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Introduction

Welcome to the Affymetrix® 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

Legend:
- RNA
- DNA
- T7 promoter
- Biotin
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 (refer to Chapter 5).
7. Wash and stain HT Array Plates using the Array Station (refer to Chapter 6).
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, please 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](image-url)
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. Affymetrix GeneChip® Operating Software User’s Guide (P/N 701439): if you are using Affymetrix GeneChip® analysis software, you should be familiar with Affymetrix’ GeneChip® Operating System in order to interpret the assay results.
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**

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 on page 15 for details.

**PRECIPITATION PROCEDURE**

1. Add 1/10 volume 3M NaOAc, pH 5.2, and 2.5 volumes ethanol.*
2. Mix and incubate at \(-20^\circ\text{C}\) for at least 1 hour.
3. Centrifuge at \(\geq 12,000 \times g\) 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 H\(\text{2O}\). 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 A_{260}/A_{280} 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.

![Electropherogram](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: Affymetrix, 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

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<tr>
<th>Poly-A RNA Spike</th>
<th>Final Dilution (estimated ratio of copy number)</th>
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<tr>
<td><em>lys</em></td>
<td>1:100,000</td>
</tr>
<tr>
<td><em>phe</em></td>
<td>1:50,000</td>
</tr>
<tr>
<td><em>thr</em></td>
<td>1:25,000</td>
</tr>
<tr>
<td><em>dap</em></td>
<td>1:6,667</td>
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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

<table>
<thead>
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<th>Serial Dilutions</th>
<th>Spike-in Volume</th>
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<tr>
<td>Total RNA mRNA</td>
<td>First Second Third</td>
</tr>
<tr>
<td>1 μg</td>
<td>1:20 1:50 1:50</td>
</tr>
<tr>
<td>2 μg</td>
<td>1:20 1:50 1:25</td>
</tr>
</tbody>
</table>

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

Affymetrix highly recommends 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.
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.

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 by Affymetrix. 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: Affymetrix, P/N 900687
- GeneChip® HT IVT Labeling Kit; Affymetrix: P/N 900688
- GeneChip® Eukaryotic Hybridization Control Kit: Affymetrix, P/N 900454 (30 reactions) or P/N 900457 (150 reactions), contains Control cRNA and Control Oligo B2
- Control Oligo B2 (3 nM): Affymetrix, 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

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.
• 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
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.

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

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.

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

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Rxns</td>
<td>48 Rxns</td>
</tr>
<tr>
<td>T7-Oligo(dT) Primer, 50 µM</td>
<td>1.0 µL</td>
<td>32.0 µL</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>4.0 µL</td>
<td>127.9 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>5.0 µL</td>
<td>159.9 µL</td>
</tr>
<tr>
<td>Volume per Strip Tube Well</td>
<td>18.5 µL</td>
<td>34.0 µL</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Rxns</td>
<td>48 Rxns</td>
<td>96 Rxns</td>
<td></td>
</tr>
<tr>
<td>5X 1st Strand Reaction Mix</td>
<td>4.0 µL</td>
<td>114.0 µL</td>
<td>228.0 µL</td>
<td>456.0 µL</td>
</tr>
<tr>
<td>DTT, 0.1 M</td>
<td>2.0 µL</td>
<td>57.0 µL</td>
<td>114.0 µL</td>
<td>228.0 µL</td>
</tr>
<tr>
<td>dNTP Mix, 10 mM</td>
<td>1.0 µL</td>
<td>28.5 µL</td>
<td>57.0 µL</td>
<td>114.0 µL</td>
</tr>
<tr>
<td>SuperScript™ II</td>
<td>1.0 µL</td>
<td>28.5 µL</td>
<td>57.0 µL</td>
<td>114.0 µL</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>2.0 µL</td>
<td>57.0 µL</td>
<td>114.0 µL</td>
<td>228.0 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>10.0 µL</td>
<td>285.0 µL</td>
<td>570.0 µL</td>
<td>1,140.0 µL</td>
</tr>
<tr>
<td>Volume per Strip Tube Well</td>
<td>33.5 µL</td>
<td>69.3 µL</td>
<td>140.5 µL</td>
<td></td>
</tr>
</tbody>
</table>
Procedure 3: Second-Strand cDNA Synthesis Master Mix

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.

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

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

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 Rxns</td>
<td>48 Rxns</td>
<td>96 Rxns</td>
</tr>
<tr>
<td>T4 DNA Polymerase</td>
<td>2.0 µL</td>
<td>69.3 µL</td>
<td>118.0 µL</td>
<td>236.0 µL</td>
</tr>
<tr>
<td>1X T4 DNA Polymerase Buffer</td>
<td>2.0 µL</td>
<td>69.3 µL</td>
<td>118.0 µL</td>
<td>236.0 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>4.0 µL</td>
<td>138.6 µL</td>
<td>236.0 µL</td>
<td>472.0 µL</td>
</tr>
<tr>
<td>Volume per Strip Tube Well</td>
<td>16.0 µL</td>
<td>27.8 µL</td>
<td>57.0 µL</td>
<td></td>
</tr>
</tbody>
</table>
Procedure 5: IVT Master Mix

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>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 Rxns</td>
<td>48 Rxns</td>
<td>96 Rxns</td>
<td></td>
</tr>
<tr>
<td>10X IVT Buffer</td>
<td>6.0 µL</td>
<td>168.0 µL</td>
<td>336.0 µL</td>
<td>672.0 µL</td>
<td></td>
</tr>
<tr>
<td>IVT Labeling NTP Mix</td>
<td>18.0 µL</td>
<td>504.0 µL</td>
<td>1,008.0 µL</td>
<td>2,016.0 µL</td>
<td></td>
</tr>
<tr>
<td>IVT Labeling Enzyme Mix</td>
<td>6.0 µL</td>
<td>168.0 µL</td>
<td>336.0 µL</td>
<td>672.0 µL</td>
<td></td>
</tr>
<tr>
<td>T7 RNA Polymerase</td>
<td>1.0 µL</td>
<td>28.0 µL</td>
<td>56.0 µL</td>
<td>112.0 µL</td>
<td></td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>7.0 µL</td>
<td>196.0 µL</td>
<td>392.0 µL</td>
<td>784.0 µL</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>38.0 µL</td>
<td>1,064.0 µL</td>
<td>2,128.0 µL</td>
<td>4,256.0 µL</td>
<td></td>
</tr>
<tr>
<td>Volume per Strip Tube Well</td>
<td>131.0 µL</td>
<td>264.0 µL</td>
<td>529.0 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Procedure 6: Fragmentation Buffer

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

<table>
<thead>
<tr>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Rxns</td>
</tr>
<tr>
<td>5X Fragmentation Buffer (Volume per Strip Tube Well)</td>
<td>7.5 µL</td>
</tr>
</tbody>
</table>
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.

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:
- 5.04 mL of 12X MES Stock Buffer
- 30.73 mL of 5M TMAC
- 2.46 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

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

The recipes in Table 3.7 and Table 3.8 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.

Refer to Table 3.7 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 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.

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 below. For hybridizing to two HT Array Plates, follow Step 3B below.

   A. Referring 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. Referring 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.

4. Aliquot the appropriate volumes of the master mix as indicated in Table 3.7 (or Table 3.8) into each well of the QIAGEN Elution Strip Tube(s).

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.

**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 Hybridization Cocktail Master Mix for Cold Reagent Block - 1 HT Array Plate

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

### Table 3.8
200 µL Hybridization Cocktail Master Mix for Cold Reagent Block - 2 HT Array Plates

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 Rxns</td>
</tr>
<tr>
<td>20X Hybridization Control Stock</td>
<td>10.0 µL</td>
<td>272.5 µL</td>
</tr>
<tr>
<td>3 nM B2 Oligo</td>
<td>3.3 µL</td>
<td>89.9 µL</td>
</tr>
<tr>
<td>HS DNA (10 mg/mL)</td>
<td>2.0 µL</td>
<td>54.5 µL</td>
</tr>
<tr>
<td>Acetylated BSA (50 mg/mL)</td>
<td>2.0 µL</td>
<td>54.5 µL</td>
</tr>
<tr>
<td>1.23X Hybridization Buffer</td>
<td>162.7 µL</td>
<td>4,433.6 µL</td>
</tr>
<tr>
<td>Total Volume</td>
<td>180.0 µL</td>
<td>4,905.0 µL</td>
</tr>
<tr>
<td># of QIAGEN Strips Used</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Volume per Well in 1st Strip</td>
<td>408.0 µL</td>
<td>408.0 µL</td>
</tr>
<tr>
<td>Volume per Well in 2nd Strip</td>
<td>204.0 µL</td>
<td>408.0 µL</td>
</tr>
<tr>
<td>Volume per Well in 3rd Strip</td>
<td>408.0 µL</td>
<td>408.0 µL</td>
</tr>
<tr>
<td>Volume per Well in 4th Strip</td>
<td>408.0 µL</td>
<td></td>
</tr>
<tr>
<td>Volume per Well in 5th Strip</td>
<td>408.0 µL</td>
<td></td>
</tr>
<tr>
<td>Volume per Well in 6th Strip</td>
<td>408.0 µL</td>
<td></td>
</tr>
</tbody>
</table>
Additional Reagent Preparation Steps Required

SETUP 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.

SETUP H2O 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.
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.

![Automated Target Preparation Schematic](image)

**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 4.15
Liquid waste drainage
2. Ensure that all 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.

   Clean out empty tip boxes in Twister II® Rack 2.

   Do not remove tip boxes while run is in progress.

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

   **Table 4.1**

   Pipette Tip Usage

<table>
<thead>
<tr>
<th>Number of Rxns</th>
<th>8</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
<th>48</th>
<th>56</th>
<th>64</th>
<th>72</th>
<th>80</th>
<th>88</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Tip Boxes Needed</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>21</td>
</tr>
</tbody>
</table>

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 on page 55 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 AFFYMETRIX® GENE EXPRESSION TARGET PREPARATION (TP) PROTOCOL ON THE GENECHIP® ARRAY STATION

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

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 Affymetrix 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**

Affymetrix 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 Affymetrix 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 on page 88).

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 on page 88).
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. Affymetrix strongly suggests you utilize 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. Please contact Affymetrix personnel for assistance with configuring email or pager notification.

K. 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.
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 on page 88 for an example of this report). After verifying you have correctly set up the Reagent Block, click Next.
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:

![User Message window if holding at 4°C after IVT.](image)

**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. First 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.
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 \textit{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 $\mu$g/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).

\textbf{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.

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.
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.

8. Remove the optical plate and place in the spectrophotometer.


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.


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](image)

Figure 4.39
Graphic displaying cRNA yields (in µg)
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For HT Array Plates Using the GeneChip® Array Station

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

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.

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 on page 83.
chapter 4 | Array Station Setup and Target Preparation

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.
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.
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](image)

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.
2. 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, A260/A280 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 (25ug to 250ug)**
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 cleanup 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 Setup Protocol and Hybridization
Introduction

This chapter 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 5.1).

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.

AUTOMATED HYBRIDIZATION SETUP SCHEMATIC

This section summarizes the Hybridization Setup protocol steps.

Figure 5.1
Steps 49 to 54: Hybridization Setup and Hybridization
Reagents and Materials Required

The reagents and materials required in the Hybridization Setup protocol have been tested and evaluated by Affymetrix. 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.

REAGENTS AND MATERIALS

- 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, page 36

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.
Pre-Hybridization Buffer for HT Array Plates

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 5.1.
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 5.1.
2. Pour 20 mL into the VWR Omnitray.

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 5.1
Pre-Hybridization Buffer Recipe

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

*The 1.23X hybridization buffer is TMAC containing buffer. See Table 5.2 for recipe.
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.

Table 5.2
1.23X Hybridization Buffer recipe.

<table>
<thead>
<tr>
<th></th>
<th>50 mL</th>
<th>1 L</th>
<th>Final 1X Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>12X MES</td>
<td>5.04 mL</td>
<td>100.8 mL</td>
<td>100 mM</td>
</tr>
<tr>
<td>5M TMAC</td>
<td>30.73 mL</td>
<td>614.6 mL</td>
<td>2.5M</td>
</tr>
<tr>
<td>0.5M EDTA</td>
<td>2.46 mL</td>
<td>49.2 mL</td>
<td>20 mM</td>
</tr>
<tr>
<td>10% Tween-20</td>
<td>0.06 mL</td>
<td>1.2 mL</td>
<td>0.01%</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>11.71 mL</td>
<td>234.2 mL</td>
<td></td>
</tr>
<tr>
<td>Total Volume</td>
<td>50 mL</td>
<td>1 L</td>
<td></td>
</tr>
</tbody>
</table>
Beginning a Run – Hybridization Setup Deck Layout

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

1. Setup the deck with the appropriate consumables. Refer to Figure 5.2.
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 5.2
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.
 chapter 5 | Hybridization Setup Protocol and Hybridization

Running Affymetrix® Gene Expression Hybridization Setup Protocol on the GeneChip® Array Station

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 5.3).

![Figure 5.3](Sciclone Workstation Software Login window)

3. Enter your User Name and Password and click OK. The Operator Runtime interface window appears (Figure 5.4).
4. Select **File → Open** to access the load application window (Figure 5.5).
Select HYB_0001 and click **Open**.

The hybridization setup application is loaded into Sciclone Workstation Software.
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 5.7.
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.

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 5.11 on page 108 for a description of the Summary Report).

**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.
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 5.8**
Hybridization Deck Layout when **Run hybridization mix transfer method** is 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 5.10). Click OK.

You must place the HT Array Plate hybridization sandwich in an incubator equilibrated to 48°C. Please refer to page 110.

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 5.11 shows an example of a Hybridization Setup Summary Report.
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 5.12.
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.
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.

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.
Chapter 6

Wash and Stain
Introduction

This chapter 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 6.1 to Figure 6.6).

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.

Automated Wash and Stain Schematic

This section summarizes the Wash and Stain protocol steps.

Figure 6.1
Step 1: Fill HT Wash Trays with Low Stringency Wash (LSW) Buffer
Figure 6.2
Step 2: Low Stringency Wash (LSW)

Figure 6.3
Step 3: High Stringency Wash (HSW); Transfer of Hybridization Cocktail; Drain/Refill HT Wash Trays

Figure 6.4
Step 4: First Stain (Phycoerythrin); Low Stringency Wash; Drain/Refill HT Wash Trays
Figure 6.5
Step 5: Second Stain (Ab); Low Stringency Wash; Drain/Refill HT Wash Tray

Figure 6.6
Step 6: Third Stain (Streptavidin-Phycoerythrin); Low Stringency Wash

Figure 6.7
Step 7: Filling HT Scan Tray with Holding Buffer and Insertion of the HT Array Plate
Reagents and Materials Required

The following reagents and materials required in the Wash and Stain protocol have been tested and evaluated by Affymetrix. 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.

REAGENTS AND MATERIALS

- Nuclease-free Water: Ambion, P/N 9932
- 20X SSPE (3M NaCl, 0.2M NaH₂PO₄, 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-Phycerylhrin Streptavidin: Molecular Probes, P/N S-866
- Surfact-Amps® 20 (Tween-20), 10%: Pierce Chemical, P/N 28320
Reagent 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.

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.

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 1 AND STAIN 3

1. In a 50 mL conical tube, add the components listed in the Stain 1 + Stain 3 column of Table 6.1, and mix well.

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

3. Aliquot 80 μL of the Stain1 + Stain 3 mixture 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.

Table 6.1
Stain 1 and Stain 3 Recipe

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

1. In a 14 mL conical tube, add the components listed in the 24 wells or 96 wells column of Table 6.2 as appropriate, and mix well.

2. Obtain one HT Stain tray and label it “Stain 2.”

3. Aliquot 80 μL of the Stain 2 mixture into the appropriate wells of the labeled HT Stain Tray.

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

Table 6.2
Stain 2 Synthesis

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

*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.
LOW STRINGENCY BUFFER

1. Prepare 2,000 mL of Low Stringency Wash Buffer.

2. Connect Bulk Dispenser Buffer Line to container containing Low Stringency Wash Buffer.

Low Stringency Wash Buffer Recipe

(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 BUFFER

1. Prepare 1 L of High Stringency Wash Buffer.
2. Pour 94 mL into one HT Wash Tray.

High Stringency Buffer Recipe

(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**

1. Prepare 1 L of MES Holding Buffer.
2. Pour 20 mL into a VWR Omnitray.

**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.

**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 – 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 6.8.

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 6.9 and Figure 6.10.

4. Set the temperature of the Watlow Temperature Controller to 48°C. Refer to Figure 6.11.

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 6.8**
The deck layout for the wash and stain protocol—load at least three tip racks in Rack 1.

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>HT Wash Tray</td>
<td>HT Wash Tray</td>
<td>HT Wash Tray</td>
<td>Base for stackable tip rack</td>
</tr>
<tr>
<td>B1</td>
<td>B2</td>
<td>B3</td>
<td>B4</td>
<td>B5</td>
</tr>
<tr>
<td>Tip Rack</td>
<td>HT Wash Tray</td>
<td>Bio-Rad Plate with lid</td>
<td>Red Tip Rack</td>
<td>Waste Chute</td>
</tr>
<tr>
<td>C1</td>
<td>C2</td>
<td>C3</td>
<td>C4</td>
<td>C5</td>
</tr>
<tr>
<td>Lubrication Block</td>
<td>Stack of 3 HT Stain Trays</td>
<td>HT Array Plate and HT Hyb Tray after hybridisation</td>
<td>HT Wash Tray with flat lid contains 84 mL of Wash Buffer on a flat Peltier adaptor set at 48°C</td>
<td>HT Array Plate Clamp Release</td>
</tr>
<tr>
<td>D1</td>
<td>D2</td>
<td>D3</td>
<td>D4</td>
<td>D5</td>
</tr>
<tr>
<td>Tip Rack</td>
<td>Clamping Locator Empty</td>
<td>HT Scan Tray on Black Spacer Tray</td>
<td>Liquid Waste Drain</td>
<td>VWR Omnitray Fill with ~20 mL MES holding buffer</td>
</tr>
</tbody>
</table>

**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 6.9
Installing the hybridization block fixture

Figure 6.10
Securing the hybridization block fixture
Figure 6.11
Setting the 48°C temperature on the Watlow Temperature Controller
Running Affymetrix® Gene Expression Wash and Stain Protocol on the GeneChip® Array Station

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 6.12).

4. Enter your User name and Password and click OK. The Operator Runtime interface appears (Figure 6.13).
5. Select **File → Open** to access the load application window (Figure 6.14).
6. Select WS_0001 and click Open.

The Affymetrix Wash Stain Protocol is loaded into Sciclone Workstation software (Figure 6.15).
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 6.16.
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 6.17).

![Figure 6.17](image-url)

Wash/Stain Deck Layout Setup window
11. 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.

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 6.18).

![Figure 6.18](image)

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.

16. At the end of the protocol, The End message appears (Figure 6.19). Click OK.
17. 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 6.20 shows an example of a Summary Report.
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 6.21.

![Figure 6.21](image)

**Figure 6.21**
LIMS-friendly Report Example
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**

<table>
<thead>
<tr>
<th>Volume per Rxn</th>
<th>8 Rxns</th>
<th>16 Rxns</th>
<th>24 Rxns</th>
<th>32 Rxns</th>
<th>40 Rxns</th>
<th>48 Rxns</th>
<th>56 Rxns</th>
<th>64 Rxns</th>
<th>72 Rxns</th>
<th>80 Rxns</th>
<th>88 Rxns</th>
<th>96 Rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>T7-Oligo(dT) Primer, 50 µM</td>
<td>1.0</td>
<td>5.0</td>
<td>10.0</td>
<td>15.0</td>
<td>21.0</td>
<td>26.0</td>
<td>32.0</td>
<td>38.0</td>
<td>44.0</td>
<td>50.0</td>
<td>56.0</td>
<td>62.0</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>11.0</td>
<td>22.0</td>
<td>33.0</td>
<td>44.0</td>
<td>55.0</td>
<td>66.0</td>
<td>77.0</td>
<td>88.0</td>
<td>99.0</td>
<td>110.0</td>
<td>121.0</td>
<td>132.0</td>
</tr>
<tr>
<td>SuperScript II</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>DTT, 0.1 M</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>dNTP Mix, 10 mM</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>SuperScript II</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
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<tr>
<td>Nuclease-free Water</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>8.0</td>
<td>10.0</td>
<td>12.0</td>
<td>14.0</td>
<td>16.0</td>
<td>18.0</td>
<td>20.0</td>
<td>22.0</td>
<td>24.0</td>
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<tr>
<td>Total Volume</td>
<td>8.0</td>
<td>16.0</td>
<td>24.0</td>
<td>32.0</td>
<td>40.0</td>
<td>48.0</td>
<td>56.0</td>
<td>64.0</td>
<td>72.0</td>
<td>80.0</td>
<td>88.0</td>
<td>96.0</td>
</tr>
</tbody>
</table>

### Table A.2

**First-Strand cDNA Synthesis Cocktail Master Mix for Cold Reagent Block**

<table>
<thead>
<tr>
<th>Volume per Rxn</th>
<th>8 Rxns</th>
<th>16 Rxns</th>
<th>24 Rxns</th>
<th>32 Rxns</th>
<th>40 Rxns</th>
<th>48 Rxns</th>
<th>56 Rxns</th>
<th>64 Rxns</th>
<th>72 Rxns</th>
<th>80 Rxns</th>
<th>88 Rxns</th>
<th>96 Rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X 1st Strand Rxn Mix</td>
<td>4.0</td>
<td>8.0</td>
<td>12.0</td>
<td>16.0</td>
<td>20.0</td>
<td>24.0</td>
<td>28.0</td>
<td>32.0</td>
<td>36.0</td>
<td>40.0</td>
<td>44.0</td>
<td>48.0</td>
</tr>
<tr>
<td>DTT, 0.1 M</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>8.0</td>
<td>10.0</td>
<td>12.0</td>
<td>14.0</td>
<td>16.0</td>
<td>18.0</td>
<td>20.0</td>
<td>22.0</td>
<td>24.0</td>
</tr>
<tr>
<td>dNTP Mix, 10 mM</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>SuperScript II</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
<td>8.0</td>
<td>10.0</td>
<td>12.0</td>
<td>14.0</td>
<td>16.0</td>
<td>18.0</td>
<td>20.0</td>
<td>22.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Total Volume</td>
<td>10.0</td>
<td>20.0</td>
<td>30.0</td>
<td>40.0</td>
<td>50.0</td>
<td>60.0</td>
<td>70.0</td>
<td>80.0</td>
<td>90.0</td>
<td>100.0</td>
<td>110.0</td>
<td>120.0</td>
</tr>
</tbody>
</table>

| Vol per Strip Tube Well | 10.0 | 20.0 | 30.0 | 40.0 | 50.0 | 60.0 | 70.0 | 80.0 | 90.0 | 100.0 | 110.0 | 120.0 |

### Table A.3

**3' Primer Cocktail Master Mix for Cold Reagent Block**

<table>
<thead>
<tr>
<th>Volume per Rxn</th>
<th>8 Rxns</th>
<th>16 Rxns</th>
<th>24 Rxns</th>
<th>32 Rxns</th>
<th>40 Rxns</th>
<th>48 Rxns</th>
<th>56 Rxns</th>
<th>64 Rxns</th>
<th>72 Rxns</th>
<th>80 Rxns</th>
<th>88 Rxns</th>
<th>96 Rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Primer Mix</td>
<td>40.0</td>
<td>80.0</td>
<td>120.0</td>
<td>160.0</td>
<td>200.0</td>
<td>240.0</td>
<td>280.0</td>
<td>320.0</td>
<td>360.0</td>
<td>400.0</td>
<td>440.0</td>
<td>480.0</td>
</tr>
<tr>
<td>dNTP Mix, 10 mM</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>SuperScript II</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>5.0</td>
<td>6.0</td>
<td>7.0</td>
<td>8.0</td>
<td>9.0</td>
<td>10.0</td>
<td>11.0</td>
<td>12.0</td>
</tr>
<tr>
<td>Nuclease-free Water</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total Volume</td>
<td>41.0</td>
<td>82.0</td>
<td>123.0</td>
<td>164.0</td>
<td>205.0</td>
<td>246.0</td>
<td>287.0</td>
<td>328.0</td>
<td>369.0</td>
<td>410.0</td>
<td>451.0</td>
<td>492.0</td>
</tr>
</tbody>
</table>

| Vol per Strip Tube Well | 10.0 | 20.0 | 30.0 | 40.0 | 50.0 | 60.0 | 70.0 | 80.0 | 90.0 | 100.0 | 110.0 | 120.0 |

**Vol per Strip Tube Well**

10.0 20.0 30.0 40.0 50.0 60.0 70.0 80.0 90.0 100.0 110.0 120.0
Table A.3
Second-Strand cDNA Synthesis Cocktail Master Mix for Cold Reagent Block

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes (μL):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 Rxns</td>
<td>16 Rxns</td>
</tr>
<tr>
<td>5X 2nd Strand Rxn Mix</td>
<td>30.0</td>
<td>270.0</td>
</tr>
<tr>
<td>dNTP Mix, 10 mM</td>
<td>3.0</td>
<td>27.0</td>
</tr>
<tr>
<td>DNA Ligase, 10 unit/μL</td>
<td>1.0</td>
<td>9.0</td>
</tr>
<tr>
<td>DNA Polymerase I, 10 unit/μL</td>
<td>4.0</td>
<td>36.0</td>
</tr>
<tr>
<td>RNase H, 2 unit/μL</td>
<td>1.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Total Volume</td>
<td>39.0</td>
<td>351.0</td>
</tr>
<tr>
<td>Vol per Strip Tube Well</td>
<td>41.0</td>
<td>85.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>Volume per Rxn</th>
<th>Adjusted Volumes (μL):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8 Rxns</td>
<td>16 Rxns</td>
</tr>
<tr>
<td>T4 DNA Polymerase</td>
<td>2.0</td>
<td>30.7</td>
</tr>
<tr>
<td>1X T4 DNA Polymerase Buffer</td>
<td>2.0</td>
<td>30.7</td>
</tr>
<tr>
<td>Total Volume</td>
<td>4.0</td>
<td>61.4</td>
</tr>
<tr>
<td>Vol per Strip Tube Well</td>
<td>7.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>
### Table A.5

<table>
<thead>
<tr>
<th>Volume per Rxn</th>
<th>Adjusted Volumes (μL):</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 16 32 48 72 96</td>
<td>96 90.4 89.6 88 80 72</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8 Rxns</th>
<th>16 Rxns</th>
<th>24 Rxns</th>
<th>32 Rxns</th>
<th>40 Rxns</th>
<th>48 Rxns</th>
<th>56 Rxns</th>
<th>64 Rxns</th>
<th>72 Rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.0</td>
<td>16.0</td>
<td>24.0</td>
<td>32.0</td>
<td>40.0</td>
<td>48.0</td>
<td>56.0</td>
<td>64.0</td>
<td>72.0</td>
</tr>
</tbody>
</table>

- **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

### Table A.6

<table>
<thead>
<tr>
<th>Volume per Strip Tube Well</th>
<th>Adjusted Volumes (μL):</th>
</tr>
</thead>
<tbody>
<tr>
<td>10x IVT Buffer</td>
<td>96 88 72 48 32 24 16 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>8 Rxns</th>
<th>16 Rxns</th>
<th>24 Rxns</th>
<th>32 Rxns</th>
<th>40 Rxns</th>
<th>48 Rxns</th>
<th>56 Rxns</th>
<th>64 Rxns</th>
<th>72 Rxns</th>
</tr>
</thead>
<tbody>
<tr>
<td>43.0</td>
<td>87.0</td>
<td>131.0</td>
<td>174.0</td>
<td>219.0</td>
<td>264.0</td>
<td>308.0</td>
<td>352.0</td>
<td>396.0</td>
</tr>
</tbody>
</table>

- **5X Fragmentation Buffer**
  - 3.1 4.7 6.3 8.0 9.7 11.4 13.0 14.7 16.4 18.1 19.8 21.4 23.1

- **Nuclease-free Water**
  - 70.2 112.0 163.8 215.6 267.4 319.2 371.0 422.8 474.6 526.4 578.2 630.0 681.8

- **IVT Fragmentation 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

- **IVT Fragmentation NTP Mix**
  - 117.0 234.0 351.0 468.0 585.0 702.0 819.0 936.0 1,053.0 1,170.0 1,287.0 1,404.0

- **IVT Fragmentation Nuclease-free Water**
  - 96.0 112.0 128.0 144.0 160.0 176.0 192.0 208.0 224.0 240.0 256.0 272.0 288.0

- **IVT Fragmentation Total Volume**
  - 272.0 544.0 816.0 1,088.0 1,360.0 1,632.0 1,904.0 2,176.0 2,448.0 2,720.0 2,992.0 3,264.0 3,536.0
### Table A.7

1 HT Array Plate
100 µL TMAC Hybridization Cocktail Master Mix for Cold Reagent Block

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

<table>
<thead>
<tr>
<th>Volume per Well</th>
<th>1st Strip</th>
<th>2nd Strip</th>
<th>3rd Strip</th>
<th>4th Strip</th>
<th>5th Strip</th>
<th>6th Strip</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adj. Volumes (μL):</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>8 Rxns</td>
<td>16</td>
<td>32</td>
<td>48</td>
<td>64</td>
<td>80</td>
<td>96</td>
</tr>
<tr>
<td>16 Rxns</td>
<td>32</td>
<td>64</td>
<td>96</td>
<td>128</td>
<td>160</td>
<td>192</td>
</tr>
<tr>
<td>24 Rxns</td>
<td>48</td>
<td>96</td>
<td>144</td>
<td>192</td>
<td>240</td>
<td>288</td>
</tr>
<tr>
<td>32 Rxns</td>
<td>64</td>
<td>128</td>
<td>192</td>
<td>256</td>
<td>320</td>
<td>384</td>
</tr>
<tr>
<td>40 Rxns</td>
<td>80</td>
<td>160</td>
<td>240</td>
<td>320</td>
<td>400</td>
<td>480</td>
</tr>
<tr>
<td>48 Rxns</td>
<td>96</td>
<td>192</td>
<td>288</td>
<td>384</td>
<td>480</td>
<td>576</td>
</tr>
<tr>
<td>56 Rxns</td>
<td>112</td>
<td>224</td>
<td>336</td>
<td>448</td>
<td>560</td>
<td>672</td>
</tr>
<tr>
<td>64 Rxns</td>
<td>128</td>
<td>256</td>
<td>384</td>
<td>512</td>
<td>640</td>
<td>768</td>
</tr>
</tbody>
</table>

# of QIAGEN Strips Used

<table>
<thead>
<tr>
<th>Total</th>
<th>1 2 3 4 5 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>90.8 181.7 272.5 363.3 454.2 545.0</td>
</tr>
<tr>
<td>HS DNA (10 mg/mL)</td>
<td>2.0 18.2 36.3 54.5 72.7 90.8 109.0</td>
</tr>
<tr>
<td>Acetylated BSA (50 mg/mL)</td>
<td>2.0 18.2 36.3 54.5 72.7 90.8 109.0</td>
</tr>
<tr>
<td>3nM B2 Oligo</td>
<td>3.3 30.0 60.0 90.0 120.0 150.0</td>
</tr>
<tr>
<td>1.23x Hybridization Buffer</td>
<td>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</td>
</tr>
</tbody>
</table>

Volume Adjusted Volumes (μL): per 8 16 24 32 48 64 96 80 72 64 56 48 40 32 24 16 8 2
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.
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.
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:

1) If using barcodes, select the barcode option. Please refer to
Chapter 4, page 70, substep F for a description on how to use barcoded plates.

2) Select the **Perform automatic sample transfer** option.

3) 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 on page 75.

4) Select the option, **Twister II tip rack spacer plates are in use** when using the spacer plates if there is a static electricity concern.

5) 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.

---

**NOTE**

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.
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**.
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](image)

**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. Please 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. In order to run customized applications click the Customize run button as shown in Figure C.1.

![Figure C.1](image-url)
You are prompted for information on the Run Type as shown in Figure C.2.

![Customized Run Setup window](image)

**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. Once 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

<table>
<thead>
<tr>
<th>Starting Selection</th>
<th>Deck Layout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Target Prep Starting Layout – Manual Transfer</td>
<td>Layout A</td>
</tr>
<tr>
<td>Full Target Prep Starting Layout – Automated Transfer</td>
<td>Layout B</td>
</tr>
<tr>
<td>First Half of Target Prep Starting Layout – Manual Transfer</td>
<td>Layout A</td>
</tr>
<tr>
<td>First Half of Target Prep Starting Layout – Automated Transfer</td>
<td>Layout B</td>
</tr>
<tr>
<td>Second Half of Target Prep Starting Layout</td>
<td>Layout C</td>
</tr>
<tr>
<td>Fragmentation Starting Layout</td>
<td>Layout D</td>
</tr>
</tbody>
</table>
Table C.2
The Array Station Starting Deck Layouts for Custom Selection of Run Steps

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

Table C.3
Deck Layout for User Intervention

<table>
<thead>
<tr>
<th>Starting Selection</th>
<th>Deck Layout</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Target Prep User Intervention Layout</td>
<td>Layout O</td>
</tr>
</tbody>
</table>
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

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>EMPTY</td>
<td>RNAClean™ in low profile lidded reservoir plate</td>
<td>75% EtOH in lidded reservoir</td>
<td>Base for stackable tip rack</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>Red Tip Rack</td>
<td>EMPTY</td>
<td>Nuclease free H₂O in lidded reservoir</td>
<td>Waste Chute</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubrication Block</td>
<td>Corning Polystyrene 3795 U bottom plate on Mag Separator. Label as “cDNA Cleanup”</td>
<td>Bio-Rad Plate with lid. Label as “Purified cDNA”</td>
<td>Lidded Cold Block for Reagents</td>
<td>Corning Polystyrene 3795 U-bottom plate. Label as “cRNA Cleanup”</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>Bio-Rad Plate with lid. Label as “un-frag cRNA”</td>
<td>Bio-Rad Plate with lid Manual prep: contains 5µL total RNA in each well. Label as “Total RNA”</td>
<td>Liquid Waste Drain</td>
<td>EMPTY</td>
</tr>
</tbody>
</table>

This location will be used for Twister access.
### Array Station Deck Layout Configuration B

<table>
<thead>
<tr>
<th>Column</th>
<th>Row</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>1</td>
<td>Tip Rack</td>
</tr>
<tr>
<td>A2</td>
<td>1</td>
<td>EMPTY</td>
</tr>
<tr>
<td>A3</td>
<td>1</td>
<td>RNA Clean™ in low profile lidded reservoir</td>
</tr>
<tr>
<td>A4</td>
<td>1</td>
<td>75% EtOH in lidded reservoir</td>
</tr>
<tr>
<td>A5</td>
<td>1</td>
<td>Base for stackable tip rack</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td>Tip Rack</td>
</tr>
<tr>
<td>B2</td>
<td>2</td>
<td>Red Tip Rack</td>
</tr>
<tr>
<td>B3</td>
<td>2</td>
<td>EMPTY</td>
</tr>
<tr>
<td>B4</td>
<td>2</td>
<td>Nuclease free H₂O in lidded reservoir</td>
</tr>
<tr>
<td>B5</td>
<td>2</td>
<td>Waste Chute</td>
</tr>
<tr>
<td>C1</td>
<td>3</td>
<td>Lubrication Block</td>
</tr>
<tr>
<td>C2</td>
<td>3</td>
<td>Corning Polystyrene 3796 U-bottom plate on Mag Separator. Label as “cDNA Cleanup”</td>
</tr>
<tr>
<td>C3</td>
<td>3</td>
<td>Bio-Rad Plate with lid Label as “Purified cDNA”</td>
</tr>
<tr>
<td>C4</td>
<td>3</td>
<td>Lidded Cold Block for Reagents</td>
</tr>
<tr>
<td>C5</td>
<td>3</td>
<td>Corning Polystyrene 3796 U-bottom plate. Label as “cRNA Cleanup”</td>
</tr>
<tr>
<td>D1</td>
<td>4</td>
<td>Tip Rack</td>
</tr>
<tr>
<td>D2</td>
<td>4</td>
<td>Greiner Polypropylene U-bottom plate for Hyb Label as “Starting Sample” at least 20 µL</td>
</tr>
<tr>
<td>D3</td>
<td>4</td>
<td>Bio-Rad Plate with lid Label as “Total RNA”</td>
</tr>
<tr>
<td>D4</td>
<td>4</td>
<td>Liquid Waste Drain</td>
</tr>
</tbody>
</table>
| D5     | 4   | EMPTY       

**Note:** This location will be used for Twister access.
Figure C.5
Array Station Deck Layout Configuration C

- **A1**: Tip Rack
- **A2**: Greiner Polystyrene U-bottom plate. Label as "normalized cRNA"
- **A3**: Greiner Polystyrene U-bottom plate for Hyb. Label as "Hyb-ready sample"
- **A4**: Base for stackable tip rack
- **A5**: Empty

- **B1**: Tip Rack
- **B2**: Red Tip Rack
- **B3**: Empty
- **B4**: Nuclease free H₂O in lidded reservoir
- **B5**: Waste Chute

- **C1**: Lubrication Block
- **C2**: Greiner Polystyrene UV transparent optical plate
- **C3**: Two Plastic Lids - stacked
- **C4**: Lidded Cold Block for Reagents
- **C5**: HT Array Plate Release Clamp Station

- **D1**: Tip Rack
- **D2**: Bio-Rad Plate with lid. Label as "frag cRNA"
- **D3**: Bio-Rad Plate with lid. Label as "un-frag cRNA." Wells contain 40 µL clean cRNA
- **D4**: Liquid Waste Drain
- **D5**: Empty

This location will be used for Twister access.
**Figure C.6**

Array Station Deck Layout Configuration D

- **A1**: Tip Rack
- **A2**: Greiner Polypropylene U-bottom plate. Label as "normalized cRNA"
- **A3**: Greiner Polypropylene U-bottom plate for Hyb. Label as "Hyb-ready sample"
- **A4**: EMPTY
- **A5**: Base for stackable tip rack
- **B1**: Tip Rack
- **B2**: Red Tip Rack
- **B3**: Bio-Rad Plate with lid. Label as "un-frag cRNA"
- **B4**: EMPTY
- **B5**: Waste Chute
- **C1**: Lubrication Block
- **C2**: EMPTY
- **C3**: Two Plastic Lids - stacked
- **C4**: Lidded Cold Block for Reagents
- **C5**: HT Array Plate Release Clamp Station
- **D1**: Tip Rack
- **D2**: EMPTY
- **D3**: Bio-Rad Plate with lid. Label as "frag cRNA." Wells contain normalized cRNA at 0.625 µg/µL
- **D4**: Liquid Waste Drain
- **D5**: EMPTY

This location will be used for Twister access.

Bio-Rad Plate with lid. Label as "un-frag cRNA".
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

- **Tip Rack**
- **Red Tip Rack**
- **Lubrication Block**
- **Bio-Rad Plate with lid. Label as "un-frag cRNA"**
- **Bio-Rad Plate with lid. Wells contain 13 μL of completed cDNA synthesis reaction. Label as "Total RNA"**
- **Bio-Rad Plate with lid on Mag Separator. Label as "cDNA Cleanup"**
- **RNAClean™ in low profile lidded reservoir plate**
- **75% EtOH in lidded reservoir**
- **Lidded Cold Block for Reagents**
- **Bio-Rad Plate with lid. Label as "Purified cDNA"**
- **Corning Polystyrene 3795 U-bottom plate on Mag Separator. Label as "cRNA Cleanup"**
- **Corning Polystyrene 3795 U-bottom plate. Label as "cDNA Cleanup"**
- **Nuclease free H₂O in lidded reservoir**
- **Waste Chute**
- **Liquid Waste Drain**
- **This location will be used for Twister access**
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

- **A1**: Tip Rack
- **A2**: Corning Polystyrene 3796 U-bottom plate. Label as "cRNA Cleanup"
- **A3**: RNAClean™ in low profile lidded reservoir plate
- **A4**: 75% EtOH in lidded reservoir
- **A5**: Base for stackable tip rack

- **B1**: Tip Rack
- **B2**: Red Tip Rack
- **B3**: Bio-Rad Plate with lid. Label as "total RNA"
- **B4**: Nuclease free H₂O in lidded reservoir
- **B5**: Waste Chute

- **C1**: Lubrication Block
- **C2**: Mag Separator
- **C3**: EMPTY
- **C4**: Lidded Cold Block for Reagents
- **C5**: Corning Polystyrene 3796 U-bottom plate. Label as "cDNA Cleanup"

- **D1**: Tip Rack
- **D2**: Bio-Rad Plate with lid. Label as "un-frag cRNA"
- **D3**: Bio-Rad Plate with lid. Wells contain 60 µL of completed IVT reaction. Label as "Purified cDNA"
- **D4**: Liquid Waste Drain
- **D5**: EMPTY

This location will be used for Twister access.
Figure C.14
Array Station Deck Layout Configuration L

<table>
<thead>
<tr>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>Corning Polystyrene 3795 U-bottom plate. Label as “cRNA Cleanup” Plate contains bound, washed cRNA</td>
<td>RNAClean™ in low profile lidded reservoir plate</td>
<td>75% EtOH in lidded reservoir</td>
<td>Base for stackable tip rack</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>Red Tip Rack</td>
<td>Bio-Rad Plate with lid. Label as “Total RNA”</td>
<td>Nuclease free H2O in lidded reservoir</td>
<td>Waste Chute</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lubrication Block</td>
<td>Mag Separator</td>
<td>Bio-Rad Plate with lid. Label as “Purified cDNA”</td>
<td>Lidded Cold Block for Reagents</td>
<td>Corning Polystyrene 3795 U-bottom plate. Label as “cDNA Cleanup”</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip Rack</td>
<td>EMPTY</td>
<td>Bio-Rad Plate with lid. Label as “un-frag cRNA”</td>
<td>Liquid Waste Drain</td>
<td>EMPTY</td>
</tr>
</tbody>
</table>

This location will be used for Twister access.
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

<table>
<thead>
<tr>
<th>User Group</th>
<th>Permissions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sciclone Operators</td>
<td>Access Runtime Window, Run Methods, Access Direct Control Window, Error Recovery</td>
</tr>
<tr>
<td>Sciclone Method Developers</td>
<td>All Operator Privileges, Access Method Editor Window, Create, Edit, Save and Delete Methods, Layouts and Liquid Classes, Import and Export Applications and Methods</td>
</tr>
<tr>
<td>Sciclone Administrators</td>
<td>Access all Sciclone systems</td>
</tr>
</tbody>
</table>

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 Affymetrix 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 Affymetrix applications you should 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
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

<table>
<thead>
<tr>
<th>Line Number</th>
<th>Contents</th>
<th>Possible Values or Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Application name</td>
<td>TP_0001</td>
</tr>
<tr>
<td>2</td>
<td>Name of Array Station user</td>
<td>Doe, John</td>
</tr>
<tr>
<td>3</td>
<td>Run start time</td>
<td>4:30:31 PM</td>
</tr>
<tr>
<td>4</td>
<td>Run start date</td>
<td>Monday, April 03, 2006</td>
</tr>
<tr>
<td>5</td>
<td>Run end time</td>
<td>8:53:41 PM</td>
</tr>
<tr>
<td>6</td>
<td>Run duration</td>
<td>4:23:10</td>
</tr>
<tr>
<td>7</td>
<td>Tracking ID</td>
<td>User-entered string*</td>
</tr>
<tr>
<td>8</td>
<td>Tip-spacers-used flag</td>
<td>YES or NO</td>
</tr>
<tr>
<td>9</td>
<td>Number of samples</td>
<td>8, 16, 24, ..., 96</td>
</tr>
<tr>
<td>10</td>
<td>In-vitro transcription time</td>
<td>4, 8, or 16</td>
</tr>
<tr>
<td>11</td>
<td>Target destination</td>
<td>One HT array plate</td>
</tr>
<tr>
<td>12</td>
<td>First run step</td>
<td>Primer annealing [ 2 †]</td>
</tr>
<tr>
<td>13</td>
<td>Last run step</td>
<td>Hybridization mix [ 15 †]</td>
</tr>
<tr>
<td>14</td>
<td>Initial sample transfer</td>
<td>Manual or Automatic</td>
</tr>
<tr>
<td>15</td>
<td>Hold-after-IVT flag</td>
<td>YES or NO</td>
</tr>
<tr>
<td>16</td>
<td>cDNA kit lot number</td>
<td>User-entered value‡</td>
</tr>
<tr>
<td>17</td>
<td>IVT kit lot number</td>
<td>User-entered value§</td>
</tr>
<tr>
<td>18</td>
<td>Barcode-reader-enabled flag</td>
<td>YES or NO</td>
</tr>
</tbody>
</table>
Table E.1 (Continued)
Contents of Summary Report File for Target Preparation 0001

<table>
<thead>
<tr>
<th>Line Number</th>
<th>Contents</th>
<th>Possible Values or Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Total RNA barcode</td>
<td>Value read during run**</td>
</tr>
<tr>
<td>20</td>
<td>Unfragmented cRNA barcode</td>
<td>Value read during run**</td>
</tr>
<tr>
<td>21</td>
<td>Normalized cRNA barcode</td>
<td>Value read during run**</td>
</tr>
<tr>
<td>22</td>
<td>Fragmented cRNA barcode</td>
<td>Value read during run**</td>
</tr>
<tr>
<td>23</td>
<td>Hybridization-ready barcode</td>
<td>Value read during run**</td>
</tr>
<tr>
<td>24</td>
<td>Limits for pre-normalization yields</td>
<td>25&lt;tab&gt;250</td>
</tr>
<tr>
<td>25</td>
<td>Wells outside pre-normalization limits</td>
<td>A01&lt;tab&gt;C12&lt;tab&gt;E05</td>
</tr>
<tr>
<td>26</td>
<td>Robot errors</td>
<td>Tab-delimited set of numbers</td>
</tr>
</tbody>
</table>

*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.
†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

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

*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 placeholder 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

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

*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 F

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**

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Supplier</th>
<th>P/N</th>
<th>Quantity / Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneChip® Array Station</td>
<td>Affymetrix</td>
<td>00-0162 (110V) or 00-0235 (220v)</td>
<td>1</td>
</tr>
<tr>
<td>GeneChip® HT Scanner</td>
<td>Affymetrix</td>
<td>00-0172</td>
<td>1</td>
</tr>
<tr>
<td>Allen wrench (2.5mm)</td>
<td>Multiple</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Dual Channel Thermocouple</td>
<td>VWR International</td>
<td>61220-605</td>
<td>1</td>
</tr>
<tr>
<td>12-multichannel 20 µL Pipetman</td>
<td>Rainin</td>
<td>L12-20</td>
<td>1</td>
</tr>
<tr>
<td>12-multichannel 200 µL Pipetman</td>
<td>Rainin</td>
<td>L12-200</td>
<td>1</td>
</tr>
<tr>
<td>8-multichannel 20 µL Pipetman</td>
<td>Rainin</td>
<td>L8-20</td>
<td>1</td>
</tr>
<tr>
<td>8-multichannel 200 µL Pipetman</td>
<td>Rainin</td>
<td>L8-200</td>
<td>1</td>
</tr>
<tr>
<td>Auto-sealing microplate lid, arched, wide tab</td>
<td>Bio-Rad</td>
<td>MSL 2032</td>
<td>4</td>
</tr>
<tr>
<td>Gripper Pads</td>
<td>Caliper LifeSciences</td>
<td>52071</td>
<td>8</td>
</tr>
<tr>
<td>Heatblock</td>
<td>Multiple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microseal P pads ADHESIVE</td>
<td>Bio-Rad</td>
<td>MSP-1001</td>
<td>1 (10 seals)</td>
</tr>
<tr>
<td>Multidispensing pipette - 1,000 µL</td>
<td>Rainin</td>
<td>E3-1000</td>
<td></td>
</tr>
<tr>
<td>Multidispensing pipette - 200 µL</td>
<td>Rainin</td>
<td>E3-200</td>
<td></td>
</tr>
<tr>
<td>Sealing Roller</td>
<td>Bio-Rad</td>
<td>MSR-0001</td>
<td></td>
</tr>
<tr>
<td>Zerostat Anti-Static Gun</td>
<td>Audioadvisor.com</td>
<td>Zerostat</td>
<td></td>
</tr>
</tbody>
</table>
## CONSUMABLES – TARGET PREPARATION

### Table F.2
Consumables List for Target Preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
<th>P/N</th>
<th>Quantity / Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2 mL Square Well Storage Plate, Low Profile</td>
<td>ABGene/Marsh</td>
<td>AB-1127</td>
<td>1</td>
</tr>
<tr>
<td>96 Wells High Profile 300 mL Reservoir</td>
<td>E&amp;K Scientific Products</td>
<td>EK-2035</td>
<td>2</td>
</tr>
<tr>
<td>96-Well Hard-Shell PCR Plate</td>
<td>Bio-Rad</td>
<td>HSP-9601</td>
<td>4</td>
</tr>
<tr>
<td>Aluminum Foil Lids*</td>
<td>Beckman</td>
<td>538619</td>
<td>1 pack</td>
</tr>
<tr>
<td>BD Falcon™ Test Tube, 14 mL</td>
<td>VWR International</td>
<td>60819-761</td>
<td>2</td>
</tr>
<tr>
<td>BD Falcon™ Test Tube, 5 mL*</td>
<td>VWR International</td>
<td>60819-728</td>
<td>2</td>
</tr>
<tr>
<td>Corning Polystyrene Round Bottom Plates</td>
<td>Fisher Scientific</td>
<td>CLS3795</td>
<td>2</td>
</tr>
<tr>
<td>DNAZap™</td>
<td>Ambion</td>
<td>9890</td>
<td>1 (2 bottles)</td>
</tr>
<tr>
<td>Elution Strip Tubes, 0.85 mL</td>
<td>QIAGEN</td>
<td>19588</td>
<td>6 strips</td>
</tr>
<tr>
<td>Greiner round bottom clear polypropylene plate</td>
<td>E&amp;K Scientific Products</td>
<td>20261</td>
<td>2</td>
</tr>
<tr>
<td>Greiner UV transparent Optical Plates</td>
<td>E&amp;K Scientific Products</td>
<td>AB-1127</td>
<td>2</td>
</tr>
<tr>
<td>KimWipes®</td>
<td>VWR International</td>
<td>34256</td>
<td></td>
</tr>
<tr>
<td>Low-Profile 0.2 mL PCR 8-Tube Strips</td>
<td>Bio-Rad</td>
<td>TLS-0801</td>
<td>4 strips</td>
</tr>
<tr>
<td>Microseal ‘F’ Adhesive Foil</td>
<td>Bio-Rad</td>
<td>MSF-1001</td>
<td></td>
</tr>
<tr>
<td>Microtiter plate lids</td>
<td>Phenix</td>
<td>ML-5009</td>
<td>5</td>
</tr>
<tr>
<td>Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL</td>
<td>VWR International</td>
<td>20171-028</td>
<td>2</td>
</tr>
<tr>
<td>RNase-Free 1.5 mL Microfuge tube*</td>
<td>Ambion</td>
<td>12400</td>
<td>6</td>
</tr>
<tr>
<td>RNaseZap® wipes</td>
<td>Ambion</td>
<td>9786</td>
<td>1 (100 wipes)</td>
</tr>
<tr>
<td>Stacker tips 200 µL non-sterile</td>
<td>Caliper LifeSciences</td>
<td>78641</td>
<td>1 box</td>
</tr>
</tbody>
</table>

* or equivalent
CONSUMABLES – HYBRIDIZATION SETUP

Table F.3
Consumables List for Hybridization Setup

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
<th>P/N</th>
<th>Quantity / Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT Hybridization Tray*</td>
<td>Affymetrix</td>
<td>202111</td>
<td>1</td>
</tr>
<tr>
<td>HT Stain Tray*</td>
<td>Affymetrix</td>
<td>900745</td>
<td>1</td>
</tr>
<tr>
<td>96-Well Hard-Shell PCR Plate</td>
<td>Bio-Rad</td>
<td>HSP-9601</td>
<td>1</td>
</tr>
<tr>
<td>Omnitray or Greiner Round Bottom Clear Polypropylene Plate</td>
<td>VWR or E&amp;K Scientific Products</td>
<td>4660-638 or 20261</td>
<td>1</td>
</tr>
<tr>
<td>Stacker Tips 200 µL non-sterile</td>
<td>Caliper LifeSciences</td>
<td>78642</td>
<td>3</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Item</th>
<th>Source</th>
<th>P/N</th>
<th>Quantity / Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT Scan Tray*</td>
<td>Affymetrix</td>
<td>900746</td>
<td>1</td>
</tr>
<tr>
<td>HT Stain Tray*</td>
<td>Affymetrix</td>
<td>900745</td>
<td>3</td>
</tr>
<tr>
<td>HT Wash Tray*</td>
<td>Affymetrix</td>
<td>900752</td>
<td>5</td>
</tr>
<tr>
<td>Aluminum Foil Lids</td>
<td>Beckman</td>
<td>538619</td>
<td>1 pack</td>
</tr>
<tr>
<td>BD Falcon™ Test Tube, 14 mL</td>
<td>VWR International</td>
<td>60819-761</td>
<td>2</td>
</tr>
<tr>
<td>BD Falcon™ Test Tube, 5 mL†</td>
<td>VWR International</td>
<td>60819-728</td>
<td>1</td>
</tr>
<tr>
<td>Polypropylene Centrifuge Tubes with Caps, Sterile 50 mL</td>
<td>VWR International</td>
<td>20171-028</td>
<td>2</td>
</tr>
<tr>
<td>RNase-Free 1.5 mL Microfuge tube†</td>
<td>Ambion</td>
<td>12400</td>
<td>1</td>
</tr>
<tr>
<td>Round Bottom Clear Polypropylene Plate</td>
<td>E&amp;K Scientific Products</td>
<td>20261</td>
<td>1</td>
</tr>
<tr>
<td>Stacker Tips 200 µL non-sterile</td>
<td>Caliper LifeSciences</td>
<td>78641</td>
<td>1 Box</td>
</tr>
<tr>
<td>96-Well Hard-Shell PCR Plate</td>
<td>Bio-Rad</td>
<td>HSP-9601</td>
<td>1</td>
</tr>
<tr>
<td>Omnitray</td>
<td>VWR International</td>
<td>4660-638</td>
<td>2</td>
</tr>
</tbody>
</table>

*HT Stain, Wash, and Scan Trays are packaged with the HT Array Plates.
†or equivalent
# REAGENTS – TOTAL RNA ISOLATION

Table F.5
Reagent List for Total RNA Isolation

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRizol® Reagent</td>
<td>Invitrogen Life Technologies</td>
<td>15596-018</td>
</tr>
<tr>
<td>Ethanol, 80% (stored at -20°C)</td>
<td>Multiple</td>
<td></td>
</tr>
<tr>
<td>Glycogen (optional)</td>
<td>Ambion</td>
<td>9510</td>
</tr>
<tr>
<td>Pellet Paint® (optional)</td>
<td>Novagen</td>
<td>69049-3</td>
</tr>
<tr>
<td>QIAzol™ Lysis Reagent</td>
<td>QIAGEN</td>
<td>79306</td>
</tr>
<tr>
<td>RNeasy® Mini Kit</td>
<td>QIAGEN</td>
<td>74104</td>
</tr>
<tr>
<td>Sodium Acetate (NaOAc), 3M</td>
<td>Sigma-Aldrich</td>
<td>S7899</td>
</tr>
</tbody>
</table>
### REAGENTS – TARGET PREPARATION

**Table F.6**  
Reagent List for Target Preparation

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneChip® HT One-Cycle Target Labeling and Controls Kit (96 samples)</td>
<td>Affymetrix</td>
<td>900686</td>
</tr>
<tr>
<td>containing:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• GeneChip® HT One-Cycle cDNA Synthesis Kit 96-Reactions, P/N 900687</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• GeneChip® HT IVT Labeling Kit 96-Reactions, P/N 900688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• GeneChip® Eukaryotic Poly-A RNA Control Kit 100-Reactions, P/N 900433</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• GeneChip® Eukaryotic Hybridization Control Kit 150-Reactions, P/N 900457</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GeneChip® Eukaryotic Poly-A RNA Control Kit containing:</td>
<td>Affymetrix</td>
<td>900433</td>
</tr>
<tr>
<td>• Poly-A Control Stock (16 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Poly-A Control Dil Buffer (3.8 mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GeneChip® HT One-Cycle cDNA Synthesis Kit containing:</td>
<td>Affymetrix</td>
<td>900687</td>
</tr>
<tr>
<td>• T7-Oligo(dT) Primer, 50 µM (130 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 5X 1st Strand Reaction Mix (460 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• DTT, 0.1M (230 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• dNTP, 10 mM (460 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• SuperScript™ II (120 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 5X 2nd Strand Reaction Mix (3,300 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• E. coli DNA Polymerase I (440 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• E. coli DNA Ligase (110 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• RNase H (110 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• T4 DNA Polymerase (280 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 5X T4 DNA Polymerase Buffer (60 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GeneChip® HT IVT Labeling Kit containing:</td>
<td>Affymetrix</td>
<td>900688</td>
</tr>
<tr>
<td>• 10X IVT Labeling Buffer, 1 tube (675 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• IVT Labeling Enzyme Mix, 1 tube (675 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• IVT Labeling NTP Mix, 2 tubes (1,010 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 3’-Labeling Control (0.5 µg/µL), 1 tube (10 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• T7 RNA Polymerase, 1 tube (115 µL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 5X Fragmentation Buffer, 1 tube (855 µL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table F.6 (Continued)
Reagent List for Target Preparation

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free Water</td>
<td>Ambion</td>
<td>9932</td>
</tr>
<tr>
<td>RNAClean 60 mL</td>
<td>Agencourt</td>
<td>000494</td>
</tr>
</tbody>
</table>

Table F.7
Reagent List for Hybridization Setup

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine Serum Albumin (BSA) solution (50 mg/mL)</td>
<td>Invitrogen Life Technologies</td>
<td>15561-020</td>
</tr>
<tr>
<td>EDTA Disodium Salt, 0.5M solution (100 mL)</td>
<td>Sigma-Aldrich</td>
<td>E7889</td>
</tr>
<tr>
<td>Herring Sperm DNA</td>
<td>Promega Corporation</td>
<td>D1811</td>
</tr>
<tr>
<td>MES hydrate SigmaUltra</td>
<td>Sigma-Aldrich</td>
<td>M5287</td>
</tr>
<tr>
<td>MES Sodium Salt</td>
<td>Sigma-Aldrich</td>
<td>M5057</td>
</tr>
<tr>
<td>Nuclease-free Water, 1 L</td>
<td>Ambion</td>
<td>9932</td>
</tr>
<tr>
<td>Surfact-Amps® 20 (Tween-20), 10%</td>
<td>Pierce Chemical</td>
<td>28320</td>
</tr>
<tr>
<td>TMAC (5M)</td>
<td>Sigma-Aldrich</td>
<td>T3411</td>
</tr>
</tbody>
</table>
REAGENTS – WASH AND STAIN

Table F.8
Reagent List for Wash and Stain

<table>
<thead>
<tr>
<th>Material</th>
<th>Source</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free Water</td>
<td>Ambion</td>
<td>9932</td>
</tr>
<tr>
<td>20X SSPE (3M NaCl, 0.2M NaH₂PO₄, 0.02M EDTA)</td>
<td>Cambrex</td>
<td>51214</td>
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<td>5M NaCl, RNase-free, DNase-free</td>
<td>Ambion</td>
<td>9760G</td>
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<tr>
<td>Anti-Streptavidin Antibody (Goat), Biotinylated</td>
<td>Vector Laboratories</td>
<td>BA-0500</td>
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<td>Bovine Serum Albumin (BSA) solution (50 mg/mL)</td>
<td>Invitrogen Life Technologies</td>
<td>15561-020</td>
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<tr>
<td>Distilled Water</td>
<td>Invitrogen Life Technologies</td>
<td>15230-147</td>
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<tr>
<td>Goat IgG, Reagent Grade</td>
<td>Sigma-Aldrich</td>
<td>I 5256</td>
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<tr>
<td>PBS, pH 7.2</td>
<td>Invitrogen Life Technologies</td>
<td>20012-027</td>
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<tr>
<td>R-Phycoerythrin Streptavidin</td>
<td>Molecular Probes</td>
<td>S-866</td>
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<tr>
<td>Surfact-Amps® 20 (Tween-20), 10%</td>
<td>Pierce Chemical</td>
<td>28320</td>
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## Supplier Contact Information

<table>
<thead>
<tr>
<th>Source</th>
<th>Web Site</th>
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<tr>
<td>ABGene/Marsh</td>
<td><a href="http://www.marshbio.com">www.marshbio.com</a></td>
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<tr>
<td>Ambion</td>
<td><a href="http://www.ambion.com">www.ambion.com</a></td>
</tr>
<tr>
<td>Affymetrix</td>
<td><a href="http://www.affymetrix.com">www.affymetrix.com</a></td>
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<td>Agencourt</td>
<td><a href="http://www.agencourt.com">www.agencourt.com</a></td>
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<tr>
<td>Aldrich</td>
<td><a href="http://www.sigmaaldrich.com">www.sigmaaldrich.com</a></td>
</tr>
<tr>
<td>Ambion</td>
<td><a href="http://www.ambion.com">www.ambion.com</a></td>
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<tr>
<td>Audioadvisor.com</td>
<td><a href="http://www.audioadvisor.com">www.audioadvisor.com</a></td>
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<td>Beckman</td>
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<td>Bio-Rad</td>
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<td>Caliper</td>
<td><a href="http://www.caliperls.com">www.caliperls.com</a></td>
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<td>Cambrex</td>
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<tr>
<td>Cole-Parmer</td>
<td><a href="http://www.coleparmer.com">www.coleparmer.com</a></td>
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<td>E&amp;K Scientific Products</td>
<td><a href="http://www.eandkscientific.com">www.eandkscientific.com</a></td>
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<td>Fisher Scientific</td>
<td><a href="http://www.fishersci.com">www.fishersci.com</a></td>
</tr>
<tr>
<td>Invitrogen Life Technologies</td>
<td><a href="http://www.invitrogen.com">www.invitrogen.com</a></td>
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<td>Molecular Probes</td>
<td><a href="http://www.probes.com">www.probes.com</a></td>
</tr>
<tr>
<td>Novagen</td>
<td><a href="http://www.emdbiosciences.com/html/NVG/home.html">www.emdbiosciences.com/html/NVG/home.html</a></td>
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<tr>
<td>Phenix</td>
<td><a href="http://www.phenix1.com">www.phenix1.com</a></td>
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<tr>
<td>Pierce Chemical</td>
<td><a href="http://www.piercenet.com">www.piercenet.com</a></td>
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<td>Promega Corporation</td>
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<td><a href="http://www.qiagen.com">www.qiagen.com</a></td>
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<td>Rainin</td>
<td><a href="http://www.rainin-global.com">www.rainin-global.com</a></td>
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Table F.9 (Continued)
Supplier Contact Information

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<td>Sigma-Aldrich</td>
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<td>USA Scientific</td>
<td><a href="http://www.usascientific.com">www.usascientific.com</a></td>
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<tr>
<td>Vector Laboratories</td>
<td><a href="http://www.vectorlabs.com">www.vectorlabs.com</a></td>
</tr>
<tr>
<td>VWR International</td>
<td><a href="http://www.vwr.com">www.vwr.com</a></td>
</tr>
</tbody>
</table>
Appendix G

Affymetrix Contact Information
When to Contact Technical Support

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

IMPORTANT

Make sure you have the model and serial number.

Affymetrix, Inc.
3420 Central Expressway
Santa Clara, CA 95051
USA

E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd
Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom

E-mail: supporteurope@affymetrix.com
UK and Others Tel: +44 (0) 1628 552550
France Tel: 0800919505
Germany Tel: 01803001334
Fax: +44 (0) 1628 552585
Affymetrix Japan, K. K.
Mita NN Bldg
16 Floor, 4-1-23 Shiba,
Minato-ku, Tokyo 108-0014
Japan
Tel: (03) 5730-8200
Fax: (03) 5730-8201