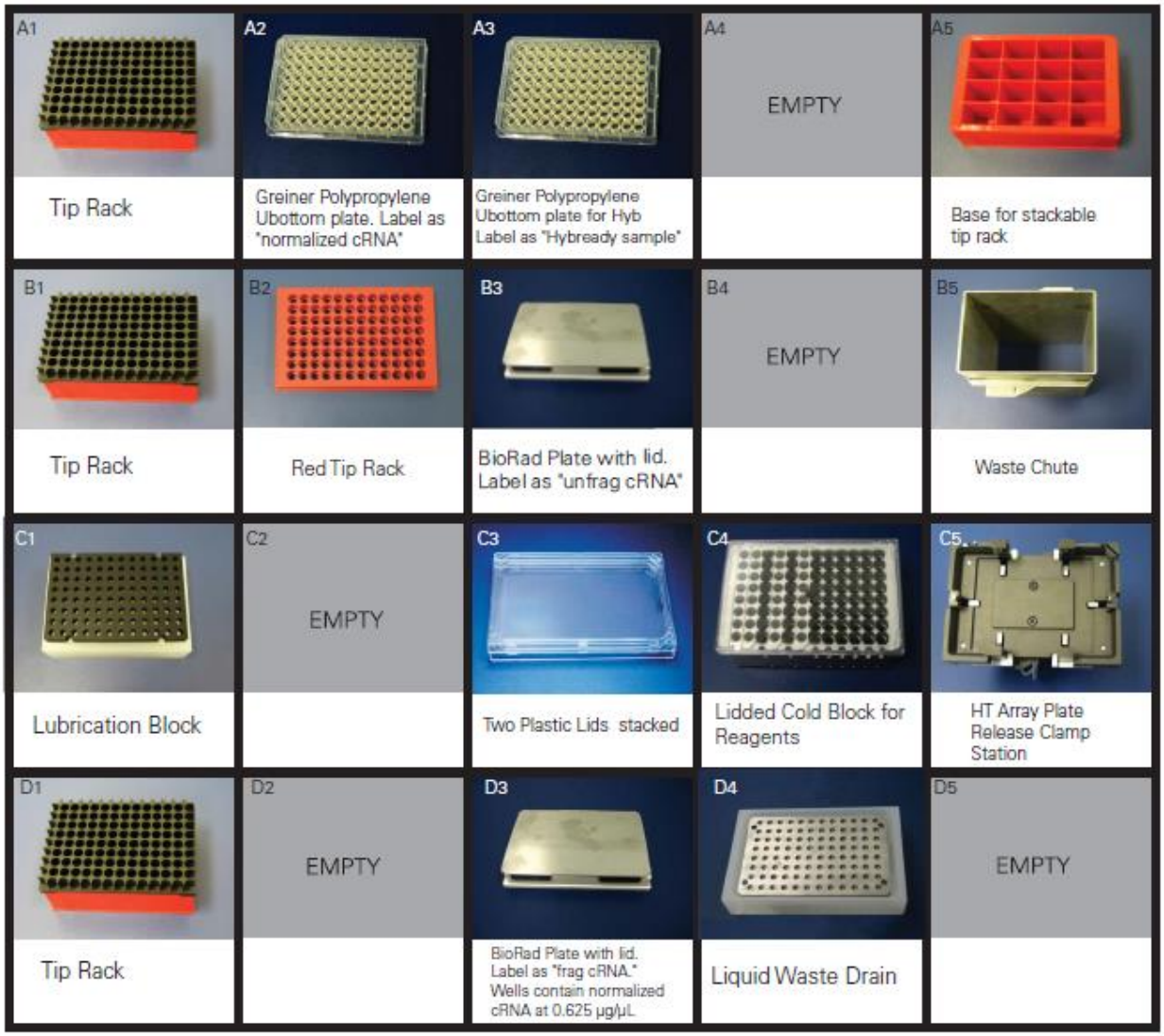


Figure C.6 Array Station Deck Layout Configuration D

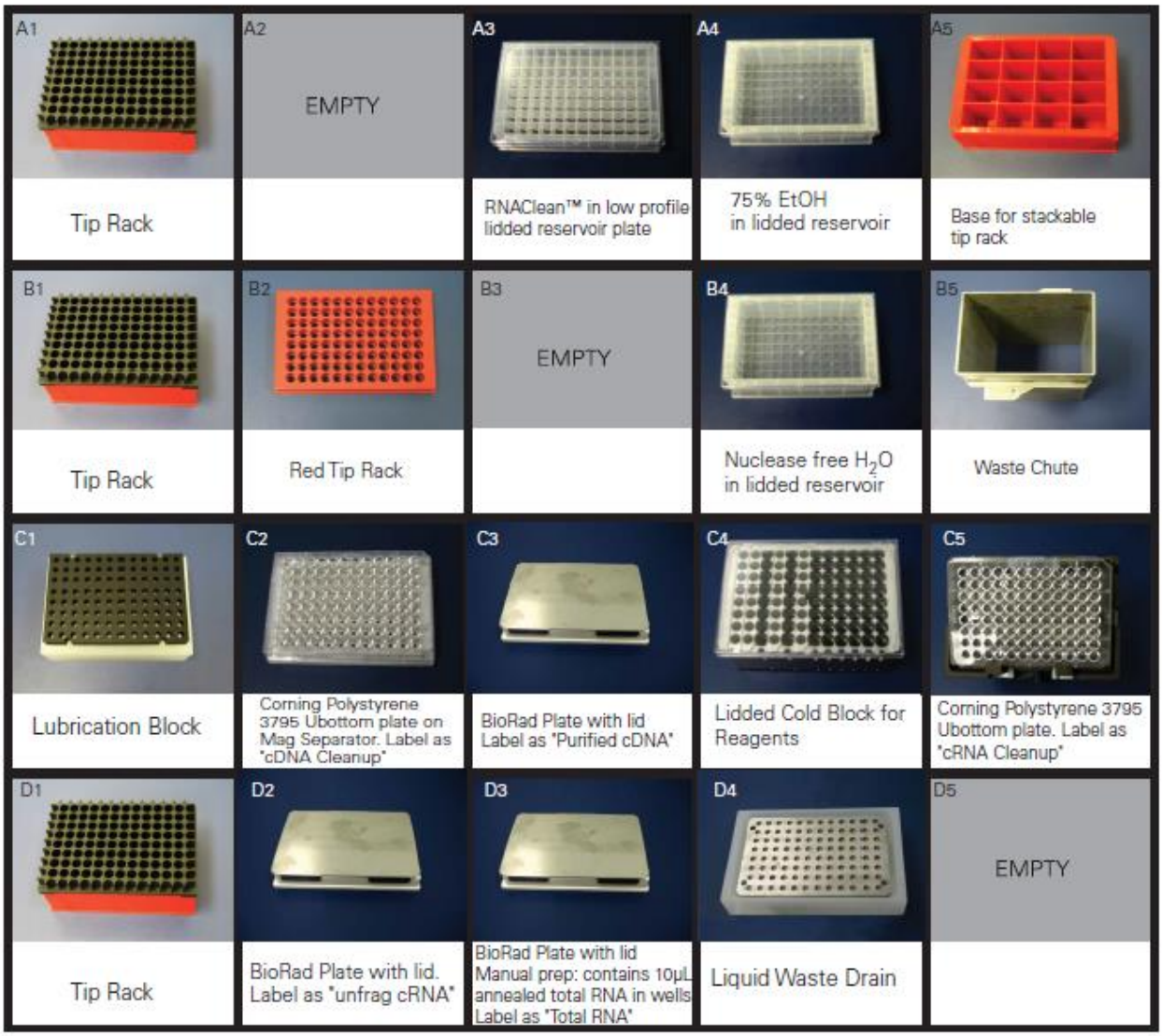


Figure C.7 Array Station Deck Layout Configuration E

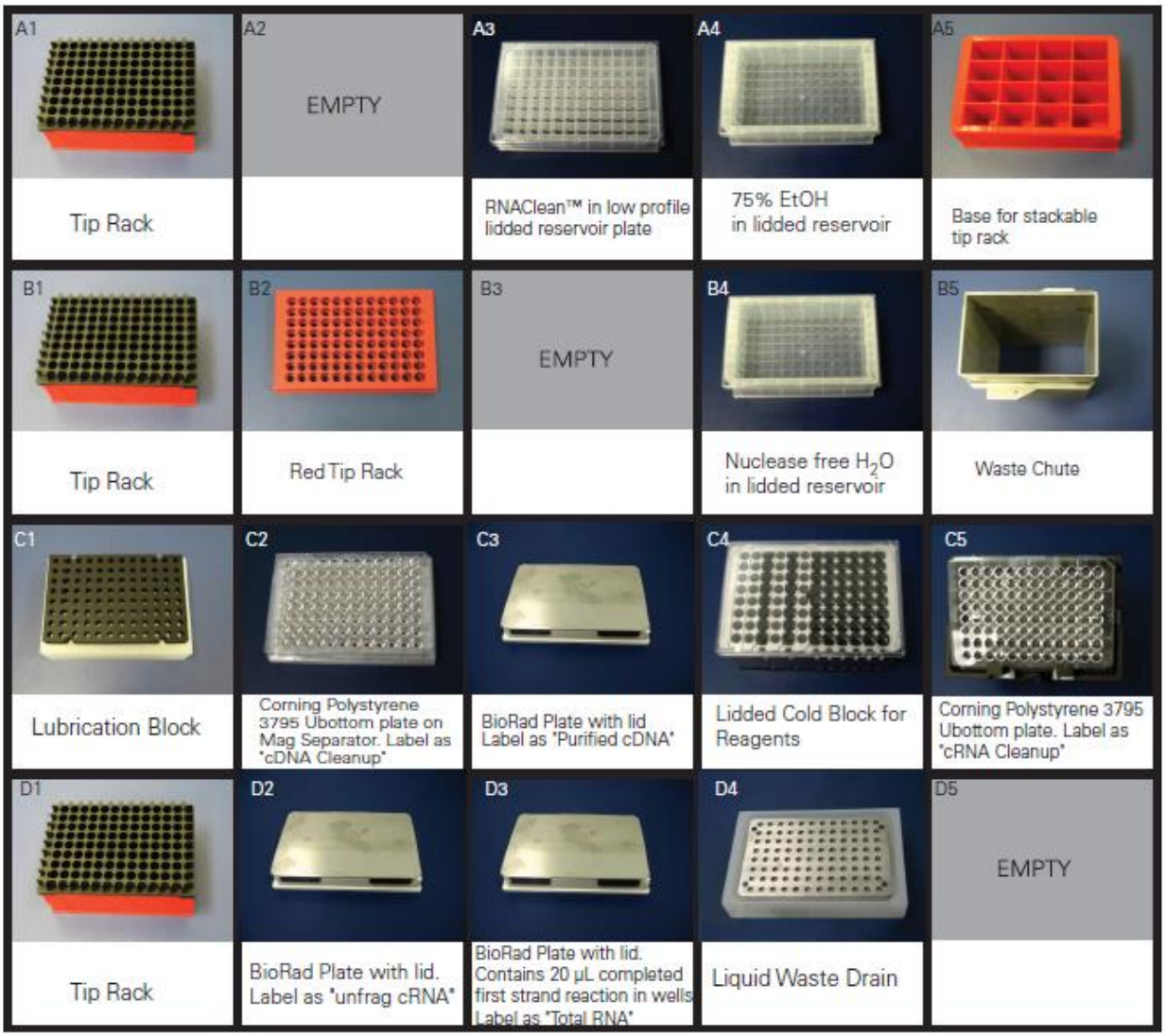


Figure C.8 Array Station Deck Layout Configuration F

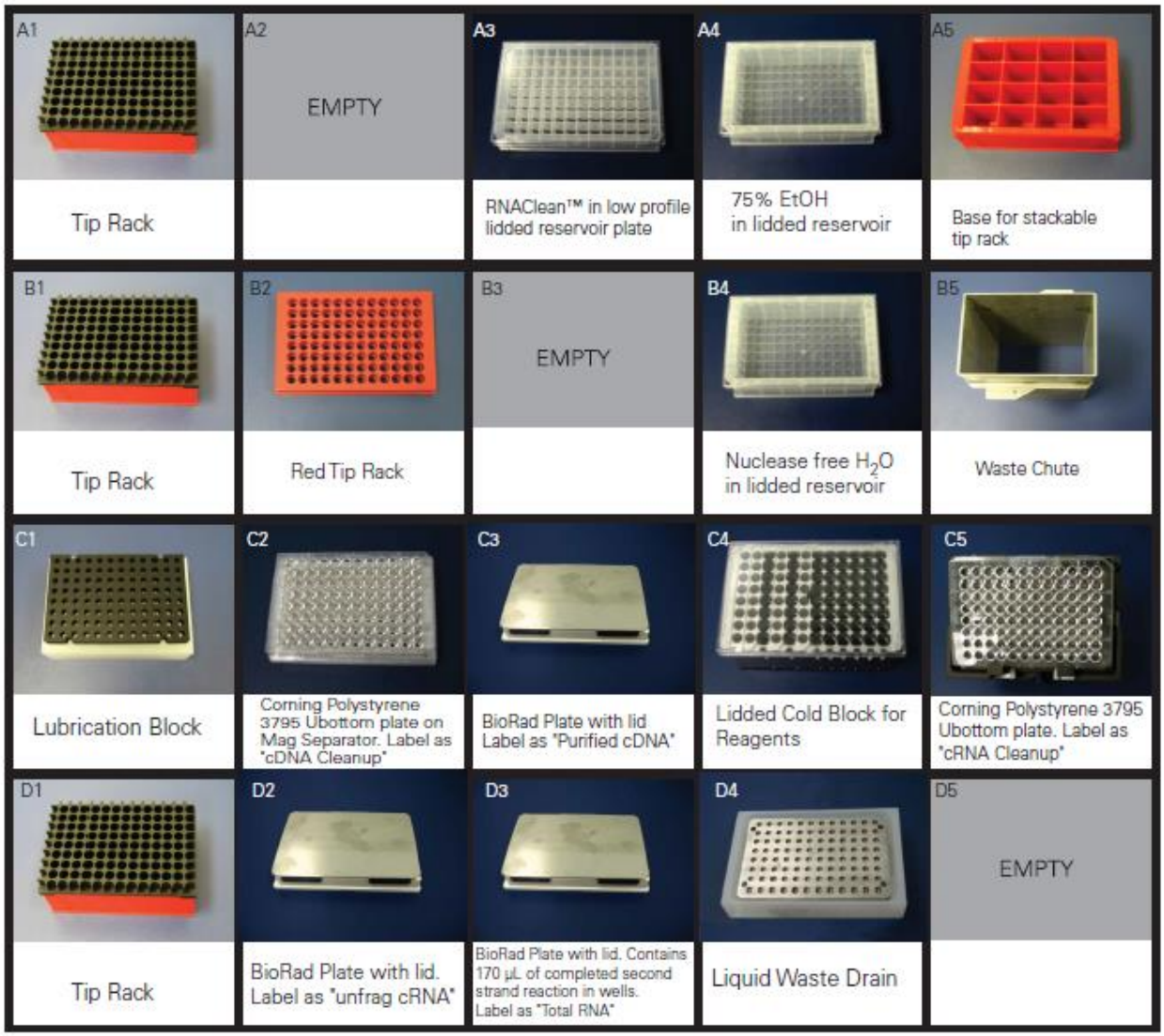


Figure C.9 Array Station Deck Layout Configuration G

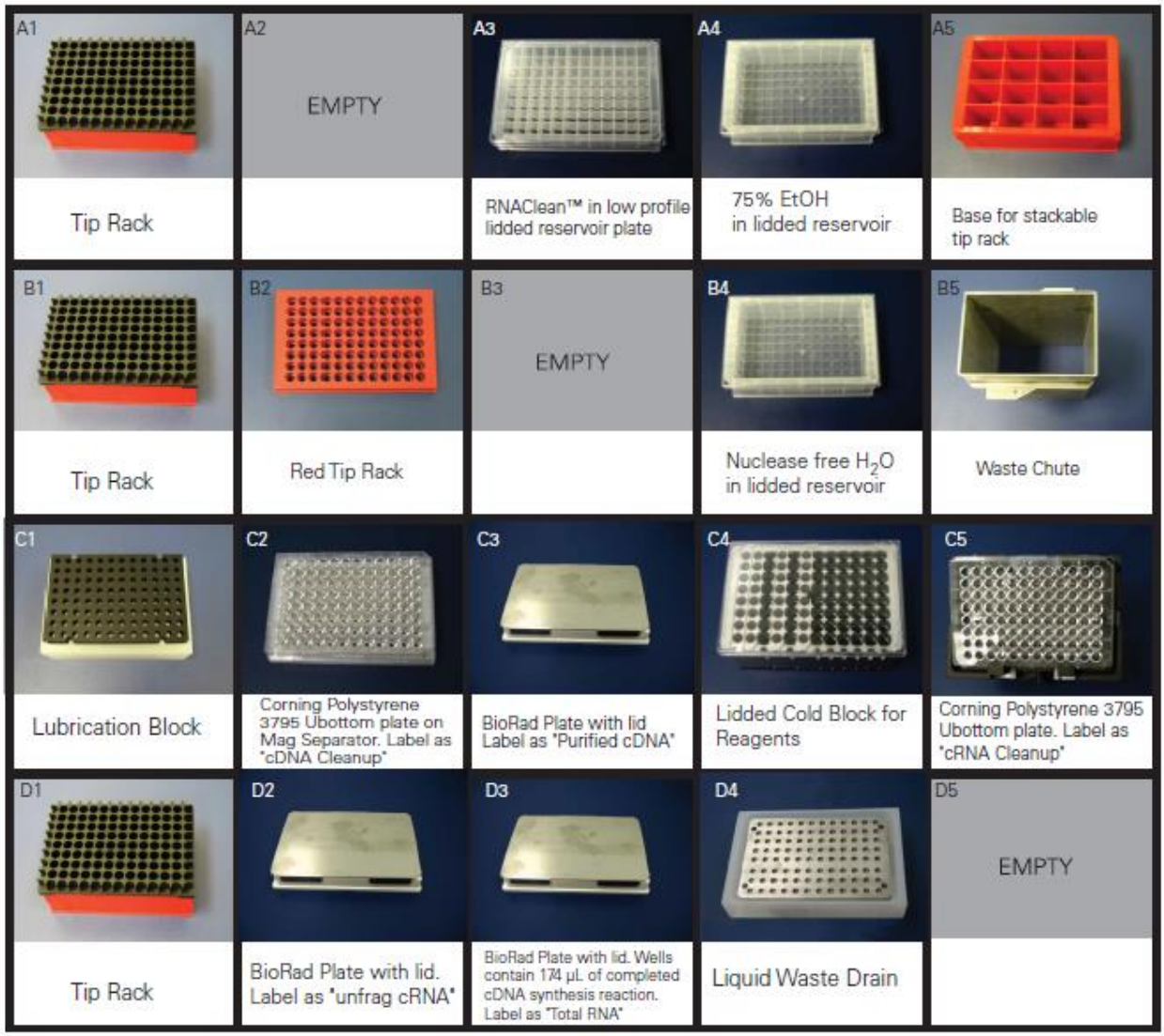


Figure C.10 Array Station Deck Layout Configuration H

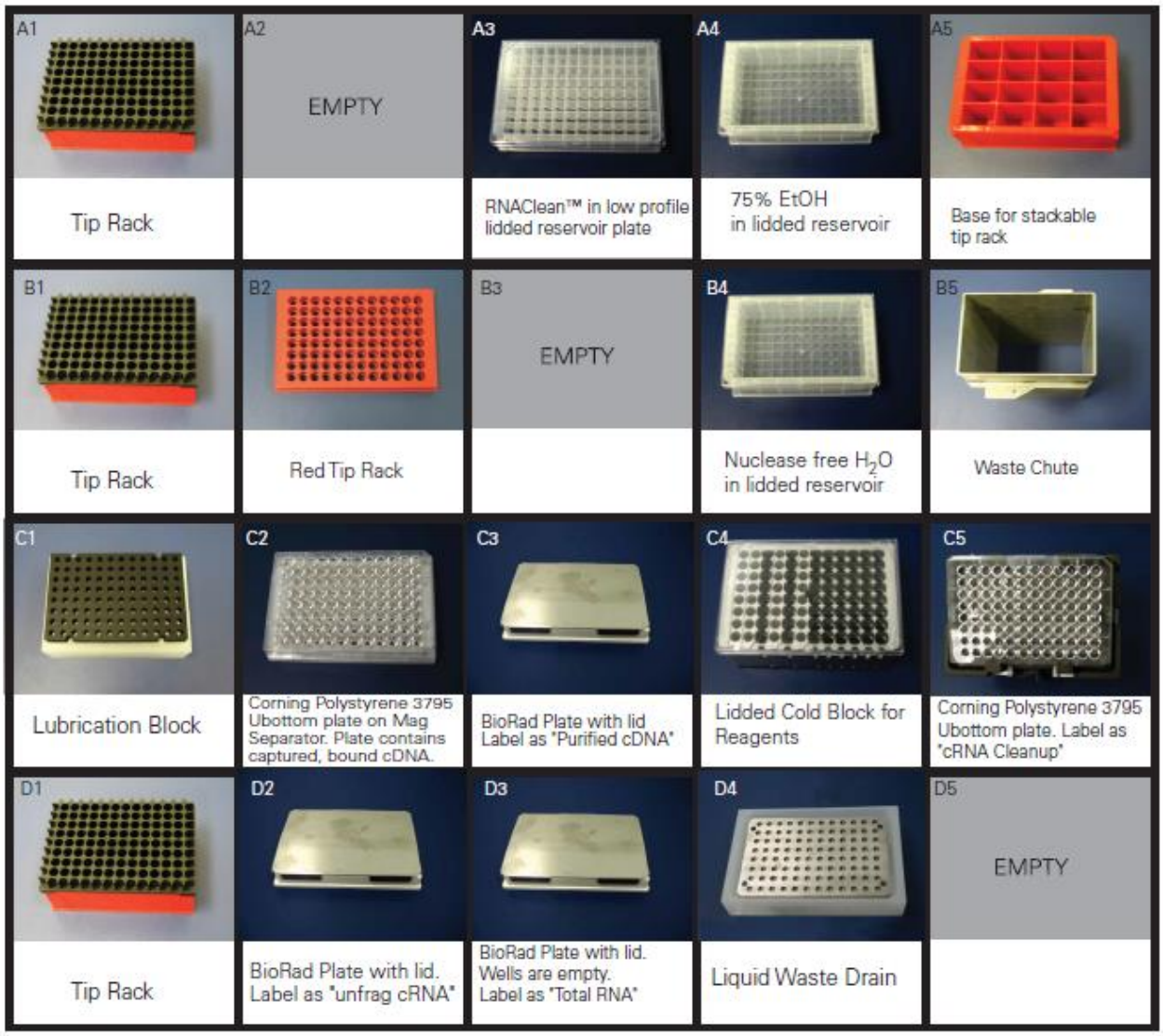


Figure C.11 Array Station Deck Layout Configuration I

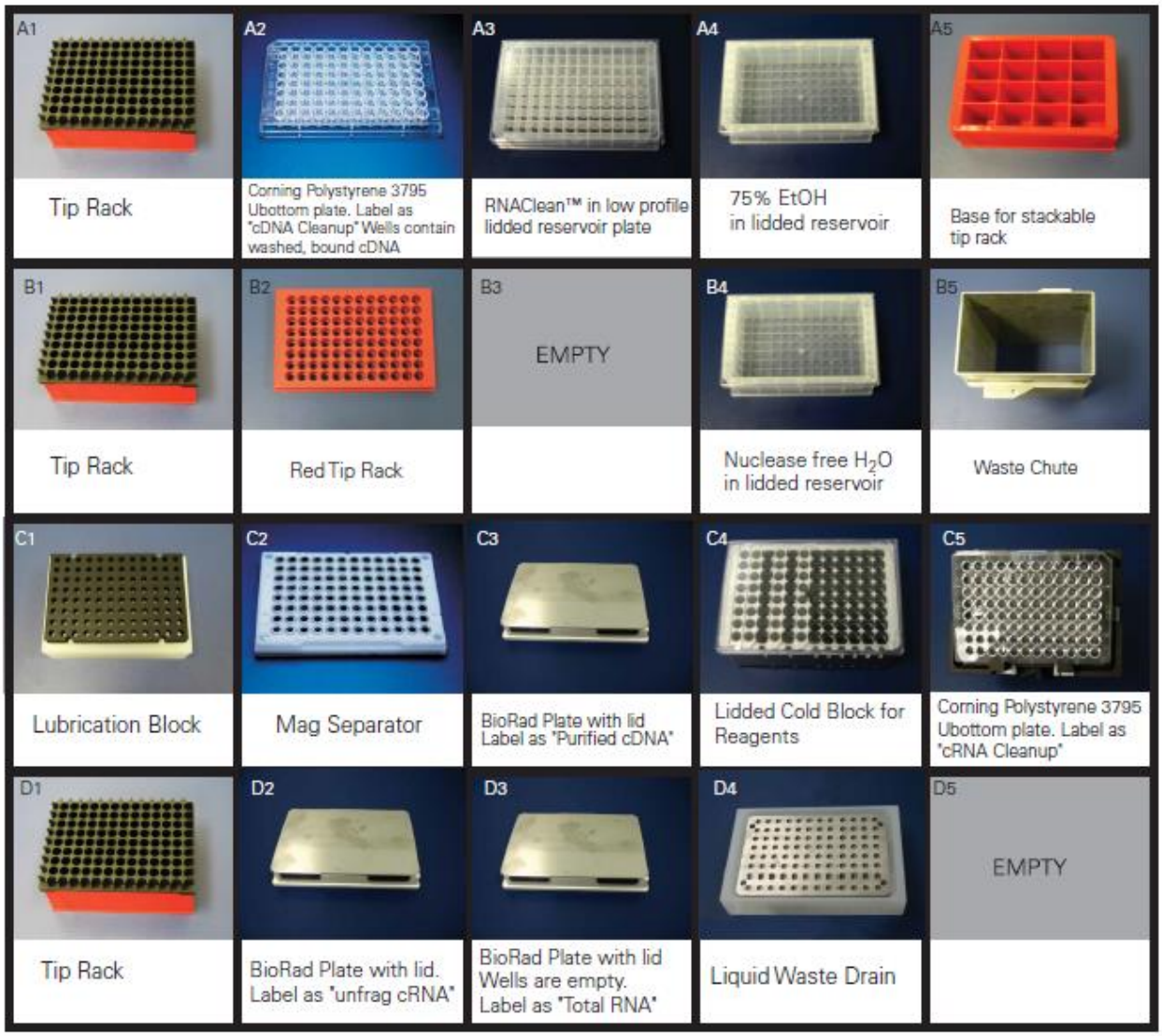


Figure C.12 Array Station Deck Layout Configuration J

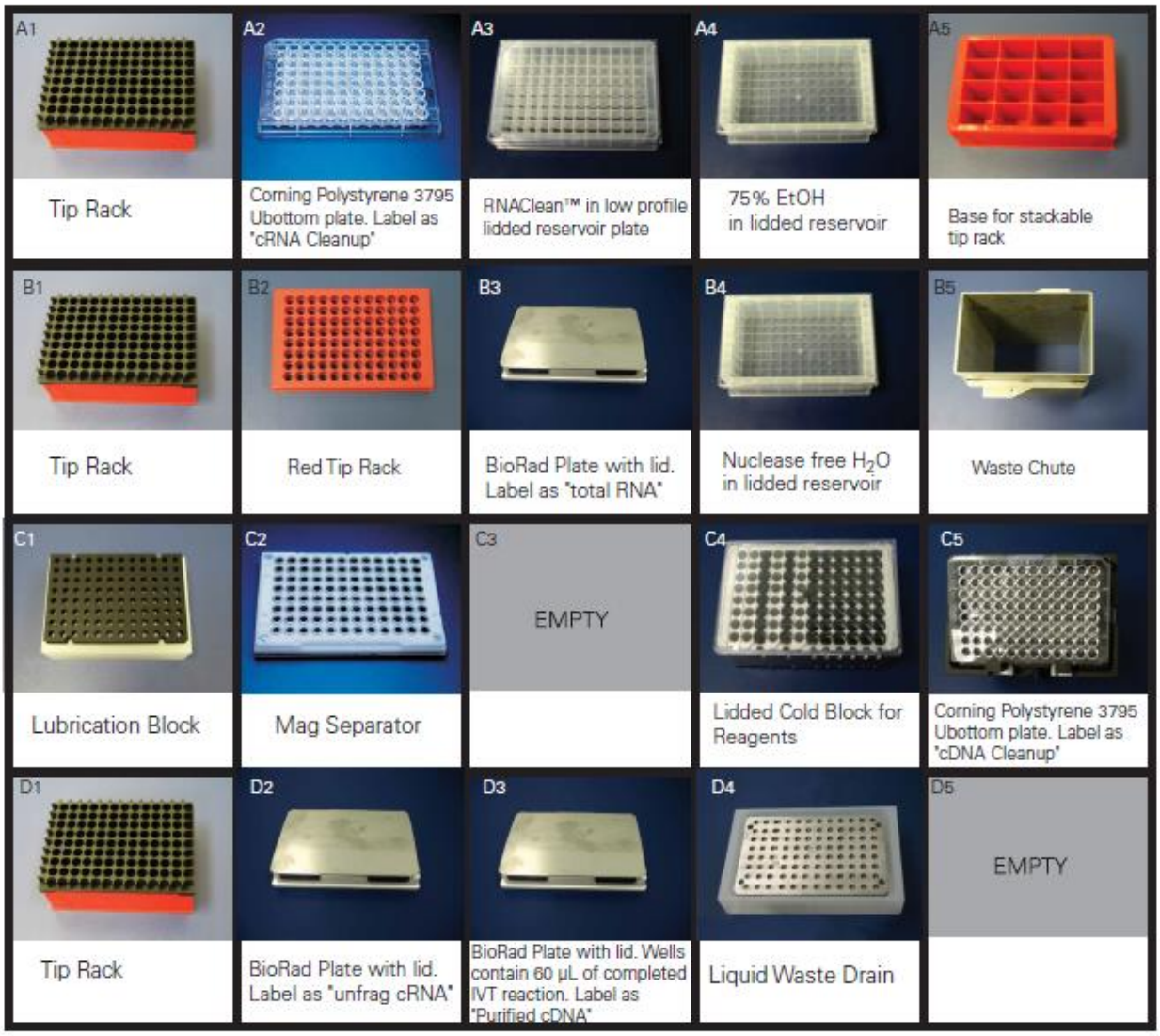


Figure C.13 Array Station Deck Layout Configuration K

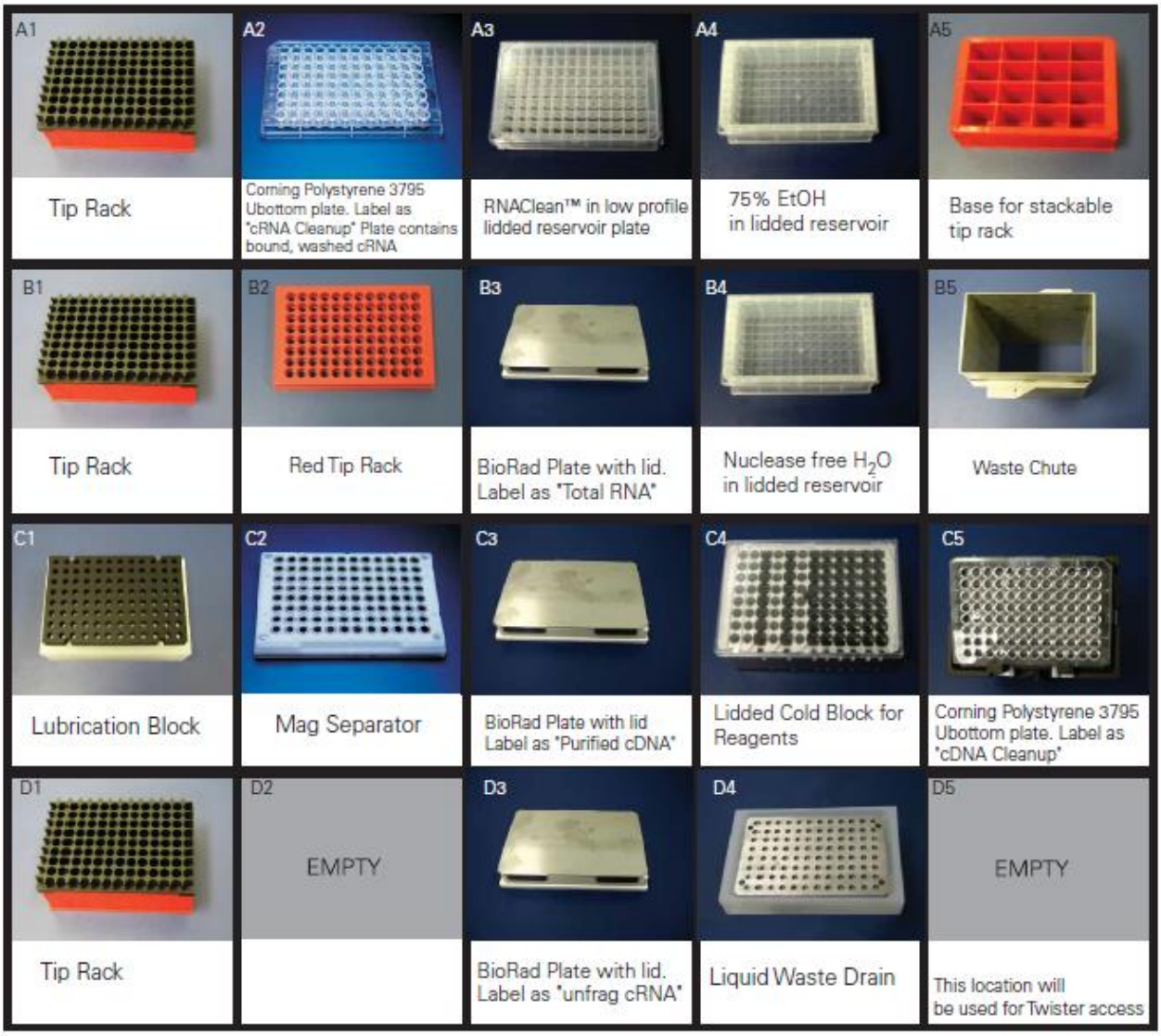


Figure C.14 Array Station Deck Layout Configuration L

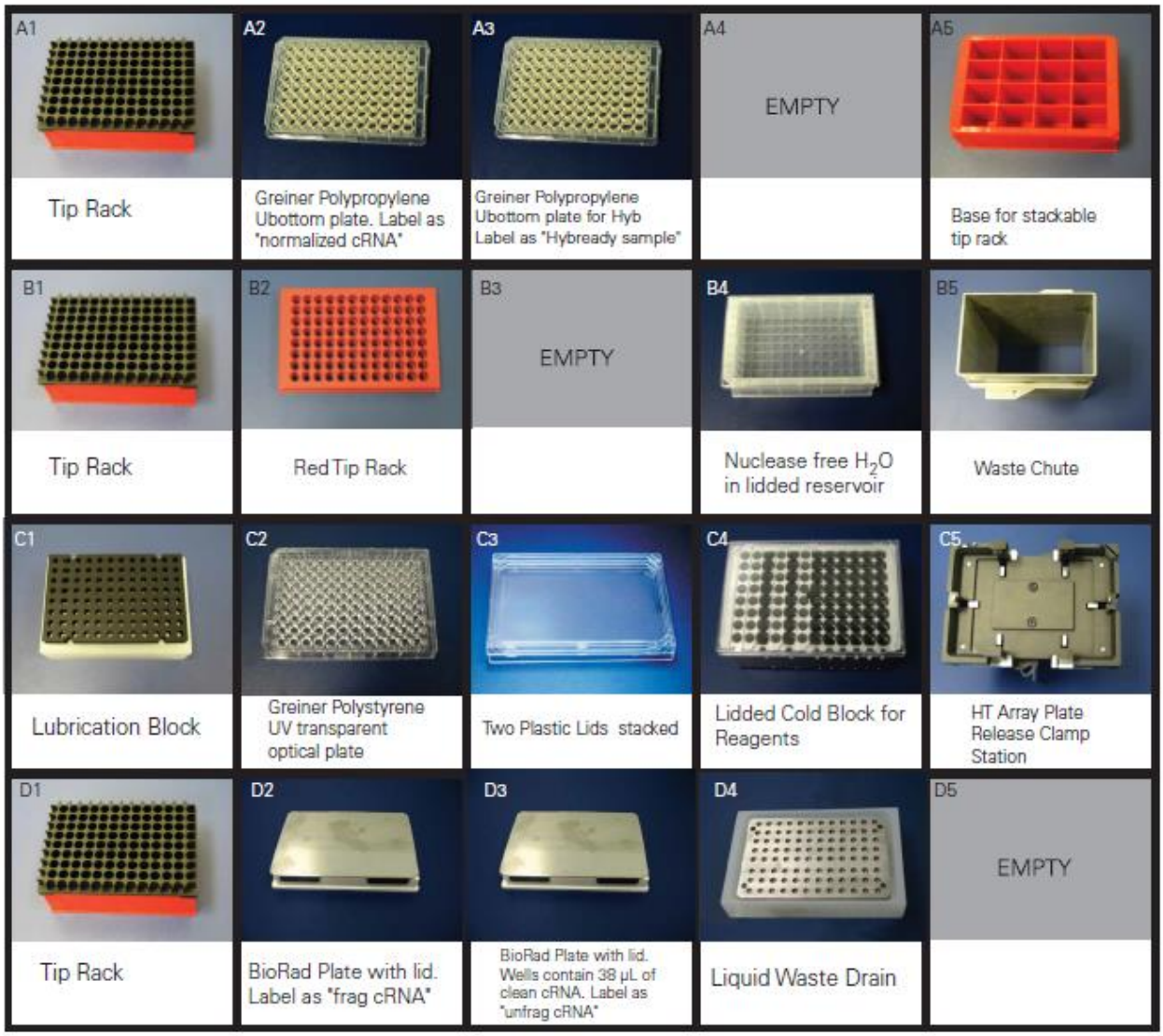


Figure C.15 Array Station Deck Layout Configuration M

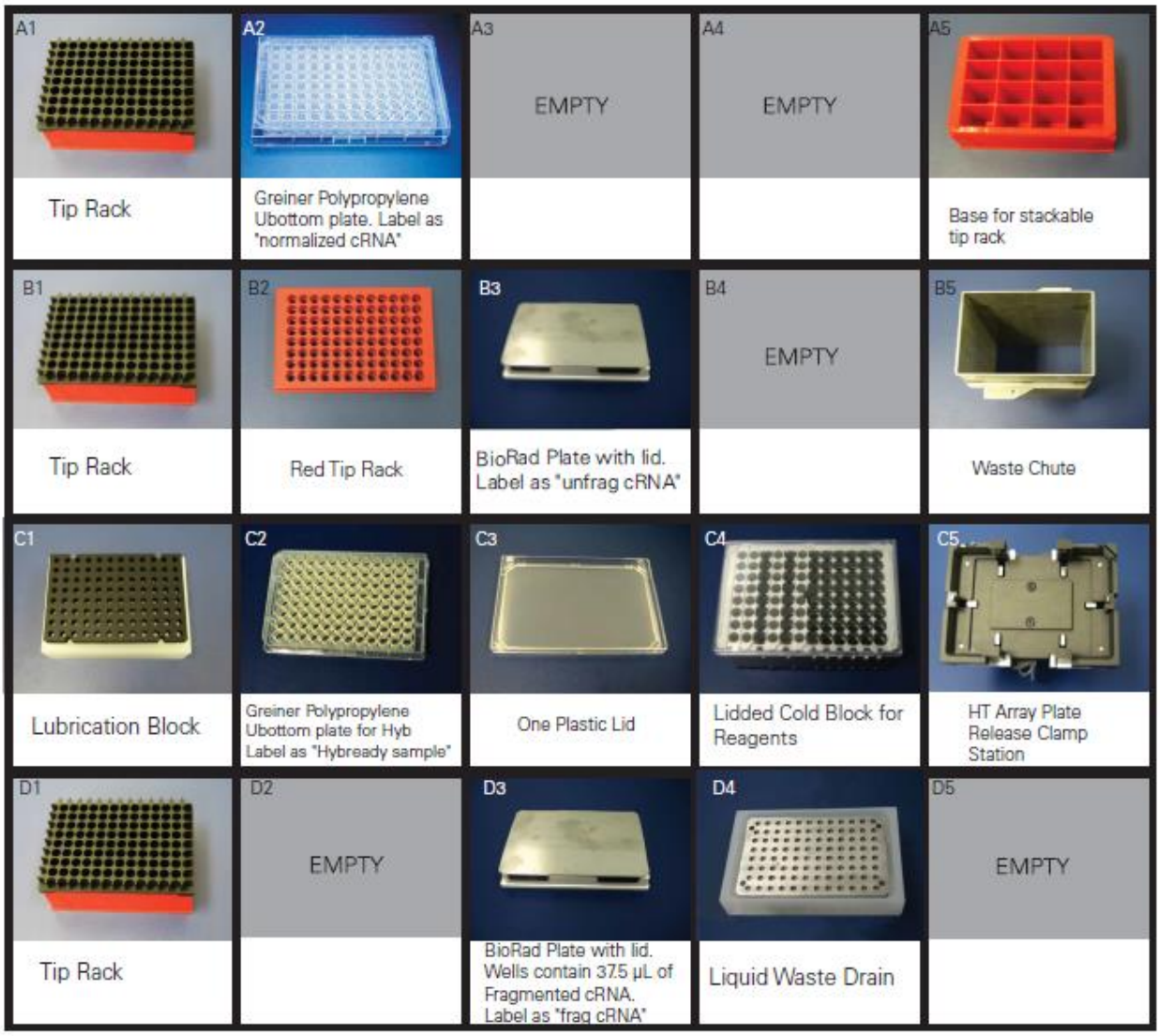


Figure C.16 Array Station Deck Layout Configuration N

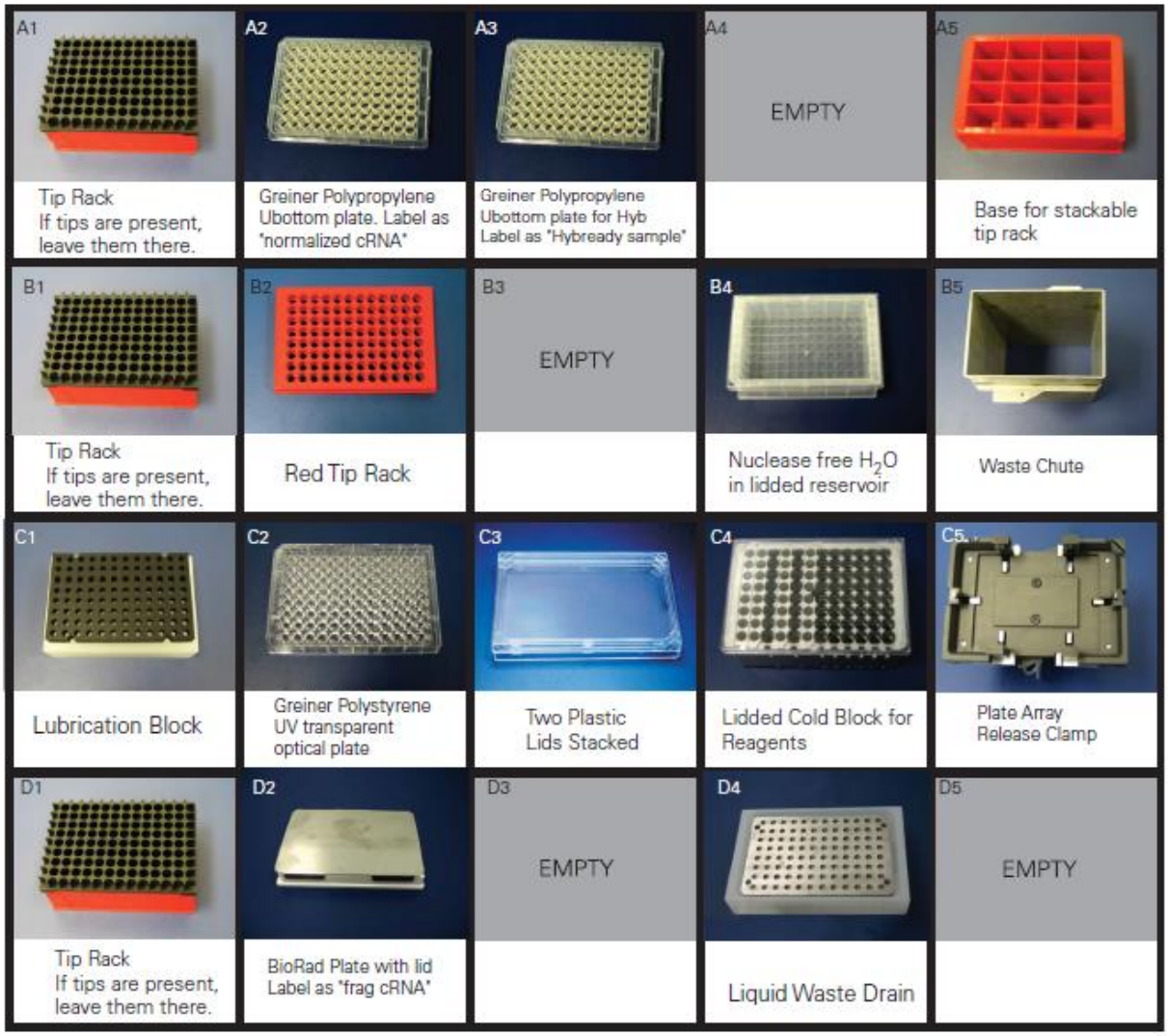


Figure C.17 Array Station Deck Layout Configuration O

Appendix D Sciclone™ Workstation Software User Information

User Level Configuration

Sciclone™ Workstation Software has three user groups with different permissions. These groups are Operators, Developers and Administrators.

Table D.1 Permissions for Sciclone Workstation Software

User Group	Permissions
Sciclone™ Operators	Access Runtime Window, Run Methods, Access Direct Control Window, Error Recovery
Sciclone™ Method Developers	All Operator Privileges, Access Method Editor Window, Create, Edit, Save and Delete Methods, Layouts and Liquid Classes, Import and Export Applications and Methods
Sciclone™ Administrators	Access all Sciclone™ systems

The level of access is determined at User Login. A **User Name** and **Password** is required at login (see Figure D.1). The level of access for each authorized user will be set up during installation of your Sciclone™ Workstation Software by support personnel.

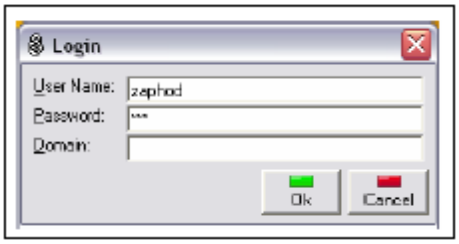


Figure D.1 Sciclone™ Workstation Software Login window

If the User logged into the system is in the Operator User Group, he will have access to the Runtime window (Figure D.2).

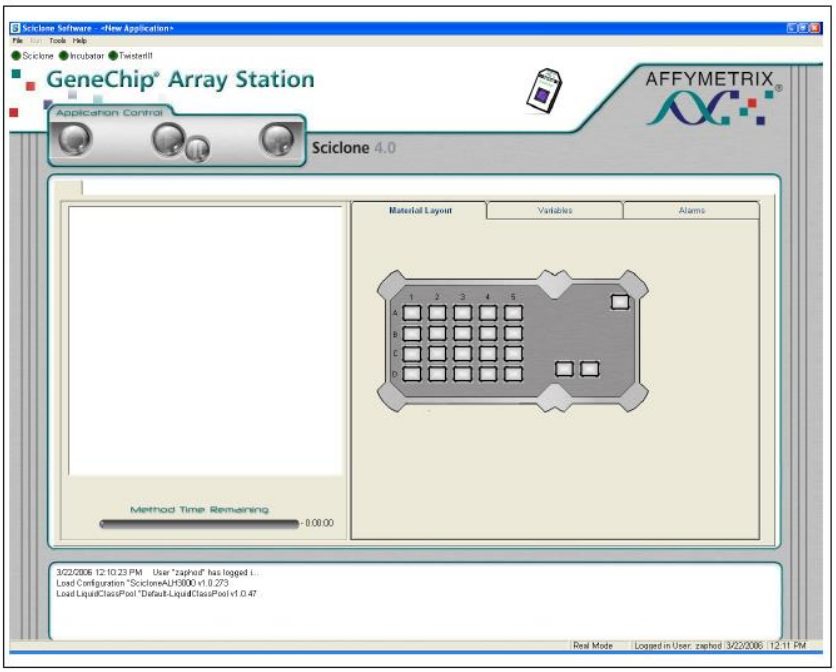


Figure D.2 Sciclone Workstation Software Operator window

If the User logged into the system is in the Developer or Administrator User Group, he will have access to the Method Editor window (Figure D.3).

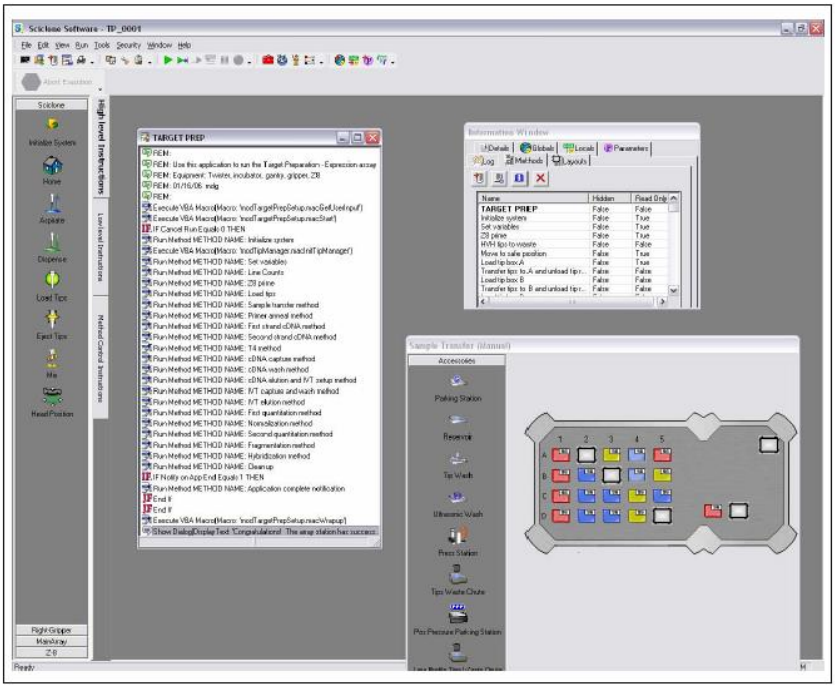


Figure D.3 Sciclone Workstation Software Developer window

NOTE: When running the applications, login as an Operator and run the applications from the Operator window.

More information on the User groups can be found in the Sciclone Users Manual.

Appendix E Formats Of Summary Reports From The Genechip™ Array Station

Formats of Summary Reports from the GeneChip™ Array Station

Introduction

Each of the applications being written for the GeneChip™ Array Station produces a summary report suitable for importing into a Laboratory Information Management System (LIMS). The report will tell how the operator chose to set up the run; what the plate barcodes were (if the operator turned barcode reading on); what errors occurred during the run; and, where applicable, which wells in the plate being processed are outside an acceptance criteria. To make importation uncomplicated, the report is a text file in a standard format easily parsed by LIMS software. Because some of the items in the report are expected to contain commas, using commas as delimiters would be undesirable, so the tab-delimited text-file format will be utilized over the comma-separated-variables format. In that format the text is divided into lines, and fields within a line are separated from each other by tab characters.

File Names

The summary-report files are given names that identify the application that generates them as well as the particular run of that application being summarized. Accordingly, the file names are constructed by appending the application name to "SummaryReport_", following that with a date-and-time stamp constructed out of the starting time of the run, and appending ".txt" as the file extension. For example, the name of a report file generated by a run of application TP_0001 on April 3, 2006, at 4:30:31pm would be "SummaryReport_TP_0001_4_3_06_4_30_31_PM.txt" on a workstation set up to use the North American date-and-time format. Note that the underscore character is used in place of other punctuation to ensure that the file name conforms to operating-system rules.

File Contents

Thus far three applications have been written, all for gene expression: Target Preparation 0001, Hybridization Setup 0001, and Wash Stain 0001. The contents of the report files from all three applications are found in the following Tables.

Table E.1 Contents of Summary Report File for Target Preparation 0001

Line Number	Contents	Possible Values or *Example*
1	Application name	TP_0001
2	Name of Array Station user	Doe, John
3	Run start time	4:30:31 PM
4	Run start date	Monday, April 03, 2006
5	Run end time	8:53:41 PM
6	Run duration	4:23:10
7	Tracking ID	User-entered string*
8	Tip-spacers-used flag	YES or NO
9	Number of samples	8, 16, 24, …, 96
10	In-vitro transcription time	4, 8, or 16
11	Target destination	One HT array plate
12	First run step	Primer annealing [2]†
13	Last run step	Hybridization mix [15]†
14	Initial sample transfer	Manual or Automatic
15	Hold-after-IVT flag	YES or NO
16	cDNA kit lot number	User-entered value‡
17	IVT kit lot number	User-entered value‡
18	Barcode-reader-enabled flag	YES or NO
19	Total RNA barcode	Value read during run**
20	Unfragmented cRNA barcode	Value read during run**
21	Normalized cRNA barcode	Value read during run**

Table E.1 (Continued) Contents of Summary Report File for Target Preparation 0001

Line Number	Contents	Possible Values or *Example*
22	Fragmented cRNA barcode	Value read during run**
23	Hybridization-ready barcode	Value read during run**
24	Limits for pre-normalization yields	25<tab>250
25	Wells outside pre-normalization limits	A01<tab>C12<tab>E05
26	Robot errors	Tab-delimited set of numbers

* The Tracking ID is a string entered by the user when the run is being set up. It may have up to 40 characters and may also be blank.
† The names of the run steps are followed by their step numbers enclosed in square brackets.
‡ Kit lot numbers will typically be in barcodes read from the kits with a hand-held barcode reader attached to the workstation.
**These barcodes will be read from the indicated items during the run by a barcode reader on the deck of the robot provided that the barcode-reader-enabled flag is set to YES. If the flag is set to NO or if the attempt to read the barcode failed, a phrase to that effect will replace the barcode on the corresponding line of the report.

Table E.2 Contents of Summary Report File for Hybridization Setup 0001

Line Number	Contents	Possible Values or *Example*
1	Application name	HYB_0001
2	Name of Array Station user	Doe, John
3	Run start time	4:30:31 PM
4	Run start date	Monday, April 03, 2006
5	Run end time	8:53:41 PM
6	Run duration	4:23:10
7	Tracking ID	User-entered string*
8	Tip-spacers-used flag	YES or NO
9	Number of arrays	24 or 96
10	Barcode-reader-enabled flag	YES or NO
11	Hybridization sample barcode	Value read during run†
12	HT array plate A barcode	Value read during run†
13	HT array plate B barcode	No plate B‡
14	Pre-hybridization flag	YES or NO
15	Hybridization-mix-transfer flag	YES or NO
16	Robot errors	Tab-delimited set of numbers

* The Tracking ID is a string entered by the user when the run is being set up. It may have up to forty characters and may also be blank.
† These barcodes will be read from the indicated items during the run by a barcode reader on the deck of the robot provided that the barcode-reader-enabled flag is set to YES. If the flag is set to NO or if the attempt to read the barcode failed, a phrase to that effect will replace the barcode on the corresponding line of the report.
‡ This is a place holder for a future application that will perform hybridization setup for two HT array plates in one run.

Table E.3 Contents of Summary Report File for Wash Stain 0001

Line Number	Contents	Possible Values or *Example*
1	Application name	WS_0001
2	Name of Array Station user	Doe, John
3	Run start time	4:30:31 PM
4	Run start date	Monday, April 03, 2006
5	Run end time	8:53:41 PM
6	Run duration	4:23:10
7	Tracking ID	User-entered string*
8	Tip-spacers-used flag	YES or NO
9	Number of arrays	24 or 96
10	Barcode-reader-enabled flag	YES or NO
11	HT array plate barcode	Value read during run†
12	Recovered sample plate barcode	Value read during run†
13	Robot errors	Tab-delimited set of numbers

* The Tracking ID is a string entered by the user when the run is being set up. It may have up to forty characters and may also be blank.
† These barcodes will be read from the indicated items during the run by a barcode reader on the deck of the robot provided that the barcode-reader-enabled flag is set to YES. If the flag is set to NO or if the attempt to read the barcode failed, a phrase to that effect will replace the barcode on the corresponding line of the report.

File Location

By convention, the applications will write the summary files into C:\Affymetrix\Reports\Data.

Appendix Reagents, Equipment, and Supplier Contact Information

Master List - Consumables, Reagents, and Equipment

You will need the following reagents and supplies to complete the target preparation on the Array Station system. The reagent quantities listed are for one plate of 96 well reactions.

IMPORTANT: All labware, including pipettes, must be RNase/DNase-free.

Instruments

Table F.1 Instruments

Instruments	Supplier	P/N	Quantity / Run
GeneChip™ Array Station	Thermo Fisher Scientific™	00-0162 (110V) or 00-0235 (220v)	1
GeneChip™ HT Scanner	Thermo Fisher Scientific™	00-0172	1
Allen wrench (2.5mm)	Multiple		1
Dual Channel Thermocouple	VWR™ International	61220-605	1
12-multichannel 20 µL Pipetman	Rainin™	L12-20	1
12-multichannel 200 µL Pipetman	Rainin™	L12-200	1
8-multichannel 20 µL Pipetman	Rainin™	L8-20	1
8-multichannel 200 µL Pipetman	Rainin™	L8-200	1
Auto-sealing microplate lid, arched, wide tab	Bio-Rad	MSL 2032	4
Gripper Pads	Caliper LifeSciences	52071	8

Table F.1 (Continued) Instruments

Instruments	Supplier	P/N	Quantity / Run
Heatblock	Multiple		
Microseal P pads ADHESIVE	Bio-Rad™	MSP-1001	1 (10 seals)
Multidispensing pipette - 1,000 µL	Rainin™	E3-1000	
Multidispensing pipette - 200 µL	Rainin™	E3-200	
Sealing Roller	Bio-Rad™	MSR-0001	
Zerostat Anti-Static Gun	Audioadvisor.com	Zerostat	

Consumables – Target Preparation

Table F.2 Consumables List for Target Preparation

Item	Source	P/N	Quantity / Run
1.2 mL Square Well Storage Plate, Low Profile	ABGene/Marsh	AB-1127	1
96 Wells High Profile 300 mL Reservoir	E&K Scientific Products	EK-2035	2
96-Well Hard-Shell PCR Plate	Bio-Rad	HSP-9601	4
Aluminum Foil Lids*	Beckman Coulter	538619	1 pack
BD Falcon™ Test Tube, 14 mL	VWR™ International	60819-761	2
BD Falcon™ Test Tube, 5 mL*	VWR™ International	60819-728	2
Corning Polystyrene Round Bottom Plates	Fisher Scientific	CLS3795	2
DNAZap™	Ambion™	9890	1 (2 bottles)
Elution Strip Tubes, 0.85 mL	QIAGEN	19588	6 strips
Greiner round bottom clear polypropylene plate	E&K Scientific Products	20261	2
Greiner UV transparent Optical Plates	E&K Scientific Products	AB-1127	2
KimWipes™	VWR™ International	34256	
Low-Profile 0.2 mL PCR 8-Tube Strips	Bio-Rad™	TLS-0801	4 strips
Microseal 'F' Adhesive Foil	Bio-Rad™	MSF-1001	
Microtiter plate lids	Phenix	ML-5009	5

Table F.2 (Continued) Consumables List for Target Preparation

Item	Source	P/N	Quantity / Run
Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL	VWR™ International	20171-028	2
RNase-Free 1.5 mL Microfuge tube, or equivalent	Ambion™	12400	6
RNaseZap™ wipes	Ambion™	9786	1 (100 wipes)
Stacker tips 200 µL non-sterile	Caliper LifeSciences	78641	1 box

Consumables – Hybridization Setup

Table F.3 Consumables List for Hybridization Setup

Item	Source	P/N	Quantity / Run
HT Hybridization Tray*	Thermo Fisher Scientific™	202111	1
HT Stain Tray*	Thermo Fisher Scientific™	900745	1
96-Well Hard-Shell PCR Plate	Bio-Rad™	HSP-9601	1
Omnitray or Greiner Round Bottom Clear Polypropylene Plate	VWR™ E&K Scientific Products	4660-638 20261	1 1
Stacker Tips 200 µL non-sterile	Caliper LifeSciences	78642	3

* Stain and Hybridization Tray are packaged with the HT Array Plates.

Consumables – Wash and Stain

Table F.4 Consumables List for Wash and Stain of HT Array Plate

Item	Source	P/N	Quantity / Run
HT Scan Tray*	Thermo Fisher Scientific™	900746	1
HT Stain Tray*	Thermo Fisher Scientific™	900745	3
HT Wash Tray*	Thermo Fisher Scientific™	900752	5
Aluminum Foil Lids	Beckman Coulter™	538619	1 pack
Quarter Reservoir Modules	Beckman Coulter™	372790	2
Quarter Reservoir Frame	Beckman Coulter™	372795	1
BD Falcon™ Test Tube, 14 mL	VWR™ International	60819-761	2
BD Falcon™ Test Tube, 5 mL (or equivalent)	VWR™ International	60819-728	1
Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL	VWR™ International	20171-028	2
RNase-Free 1.5 mL Microfuge tube†	Ambion™	12400	1
Round Bottom Clear Polypropylene Plate	E&K Scientific Products	20261	1
Stacker Tips 200 µL non-sterile	Caliper LifeSciences	78641	1 Box
96-Well Hard-Shell PCR Plate	Bio-Rad™	HSP-9601	1
Omnitray	VWR™ International	4660-638	2

* HT Stain, Wash, and Scan Trays are packaged with the HT Array Plates.

Reagents – Total RNA Isolation

Table F.5 Reagent List for Total RNA Isolation

Material	Source	P/N
TRIzolTM Reagent	InvitrogenTM Life Technologies	15596-018
Ethanol, 80% (stored at –20°C)	Multiple	
Glycogen (optional)	AmbionTM	9510
Pellet PaintTM (optional)	NovagenTM	69049-3
QIAzol™ Lysis Reagent	QIAGENTM	79306
RNeasyTM Mini Kit	QIAGENTM	74104
Sodium Acetate (NaOAc), 3M	Sigma-AldrichTM	S7899

Reagents – Target Preparation

Table F.6 Reagent List for Target Preparation

Material	Source	P/N
GeneChip™ HT One-Cycle Target Labeling and Controls Kit (96 samples) containing: • GeneChip™ HT One-Cycle cDNA Synthesis Kit 96-Reactions, P/N 900687 • GeneChip™ HT IVT Labeling Kit 96-Reactions, P/N 900688 • GeneChip™ Eukaryotic Poly-A RNA Control Kit 100-Reactions, P/N 900433 • GeneChip™ Eukaryotic Hybridization Control Kit 150-Reactions, P/N 900457	Thermo Fisher Scientific™	900686
GeneChip™ Eukaryotic Poly-A RNA Control Kit containing: • Poly-A Control Stock (16 µL) • Poly-A Control Dil Buffer (3.8 mL)	Thermo Fisher Scientific™	900433
GeneChip™ HT One-Cycle cDNA Synthesis Kit containing: • T7-Oligo(dT) Primer, 50 µM (130 µL) • 5X 1st Strand Reaction Mix (460 µL) • DTT, 0.1M (230 µL) • dNTP, 10 mM (460 µL) • SuperScript™ II (120 µL) • 5X 2nd Strand Reaction Mix (3,300 µL) • E. coli DNA Polymerase I (440 µL) • E. coli DNA Ligase (110 µL) • RNase H (110 µL) • T4 DNA Polymerase (280 µL) • 5X T4 DNA Polymerase Buffer (60 µL)	Thermo Fisher Scientific™	900687
GeneChip™ HT IVT Labeling Kit containing: • 10X IVT Labeling Buffer, 1 tube (675 µL) • IVT Labeling Enzyme Mix, 1 tube (675 µL) • IVT Labeling NTP Mix, 2 tubes (1,010 µL) • 3'-Labeling Control (0.5 µg/µL), 1 tube (10 µL) • T7 RNA Polymerase, 1 tube (115 µL) • 5X Fragmentation Buffer, 1 tube (855 µL)	Thermo Fisher Scientific™	900688
Nuclease-free Water	Ambion	9932
RNAClean 60 mL	Agencourt	000494

Reagents – Hybridization, Wash and Stain

Table F.7 Reagent List for Hybridization, Wash, and Stain

Material	Source	P/N
GeneChip™ HT Hybridization, Wash, and Stain Kit containing: • Box 1: HT Hybridization Mixes and HT Stain Module • Box 2: HT Wash Buffers A and B • GeneChip™ *HT Wash Buffers A and B* (P/N 901220) • GeneChip™ *HT Array Holding Buffer* (P/N 901218)	Thermo Fisher Scientific™	901219
GeneChip™ Eukaryotic Hybridization Control Kit (). Kit containing: • GeneChip™ Control Oligo B2 (P/N 900301) • 20X Hybridization Controls		30 Rxns P/N 900454; 150 Rxns P/N 900457
Bovine Serum Albumin (BSA) solution (50 mg/mL)	Invitrogen Life Technologies	15561-020
EDTA Disodium Salt, 0.5M solution (100 mL)	Sigma-Aldrich	E7889
Herring Sperm DNA	Promega Corporation	D1811
MES hydrate SigmaUltra	Sigma-Aldrich	M5287
MES Sodium Salt	Sigma-Aldrich	M5057
Nuclease-free Water, 1 L	Ambion	9932
Surfact-Amps™ 20 (Tween-20), 10%	Pierce Chemical	28320
TMAC (5M)	Sigma-Aldrich	T3411
Nuclease-free Water	Ambion	9932
20X SSPE (3M NaCl, 0.2M NaH_2PO_4, 0.02M EDTA)	Cambrex	51214
5M NaCl, RNase-free, DNase-free	Ambion	9760G
Anti-Streptavidin Antibody (Goat), Biotinylated	Vector Laboratories	BA-0500
Bovine Serum Albumin (BSA) solution (50 mg/mL)	Invitrogen Life Technologies	15561-020
Distilled Water	Invitrogen Life Technologies	15230-147
Goat IgG, Reagent Grade	Sigma-Aldrich	I 5256

Table F.7 (Continued) Reagent List for Hybridization, Wash, and Stain

Material	Source	P/N
PBS, pH 7.2	Invitrogen Life Technologies	20012-027
R-Phycoerythrin Streptavidin	Molecular Probes	S-866
Surfact-Amps™ 20 (Tween-20), 10%	Pierce Chemical	28320

Supplier Contact Information

Table F.8 Supplier Contact Information

Source	Web Site
ABGene™/Marsh	www.marshbio.com
Ambion™	www.ambion.com
Thermo Fisher Scientific™	www.Thermo Fisher.com
Agencourt™	www.agencourt.com
Aldrich	www.sigmaaldrich.com
Ambion	www.ambion.com
Audioadvisor.com	www.audioadvisor.com
Beckman Coulter™	www.beckman.com
Bio-Rad™	www.bio-rad.com
Caliper	www.caliperls.com
Cambrex	www.cambrex.com
Cole-Parmer™	www.coleparmer.com
E&K Scientific Products	www.eandkscientific.com
Fisher Scientific	www.fishersci.com
Invitrogen Life Technologies	www.invitrogen.com
Molecular Probes	www.probes.com
Novagen	www.emdbiosciences.com/html/NVG/home.html
Phenix™	www.phenix1.com
Pierce™ Chemical	www.piercenet.com
Promega Corporation	www.promega.com
QIAGEN™	www.qiagen.com
Rainin™	www.rainin-global.com
Sigma-Aldrich™	www.sigma-aldrich.com
USA Scientific™	www.usascientific.com
Vector™ Laboratories	www.vectorlabs.com
VWR™ International	www.vwr.com

Appendix G Contact Information

When to Contact Technical Support

Under any of the following conditions, unplug the instrument from the power source and contact Technical Support:

- when the power cord is damaged or frayed;
- if any liquid has penetrated the instrument;

If the instrument must be returned for repair, call Technical Support.

IMPORTANT: Make sure you have the model and serial number.

Documentation and support

Obtaining support

Technical support

For the latest services and support information for all locations, visit **www.thermofisher.com**.

At the website, you can:

Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities

Search through frequently asked questions (FAQs)

Submit a question directly to Technical Support (**thermofisher.com/support)**

Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents

Obtain information about customer training

Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.