One-Color Microarray-Based Gene Expression Analysis (Quick Amp Labeling) with Tecan HS Pro Hybridization

Protocol

For use with Agilent Gene Expression oligo microarrays

Version 5.7, May 2008

Microarrays manufactured with Agilent SurePrint Technology

Research Use Only. Not for use in Diagnostic Procedures.

Agilent Technologies

Notices

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www.agilent.com/chem/contactus

or send an email to:
lsca-ibs-support@agilent.com

For Tecan Hybridization Station support, contact your Tecan representative.

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CAUTION

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A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

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 analysis.

If you have comments about this protocol, send an e-mail to feedback_genomics@agilent.com.

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.

3 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.

4 Supplemental Procedures

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

5 Reference

This chapter contains reference information related to the protocol.

What's New in This Protocol?

This new protocol is based on version 5.7 of the *One-Color Microarray-Based Gene Expression Analysis (Quick Amp Labeling)* with the following differences:

- Hybridization is done with the Tecan HS Pro Hybridization Station instead of the Agilent SureHyb chamber.
- Only the 4-pack microarrays are supported.

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 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.
  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.
- 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.**
- **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

- Four microarrays printed on each 1-inch × 3-inch glass slides, of a five slide kit.
- CD containing microarray design files in various file formats

Custom microarray kits

- Four 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 G2565BA
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 80°C, 40°C, 65°C, 60°C, and 37°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
Quick Amp Labeling Kit, One-Color	Agilent p/n 5190-0442
RNA Spike-In Kit, One-Color	Agilent p/n 5188-5282
Gene Expression Hybridization Kit	Agilent p/n 5188-5242
Gene Expression Wash Buffer 1	Agilent p/n 5188-5325
Gene Expression Wash Buffer 2	Agilent p/n 5188-5326
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
Microarray Wash Buffer Additive (25 mL)	Agilent p/n 5190-0401
Pre-hybridization buffer (4 L)	Agilent p/n 5190-0402

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
Acetonitrile	Sigma p/n 271004-1L

Required Hardware and Software

Description
Pentium III 800 MHz or higher (Pentium IV 1.5 GHz or higher recommended)
Agilent's Scan Control software, version A. 7.0.1 (includes XDR functionality)
512 MB RAM (1 GB recommended)
20 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 9.5.3
Internet Explorer 5.5 or later
Adobe Acrobat Reader 4.0 or later
Tecan HS Pro 400 or 4800 with HS Pro Control Manager Software 3.0 or later

Optional Software

Description
GeneSpring GX 9.0 or later

2 Equipment and Reagent Preparation

This chapter describes the preparation steps that you need to take before hybridization with the Tecan HS 4800 Pro and 400 Pro Hybridization Stations.

To test and prepare the equipment for first use

CAUTION Before you begin, make sure you have read and understand operating, maintenance and safety instructions for using your Tecan hybridization station. Refer to the documentation that came with your hybridization station.

1. Set up the hybridization station, including computer, all tubes, reagents, waste, and pressure bottles, power, and software.
2. Carefully check O-rings for damage.
3. Clean the hybridization chambers with water and then dry with compressed nitrogen.
4. Insert hybridization chambers into chamber frames.
5. Place the adapter with dummy slides onto the heating plates and close the chamber carefully.
6. Flush the delivered reagent bottles with distilled water before use.
7. Run a rinsing procedure with the Cleaning and Conditioning Solution, instead of water.

   To prepare the Cleaning and Conditioning Solution:

   - In the Tecan wash bottle, add 1 mL of the Agilent Microarray Wash Additive (5190-0401) to 1 L of the Agilent Gene Expression Wash Buffer 2. Mix thoroughly by shaking.

NOTE Use the solution to rinse the instrument at regular intervals of approximately every 2 weeks to coincide with the replacement of the hybridization chamber O-rings.

8. Run a rinsing procedure with distilled water.
9. For conditioning the hybridization chambers, start a hybridization run (for example 12 hours) at the temperature used in the protocol (65°C) with 1x Hybridization buffer and dummy glass.
10. Run a rinsing procedure with Final System Drying to dry the chambers.

    This step is described in the Tecan operating guide.

To set up the reagent bottles

- Prepare the six Wash Bottles according to Table 1.

Table 1

Wash Bottle	Reagent	Heated
1	Agilent Prehybridization Buffer	Yes
2	Gene Expression Wash Buffer 1	No
3	Gene Expression Wash Buffer 2 w/ 0.01% Wash Buffer Additive*	Yes
4	Empty	N/A
5	Water	No
6	Cleaning and Conditioning Solution	No

* Spike the Wash Buffer Additive into the Gene Expression Wash Buffer 2 (5188-5326) at the ratio of 100 µL per 1 liter of wash buffer

WARNING **Never add Agilent Stabilization and Drying Solution to a Tecan reagent bottle for use inside the HS Pro Hybridization Station. The Agilent Stabilization and Drying Solution is toxic and flammable.**

3 Procedures

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

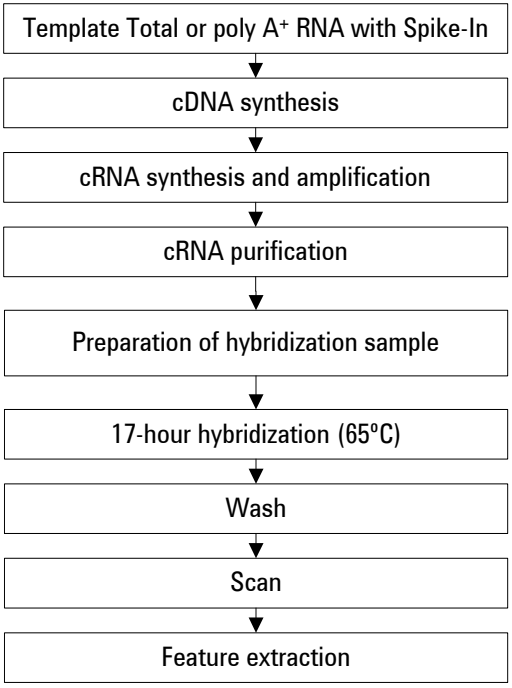


Figure 1 Workflow for sample preparation and array processing.

Sample Preparation

Agilent's Quick Amp Labeling Kit generates fluorescent cRNA (complimentary RNA) with a sample input RNA range between 200 ng and 1 µg of total RNA or a minimum of 10 ng of poly A^+ RNA for one-color processing. The method uses T7 RNA polymerase, which simultaneously amplifies target material and incorporates cyanine 3-labeled CTP. There is routinely at least a 100-fold RNA amplification with use of this kit.

NOTE

For optimal performance, use high quality, intact template total or poly A+ RNA. Please refer to "Quality Assessment of Template RNA and Labeled cRNA" on page 46 for general guidance and procedural recommendations on quality assessment of template RNA.

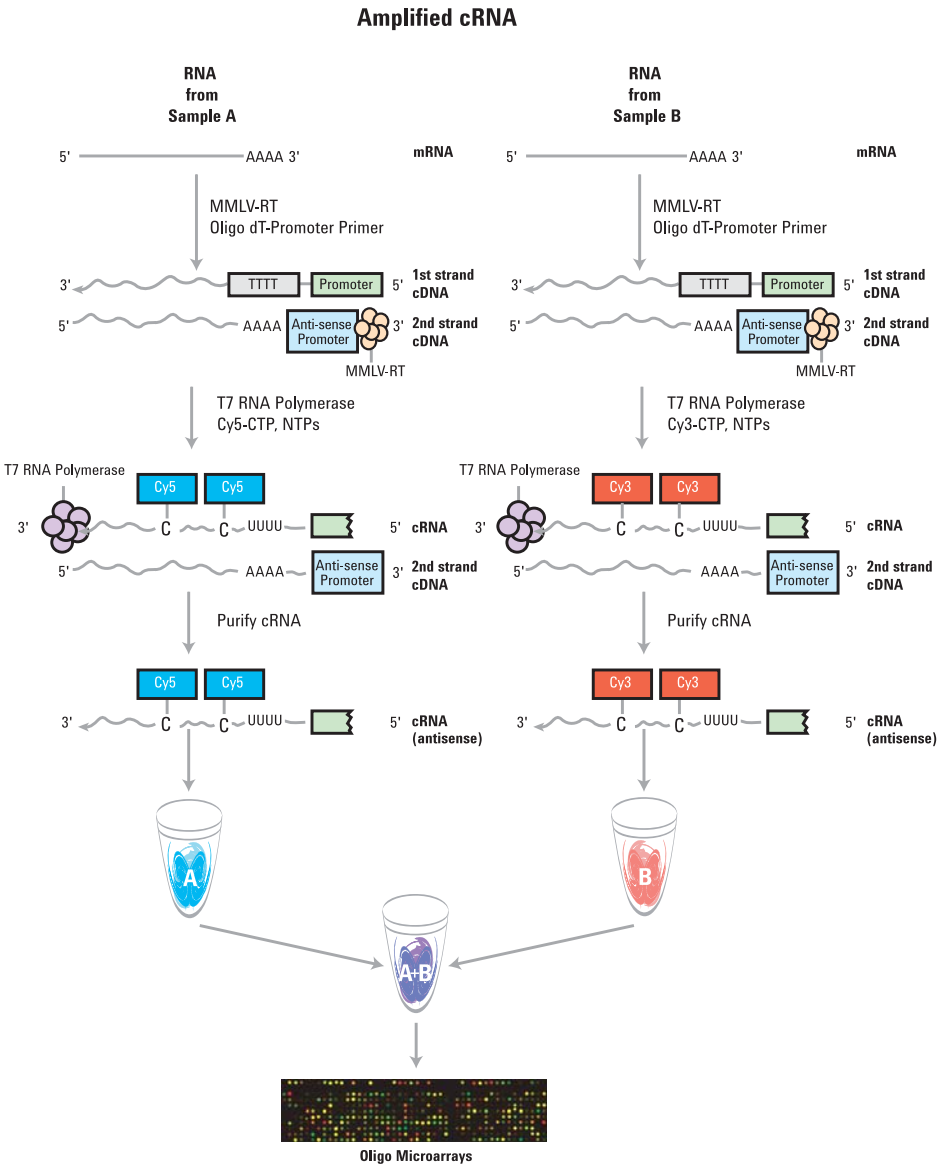


Figure 2 Schematic of amplified cRNA procedure. Generation of cRNA for a two-color microarray experiment is shown. When you generate targets for a one-color microarray experiment, only the Cy3-labeled "B" sample is produced and hybridized.

Step 1. Prepare One-Color Spike-Mix

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-ins. 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, 40°C, and 80°C.
2. Mix the One-Color Spike-Mix stock solution vigorously on a vortex mixer.
3. Heat at 37°C for 5 minutes, and mix on a vortex mixer once more.
4. Briefly 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 2 provides the dilutions of Agilent One-Color Spike-Mix for a range of total RNA input amounts. These are diluted such that 1 µL of One-Color Spike-Mix is added for every 100 ng of total RNA in the labeling reaction up to 500 ng, and 0.5 µL added for every 100 ng total RNA for input amounts greater than 500 ng. If you start with the minimum 10 ng mRNA as the input mass, follow the dilution scheme as described for the input mass of 1000 ng total RNA.

Table 2 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)	PolyA RNA (ng)	Maximum Volume of RNA (µL)	First	Second	Third	
200		8.3	1:20	1:25	1:10	2
300		7.3	1:20	1:25	1:10	3
400		6.3	1:20	1:25	1:10	4
500		5.3	1:20	1:25	1:10	5
600		7.3	1:20	1:25	1:5	3
700		6.8	1:20	1:25	1:5	3.5
800		6.3	1:20	1:25	1:5	4
900		5.8	1:20	1:25	1:5	4.5
1000	10	5.3	1:20	1:25	1:5	5

NOTE Use RNase-free microfuge tubes and tips. Avoid pipetting volumes less than 2 µL to ensure accuracy.

For example, to prepare the Agilent One-Color Spike-Mix dilution appropriate for 200 ng of total RNA starting sample:

1. Mix the thawed Agilent One-Color Spike-Mix concentrate vigorously on a vortex mixer.
2. Heat at 37°C in a circulating water bath for 5 minutes.
3. Mix the Agilent One-Color Spike-Mix tube vigorously again on a vortex mixer.
4. Spin briefly in a centrifuge to separate contents to the bottom of the tube.
5. Add 2 µL of Agilent One-Color Spike-Mix stock to 38 µL of Dilution Buffer provided in the kit (1:20).
6. 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.

7 Add 2 µL of First Dilution to 48 µL of Dilution Buffer for the Second Dilution (1:25).

8 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.

9 Add 4 µL of Second Dilution to 36 µL of Dilution Buffer for the Third Dilution (1:10).

10 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 Third Dilution (now at a 5000-fold final dilution).

11 Add 2 µL of Third Dilution to 200 ng of sample total RNA and continue with cyanine 3 labeling using the Agilent Low Input Linear Amplification Kit protocol as specified.

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 and third dilution tubes.

Step 2. Prepare labeling reaction

1. Add 200 to 1000 ng of total RNA to a 1.5-mL microcentrifuge tube in an appropriate volume (see Table 3). Samples should be diluted such that at least 2 µL of sample is pipetted into the tube.
2. Add 1.2 µL of T7 Promoter Primer (from the Agilent Quick Amp Kit, One-Color). See Table 3.
3. Add the appropriate volume of diluted Spike-Mix as indicated in Table 3.

Table 3 Template and T7 Promoter Primer Mix

Total RNA input (ng)	PolyA RNA (ng)	Max RNA volume (µL)	Third Dilution of Spike-Mix volume (µL)	T7 Promoter primer (µL)	Total volume (µL)
200		8.3	2	1.2	11.5
300		7.3	3	1.2	11.5
400		6.3	4	1.2	11.5
500		5.3	5	1.2	11.5
600		7.3	3	1.2	11.5
700		6.8	3.5	1.2	11.5
800		6.3	4	1.2	11.5
900		5.8	4.5	1.2	11.5
1000	10	5.3	5	1.2	11.5

4. Use nuclease-free water to bring the total reaction volume to 11.5 µL.
5. Denature the primer and the template by incubating the reaction at 65°C in a circulating water bath for 10 minutes.
6. Place the reactions on ice and incubate for 5 minutes.
7. Immediately prior to use, gently mix the components listed in Table 4 for the cDNA Master Mix by adding in the order indicated, and put on ice.

8 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.

MMLV-RT and RNaseOUT are enzymes, which need to be kept on ice and are to be added to the cDNA Master Mix just before starting the reactions.

Be sure to use the 10 mM dNTP mix tube from the kit.

Table 4 cDNA Master Mix

Component	Volume (µL) per reaction	Volume (µL) per 4.5 reactions
5X First Strand Buffer	4	18
0.1 M DTT	2	9
10 mM dNTP mix	1	4.5
MMLV-RT	1	4.5
RNaseOut	0.5	2.3
Total Volume	8.5	38.3

9 Briefly spin each sample tube in a microcentrifuge to drive down the contents from the tube walls and the lid. Return the tubes to ice.

10 Add 8.5 µL of cDNA Master Mix to each sample tube and mix by pipetting up and down.

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

12 Move samples to a 65°C circulating water bath and incubate for 15 minutes.

13 Move samples to ice. Incubate for 5 minutes.

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

15 Immediately prior to use, gently mix the components listed in Table 5 in the order indicated for the Transcription Master Mix by pipetting at room temperature.

16 Prewarm the 50% PEG solution at 40°C for 1 minute. 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. Careful pipetting is required to ensure accurate volume. Keep at room temperature until needed.

RNaseOUT, inorganic pyrophosphatase, and T7 RNA polymerase are enzymes, which need to be kept on ice and should be added to the Transcription Master Mix just before starting the reactions.

Table 5 Transcription Master Mix

Component	Volume (µL) per reaction	Volume (µL) per 4.5 reactions
Nuclease-free water	15.3	68.9
4X Transcription Buffer	20	90
0.1 M DTT	6	27
NTP mix	8	36
50% PEG	6.4	28.8
RNaseOUT	0.5	2.3
Inorganic pyrophosphatase	0.6	2.7
T7 RNA Polymerase	0.8	3.6
Cyanine 3-CTP	2.4	10.8
Total Volume	60	270

17 Add 60 µL of Transcription Master Mix to each sample tube. Gently mix by pipetting.

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

Step 3. Purify the labeled/amplified RNA

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

NOTE Ensure that ethanol was added to the RPE buffer as specified in the Qiagen manual before proceeding.

1. Add 20 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, 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.
8. Elute the cleaned 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.

CAUTION *Do not discard the final flow-through*. It now contains the cRNA sample.

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:

(*Concentration of cRNA*) * 30 µL (*elution volume*) / 1000 = µg of cRNA.

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

(*Concentration of Cy3*) / (*Concentration of cRNA*) * 1000 = pmol Cy3 per µg cRNA

11 Examine the yield and specific activity results.

CAUTION If the yield is <1.65 µg and the specific activity is <9.0 pmol Cy3 per µg cRNA do not proceed to the hybridization step. Repeat cRNA preparation.

NOTE Please refer to "Quality Assessment of Template RNA and Labeled cRNA" on page 46 for general guidance and procedural recommendations on quality assessment of labeled cRNA.

NOTE You may need to use a Speed Vac to dry the sample to load 1.65 µg/channel in a volume of 22.8 µL.

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 Table 6 to a 1.5 mL nuclease-free microfuge tube.

3 Mix well but gently on a vortex mixer.

Table 6 Fragmentation mix

Components	Volume/Mass
cyanine 3-labeled, linearly amplified cRNA	1.65 µg
10X Blocking Agent	6 µL
Nuclease-free water	bring volume to 28.8 µL
25X Fragmentation Buffer	1.2 µL
Total Volume	30 µL

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

CAUTION Do not exceed 30 minutes. Adding the 2X Hybridization Buffer will stop the fragmentation reaction.

5 Add 2x GEx Hybridization Buffer HI-RPM to the fragmentation mix at the appropriate volume to stop the fragmentation reaction. See Table 7.

Table 7 Hybridization mix

Components	Volumes per hybridization
cRNA from Fragmentation Mix	30 µL
2x GEx Hybridization Buffer HI-RPM	30 µL

6 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.

7 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.

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

Refer to "Microarray Handling Tips" on page 59 for information on how to safely handle microarrays.

Step 3. Tecan Hybridization

In this step, you hybridize the microarrays with the Tecan HS Pro hybridization station. For support on the Tecan HS Pro hybridization station, contact your Tecan representative.

1 Set up the Tecan HS Pro hybridization station. Follow the instructions in *Instructions for use for HS 4800/HS400 Pro Hybridization Station* to:

- Set up the reagent bottles (see Table 1 on page 17).
- Load slides.
- Inject samples.

Note the reverse order of the Tecan chamber designations and the array numbers in Agilent's Feature Extraction Software. The Tecan Quad chamber labeled "A" will be loaded first and corresponds to array number 4. The Tecan Quad chamber labeled "D" will be loaded last and corresponds to array number 1.

2 Open the Tecan HS Pro Control Manager software, then run the Agilent Gene Expression program.

You can download the Agilent Gene Expression program from http://www.opengenomics.com/Hardware.aspx.

The Gene Expression Program contains the steps in Table 8. See "Agilent Gene Expression Program Settings" on page 65 for more information.

3 Turn on the reagent bottle heating.

4 Click **Go**.

Table 8 Gene Expression Program

Step Number	Program Step	Step Action
1	Wash	Agilent Prehybridization Buffer at 65°C
2	Sample Injection	Sample Loading (55 µL with A4X44k)
3	Hybridization	65°C for 17 hours
4	Wash	GE Wash Buffer 1 at Room Temp
5	Wash	GE Wash Buffer 2 at 37°C
6	Drying	2 minutes at 30°C

5 Follow the instructions in *Instructions for use for HS 4800/HS400 Pro Hybridization Station* to inject the samples.

6 When the program is finished, remove the MTP Slide adapter from the instrument.

You can now remove the slides to scan in the Agilent scanner.

At this point, you can process the slides with the Agilent Stabilization and Drying Solution to protect the dyes against ozone degradation. See "Step 2. Wash with Stabilization and Drying Solution" on page 53.

After you remove the MTP slide adapter from the instrument, a small amount of adhesive or ink from the barcode may remain on the heating block. Remove the adhesive and ink with a 70% ethanol solution.

Scanning and Feature Extraction

Step 1. Scan the slides

Agilent Scanner Settings

1 Assemble the slides into an appropriate slide holder, either version B or A. Place the slides into the slide holder such that the numeric barcode side is visible (*not* the "Agilent"-labeled barcode side). Refer to "General Microarray Layout and Orientation" on page 60.

2 Place assembled slide holders into scanner carousel.

3 Verify scan settings for one-color scans.

Table 9 Scan Settings

	Settings
Scan region	Scan Area (61 x 21.6 mm)
Scan resolution (µm)	5
5µm scanning mode	Single Pass
eXtended Dynamic range	(selected)
Dye channel	Green
Green PMT	XDR Hi 100% XDR Lo 10%

To change any settings, click **Settings > Modify Default Settings**. A window pops up from which you can change the settings.

4 Select settings for the automatic file naming

- **Prefix 1** is set to **Instrument Serial Number**
- **Prefix 2** is set to **Array Barcode**

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

6 Click **Scan Slot *m*-*n*** on the Scan Control main window where the letter ***m*** represents the Start slot where the first slide is located and the letter ***n*** represents the End slot where the last slide is located.

Gene Pix scanner settings

Only GenePix 4000A and 4000B scanners are supported for scanning Agilent gene expression microarrays.

Refer to the manufacturer's user guide for appropriate scanner settings.

Refer to "General Microarray Layout and Orientation" on page 60 for appropriate slide layout and orientation in GenePix scanner.

NOTE Agilent 4x44K microarrays require 5 µm scan resolution, which is only supported in GenePix 4000B.

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

Feature Extraction (FE) 9.5.3 supports extraction of one-color .tif images of Agilent microarrays scanned on Agilent Scanner or GenePix (Axon/Molecular Devices) scanner.

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

1 Open the Agilent Feature Extraction (FE) software version 9.5.3.

   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 or GenePix (4000A or 4000B) 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 3 below are selected.

d In the **Other** section, choose a QC Metric Set for the project. For Agilent one-color microarrays, select **GE1_QCM_Feb07.**

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
Visual Results	Local file only
Grid	Local file only
QC Report	Local 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	True
⊟ Other	
QC Metric Set	
External DyeNorm List File	
Overwrite Previous Results	False

Figure 3 Default settings

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-v5_95_Feb07**.

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.

NOTE If scans are done with an Agilent scanner in XDR mode, the High and Low images are automatically combined when imported into the Feature Extraction software version 9.1 or newer. Images are not combined with non-Agilent scanned images.

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.

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.

4 Supplemental Procedures

The procedures in this chapter are optional but recommended.

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.

The integrity of the input template RNA, as well as labeled cRNA should be determined prior to labeling/amplification and hybridization respectively, using 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 10 and Table 11 to make sure that you have the appropriate analyzer, kits, and compatible assays.

Table 10 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

Table 11 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 4.

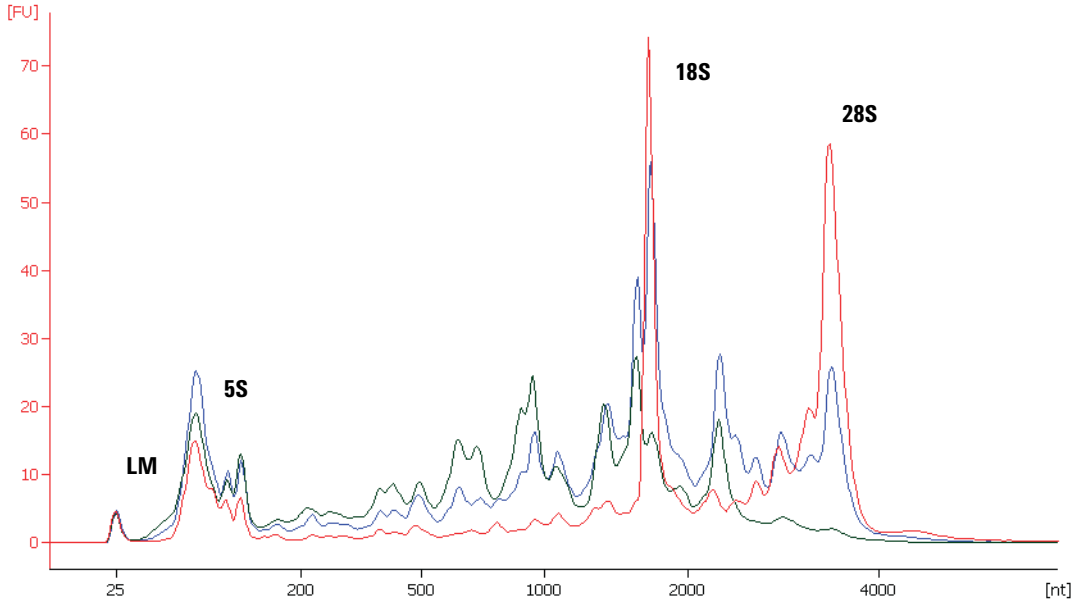


Figure 4 Analysis of (human) total RNA with the Eukaryote total RNA Nano assay using three different samples with decreasing integrity: Red, RIN 8.4; Blue, RIN 5.9; Green, RIN 3,6. Characteristic regions for ribosomal peaks and the lower marker (LM) are displayed.

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 no bands are in this range, and distinct bands less than 200 nucleotides in length exist, *do not* proceed with that sample because it has likely been degraded and will not provide accurate results. See Figure 5.

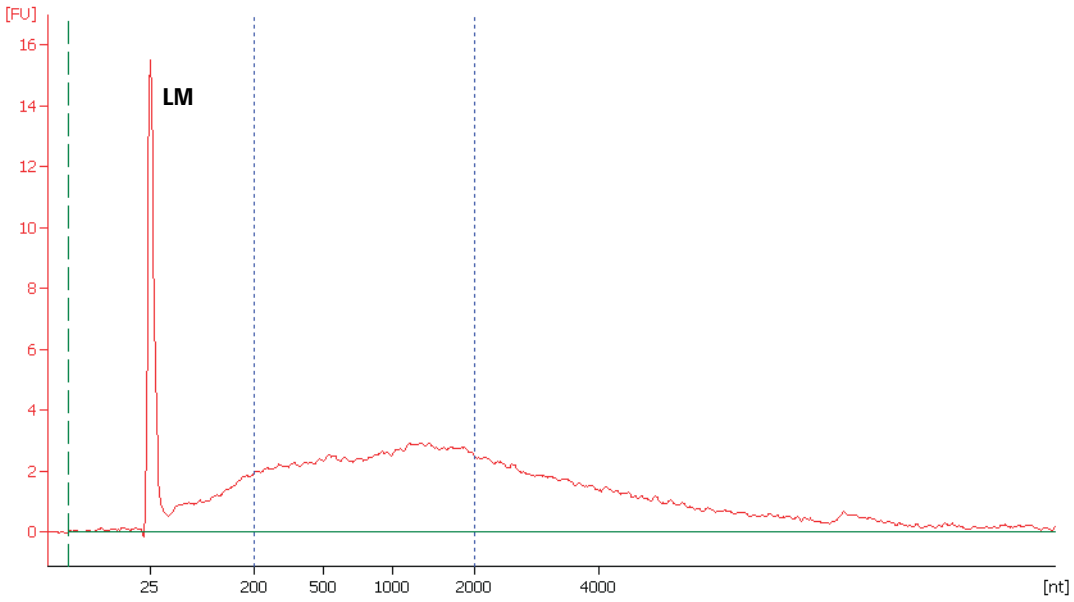


Figure 5 Smear analysis on non-fragmented Cy3 labeled cRNA allows determination of relative concentration of products within size regions. For example, 52 % of overall RNA signal results from fragments with size from 200 to 2000 nt.

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.

Preventing Ozone-Related Problems

While cyanine 3 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 cyanine 3 signal and compromise microarray performance. The Agilent Stabilization and Drying Solution is designed to protect against ozone-induced degradation of cyanine dyes and is recommended when using Agilent oligo-based microarrays in high-ozone environments. In addition, the organic solvent based wash described in the following wash procedure can reduce background variability produced by wash artifacts.

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

Cyanine 3 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 our technical note on **Improving Microarray Results by Preventing Ozone-Mediated Fluorescent Signal Degradation** (publication 5989-0875EN).

NOTE 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. Gloves and eye/face protection should be used in every step of the warming procedures.**

Table 12 lists the wash conditions for the wash procedure with Stabilization and Drying Solution.

Table 12 Wash conditions

	Dish	Wash Buffer	Temperature	Time
Acetonitrile Wash	1	Acetonitrile	Room temperature	10 seconds
3rd wash	2	Stabilization and Drying Solution	Room temperature	30 seconds

1 1 In the fume hood, fill slide-staining dish #1 approximately three-fourths full with acetonitrile. Add a magnetic stir bar and place this dish on a magnetic stir plate.

2 In the fume hood, fill slide-staining dish #2 approximately three-fourths full with Stabilization and Drying Solution. Add a magnetic stir bar and place this dish on a magnetic stir plate.

3 If you haven't already done so, remove the MTP slide adapter from the Tecan HS Pro hybridization station.

4 Remove the slides from the MTP slide adapter and put them in a slide rack.

5 Immediately transfer the slide rack to slide-staining dish #1 containing acetonitrile, and stir using setting 4 for 10 seconds.

6 Transfer slide rack to slide-staining dish #2 filled with Stabilization and Drying Solution, and stir using setting 4 for 30 seconds.

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

8 Scan slides immediately to minimize impact of environmental oxidants on signal intensities. If necessary, store slides in original slide boxes in a N2 purge box, in the dark.

9 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 9.0.

Note that the default normalization scheme for Agilent one-color data in the GeneSpring GX 9 program is 75th percentile normalization.

For more information on the GeneSpring GX 9 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.

5 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.

G2566-90009 Agilent G2565AA and G2565BA Microarray Scanner System User Manual

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.

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 (G2565BA)

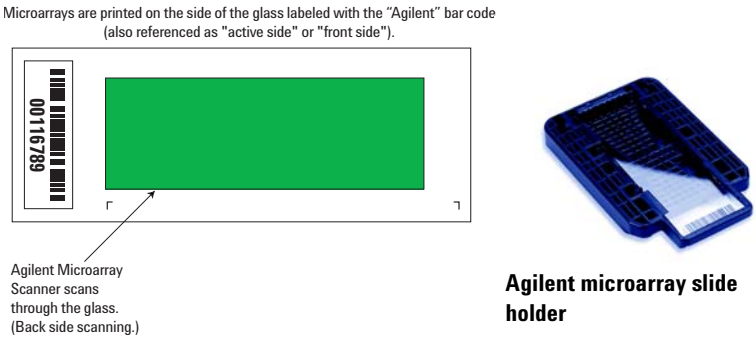


Figure 6 Agilent microarray slide and slide holder. The opposite or "non-active" numerically barcoded side is shown.

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 6 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 7).

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.

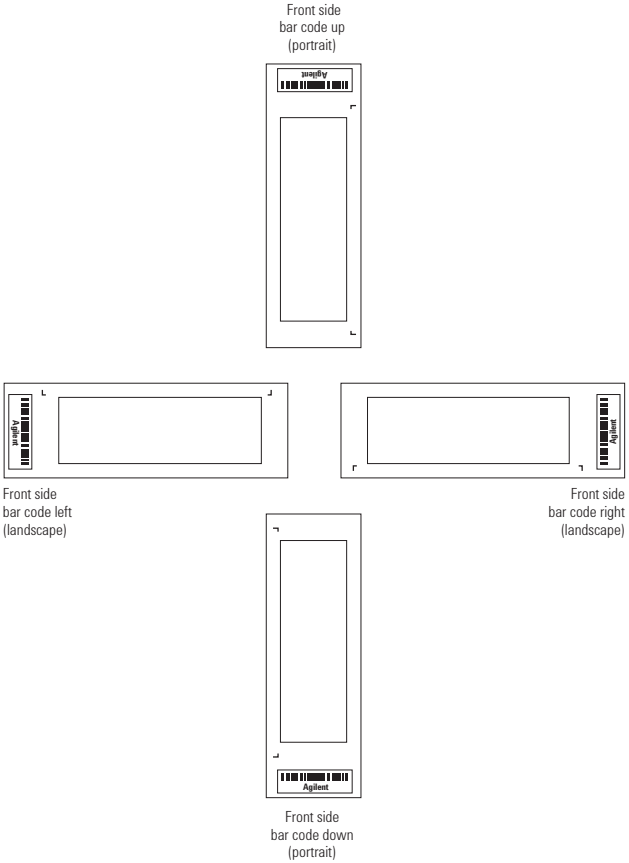


Figure 7 Microarray slide orientation

Array/Sample tracking on a 4x44K array slide

Use the form below to make notes to track your samples on a 4-pack array slide.

Arrays

	Array 1_1	Array 1_2	Array 1_3	Array 1_4
BARCODE	**Sample:** _____ _____ _____	**Sample:** _____ _____ _____	**Sample:** _____ _____ _____	**Sample:** _____ _____ _____

Barcode number _____

Related Microarray Reagents

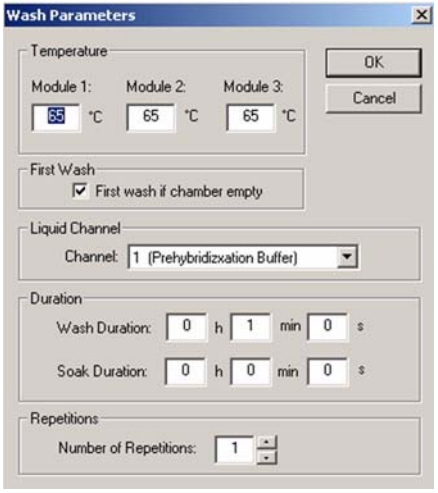
Description	Vendor and part number
Universal Human Reference RNA	Stratagene p/n 740000
Universal Mouse Reference RNA	Stratagene p/n 740100
Universal Rat Reference RNA	Stratagene p/n 740200
Fairplay III Microarray Labeling Kit	Stratagene p/n 252012

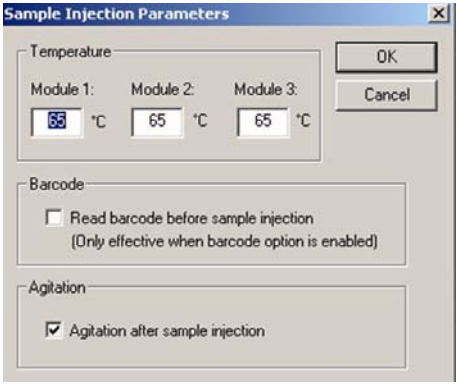
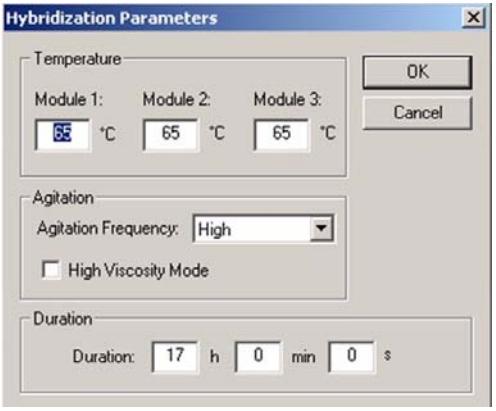
Agilent Gene Expression Program Settings

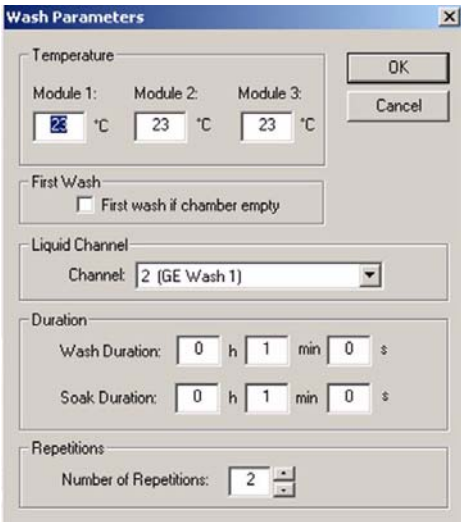
The Agilent Gene Expression program is run by the Tecan HS Pro Control Manager software to hybridize Agilent microarrays. The program contains appropriate defaults for Agilent microarrays.

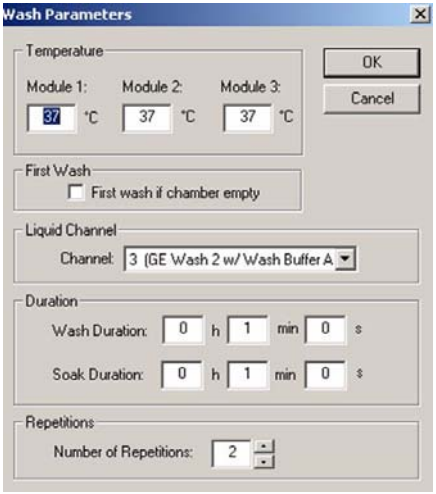
You can download the Agilent Gene Expression program at http://www.opengenomics.com/Hardware.aspx.

The steps in the program are described here. The graphics in the table contain the specific parameters that are contained in the program.

Step Number	Program Step	Action
1	**Wash (Pre-hybridization)**	**Agilent Prehybridization Buffer at 65° C** The first wash step prepares the slides for sample injection using the Agilent Prehybridization Buffer with the parameters shown below. 

Step Number	Program Step	Action
2	**Sample Injection**	**Sample Loading (55 µL)** Refer to the *Instructions for use for HS 4800 Pro/HS 400 Pro Hybridization* Station for proper technique of sample injection. 
3	**Hybridization**	**65° C for 17 hours** The hybridization parameters include a 17 hour hybridization at 65°C with High agitation frequency. 

Step Number	Program Step	Action
4	Wash	**Gene Expression Wash Buffer 1 at Room Temp** The wash 1 step is 2 cycles at room temperature using Gene Expression Wash Buffer 1. 

Step Number	Program Step	Action
5	Wash	**Gene Expression Wash Buffer 2 at 37°C** The wash 2 step is 2 cycles at 37°C using Gene Expression Wash Buffer 2 with 0.01% Wash Buffer Additive 
6	Drying	**2 minutes at 30°C** The 2 minute drying step prepares the slides for immediate scanning or the optional use of the Stabilization and Drying Solution. 

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In This Book

This guide contains information to run the One-Color Microarray-Based Gene Expression Analysis (Quick Amp Labeling) with Tecan HS Pro Hybridization protocol.

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