# Agilent One-Color Microarray-Based Exon Analysis

## Low Input Quick Amp WT Labeling

## Protocol

For use with Agilent Gene Expression Exon microarrays

**Version 1.0, November 2010**

**Microarrays manufactured with Agilent SurePrint Technology**

**Research Use Only. Not for use in Diagnostic Procedures.**

Agilent Technologies

# Notices



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# In this Guide...

This document describes Agilent's recommended procedures for the preparation and labeling of complex biological targets and hybridization, washing, scanning, and feature extraction of Agilent 60-mer oligonucleotide microarrays for microarray-based one-color gene expression exon analysis.

### 1 Before You Begin

This chapter contains information (such as procedural notes, safety information, required reagents and equipment) that you should read and understand before you start an experiment.

### 2 Procedures

This chapter describes the steps to prepare samples, hybridize, wash and scan gene expression microarrays, and to extract data using the Agilent Feature Extraction Software.

### 3 Supplemental Procedures

This chapter contains instructions for quality assessment of template RNA and labeled cRNA, and steps to prevent ozone-related problems.

### 4 Reference

This chapter contains reference information related to the protocol.

## What's in This Protocol?

This protocol is based on the *One-Color Microarray-Based Gene Expression Analysis (Low Input Quick Amp Labeling) Protocol*, version 6.3, but with these changes:

- Low Input Quick Amp WT Labeling kit, with a new primer, is used instead of the Low Input Quick Amp Labeling kit.
- Gene Expression Exon Microarrays and whole transcript labeling are supported.
- The WT Primer Mix replaces the T7 promoter primer.
- The starting input requirement for the 2-pack, 4-pack and 8-pack microarrays is 25 ng of total RNA (50 ng is recommended).
- The 1-pack microarray input requirement is 100 ng of total RNA.
- The recommended Specific Activity for hybridization is increased to 15 pmol cyanine 3 per µg cRNA.
- The C scanner is required, as Agilent Exon Microarrays are available only on the Agilent SurePrint G3 microarray formats.

# Content

# 1 Before You Begin



Make sure you read and understand the information in this chapter and have the necessary equipment and reagents listed before you start an experiment.

**NOTE** Agilent cannot guarantee microarray performance and does not provide technical support to those who use non-Agilent protocols in processing Agilent's microarrays.

## Procedural Notes

- Determine the integrity and purity of the input RNA for labeling and hybridization prior to use to increase the likelihood of a successful experiment.
- To prevent contamination of reagents by nucleases, always wear powder-free laboratory gloves, and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips.
- Maintain a clean work area.
- When preparing frozen reagent stock solutions for use:
  1. Thaw the aliquot as rapidly as possible without heating above room temperature, unless otherwise indicated.
  2. Mix briefly on a vortex mixer, then centrifuge for 5 to 10 seconds to drive the contents off of walls and lid.
  3. Store on ice or in a cold block until use, unless otherwise indicated.
- In general, follow Biosafety Level 1 (BL1) safety rules.

## Safety Notes

**CAUTION**

- Inspect the Stabilization and Drying Solution bottle for chips or cracks prior to use. Failure to do so may result in bottle breakage.
- Wear appropriate personal protective equipment (PPE) when working in the laboratory.

**WARNING**

- **Cyanine 3-CTP is a potential carcinogen. Avoid inhalation, swallowing, or contact with skin.**
- **LiCl is toxic and a potential teratogen. May cause harm to breastfed babies. Possible risk of impaired fertility. Harmful if inhaled, swallowed, or contacts skin. Target organ: central nervous system. Wear suitable PPE. LiCl is a component of Agilent's 2X Hybridization Buffer.**
- **Lithium dodecyl sulfate (LDS) is harmful by inhalation and irritating to eyes, respiratory system and skin. Wear suitable PPE. LDS is a component of Agilent's 2X Hybridization Buffer.**
- **Triton is harmful if swallowed. Risk of serious damage to eyes. Wear suitable PPE. Triton is a component of Agilent's 2X Hybridization Buffer and is an additive in wash buffers.**
- **Acetonitrile is a flammable liquid and vapor. Harmful if inhaled, swallowed, or contacts skin. Target organs: liver, kidneys, cardiovascular system and CNS.**
- **Agilent Stabilization and Drying Solution is toxic and flammable and must be used in a suitable fume hood. This solution contains acetonitrile and must be disposed of in a manner consistent with disposal of like solvents. Gloves and eye/face protection should be used during every step of this protocol, especially when handling acetonitrile and the Stabilization and Drying Solution.**

# Agilent Oligo Microarray Kit Contents

Check the Agilent Web site at www.agilent.com/chem/dualmode for the most up to date list of supported microarray designs.

### Catalog microarray kits

- Two or four microarrays printed on each 1-inch × 3-inch glass slides, of a three slide kit.
- DVD containing microarray design files in various file formats

### Custom microarray kits

- One to eight microarrays printed on each 1-inch × 3-inch glass slide
- Number of microarrays varies per kit and per order

**NOTE**

Store entire kit at room temperature. After breaking foil on microarray pouch, store microarray slides at room temperature (in the dark) under a vacuum dessicator or nitrogen purge box. Do not store microarray slides in open air after breaking foil.

# Required Equipment

| Description | Vendor and part number |
|---|---|
| Agilent Microarray Scanner | Agilent p/n G2565CA |
| Hybridization Chamber, stainless | Agilent p/n G2534A |
| Hybridization Chamber gasket slides | |
| 1 microarray/slide, 5 slides/box | Agilent p/n G2534-60003 |
| 2 microarrays/slide, 5 slides/box | Agilent p/n G2534-60002 |
| 4 microarrays/slide, 5 slides/box | Agilent p/n G2534-60011 |
| 8 microarrays/slide, 5 slides/box | Agilent p/n G2534-60014 |
| Hybridization oven; temperature set at 65°C | Agilent p/n G2545A |
| Hybridization oven rotator for Agilent Microarray Hybridization Chambers | Agilent p/n G2530-60029 |
| Nuclease-free 1.5 mL microfuge tubes | Ambion p/n 12400 or equivalent |
| Magnetic stir bar (×2) | Corning p/n 401435 or equivalent |
| Magnetic stir plate (×2) | Corning p/n 6795-410 or equivalent |
| Microcentrifuge | Eppendorf p/n 5417R or equivalent |
| NanoDrop ND-1000 UV-VIS spectrophotometer | NanoDrop p/n ND-1000 or equivalent |
| Slide-staining dish, with slide rack (×3) | Thermo Shandon p/n 121 or equivalent |
| Circulating water baths or heat blocks set to 37°C, 65°C, 80°C, 40°C, 70°C, and 60°C | |
| Clean forceps | |
| Ice bucket | |
| Pipetman micropipettors, (P-10, P-20, P-200, P-1000) or equivalent | |
| Powder-free gloves | |
| Sterile, nuclease-free aerosol barrier pipette tips | |
| Vortex mixer | |
| Timer | |
| Nitrogen purge box for slide storage | |

## Required Reagents

| Description | Vendor and part number |
|---|---|
| Low Input Quick Amp WT Labeling Kit, One-Color | Agilent p/n 5190-2943 |
| RNA Spike-In Kit, One-Color | Agilent p/n 5188-5282 |
| Gene Expression Hybridization Kit | Agilent p/n 5188-5242 |
| Gene Expression Wash Buffer Kit | Agilent p/n 5188-5327 |
| DNase/RNase-free distilled water | Invitrogen p/n 10977-015 |
| Milli-Q water or equivalent | |
| RNeasy Mini Kits (50 columns or 250 columns) | Qiagen p/n 74104 or 74106 |
| 100% Ethanol | Amresco p/n E193 |

## Optional Equipment/Reagents

| Description | Vendor and part number |
|---|---|
| 2100 Bioanalyzer | Agilent p/n G2938A |
| RNA 6000 Nano Assay Kit (RNA Series II Kit) | Agilent p/n 5067-1511 |
| Slide box | Corning p/n 07201629 |
| Stabilization and Drying Solution | Agilent p/n 5185-5979 |
| Ozone-Barrier Slide Cover | Agilent p/n G2505-60550 |
| Acetonitrile | Sigma p/n 271004-1L |
| Absolutely RNA Nanoprep Kit | Stratagene p/n 400753 |
| Thermocycler | |
| PCR 96-well plate or 0.2 mL PCR tubes | |

# Required Hardware and Software

| Description |
|---|
| Pentium III 1.5 GHz or higher (Pentium IV 2.0 GHz or higher recommended) |
| Agilent Scan Control software, version 8.5 for the C Scanner |
| 2 GB RAM (4 GB recommended for 64-bit operating systems) |
| 40 GB available disk space (if saving images and results files locally) |
| Windows 2000 with SP2 or later (fully tested on SP4), Windows XP SP2 |
| Feature Extraction software 10.7.3 or later |
| Internet Explorer 6.0 or later |
| Adobe Acrobat Reader 4.0 or later |

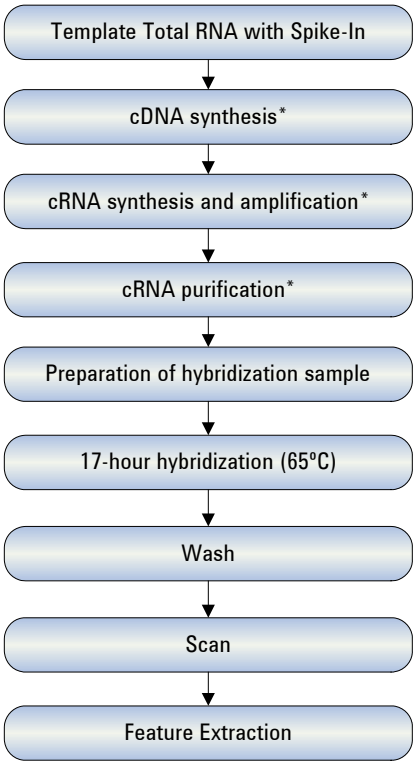
# Optional Software

| Description |
|---|
| GeneSpring GX 11.5 or later |

# 2 Procedures



Agilent's One-Color Microarray-based Exon Analysis uses cyanine 3-labeled cRNA to measure gene expression in experimental and control samples. Figure 1 is a standard workflow for sample preparation and array hybridization design.




* Samples can be stored frozen at -80°C after these steps, if needed.

**Figure 1** Workflow for sample preparation and array processing.

# Sample Preparation

Agilent's Low Input Quick Amp WT Labeling Kit generates fluorescent cRNA (complimentary RNA) with a sample input RNA range between 25 ng and 100 ng of total RNA per reaction. 50 ng is the recommended RNA input for the 2-pack, 4-pack, and 8-pack format, and 100 ng is required for the 1-pack format. The method uses T7 RNA polymerase, which simultaneously amplifies target material and incorporates cyanine 3-labeled CTP.

**NOTE**

For optimal performance, use pure high quality, intact template total RNA. RNA that is not pure, as measured by A260/A230 ratio, can lead to poor results and must be purified. Please refer to "Quality Assessment of Template RNA and Labeled cRNA" on page 54 for general guidance and procedural recommendations on quality assessment of template RNA.

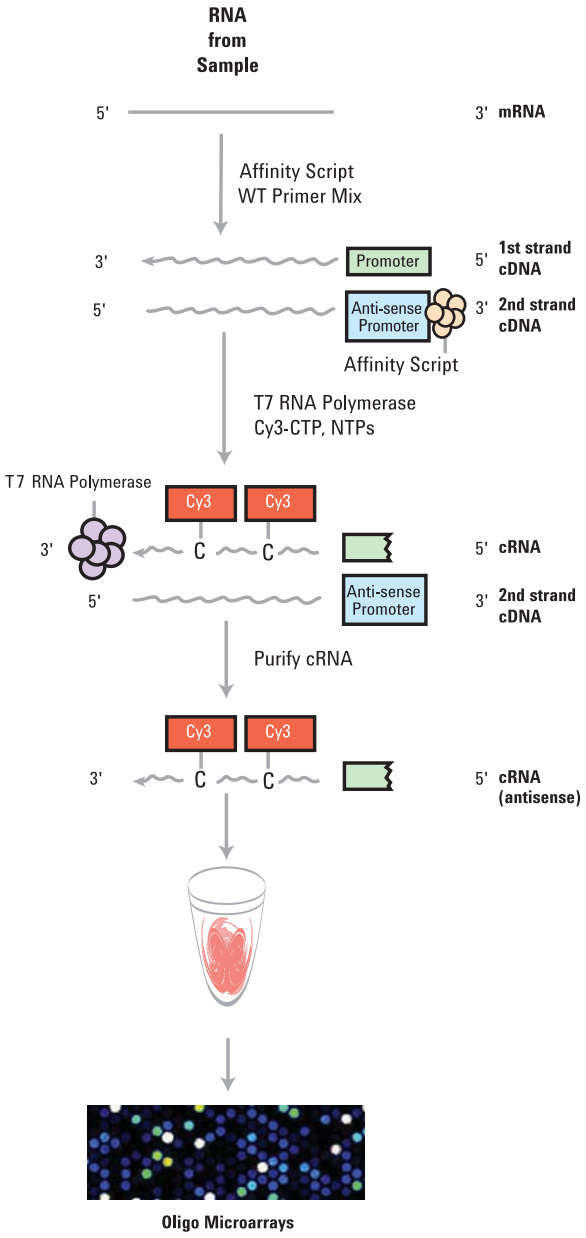



**Figure 2** Schematic of amplified cRNA procedure.

# Step 1. Prepare One-Color Spike Mix

(Time required: ~0.5 hours)

Refer to the protocol on Agilent One-Color RNA Spike-In Kit (publication 5188-5977) for in-depth instructions and troubleshooting advice on how to use one-color spike mixes. This protocol is available with the One-Color RNA Spike-In Kit and can also be downloaded from the Agilent Web site at www.agilent.com/chem/dnamanuals-protocols.

1 Equilibrate water baths to 37°C, 65°C, 80°C, 40°C and 70°C.

2 Vigorously mix the One-Color Spike Mix stock solution on a vortex mixer.

3 Heat at 37°C for 5 minutes, and mix on a vortex mixer once more.

4 Briefly spin in a centrifuge to drive contents to the bottom of the tube prior to opening. Settlement of the solution on the sides or lid of the tubes may occur during shipment and storage.

Table 1 provides the dilutions of Agilent One-Color Spike Mix for a range of total RNA input amounts. For inputs not shown Table 1, make sure that the amount of spike mix is proportional to the amount of RNA input.

**Table 1** Dilutions of Agilent One-Color Spike Mix for Cyanine 3-labeling

| Starting Amount of RNA | Serial Dilution | | | | Spike Mix Volume to be used in each labeling reaction (µL) |
|---|---|---|---|---|---|
| Total RNA (ng) | First | Second | Third | Fourth | |
| 25 | 1:20 | 1:25 | 1:20 | 1:4 | 2 |
| 50 | 1:20 | 1:25 | 1:20 | 1:2 | 2 |
| 100 | 1:20 | 1:25 | 1:20 | | 2 |

**NOTE** Use RNase-free microfuge tubes and tips. Make sure you dispense at least 2 µL with a pipette to ensure accuracy.

For example, to prepare the Agilent One-Color Spike Mix dilution appropriate for 25 ng of total RNA starting sample:

1 Create the First Dilution:

   a Label a new sterile 1.5-mL microcentrifuge tube “Spike Mix First Dilution.”

b Mix the thawed Spike Mix vigorously on a vortex mixer.

c Heat at 37°C in a circulating water bath for 5 minutes.

d Mix the Spike Mix tube vigorously again on a vortex mixer.

e Spin briefly in a centrifuge to separate contents to the bottom of the tube.

f Into the First Dilution tube, put 2 µL of Spike Mix stock.

g Add 38 µL of Dilution Buffer provided in the Spike-In kit (1:20).

h Mix thoroughly on a vortex mixer and spin down quickly to collect all of the liquid at the bottom of the tube. This tube contains the First Dilution.

2 Create the Second Dilution:

a Label a new sterile 1.5-mL microcentrifuge tube “Spike Mix Second Dilution.”

b Into the Second Dilution tube, put 2 µL of First Dilution.

c Add 48 µL of Dilution Buffer (1:25).

d Mix thoroughly on a vortex mixer and spin down quickly to collect all of the liquid at the bottom of the tube. This tube contains the Second Dilution.

3 Create the Third Dilution:

a Label a new sterile 1.5-mL microcentrifuge tube “Spike Mix Third Dilution.”

b Into the Third Dilution tube, put 2 µL of Second Dilution.

c Add 38 µL of Dilution Buffer (1:20).

d Mix thoroughly on a vortex mixer and spin down quickly to collect all the liquid at the bottom of the tube. This tube contains the Third Dilution.

4 Create the Fourth Dilution:

a Label a new sterile 1.5-mL microcentrifuge tube “Spike Mix Fourth Dilution.”

b Into the Fourth Dilution tube, add 10 µL of Third Dilution to 30 µL of Dilution Buffer for the Fourth Dilution (1:4).

c Mix thoroughly on a vortex mixer and spin down quickly to collect all of the liquid at the bottom of the tube. This tube contains the Fourth Dilution (now at a 40,000-fold final dilution).

5 Add 2 µL of Fourth Dilution to 25 ng of sample total RNA as listed in Table 1 and continue with cyanine 3 labeling using the Agilent Low Input

Quick Amp Kit protocol as described in "Step 2. Prepare labeling reaction" on page 22.

### Storage of Spike Mix dilutions

Store the Agilent RNA Spike-In Kit, One-Color at –70°C to –80°C in a non-defrosting freezer for up to 1 year from the date of receipt.

The first dilution of the Agilent One-Color Spike Mix positive controls can be stored up to 2 months in a non-defrosting freezer at -70°C to -80°C and freeze/thawed up to eight times.

After use, discard the second, third and fourth dilution tubes.

## Step 2. Prepare labeling reaction

(Time required: ~5.5 hours)

For each assay, make sure that the volume of the total RNA sample does not exceed 2.3 µL.

**NOTE** The starting input for the Low Input Quick Amp WT Labeling Kit ranges from 25 ng to 100 ng of total RNA. The recommended amount of total RNA input level for this kit is 50 ng for the 2-pack, 4-pack, and 8-pack microarrays. For the 1-pack microarrays, 100 ng is the recommended input amount.

1. Add 25 to 100 ng of total RNA to a 1.5-mL microcentrifuge tube in a final volume of 2.3 µL. If samples are concentrated, dilute with water until 25 to 100 ng of total RNA is added in a 2.3 µL volume. Dilute the total RNA just prior to use and store the total RNA at concentrations over 100 ng/µL at -80°C.
2. Prepare the WT Primer Master Mix and add to sample:
   a. Prepare the WT Primer Master Mix (with diluted RNA spike-in-controls) as listed in Table 2.

**NOTE** The Low-Input QuickAmp WT Labeling Kit contains two primers. Make sure you use the primer that is labeled "WT PRIMER".

**Table 2** WT Primer Master Mix

| Component | Volume (µL) per reaction | Volume (µL) per 5 reaction | Volume (µL) per 10 reactions |
|---|---|---|---|
| WT Primer | 1 | 5 | 10 |
| Diluted RNA spike-in controls | 2 | 10 | 20 |
| **Total Volume** | **3** | **15** | **30** |

   b. Add 3 µL of the WT Primer Master Mix to the tube that contains 2.3 µL of total RNA. Each tube now contains a total volume of 5.3 µL.
   c. Denature the primer and the template by incubating the reaction at 65°C in a circulating water bath for 10 minutes.
   d. Place the reactions on ice and incubate for 5 minutes.

3 Prewarm the 5X first strand buffer at 80°C for 3 to 4 minutes to ensure adequate resuspensions of the buffer components. For optimal resuspension, briefly mix on a vortex mixer and spin the tube in a microcentrifuge to drive down the contents from the tube walls. Keep at room temperature until needed.

4 Prepare and add cDNA Master Mix:

a Immediately prior to use, add the components for cDNA Master Mix listed in Table 3 to a 1.5-mL microcentrifuge tube, use a pipette to gently mix, and keep at room temperature.

The AffinityScript RNase Block mix is a blend of enzymes. Keep the AffinityScript RNase Block mix on ice and add to the cDNA Master Mix immediately prior to use.

**Table 3** cDNA Master Mix

| Component | Volume (µL) per reaction | Volume (µL) per 5 reaction | Volume (µL) per 10 reactions |
|---|---|---|---|
| 5X First Strand Buffer (green cap) | 2 | 10 | 20 |
| 0.1 M DTT (white cap) | 1 | 5 | 10 |
| 10 mM dNTP mix (green cap) | 0.5 | 2.5 | 5 |
| AffinityScript RNase Block Mix (violet cap) | 1.2 | 6 | 12 |
| **Total Volume** | **4.7** | **23.5** | **47** |

b Briefly spin each sample tube in a microcentrifuge to drive down the contents from the tube walls and the lid.

c Add 4.7 µL of cDNA Master Mix to each sample tube and mix by pipetting up and down. Each tube now contains a total volume of 10 µL.

d Incubate samples at 40°C in a circulating water bath for 2 hours.

e Move samples to a 70°C circulating water bath and incubate for 15 minutes.

**NOTE** Incubation at 70°C inactivates the AffinityScript enzyme.

f Move samples to ice. Incubate for 5 minutes.

g Spin samples briefly in a microcentrifuge to drive down tube contents from the tube walls and lid.

**Stopping Point** If you do not immediately continue to the next step, store the samples at -80°C.

5 Prepare and add Transcription Master Mix:

a Immediately prior to use, add the components listed in Table 4 in the order indicated into a 1.5 mL microcentrifuge tube for the Transcription Master Mix. Mix gently by pipetting at room temperature.

The T7 RNA polymerase blend is a blend of enzymes. Keep the T7 RNA polymerase on ice and add to the Transcription Master Mix just before use.

**Table 4** Transcription Master Mix

| Component | Volume (µL) per reaction | Volume (µL) per 5 reaction | Volume (µL) per 10 reactions |
|---|---|---|---|
| Nuclease-free water (white cap) | 0.75 | 3.75 | 7.5 |
| 5X Transcription Buffer (blue cap) | 3.2 | 16 | 32 |
| 0.1 M DTT (white cap) | 0.6 | 3 | 6 |
| NTP mix (blue cap) | 1 | 5 | 10 |
| T7 RNA Polymerase Blend (red cap) | 0.21 | 1.05 | 2.1 |
| Cyanine 3-CTP | 0.24 | 1.2 | 2.4 |
| **Total Volume** | **6** | **30** | **60** |

b Add 6 µL of Transcription Master Mix to each sample tube. Gently mix by pipetting. Each tube now contains a total volume of 16 µL.

c Incubate samples in a circulating water bath at 40°C for 2 hours.

**Stopping Point** If you do not immediately continue to the next step, store the samples at -80°C.

## Step 3. Purify the labeled/amplified RNA

(Time required: ~0.5 hours)

Qiagen's RNeasy mini spin columns are recommended for purification of the amplified cRNA samples.

If sample concentration causes difficulty, you can use the Stratagene Absolutely RNA Nanoprep kit as an alternative. See "Absolute RNA Nanoprep Purification" on page 48.

**NOTE** Make sure that ethanol was added to the RPE buffer as specified in the Qiagen manual before you continue.

1. Add 84 µL of nuclease-free water to your cRNA sample, for a total volume of 100 µL.
2. Add 350 µL of Buffer RLT and mix well by pipetting.
3. Add 250 µL of ethanol (96% to 100% purity) and mix thoroughly by pipetting. Do *not* centrifuge.
4. Transfer the 700 µL of the cRNA sample to an RNeasy mini column in a 2 mL collection tube. Centrifuge the sample at 4°C for 30 seconds at 13,000 rpm. Discard the flow-through and collection tube.
5. Transfer the RNeasy column to a new collection tube and add 500 µL of buffer RPE (containing ethanol) to the column. Centrifuge the sample at 4°C for 30 seconds at 13,000 rpm. Discard the flow-through. Re-use the collection tube.
6. Add another 500 µL of buffer RPE to the column. Centrifuge the sample at 4°C for 60 seconds at 13,000 rpm. Discard the flow-through and the collection tube.
7. If any buffer RPE remains on or near the frit of the column or on the outside of the column, transfer the RNeasy column to a new 1.5-mL collection tube and centrifuge the sample at 4°C for 30 seconds at 13,000 rpm to remove any remaining traces of buffer RPE. Discard this collection tube and use a fresh tube to elute the cleaned cRNA sample.

**CAUTION** *Do not discard the final flow-through in the next step*. It contains the cRNA sample.

8 Elute the purified cRNA sample by transferring the RNeasy column to a new 1.5 mL collection tube. Add 30 µL RNase-free water directly onto the RNeasy filter membrane. Wait 60 seconds, then centrifuge at 4°C for 30 seconds at 13,000 rpm.

9 Maintain the cRNA sample-containing flow-through on ice. Discard the RNeasy column.

## Step 4. Quantify the cRNA

Quantitate cRNA using NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.2.1.

1 Start the NanoDrop software.

2 Click the **Microarray Measurement** tab.

3 Before initializing the instrument as requested by the software, clean the sample loading area with nuclease-free water.

4 Load 1.0 to 2.0 µL of nuclease-free water to initialize. Then click **OK**.

5 Once the instrument has initialized, select **RNA-40** as the **Sample type** (use the drop down menu).

6 Make sure the **Recording** button is selected. If not, click **Recording** so that the readings can be recorded, saved, and printed.

**CAUTION** Failure to engage recording causes measurements to be overwritten, with no possibility of retrieval.

7 Blank the instrument by pipetting 1.0 to 2.0 µL of nuclease-free water (this can be the same water used to initialize the instrument) and click **Blank**.

8 Clean the sample loading area with a laboratory wipe. Pipette 1.0 to 2.0 µL of the sample onto the instrument sample loading area. Type the sample name in the space provided and click **Measure**.

   Be sure to clean the sample loading area between measurements and ensure that the baseline is always flat at 0, which is indicated by a thick black horizontal line. If the baseline deviates from 0 and is no longer a flat horizontal line, reblank the instrument with nuclease-free water, then remeasure the sample.

9 Print the results. If printing the results is not possible, record the following values:

   - Cyanine 3 dye concentration (pmol/µL)
   - RNA absorbance ratio (260 nm/280 nm)
   - cRNA concentration (ng/µL)

10 Determine the yield and specific activity of each reaction as follows:

   a Use the concentration of cRNA (ng/µL) to determine the µg cRNA yield as follows:

$$\frac{(\text{Concentration of cRNA}) \times 30\ \mu\text{L (elution volume)}}{1000} = \mu\text{g of cRNA}$$

**b** Use the concentrations of cRNA (ng/µL) and cyanine 3 (pmol/µL) to determine the specific activity as follows:

$$\frac{\text{Concentration of Cy3}}{\text{Concentration of cRNA}} \times 1000 = \text{pmol Cy3 per µg cRNA}$$

**11** Examine the yield and specific activity results. See Table 5 for the recommended cRNA yields and specific activities for hybridization.

**Table 5** Recommended Yields and Specific Activity

| Microarray format | Yield (µg) | Specific Activity (pmol Cy3 per µg cRNA) |
|---|---|---|
| 1-pack | 5 | 15 |
| 2-pack | 3.75 | 15 |
| 4-pack | 1.65 | 15 |
| 8-pack | 0.825 | 15 |

**NOTE**

Please refer to "Quality Assessment of Template RNA and Labeled cRNA" on page 54 for general guidance and procedural recommendations on quality assessment of labeled cRNA.

# Hybridization

## Step 1. Prepare the 10X Blocking Agent

1 Add 500 µL of nuclease-free water to the vial containing lyophilized 10X Blocking Agent supplied with the Agilent Gene Expression Hybridization Kit, *or* add 1250 µL of nuclease-free water to the vial containing lyophilized large volume 10X Blocking Agent (Agilent p/n 5188-5281).

2 Mix by gently vortexing. If the pellet does not go into solution completely, heat the mix for 4 to 5 minutes at 37°C.

3 Drive down any material adhering to the tube walls or cap by centrifuging for 5 to 10 seconds.

**NOTE** 10X Blocking Agent can be prepared in advance and stored at -20°C for up to 2 months. After thawing, repeat the vortexing and centrifugation procedures before use.

# Step 2. Prepare hybridization samples

1 Equilibrate water bath to 60°C.

2 For each microarray, add each of the components as indicated in the tables below to a 1.5-mL nuclease-free microfuge tube:

- Table 6 for 1-pack or 2-pack microarray formats
- Table 7 for 4-pack or 8-pack microarray formats

3 Mix well but gently on a vortex mixer.

**NOTE**

For 1-pack and 2-pack microarrays, if you did not generate enough labeled cRNA, add the amount of labeled cRNA to the fragmentation mix such that the same amount is used for each microarray within the same experiment (at least 1.65 µg).

**Table 6** Fragmentation mix for 1-pack or 2-pack microarray formats

| Components | Volume/Mass 1-pack microarrays | Volume/Mass 2-pack microarrays |
|---|---|---|
| Cyanine 3-labeled, linearly amplified cRNA | 5.00 µg | 3.75 µg |
| 10X Blocking Agent | 50 µL | 25 µL |
| Nuclease-free water | bring volume to 240 µL | bring volume to 120 µL |
| 25X Fragmentation Buffer | 10 µL | 5 µL |
| Total Volume | 250 µL | 125 µL |

**Table 7** Fragmentation mix for 4-pack or 8-pack microarray formats

| Components | Volume/Mass 4-pack microarrays | Volume/Mass 8-pack microarrays |
|---|---|---|
| Cyanine 3-labeled, linearly amplified cRNA | 1.65 µg | 600 ng |
| 10X Blocking Agent | 11 µL | 5 µL |
| Nuclease-free water | bring volume to 52.8 µL | bring volume to 24 µL |
| 25X Fragmentation Buffer | 2.2 µL | 1 µL |
| Total Volume | 55 µL | 25 µL |

**CAUTION** Do not incubate sample in the next step for more than 30 minutes. Cooling on ice and adding the 2x Hybridization Buffer will stop the fragmentation reaction.

4 Incubate at 60°C for exactly 30 minutes to fragment RNA.

5 Immediately cool on ice for one minute.

6 Add 2x GEx Hybridization Buffer HI-RPM to the 1-pack, 2-pack, 4-pack, and 8-pack microarray formats at the appropriate volume to stop the fragmentation reaction. See Table 8.

**Table 8** Hybridization mix

| | Volumes per hybridization | | | |
|---|---|---|---|---|
| **Components** | **1-pack** | **2-pack** | **4-pack** | **8-pack** |
| cRNA from Fragmentation Mix | 250 µL | 125 µL | 55 µL | 25 µL |
| 2x GEx Hybridization Buffer HI-RPM | 250 µL | 125 µL | 55 µL | 25 µL |

7 Mix well by careful pipetting. Take care to avoid introducing bubbles. Do not mix on a vortex mixer; mixing on a vortex mixer introduces bubbles.

8 Spin for 1 minute at room temperature at 13,000 rpm in a microcentrifuge to drive the sample off the walls and lid and to aid in bubble reduction.

   Use immediately. Do not store.

9 Place sample on ice and load onto the array as soon as possible.

Refer to "Microarray Handling Tips" on page 71 for information on how to safely handle microarrays.

## Step 3. Prepare the hybridization assembly

Refer to the *Agilent Microarray Hybridization Chamber User Guide* (G2534-90001) for in-depth instructions on how to load slides, assembly and disassembly of chambers, as well as other helpful tips. This user guide is available with the Agilent Microarray Hybridization Chamber Kit (G2534A) and can also be downloaded from the Agilent Web site at www.agilent.com/chem/dnamanuals-protocols.

1 Load a clean gasket slide into the Agilent SureHyb chamber base with the label facing up and aligned with the rectangular section of the chamber base. Ensure that the gasket slide is flush with the chamber base and is not ajar.

**NOTE**

Place unused gasket wells in the multi-pack array format at the far end opposite the barcode. In the unused wells, maintain the appropriate volume of 1x hybridization buffer for the corresponding format design. See "Array/Sample tracking microarray slides" on page 75 for 2-pack, 4-pack, and 8-pack array templates to track samples.

**CAUTION**

Do not let the pipette tip or the hybridization solution touch the gasket walls. When liquid touches the gasket wall, the likelihood of gasket leakage greatly increases.

2 Slowly dispense the volume of hybridization sample (see Table 9) onto the gasket well in a "drag and dispense" manner.

**Table 9** Hybridization Sample

| | Volumes per hybridization | | | |
|---|---|---|---|---|
| **Components** | **1-pack** | **2-pack** | **4-pack** | **8-pack** |
| Volume Prepared | 500 µL | 250 µL | 110 µL | 50 µL |
| Volume to Hybridize | 490 µL | 240 µL | 100 µL | 40 µL |

**CAUTION**

When you lower the microarray slide on top of the SureHyb gasket slide, make sure that the two slides are parallel at all times.

3 Slowly place an array "active side" down onto the SureHyb gasket slide, so that the "Agilent"-labeled barcode is facing down and the numeric barcode is facing up. Make sure the sandwich-pair is properly aligned.

**CAUTION** Do not drop the array slide onto the gasket. Doing so increases the chances of samples mixing between gasket wells.

4 Place the SureHyb chamber cover onto the sandwiched slides and slide the clamp assembly onto both pieces.

5 Hand-tighten the clamp onto the chamber.

6 Vertically rotate the assembled chamber to wet the gasket and assess the mobility of the bubbles. If necessary, tap the assembly on a hard surface to move stationary bubbles.

7 Place assembled slide chamber in rotisserie in a hybridization oven set to 65°C. Set your hybridization rotator to rotate at 10 rpm when using 2x GEx Hybridization Buffer HI-RPM.

8 Hybridize at 65°C for 17 hours.

**CAUTION** If you are not loading all the available positions on the hybridization rotator rack, be sure to balance the loaded hybridization chambers on the rack so that there are an equal number of empty positions on each of the four rows on the hybridization rack.

**NOTE** The **Gene Expression Wash Buffer 2** needs to be warmed overnight. Make sure that you prepare the wash buffer the night before you plan to do the microarray wash. See "Step 2. Prewarm Gene Expression Wash Buffer 2".

# Microarray Wash

## Step 1. Add Triton X-102 to Gene Expression wash buffers

This step is optional but highly recommended.

The addition of 0.005% Triton X-102 to the Gene Expression wash buffers reduces the possibility of array wash artifacts. Add the Triton X-102 to Gene Expression wash buffer 1 and 2 when the cubitainer of wash buffer is first opened.

Do this step to *both* Gene Expression wash buffer 1 and 2 before use.

1. Open the cardboard box with the cubitainer of wash buffer and carefully remove the outer and inner caps from the cubitainer.
2. Use a pipette to add 2 mL of the provided 10% Triton X-102 into the wash buffer in the cubitainer.
3. Replace the original inner and outer caps and mix the buffer carefully but thoroughly by inverting the container 5 to 6 times.
4. Carefully remove the outer and inner caps and install the spigot provided with the wash buffer.
5. Prominently label the wash buffer box to indicate that Triton X-102 has been added and indicate the date of addition.

Triton X-102 can be added to smaller volumes of wash buffer as long as the final dilution of the 10% Triton X-102 is 0.005% in the Gene Expression wash buffer solution.

## Step 2. Prewarm Gene Expression Wash Buffer 2

Warm the **Gene Expression Wash Buffer 2** to 37°C as follows:

1. Dispense 1000 mL of Gene Expression Wash Buffer 2 directly into a sterile 1000-mL bottle. Repeat until you have enough prewarmed Wash Buffer 2 solution for your experiment.
2. Tightly cap the 1000-mL bottle and place in a 37°C water bath the night before washing arrays. Alternatively, remove the plastic cubitainer from the box and place it in a 37°C water bath the night before washing the arrays.

## Step 3. Prepare the equipment

Always use clean equipment when doing the hybridization and wash steps. Designate and dedicate dishes to one-color experiments. The acetonitrile wash is only necessary if the staining dishes, racks and stir bars were used in previous experiments with the Agilent Stabilization and Drying Solution. Otherwise proceed to "Milli-Q water wash" on page 37.

### Acetonitrile wash

Wash staining dishes, racks and stir bars that were used in previous experiments with the Agilent Stabilization and Drying Solution with acetonitrile to remove any remaining residue.

**WARNING** **Conduct acetonitrile washes in a vented fume hood.**

1. Add the slide rack and stir bar to the staining dish.
2. Transfer the staining dish with the slide rack and stir bar to a magnetic stir plate.
3. Fill the staining dish with 100% acetonitrile.
4. Turn on the magnetic stir plate and adjust the speed to a setting of 4 (medium speed).
5. Wash for 5 minutes.
6. Discard the acetonitrile as is appropriate for your site.

7 Repeat step 1 through step 6.

8 Air dry the staining dish in the vented fume hood.

9 Proceed to “Milli-Q water wash” below.

### Milli-Q water wash

Wash all dishes, racks, and stir bars with Milli-Q water.

1 Run copious amounts of Milli-Q water through the staining dish.

2 Empty out the water collected in the dish.

3 Repeat step 1 and step 2 at least 5 times, as it is necessary to remove any traces of contaminating material.

4 Discard the Milli-Q water.

**CAUTION** Some detergents may leave fluorescent residue on the dishes. Do not use any detergent in the washing of the staining dishes. If detergent is used, all traces must be removed by copiously rinsing with Milli-Q water.

# Step 4. Wash the microarray slides

**NOTE** The microarray wash procedure for Agilent's one-color platform must be done in environments where ozone levels are 50 ppb or less. If ozone levels exceed 50 ppb in your laboratory, use the Agilent Ozone Barrier Slide Cover (described in this topic), or see"Preventing Ozone-Related Problems" on page 60.

**NOTE** When setting up the apparatus for the washes, be sure to do so near the water bath containing the pre-warmed Wash 2 solutions.

Table 10 lists the wash conditions for the wash procedure.

**Table 10** Wash conditions

| | Dish | Wash Buffer | Temperature | Time |
|---|---|---|---|---|
| Disassembly | 1 | GE Wash Buffer 1 | Room temperature | |
| 1st wash | 2 | GE Wash Buffer 1 | Room temperature | 1 minute |
| 2nd wash | 3 | GE Wash Buffer 2 | Elevated temperature | 1 minute |

1. Completely fill slide-staining dish #1 with Gene Expression Wash Buffer 1 at room temperature.
2. Place a slide rack into slide-staining dish #2. Add a magnetic stir bar. Fill slide-staining dish #2 with enough Gene Expression Wash Buffer 1 at room temperature to cover the slide rack. Place this dish on a magnetic stir plate.
3. Place the empty dish #3 on the stir plate and add a magnetic stir bar. Do not add the prewarmed (37°C) Gene Expression Wash Buffer 2 until the first wash step has begun.
4. Remove one hybridization chamber from incubator and record time. Record whether bubbles formed during hybridization and if all bubbles are rotating freely.
5. Prepare the hybridization chamber disassembly.
   a. Place the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning counterclockwise.
   b. Slide off the clamp assembly and remove the chamber cover.

c With gloved fingers, remove the array-gasket sandwich from the chamber base by grabbing the slides from their ends. Keep the microarray slide numeric barcode facing up as you quickly transfer the sandwich to slide-staining dish #1.

d Without letting go of the slides, submerge the array-gasket sandwich into slide-staining dish #1 containing Gene Expression Wash Buffer 1.

6 With the sandwich completely submerged in Gene Expression Wash Buffer 1, pry the sandwich open from the barcode end only:

a Slip one of the blunt ends of the forceps between the slides.

b Gently turn the forceps upwards or downwards to separate the slides.

c Let the gasket slide drop to the bottom of the staining dish.

d Remove the microarray slide and place into slide rack in the slide-staining dish #2 containing Gene Expression Wash Buffer 1 at room temperature. Minimize exposure of the slide to air. *Touch only the barcode portion of the microarray slide or its edges!*

7 Repeat step 4 through step 6 for up to seven additional slides in the group. For uniform washing, do up to a maximum of eight disassembly procedures yielding eight microarray slides.

8 When all slides in the group are placed into the slide rack in slide-staining dish #2, stir using setting 4 for 1 minute.

9 During this wash step, remove Gene Expression Wash Buffer 2 from the 37°C water bath and pour into the slide-staining dish #3.

**NOTE** The elevated temperature of the second wash step is usually around 31°C due to cooling by the room temperature dish and the rack of arrays.

10 Transfer slide rack to slide-staining dish #3 containing Gene Expression Wash Buffer 2 at elevated temperature. Stir using setting 4 for 1 minute.

11 Slowly remove the slide rack minimizing droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.

12 Discard used Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 2.

13 Repeat step 1 through step 12 for the next group of eight slides using fresh Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 2 pre-warmed to 37°C.

14 Put the slides in a slide holder so that the Agilent barcode faces up:

- In environments in which the ozone level exceeds 50 ppb, immediately put the slides with Agilent barcode facing up in a slide holder with an ozone-barrier slide cover on top of the array as shown in Figure 3. Refer to the *Agilent Ozone-Barrier Slide Cover User Guide* (p/n G2505-90550), included with the slide cover, for more information.

  As an alternative, use the Stabilization and Drying Solution. See "Preventing Ozone-Related Problems" on page 60.

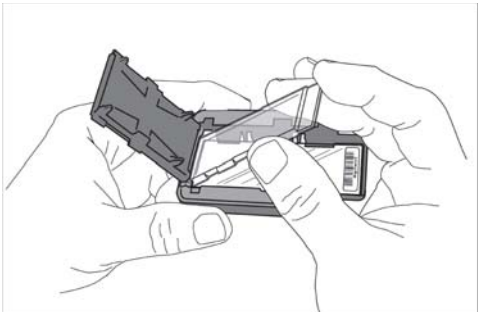


**Figure 3** Inserting the ozone-barrier slide cover

- In environments in which the ozone level is below 50 ppb, put the slides with Agilent barcode facing up in a slide holder.

**NOTE** Use fresh Gene Expression Wash Buffer 1 and 2 for each wash group (up to 8 slides).

**15** Scan slides immediately to minimize the impact of environmental oxidants on signal intensities. If necessary, store slides in orange slide boxes in a nitrogen purge box, in the dark.

# Scanning and Feature Extraction

## Step 1. Scan the slides

1 Put assembled slide holders with or without the ozone-barrier slide cover into the scanner carousel.

2 In the Scan Control main window, choose the slot number of the first slide for **Start Slot** and the slot number for the last slide for **End Slot**.

3 For 1x1M, 2x400K, 4x180K and 8x60K microarrays, select **Profile AgilentG3_GX_1Color**.

If you are unable to find the profile AgilentG3_GX_1Color from the Scan Control program, download the profile from https://www.genomics.agilent.com/GenericA.aspx?PageType=Custom&SubPageType=Custom&PageID=2074. To import the profile into the Scan Control program, click **Tools > Profile Editor** and select **Import**.

4 Verify scan settings for one-color scans. See Table 11.

**Table 11** C Scanner Scan Settings

| | For 1x1M, 2x400K, 4x180K and 8x60K G3 Microarray Formats |
|---|---|
| **Dye channel** | Green |
| **Scan region** | Scan Area (61 x 21.6 mm) |
| **Scan resolution (µm)** | 3 |
| **Tiff** | 20 bit |

5 Verify that the Scanner status in the main window says **Scanner Ready**.

6 In the Scan Control main window, click **Scan Slot *m-n*** where $m$ is the slot of the first slide, and $n$ is the slot for the last slide.

# Step 2. Extract data using Agilent Feature Extraction Software

Feature Extraction is the process by which information from probe features is extracted from microarray scan data, allowing researchers to measure gene expression in their experiments. Extraction of Gene Expression Exon microarrays is supported by Feature Extraction version 10.7.3 or later. To get the most recent Feature Extraction software for gene expression, go to the Agilent Web site at www.agilent.com/chem/fe.

After generating the microarray scan images, extract .tif images using the Feature Extraction software.

1 Open the Agilent Feature Extraction (FE) software.

   To get the most recent Feature Extraction protocols for gene expression, go to the Agilent Web site at www.agilent.com/chem/feprotocols.

2 Add the images (.tif) to be extracted to the FE Project.

   a Click **Add New Extraction Set(s)** icon on the toolbar or right-click the **Project Explorer** and select **Add Extraction...**

   b Browse to the location of the .tif files, select the .tif file(s) and click **Open**. To select multiple files, use the Shift or Ctrl key when selecting.

   The FE program automatically assigns a default grid template and protocol for each extraction set, if the following conditions are met:

   - For auto assignment of the grid template, the image must be generated from an Agilent scanner and have an Agilent barcode.
   - For auto assignment of the One-Color Gene Expression FE protocol, the **default Gene Expression protocol** must be specified in the FE Grid Template properties.

     To access the FE Grid Template properties, double-click on the grid template in the Grid Template Browser.

3 Set FE Project Properties.

   a Select the **Project Properties** tab.

   b In the **General** section, enter your name in the **Operator** text box.

   c In the **Input** section, verify that at least the following default settings as shown in Figure 4 below are selected.

   For outputs that can be imported into Rosetta Resolver, select MAGE and JPEG.

| | |
|---|---|
| ⊟ General | |
| Operator | Unknown |
| ⊟ Input | |
| Number of Extraction Sets Included | 0 |
| ⊟ Output and Data Transfer | |
| ⊟ Outputs | |
| ⊞ MAGE | None |
| JPEG | None |
| ⊟ TEXT | Local file only |
| Output Package | Compact |
| Visual Results | Local file only |
| Grid | None |
| QC Report | Local PDF file only |
| FTP Send Tiff File | False |
| ⊟ Local File Folder | |
| Same As Image | True |
| Results Folder | |
| ⊞ FTP Setting | |
| ⊟ Automatic Protocol Assignment | |
| Highest Priority Default Protocol | Grid Template Default |
| Project Default Protocol | |
| ⊟ Automatic Grid Template Assignment | |
| Use Grid file if available | False |
| External DyeNorm List File | |
| Overwrite Previous Results | True |

**Figure 4** Default settings in FE 10.7.3.

**4** Check the Extraction Set Configuration.

**a** Select the **Extraction Set Configuration** tab.

**b** Verify that the correct grid template is assigned to each extraction set in the **Grid Name** column. To assign a different grid template to an extraction set, select one from the pull down menu.

If a grid template is not available to select from the pull down menu, you must add it to the Grid Template Browser. To add, right-click inside the Grid Template Browser, select **Add**. Browse for the design file (.xml) and click **Open** to load grid template into the FE database.

To update to the latest grid templates via Online Update, right-click **Grid Template Browser** and select **Online Update**. You can also download the latest grid templates from Agilent Web site at www.agilent.com/chem/downloaddesignfiles. After downloading, you must add the grid templates to the Grid Template Browser.

After a new grid template is added to the Grid Template Browser, remember to specify the default protocol for the new grid template if you want the Feature Extraction program to automatically assign a FE protocol to an extraction set.

**c** Verify that the correct protocol is assigned to each extraction set in the **Protocol Name** column. To assign a different protocol to an extraction

set, select one from the pull down menu. For Agilent one-color microarrays, select **GE1-107_Sep09**.

The protocols automatically distinguish the formats for processing the data.

If a protocol is not available to select from the pull down menu, you must import it to the FE Protocol Browser. To import, right-click **FE Protocol Browser**, select **Import**. Browse for the FE protocol (.xml) and click **Open** to load the protocol into the FE database. Visit the Agilent Web site at www.agilent.com/chem/feprotocols to download the latest protocols.

**NOTE** These FE Protocols were optimized using data from Agilent catalog arrays, which have many replicated probes and validated Negative Control probes. If custom arrays without enough replicated probes are used, or arrays with custom probes designated as Negative Control probes are used, the default FE Protocols may not be optimal.

5 Save the FE Project (.fep) by selecting **File > Save As** and browse for desired location.

6 Verify that the icons for the image files in the FE Project Window no longer have a red X through them. A red X through the icon indicates that an extraction protocol was not selected. If needed, reselect the extraction protocol for that image file.

7 Select **Project > Start Extracting**.

8 After the extraction is completed successfully, view the QC report for each extraction set by double-clicking the QC Report link in the **Summary Report** tab. Determine whether the grid has been properly placed by inspecting Spot Finding at the Four Corners of the Array. See Figure 6.

If a QC Metric Set has been assigned to the FE Project, you can view the results of the metric evaluation in three ways:

- Project Run Summary - includes a summary sentence.
- QC Report - includes both a summary on the header and a table of metric values.
- QC Chart - includes a view of the values of each metric compared across all extractions in FE Project.

Refer to the application note on *Use of Agilent Feature Extraction Software (v8.1) QC Report to Evaluate Microarray Performance* (publication 5989-3056EN) for more details on quality assessment and troubleshooting

with the Feature Extraction QC Report. This technical note can be downloaded from the Agilent Web site at www.agilent.com/chem/dnaapplications.

### Automatic Download from eArray

Feature Extraction version 10.7.3 or higher can automatically download Grid Templates, protocols and QC metrics (QCM or QCMT). To set this up, in the eArray Login Setting dialog box, under **Advanced Options**, click **Use eArray server during extraction**. See Figure 5.

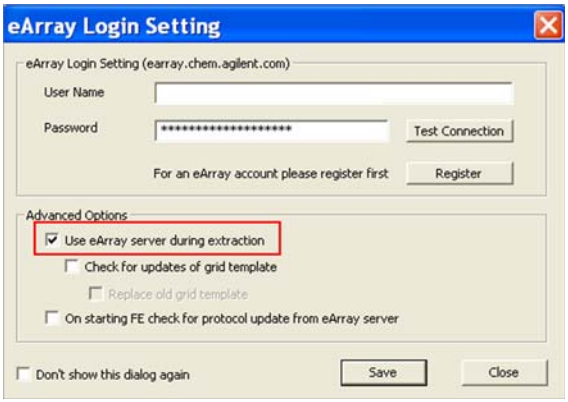
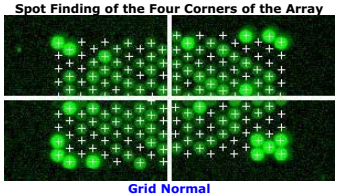



**Figure 5** eArray Login Setting

**QC Report - Agilent Technologies : 1 Color Gene Expression**

| | | | |
|---|---|---|---|
| Date | Friday, October 22, 2010 - 12:10 | Grid | 028680_D_20100527 |
| Image | US83203534_252868010157_S01 [1_1] | BG Method | No Background |
| Protocol | GE1_107_Sep09 (Read Only) | Background Detrend | On(FeatNCRange, LoPass) |
| User Name | jevenner | Multiplicative Detrend | True |
| FE Version | 10.7.3.1 | Additive Error | 2(Green) |
| Sample(red/green) | | Saturation Value | 779810 (g) |

**Spot Finding of the Four Corners of the Array**

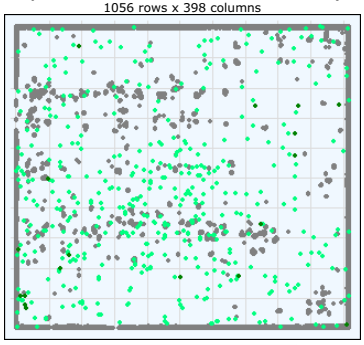


Grid Normal

| Feature | Local Background |
|---|---|
| Green | Green |
| Non Uniform 18 | 0 |
| Population 438 | 10430 |

**Spatial Distribution of All Outliers on the Array**

1056 rows x 398 columns



# FeatureNonUnif (Green) = 18(0.00%)

# GeneNonUnif (Green) = 5 (0.002 %)

BG NonUniform BG Population

Green FeaturePopulation Green Feature NonUniform

**Negative Control Stats**

| | Green |
|---|---|
| Average Net Signals | 11.61 |

**Net Signal Statistics**

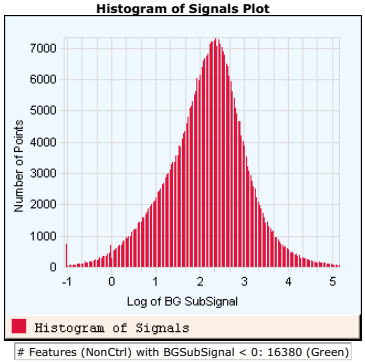
**Agilent SpikeIns:**

| | Green |
|---|---|
| # Saturated Features | 0 |
| 99% of Sig. Distrib. | 314584 |
| 50% of Sig. Distrib. | 2283 |
| 1% of Sig. Distrib. | 16 |

**Non-Control probes:**

| | Green |
|---|---|
| # Saturated Features | 0 |
| 99% of Sig. Distrib. | 15284 |
| 50% of Sig. Distrib. | 138 |
| 1% of Sig. Distrib. | 12 |

**Histogram of Signals Plot**




# Features (NonCtrl) with BGSubSignal < 0: 16380 (Green)



**Figure 6** Example of the first page of a QC Report for 2x400K microarray, generated by Feature Extraction Software

# 3 Supplemental Procedures



The procedures in this chapter are optional but recommended.

# Absolute RNA Nanoprep Purification

As an alternative to the Qiagen RNeasy purification columns, the Absolutely RNA Nanoprep kit can be used to purify the amplified cRNA after "Step 2. Prepare labeling reaction" on page 22. Use the Absolutely RNA Nanoprep Kit when it is needed or to avoid the need to concentrate purified samples. The Absolutely RNA Nanoprep Kit uses an elution volume of 20 µL.

## Step 1. Prepare the reagents

1 Prepare 80% sulfolane:

   a Incubate the 100% sulfolane in a 37°C water bath until liquefied.

   100% sulfolane is a solid at room temperature. 80% sulfolane solution is a liquid at room temperature and can be stored at room temperature for at least a month.

   b Add 1 mL of RNase-free water to 4 mL of 100% sulfolane to make 5 mL of 80% sulfolane.

   5 mL of 80% sulfolane is enough to process 50 RNA preparations (from up to 0.1 mL lysate each).

2 Prepare 1x high-salt wash buffer:

   a Add 16 mL of 100% ethanol to the bottle of 1.67X High-Salt Wash Buffer.

   b On the High-Salt Wash Buffer container, mark the check box for **1x (Ethanol Added)**.

   c Tighten the cap on the container of High-Salt Wash Buffer and store at room temperature.

3 Prepare the 1x low-salt wash buffer:

   a Add 68 mL of 100% ethanol to the bottle of 5X Low-Salt Wash Buffer.

   b On the Low-Salt Wash Buffer container, mark the check box for **1x (Ethanol Added)**.

   c Tighten the cap on the container of High-Salt Wash Buffer and store at room temperature.

## Step 2. Purify the labeled/amplified RNA

1. Add 100 µL of the Lysis Buffer to each reaction tube for a total volume of 116 µL.
2. Mix on a vortex mixer, or pipette repeatedly until homogenized.
3. Add an equal volume (116 µL) of 80% sulfolane (room temperature) to the cell lysate. Mix thoroughly on a vortex mixer for 5 seconds.

   You must use equal volumes of 80% sulfolane and cell lysate. Mix on a vortex mixer until the lysate and sulfolane are thoroughly mixed.
4. Put an RNA-binding nano-spin cup into a 2-mL collection tube.
5. Transfer the 80% sulfolane and cell lysate mixture to the RNA-binding nano-spin cup and snap the cap onto the top of the spin cup.
6. Spin the sample in a microcentrifuge at ≥12,000 x g for 60 seconds.
7. Remove and keep the spin cup. Discard the filtrate. Put the spin cup back into the same 2-mL collection tube.

   Up to this point, the RNA has been protected from RNases by the presence of guanidine thiocyanate.
8. Add 300 µL of 1x High-Salt Wash Buffer to the spin cup. Cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 x g for 60 seconds.

**CAUTION** The High-Salt Wash Buffer contains the irritant guanidine thiocyanate.

9. Remove and keep the spin cup. Discard the filtrate. Put the spin cup back into the same 2-mL collection tube.
10. Add 300 µL of 1x Low-Salt Wash Buffer to the spin cup. Cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 x g for 60 seconds.
11. Repeat step 9 and step 10 for a second low-salt wash.
12. Remove and keep the spin cup. Discard the filtrate. Put the spin cup back into the same 2-mL collection tube.
13. Add 300 µL of 1x Low-Salt Wash Buffer to the spin cup. Cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 x g for 3 minutes to dry the fiber matrix.

14 Transfer the spin cup to a fresh 2-mL collection tube.

15 Add 20 µL of Elution Buffer directly onto the fiber matrix inside the spin cup. Cap the spin cup and incubate the sample at room temperature for 2 minutes.

**NOTE** The Elution Buffer must be added directly onto the fiber matrix so that the buffer can permeate the entire fiber matrix.

To increase the RNA yield, warm the Elution Buffer to 60°C.

16 Spin the sample in a microcentrifuge at ≥12,000 x g for 5 minutes.

17 If needed, repeat the elution step (step 15 and step 16) to increase the yield of total RNA.

18 Transfer the eluate in the collection tube to a capped microcentrifuge tube to store the RNA.

The RNA can be stored at -20°C for up to one month, or at -80°C for long-term storage.

# Thermocycler Protocol

The procedure in this section is an optional thermocycler protocol for the Low Input Quick Amp Labeling Kit.

Use a thermocycler to label reactions if you have a limited number of water baths. The use of a thermocycler can slightly lower the yield of cRNA when compared to the use of water baths.

## Step 1. Program the thermocycler

- Store the following programs into your thermocycler:
  - Program 1: 65°C for 10 minutes, 4°C hold
  - Program 2: 40°C for 2 hours, 70°C for 15 minutes, 4°C hold
  - Program 3: 40°C for 2 hours, 4°C hold

Five minutes at 4°C is enough. Hold at that temperature if the reagents for the next step are not ready.

**NOTE** Use a heated lid for optimal results.

## Step 2. Synthesize cDNA from Total RNA

(Time required: ~3 hours)

1. Add 25 to 100 ng of total RNA to a 0.2 mL PCR tube or the well of a 96-well PCR plate in a volume of 2.3 µL. For optimal performance, use at least 50 ng of input total RNA for the 2-pack, 4-pack, and 8-pack format. 100 ng is required for the 1-pack format.
2. Prepare the WT Primer Master Mix as described in Table 2 on page 22.
3. Add 3 µL of WT Primer Master Mix to the tube that contains 2.3 µL of total RNA and diluted RNA spike-in controls. Each tube now contains a total volume of 5.3 µL.
4. Put the tubes in the thermocycler and run Program 1 to denature the template and anneal the primer.
5. Keep the reaction tubes in the thermocycler at 4°C, or move to benchtop rack on ice.
6. Immediately prior to use, gently mix the components in Table 3 on page 23 in the order listed by pipetting, and keep at room temperature.

**NOTE** Prewarm the 5X first strand buffer by incubating the vial in an 80°C water bath for 3 to 4 minutes to ensure adequate resuspension of the buffer components. For optimal resuspension, mix briefly on a vortex mixer and spin the tube briefly in a microcentrifuge to drive the contents off the tube walls. Keep at room temperature until use.

**NOTE** Keep the AffinityScript RNase Block Mix on ice. Do not add the AffinityScript RNase Block Mix until just before you start the reactions.

7. To each sample tube, add 4.7 µL of cDNA Master Mix for a total volume of 10 µL. Pipette up and down to mix.
8. Put reaction tubes in thermocycler and run Program 2 to synthesize double-stranded cDNA.

**NOTE** Incubation at 70°C inactivates the AffinityScript enzyme.

# Step 3. Synthesize Fluorescent cRNA Synthesis *in vitro*

(Time required: ~2.5 hours)

1 Immediately before use, make Master Mix for each cyanine dye:

   a Add the first four components listed in Table 4 on page 24 in the order shown to 1.5-mL nuclease-free microfuge tubes at room temperature.

   b Mix thoroughly on a vortex mixer.

   c Add the T7 RNA Polymerase Blend and cyanine dyes.

   d Mix gently, but completely, by pipetting up and down without introducing bubbles.

**NOTE** Do not add the T7 RNA Polymerase Blend to Transcription Master Mix until just before you do the reaction.

2 Keep the reaction tubes from step 8 above in the thermocycler at 4°C, or move to benchtop rack on ice.

3 To each sample tube, add 6 µL of Transcription Master Mix. Gently mix by pipetting up and down. The final volume of the reaction is now 16 µL.

4 Return the reaction tubes to the thermocycler and run Program 3 ("Step 1. Program the thermocycler" on page 51) to synthesize labeled cRNA.

5 Purify the labeled cRNA as described on "Step 3. Purify the labeled/amplified RNA" on page 25.

# Quality Assessment of Template RNA and Labeled cRNA

This section gives a general guideline for template RNA and labeled cRNA quality assessment before proceeding with amplification or hybridization. Although optional, this step is highly recommended.

Make sure you determine the integrity and purity of the input template RNA, as well as labeled cRNA, before you label/amplify and hybridize, respectively. Use the NanoDrop UV-VIS Spectrophotometer and the Agilent 2100 bioanalyzer. The RNA 6000 Nano LabChip kit can be used to analyze total RNA, mRNA or cRNA with the appropriate assay at the assay specified concentration. For low concentration samples consider using the RNA 6000 Pico LabChip kit.

For the assessment of total RNA quality, the Agilent 2100 Expert Software automatically provides a RNA Integrity Number (RIN). RIN provides a quantitative value for RNA integrity that facilitates the standardization of quality interpretation. Users should define a minimum threshold RIN number based on correlative data in order to eliminate experimental bias due to poor RNA quality. Analysis of single stranded RNA, e.g. mRNA and cRNA, provides information on size distribution and concentration. It allows relative quantification of fragments within a size range.

# Step 1. Prepare for quality assessment

- Refer to Table 12 and Table 13 to make sure that you have the appropriate analyzer, kits, and compatible assays.

**Table 12** Analyzer and Kits

| Description | Vendor and part number |
|---|---|
| Agilent 2100 Bioanalyzer | Agilent p/n G2938C or G2939A |
| Agilent RNA 6000 Nano LabChip Kit | Agilent p/n 5067-1511 |
| Agilent RNA 6000 Pico LabChip Kit | Agilent p/n 5067-1513 |
| NanoDrop ND-1000 UV-Vis Spectrophotometer | NanoDrop p/n ND-1000 or equivalent |

**Table 13** Compatible Assays

| Description | Compatible Assay |
|---|---|
| Agilent RNA 6000 Nano LabChip Kit | Eukaryote Total RNA Nano Assay Qualitative range 5 to 500 ng/µL |
| Agilent RNA 6000 Nano LabChip Kit | mRNA Nano Assay* Qualitative range 25 to 250 ng/µL |
| Agilent RNA 6000 Pico LabChip Kit | Eukaryote Total RNA Pico Assay Qualitative range 50 to 5000 pg/µL in water |
| Agilent RNA 6000 Pico LabChip Kit | mRNA Pico Assay* Qualitative range 250 to 5000 pg/µL in water |

* The mRNA assays are suitable for analysis of cRNA as well.

# Step 2. Assess the quality using the Agilent 2100 Bioanalyzer

1. Choose the kit and assay according to your needs. Typically the RNA Nano 6000 kit and assay will be appropriate.
2. Ensure the 2100 bioanalyzer electrodes have been cleaned as instructed in the reagent kit guide.
3. Open the Agilent 2100 expert software (version B.02.02 or higher), switch on the 2100 bioanalyzer and check communication.
4. Prepare the chip, samples and ladder as instructed in the reagent kit guide.
5. Load the prepared chip into the 2100 bioanalyzer and start the run within five minutes after preparation.
6. Within the instrument context, choose the appropriate assay from the drop down list.
7. Start the run. Enter sample names and comments in the Data and Assay context.
8. Verify the results.

### Template RNA results (total RNA)

The resulting electropherogram should have at least two distinct peaks representing the 18S and 28S ribosomal RNA. Additional bands are the lower marker, and the potentially 5S RNA. Presence of 5S RNA depends on the purification method generally showing lower abundance in column purified total RNA. See Figure 7.

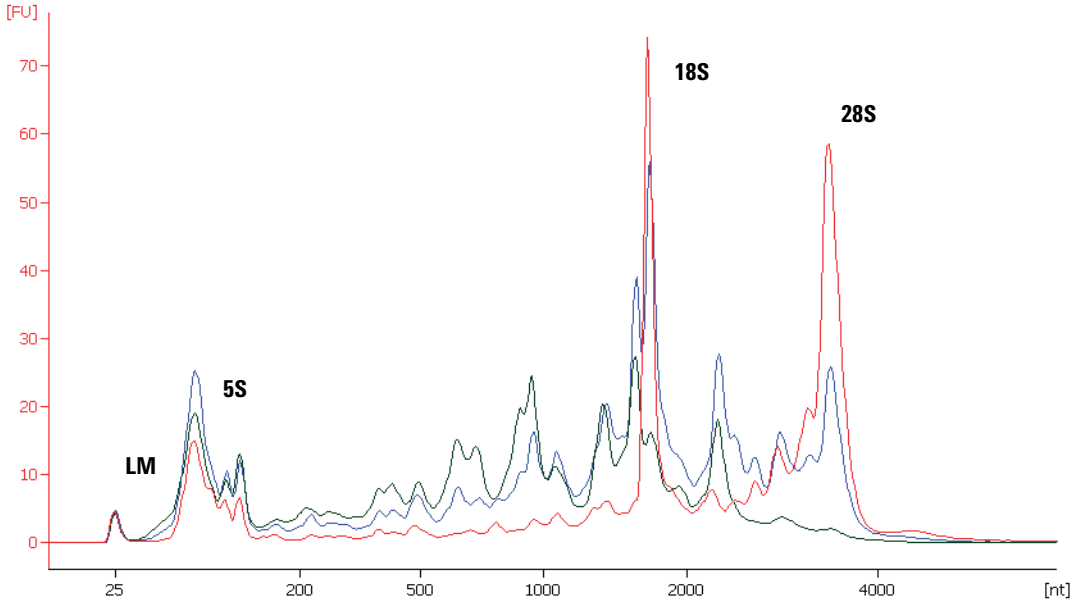



**Figure 7** Analysis of (human) total RNA with the Eukaryote total RNA Nano assay using three different samples with decreasing integrity: Red, RIN 8.4; Blue, RIN 5.9; Green, RIN 3,6. Characteristic regions for ribosomal peaks and the lower marker (LM) are displayed.

### Labeled cRNA

The resulting electropherogram should have a broad band. The majority of signal for amplified sample should fall into the size range from 200 to 2000 nucleotides. If there isn't a band in this range, and there are distinct bands less than 200 nucleotides in length, DO NOT proceed with that sample since it has likely been degraded and will not provide accurate results. See Figure 8.

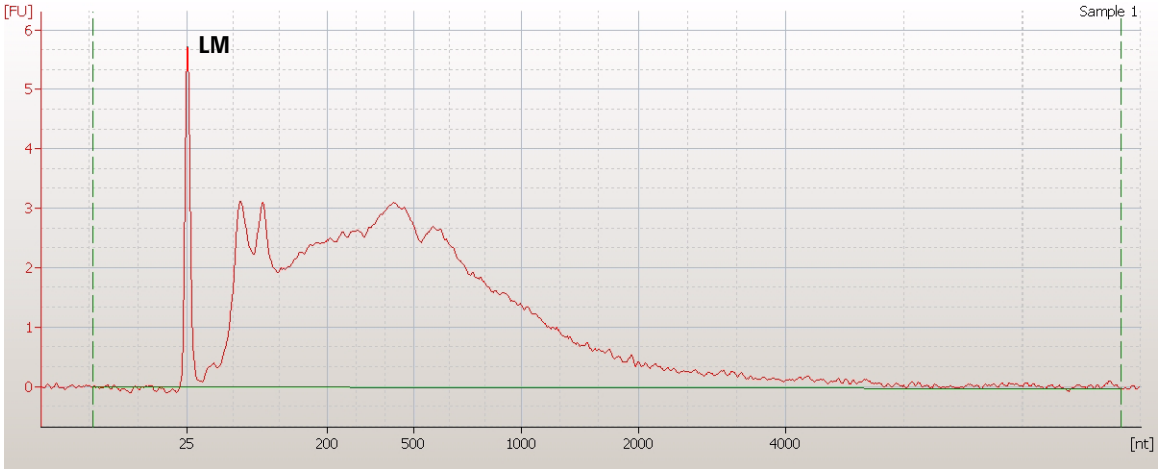



**Figure 8** Smear analysis on non-fragmented Cy3 labeled cRNA allows determination of relative concentration of products within size regions. For example, 52 % of overall RNA signal results from fragments with size from 200 to 2000 nt.

For general assistance on evaluation of total RNA with emphasis on the RNA integrity number, see the corresponding application note: “RNA integrity number (RIN) - Standardization of RNA quality control”, 5989-1165EN.

Additional information on mRNA can be found in the corresponding application notes: **Interpreting mRNA electropherograms**, publication 5988-3001EN, and **Optimizing cRNA fragmentation for microarray experiments using the Agilent 2100 bioanalyzer**, publication 5988-3119EN.

To download application notes regarding the 2100 bioanalyzer visit Agilent Web site at www.agilent.com/chem/labonachip.

# Step 3. Assess the quality using a NanoDrop Spectrophotometer

Accurate assessment of total RNA quantity and quality are crucial to the success of an Agilent Gene Expression experiment. High quality RNA should be free of contaminants such as carbohydrates, proteins, and traces of organic solvents, and should also be intact with minimal degradation.

Use the NanoDrop UV-VIS Spectrophotometer (or equivalent) to assess RNA concentration and purity.

### UV-VIS Spectrophotometry

1. In the Nanodrop program menu, select **Nucleic Acid Measurement**, then select **Sample Type** to be **RNA-40**.
2. Use 1.5 µL of nuclease-free water to blank the instrument.
3. Use 1.5 µL of each total RNA sample to measure RNA concentration. Record the RNA concentration (ng/µL) for each sample.
4. Record the $\mathbf{A_{260}/A_{280}}$ and $\mathbf{A_{260}/A_{230}}$ ratios.

   High-quality total RNA samples have an $\mathbf{A_{260}/A_{280}}$ ratio of 1.8 to 2.0, which indicates the absence of contaminating proteins. They also have an $\mathbf{A_{260}/A_{230}}$ ratio of >2.0, which indicates the absence of other organic compounds, such as guanidinium isothiocyanate, alcohol and phenol as well as cellular contaminants such as carbohydrates.

   An $\mathbf{A_{260}/A_{230}}$ ratio of <2.0 can indicate the presence of these contaminants, which can interfere with the labeling reaction or can lead to inaccurate quantification of your total RNA.

# Preventing Ozone-Related Problems

While Cy3 is sensitive to ozone degradation, the Agilent one-color platform is robust in environments where the ozone level is 50 ppb (approximately 100 µg/$m^3$) or less. Beyond this level, ozone can significantly affect Cy3 signal and compromise microarray performance. The Agilent Ozone-Barrier Slide cover is designed to protect against ozone-induced degradation of cyanine dyes and is recommended when using Agilent oligo-based microarrays in high-ozone environments. See step 14 on page 39.

As an alternative to the Ozone-Barrier Slide cover, the Agilent Stabilization and Drying Solution, which is an organic solvent based wash, can reduce background variability produced by wash artifacts.

The use of the Agilent Stabilization and Drying Solution is described in this section.

# Step 1. Prepare the Stabilization and Drying Solution

The Agilent Stabilization and Drying Solution contains an ozone scavenging compound dissolved in acetonitrile. The compound in solution is present in saturating amounts and may precipitate from the solution under normal storage conditions. If the solution shows visible precipitation, warming of the solution will be necessary to redissolve the compound. Washing slides using Stabilization and Drying Solution showing visible precipitation will have a profound adverse effect on microarray performance.

**WARNING** **The Agilent Stabilization and Drying Solution is a flammable liquid. Warming the solution will increase the generation of ignitable vapors. Gloves and eye/face protection should be used in every step of the warming procedures.**

**WARNING** **Do not use an open flame or a microwave. Do not increase temperature rapidly. Warm and mix the material away from ignition sources.**

**WARNING** **Failure to follow the outlined process will increase the potential for fire, explosion, and possible personal injury. Agilent assumes no liability or responsibility for damage or injury caused by individuals performing this process.**

1 Warm the solution slowly in a water bath or a vented conventional oven at 40°C in a closed container with sufficient head space to allow for expansion.

**NOTE** The original container can be used to warm the solution. Container volume is 700 mL and contains 500 mL of liquid. If a different container is used, maintain or exceed this headspace/liquid ratio. The time needed to completely redissolve the precipitate is dependent on the amount of precipitate present, and may require overnight warming if precipitation is heavy. DO NOT FILTER the Stabilization and Drying solution.

2 If needed, gently mix to obtain a homogenous solution.

Mix under a vented fume hood away from open flames, or other sources of ignition. Warm the solution only in a controlled and contained area that meets local fire code requirements.

3 After the precipitate is completely dissolved, let the covered solution stand at room temperature, allowing it to *equilibrate to room temperature prior to use*.

## Step 2. Wash with Stabilization and Drying Solution

Cy3 is susceptible to degradation by ozone. The following procedure is strongly recommended if the ozone levels exceed 50 ppb in your laboratory. For more information, visit www.agilent.com/chem/dnatechnicalnotes to download the technical note on **Improving Microarray Results by Preventing Ozone-Mediated Fluorescent Signal Degradation** (publication 5989-0875EN).

**NOTE**

Fresh Gene Expression Wash Buffer 1 and 2 should be used for each wash group (up to eight slides). The acetonitrile and Stabilization and Drying Solution may be reused for washing of up to three groups of slides (that is, a total of 24 slides).

**WARNING**

**The Stabilization and Drying Solution must be set-up in a fume hood. Wash 1 and Wash 2 set-up areas should be placed close to, or preferably in, the same fume hood. Gloves and eye/face protection should be used in every step of the warming procedures.**

Table 14 lists the wash conditions for the wash procedure with Stabilization and Drying Solution.

**Table 14** Wash conditions

| | Dish | Wash Buffer | Temperature | Time |
|---|---|---|---|---|
| Disassembly | 1 | GE Wash Buffer 1 | Room temperature | |
| 1st wash | 2 | GE Wash Buffer 1 | Room temperature | 1 minute |
| 2nd wash | 3 | GE Wash Buffer 2 | Elevated temperature | 1 minute |
| Acetonitrile Wash | 4 | Acetonitrile | Room temperature | 10 seconds |
| 3rd wash | 5 | Stabilization and Drying Solution | Room temperature | 30 seconds |

1. Completely fill slide-staining dish #1 with Gene Expression Wash Buffer 1 at room temperature.
2. Place a slide rack into slide-staining dish #2. Add a magnetic stir bar. Fill slide-staining dish #2 with enough Gene Expression Wash Buffer 1 at room temperature to cover the slide rack. Place this dish on a magnetic stir plate.
3. Place the empty dish #3 on the stir plate and add a magnetic stir bar. Do not add the pre-warmed (37°C) Gene Expression Wash Buffer 2 until the first wash step has begun.
4. Fill slide-staining dish #4 approximately three-fourths full with acetonitrile. Add a magnetic stir bar and place this dish on a magnetic stir plate.
5. Fill slide-staining dish #5 approximately three-fourths full with Stabilization and Drying Solution. Add a magnetic stir bar and place this dish on a magnetic stir plate.
6. Remove one hybridization chamber from incubator and record time. Record whether bubbles formed during hybridization, and if all bubbles are rotating freely.
7. Prepare the hybridization chamber disassembly.
   a. Place the hybridization chamber assembly on a flat surface and loosen the thumbscrew, turning counter-clockwise.
   b. Slide off the clamp assembly and remove the chamber cover.
   c. With gloved fingers, remove the array-gasket sandwich from the chamber base by grabbing the slides from their ends. Keep the microarray slide numeric barcode facing up as you quickly transfer the sandwich to slide-staining dish #1.
   d. Without letting go of the slides, submerge the array-gasket sandwich into slide-staining dish #1 containing Gene Expression Wash Buffer 1.
8. With the sandwich completely submerged in Gene Expression Wash Buffer 1, pry the sandwich open from the barcode end only:
   a. Slip one of the blunt ends of the forceps between the slides.
   b. Gently turn the forceps upwards or downwards to separate the slides.
   c. Let the gasket slide drop to the bottom of the staining dish.
   d. Remove the microarray slide and place into slide rack in the slide-staining dish #2 containing Gene Expression Wash Buffer 1 at room temperature. Minimize exposure of the slide to air. *Touch only the barcode portion of the microarray slide or its edges!*

9 Repeat step 6 through step 8 for up to seven additional slides in the group. A maximum of eight disassembly procedures yielding eight microarray slides is advised at one time in order to facilitate uniform washing.

10 When all slides in the group are placed into the slide rack in slide-staining dish #2, stir using setting 4 for 1 minute.

11 During this wash step, remove Gene Expression Wash Buffer 2 from the 37°C water bath and pour into the Wash 2 dish.

**NOTE** The elevated temperature of the second wash step is usually around 31°C due to cooling by the room temperature dish and the rack of arrays.

12 Transfer slide rack to slide-staining dish #3 containing Gene Expression Wash Buffer 2 at elevated temperature. Stir using setting 4 for 1 minute.

13 Remove the slide rack from Gene Expression Wash Buffer 2 and tilt the rack slightly to minimize wash buffer carry-over. Immediately transfer the slide rack to slide-staining dish #4 containing acetonitrile and stir using setting 4 for less than 10 seconds.

14 Transfer the slide rack to dish #5 filled with Stabilization and Drying Solution and stir using setting 4 for 30 seconds.

15 Slowly remove the slide rack trying to minimize droplets on the slides. It should take 5 to 10 seconds to remove the slide rack.

16 Discard used Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 2.

17 Repeat steps 1 through 16 for the next group of eight slides using fresh Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 2 pre-warmed to 37°C.

18 Scan slides immediately to minimize the impact of environmental oxidants on signal intensities. If necessary, store slides in orange slide boxes in a nitrogen purge box, in the dark.

**CAUTION** Dispose of acetonitrile and Stabilization and Drying Solution as flammable solvents.

# Normalizing Agilent One-Color Microarray Data

When comparing data across a set of one-color microarrays, a simple linear scaling of the data is usually sufficient for most experimental applications. Agilent has determined that the signal value of the 75th percentile of all of non-control probes on the microarray is a more robust and representative value of the overall microarray signal as compared to the median or 50th percentile signal. Therefore, use the 75th percentile signal value to normalize Agilent one-color microarray signals for inter-array comparisons.

## To do downstream analysis of Agilent microarray data

- Use GeneSpring GX 11.5 or later.

Note that the default normalization scheme for Agilent one-color exon data in the GeneSpring GX 11.5 (or later) program is 75th percentile scaling.

For more information on the GeneSpring GX program, go to http://www.agilent.com/chem/genespring.

## To use Feature Extraction

To normalize Agilent one-color microarray data without the GeneSpring program, use the 75th percentile value for each microarray assay in the Agilent Feature Extraction text file.

1. Generate a Feature Extraction text file.
2. Find the “STATS Table” in the middle section of the text file. This section describes the results from the array-wide statistical calculations.
3. Find the 75th percentile value of the non-control signals under the column with the heading **gPercentileIntensityProcessedSignal**.
4. Divide each of the green processed signals (**gProcessedSignal**) by the 75th percentile signal (**gPercentileIntensityProcessedSignal**) to generate the 75th percentile normalized microarray processed signals.

You can further scale the resulting 75th percentile-normalized signals by a constant, such as the average of the 75th percentile signals of the arrays in the experiment.

For more information on the output from the Agilent Feature Extraction program, please refer to the *Agilent G2567AA Feature Extraction Software Reference Guide*. You can download this guide from the Agilent Web site at www.agilent.com/chem/dnamanuals-protocols.

# 4 Reference



This chapter contains reference information related to the protocol and Feature Extraction default parameter settings

# Supplemental User Guides

First-time users of Agilent's oligo microarray system, please refer to the following user manuals for detailed descriptions and operation recommendations for each of the hardware and software components used in the one-color platform workflow. The user guides can be downloaded from the Agilent Web site at www.agilent.com/chem/dnamanuals-protocols.

G2534-90001 Agilent Microarray Hybridization Chamber User Guide

G2545-80001 G2545A Hybridization Oven User Manual

G2505-90021 Agilent G2565CA Microarray Scanner System (Scan Control Software 8.5) User Guide

Agilent G2567AA Feature Extraction Software Quick Start Guide

Agilent G2567AA Feature Extraction Software User Guide

Agilent G2567AA Feature Extraction Software Reference Guide

## Microarray Handling Tips

Each microarray is printed on the side of the glass slide containing the "Agilent"-labeled barcode. This side is called the "active" side. The numeric barcode is on the inactive side of the slide.

**CAUTION** You must familiarize yourself with the assembly and disassembly instructions for use with the Agilent Microarray Hybridization Chamber (G2534A) and gasket slides. Practice slide kits are available.

In this "processing and hybridization" procedure, the hybridization mixture is applied directly to the gasket slide, and not to the active side of the oligo microarray. Instead, the active side of the oligo microarray is placed on top of the gasket slide to form a "sandwich slide" pair.

To avoid damaging the microarray, always handle glass slides carefully by their edges. Wear powder-free gloves. Never touch the surfaces of the slides. If you do, you may cause irreparable damage to the microarray.

Never allow the microarray surface to dry out during the hybridization process and washing steps.

# General Microarray Layout and Orientation

### Agilent oligo microarray (1 microarray/slide format) as imaged on the Agilent microarray scanner (G2565CA)

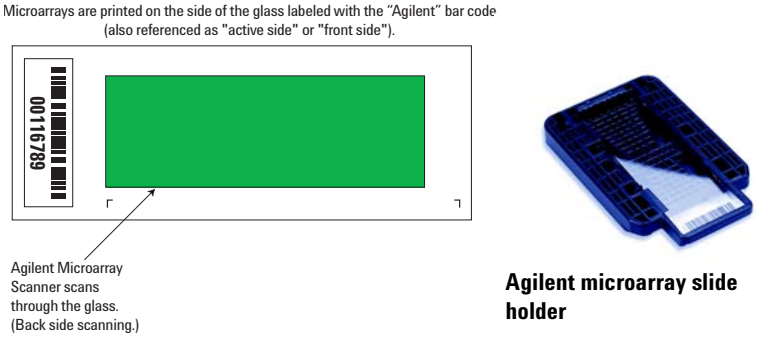



**Figure 9** Agilent microarray slide and slide holder. The opposite or "non-active" numerically barcoded side is shown.

Agilent oligo microarray formats and the resulting "microarray design files" are based on how the Agilent microarray scanner images 1-inch × 3-inch glass slides. Agilent designed its microarray scanner to scan through the glass slide (back side scanning). The glass slide is securely placed in an Agilent microarray slide holder with the "Agilent"-labeled barcode facing the inside of the slide holder. In this orientation, the "active side" containing the microarray is protected from potential damage by fingerprints and other elements. Once securely placed, the numeric barcode, non-active side of the slide, is visible from the outside of the slide holder.

Figure 9 depicts how the Agilent microarray scanner reads the microarrays and how this relates to the "microarray design files" that Agilent generates during the manufacturing process of its *in situ*-synthesized oligonucleotide microarrays. Thus, if you have a scanner that reads microarrays from the "front side" of the glass slide, the collection of microarray data points will be different in relation to the "microarray design files" supplied with the Agilent oligo microarray kit you purchased. Therefore, please take a moment to become familiar with the microarray layouts for each of the Agilent oligo microarrays and the layout information as it pertains to scanning using a "front side" scanner.

### Non-Agilent front side microarray scanners

When imaging Agilent oligo microarray slides, you must determine:

- If the scanner images microarrays by reading them on the “front side” of the glass slide (“Agilent”-labeled barcode side of the slide) and
- If the image produced by the non-Agilent scanner is oriented in a “portrait” or “landscape” mode, “Agilent”-labeled barcode left-side, right-side, up or down, as viewed as an image in the imaging software (see Figure 10).

This changes the feature numbering and location as it relates to the “microarray design files” found on the CD in each Agilent oligo microarray kit. Microarray layout maps are available from Agilent. For more information, go to www.agilent.com/chem/dnamanuals-protocols and download *Agilent Microarray Formats Technical Drawings with Tolerance* (publication G4502-90001). This document contains visual references and guides that will help you determine the feature numbering as it pertains to your particular scanner configuration.

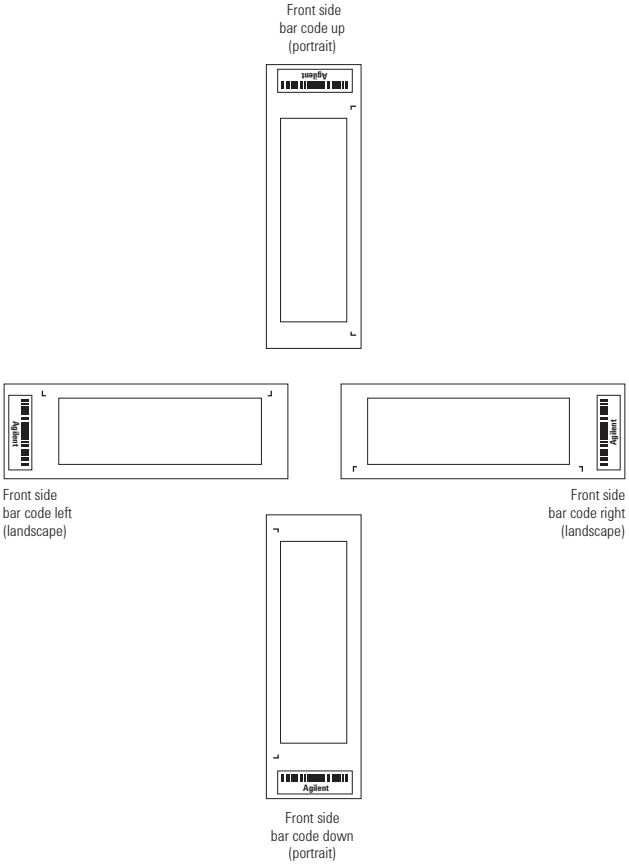



**Figure 10** Microarray slide orientation

# Array/Sample tracking microarray slides

Use the forms below to make notes to track your samples on microarray slides.

**Arrays**

| | Array 1_1 | Array 1_2 |
|---|---|---|
| BARCODE | **Sample:** | **Sample:** |

**Barcode Number** _____

**Figure 11** 2-pack microarray slides

**Arrays**

| | Array 1_1 | Array 1_2 | Array 1_3 | Array 1_4 |
|---|---|---|---|---|
| BARCODE | Sample: | Sample: | Sample | Sample: |

**Barcode Number** _____

**Figure 12** 4-pack microarray slides

**Arrays**

| | Array 1_1 | Array 1_2 | Array 1_3 | Array 1_4 |
|---|---|---|---|---|
| BARCODE | Sample: | Sample: | Sample | Sample: |
| | Sample: | Sample: | Sample | Sample: |
| | Array 2_1 | Array 2_2 | Array 2_3 | Array 2_4 |

**Barcode Number** _____

**Figure 13** 8-pack microarray slide

# Related Microarray Reagents

| Description | Vendor and part number |
|---|---|
| Universal Human Reference RNA | Stratagene p/n 740000 |
| Universal Mouse Reference RNA | Stratagene p/n 740100 |
| Universal Rat Reference RNA | Stratagene p/n 740200 |

www.agilent.com

## In This Book

This guide contains information to run the Agilent One-Color Microarray-Based Exon Analysis protocol.



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