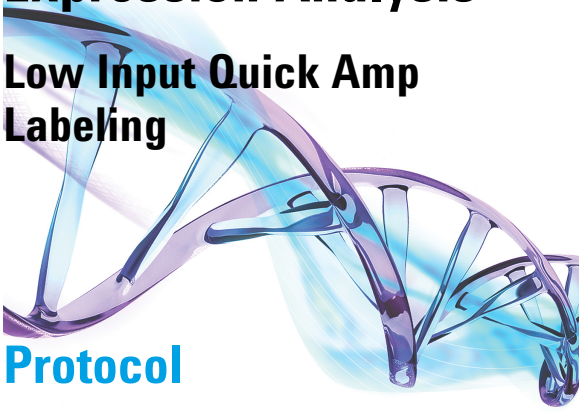
One-Color Microarray-Based Gene Expression Analysis

Low Input Quick Amp Labeling



Protocol

For use with Agilent Gene Expression oligo microarrays

Version 6.9.1, August 2015

Microarrays manufactured with Agilent SurePrint Technology

For Research Use Only. Not for use in diagnostic procedures.

Before you begin, view hands-on videos of SurePrint procedures at
http://www.agilent.com/genomics/protocolvideos.

Agilent Technologies

Notices

Manual Part Number

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CAUTION

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In this Guide...

This document describes the Agilent recommended procedures to prepare and label complex biological targets and hybridization, washing, scanning, and feature extraction of Agilent 60-mer oligonucleotide microarrays for microarray-based one-color gene expression 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 new in 6.9

- Corrected fragmentation mix table.
- Updated QC spec for specific labeling activity.
- Updated product labeling statement.

What's new in 6.8

- Updated list of supported microarray and resorted list by species.
- Expanded instructions to prepare hybridization assembly.

What's new in 6.7

- Expanded solvent wash details to prepare for microarray wash.
- Added list of supported microarrays.
- Added note to calibrate hybridization oven on a regular basis for accuracy of the collected data.
- Corrected the T7 reagent that is used in labeling reaction preparation step.
- Updated loading instructions for hybridization oven.
- Added reference to compatibility matrix for non-Agilent scanners.

What's new in 6.6

- Support for Agilent SureScan microarray scanner.

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 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 spin in a 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 dye reagents are potential carcinogens. 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 the 2× Hi-RPM 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 the 2× Hi-RPM Hybridization Buffer.**
- **Triton is harmful if swallowed. Risk of serious damage to eyes. Wear suitable PPE. Triton is a component of the 2× Hi-RPM 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.**
- **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 Microarrays

For more information on microarray designs visit the following web site: http://www.chem.agilent.com

To get design files or create a custom design, go to the Agilent eArray web site at http://earray.chem.agilent.com.

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.

Two, four or eight microarrays printed on each 1-inch × 3-inch glass slide

Catalog SurePrint HD and G3 Microarrays and Microarray Kits

Table 1 Catalog SurePrint G3 and HD Microarrays - Human

Part Number	Description
G4851C	G3 Human Gene Expression 8×60K v3 Microarray Kit (3 slides)
G4858A-072363	G3 Human Gene Expression 8×60K v3 Microarray (1 slide)
G4851B	G3 Human Gene Expression 8×60K v2 Microarray Kit (3 slides)
G4858A-039494	G3 Human Gene Expression 8×60K v2 Microarray (1 slide)
G4851A	G3 Human GE 8×60K Microarray Kit (3 slides)
G4858A-028004	G3 Human GE 8×60K Microarray (1 slide)
G4845A	HD Human GE 4×44K v2 Microarray Kit (5 slides)
G2519F-026652	HD Human GE 4×44K v2 Microarray (1 slide)

Table 2 Catalog SurePrint G3 and HD Microarrays - Mouse

Part Number	Description
G4852B	G3 Mouse GE 8×60K v2 Microarray Kit (3 slides)
G4858A-074809	G3 Mouse GE 8×60K v2 Microarray (1 slide)
G4852A	G3 Mouse GE 8×60K Microarray Kit (3 slides)
G4858A-028005	G3 Mouse GE 8×60K Microarray (1 slide)
G4846A	HD Mouse GE 4×44K v2 Microarray Kit (5-slides)
G2519F-026655	HD Mouse GE 4×44K v2 Microarray (1 slide)
G4122F	HD Whole Mouse Genome Microarray Kit, 4×44K (5 slides)
G2519F-014868	HD Whole Mouse Genome Microarray Kit, 4×44K (1 slide)

Table 3 Catalog SurePrint G3 and HD Microarrays - Rat

Part Number	Description
G4853B	G3 Rat GE 8×60K v2 Microarray Kit (3 slides)
G4858A-074036	G3 Rat GE 8×60K v2 Microarray (1 slide)
G4853A	G3 Rat GE 8×60K Microarray Kit (3 slides)
G4858A-028279	G3 Rat GE 8×60K Microarray (1 slide)
G4847B	HD Rat GE 4×44K v3 Microarray Kit (3 slides)
G2519F-028282	HD Rat GE 4×44K v3 Microarray (1 slide)

Table 4 Catalog SurePrint HD Microarrays and Microarray Kits - Model Organisms/Non-Human

Part Number	Description
G2519F-021169	Arabidopsis (V4) Gene Expression Microarray, 4×44K (1 slide)
G2519F-021623	Barley Gene Expression Microarray, 4×44K (1 slide)
G2519F-023647	Bovine (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-022520	Brassica Gene Expression Microarray, 4x44K (1 slide)
G2519F-021193	Canine (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-020186	C. elegans (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-026441	Chicken (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-022523	Cotton Gene Expression Microarray, 4×44K (1 slide)
G2519F-021791	Drosophila Gene Expression Microarray, 4×44K (1 slide)
G2519F-021322	Horse Gene Expression Microarray, 4×44K (1 slide)
G2519F-015060	Magnaporthe (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-022524	Medicago Gene Expression Microarray, 4×44K (1 slide)
G2519F-020449	Mosquito Gene Expression Microarray, 4×44K (1 slide)
G2519F-026440	Porcine (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-026806	Rhesus Macaque (V2) Gene Expression Microarray, 4×44K (1 slide)
G2519F-020908	Rabbit Gene Expression Microarray, 4×44K (1 slide)
G2519F-015241	Rice Gene Expression Microarray, 4×44K (1 slide)
G2519F-020938	Salmon Gene Expression Microarray, 4×44K (1 slide)
G4813A-019921	Sheep Gene Expression Microarray, 8×15K (1 slide)
G2519F-021113	Tobacco Gene Expression Microarray, 4×44K (1 slide)
G2519F-022297	Wheat Gene Expression Microarray, 4×44K (1 slide)
G4813A-016322	Yeast (V2) Gene Expression Microarray, 8×15K (1 slide)
G2519F-026437	Zebrafish (V3) Gene Expression Microarray, 4×44K (1 slide)

Custom Microarrays

One, two, four, or eight microarrays printed on each 1-inch × 3-inch glass slide.

Table 5 Custom SurePrint HD Microarrays

Part Number	Description
G4502A	Custom Gene Expression Microarray, 1×244K
G4503A	Custom Gene Expression Microarray, 2×105K
G2514F	Custom Gene Expression Microarray, 4×44K
G2509F	Custom Gene Expression Microarray, 8×15K

Table 6 Custom SurePrint G3 Microarrays

Part Number	Description
G4860A	SurePrint G3 Custom Gene Expression Microarray, 1×1M
G4861A	SurePrint G3 Custom Gene Expression Microarray, 2×400K
G4862A	SurePrint G3 Custom Gene Expression Microarray, 4×180K
G4102A	SurePrint G3 Custom Gene Expression Microarray, 8×60K

Required Equipment

Table 8 Required Equipment

Description	Vendor and part number
Agilent Microarray Scanner	Agilent p/n G4900DA, G2565CA or G2565BA
Hybridization Chamber, stainless	Agilent p/n G2534A
Hybridization 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
Go to www.agilent.com/genomics to see all available kit configurations.	

Table 8 Required Equipment (continued)

Description	Vendor and part number
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 tube	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
circulating water baths or heat blocks set to 37°C, 40°C, 60°C, 65°C, 70°C, and 80°C,	Corning p/n 6795-420 or equivalent
microcentrifuge	Eppendorf p/n 5417R or equivalent
sterile storage bottle	Nalgene 455-1000 or equivalent
spectrophotometer	NanoDrop p/n ND-1000 UV-VIS or equivalent
micropipettor	Pipetman P-10, P-20, P-200, P-1000 or equivalent
slide-staining dish, with slide rack (×3)	Thermo Shandon p/n 121 or equivalent
clean forceps	
ice bucket	
powder-free gloves	
sterile, nuclease-free aerosol barrier pipette tips	
vortex mixer	
timer	
nitrogen purge box for slide storage	

Required Reagents

Table 9 Required Reagents

Description	Vendor and part or catalog number
Low Input Quick Amp Labeling Kit, One-Color	Agilent p/n 5190-2305
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
RNeasy Mini Kit	Qiagen p/n 74104
ethanol (95% to 100% molecular biology grade)	Sigma-Aldrich p/n E7023-6×500ML
Milli-Q water or equivalent	
isopropyl alcohol (molecular biology grade)	

Optional Equipment/Reagents

Table 10 Optional Equipment/Reagents

Description	Vendor and part number
2100 Bioanalyzer	Agilent p/n G2939AA
RNA 6000 Nano Assay Kit (RNA Series II Kit)	Agilent p/n 5067-1511
Quick Amp Labeling Kit, One-Color	Agilent p/n 5190-0442
Stabilization and Drying Solution*	Agilent p/n 5185-5979
Ozone-Barrier Slide Cover*	Agilent p/n G2505-60550
Absolutely RNA Nanoprep Kit	Agilent p/n 400753
slide box	Corning p/n 07201629
acetonitrile	Sigma p/n 271004-1L
sulfolane	Sigma p/n T22209
thermal cycler	
PCR 96-well plate or 0.2 mL PCR tubes	

* Recommended when processing microarrays in high ozone environment.

Required Hardware and Software

Table 11

Description
Feature Extraction software 10.7.1 or later
Agilent Scan Control software. Refer to Agilent Scanner user guide for specifications.
For system and supported Internet Explorer/Adobe Reader versions, please see the System Requirements for your Feature Extraction and Scan Control Software.

Optional Software

Table 12

Description
GeneSpring GX 9.0 or later

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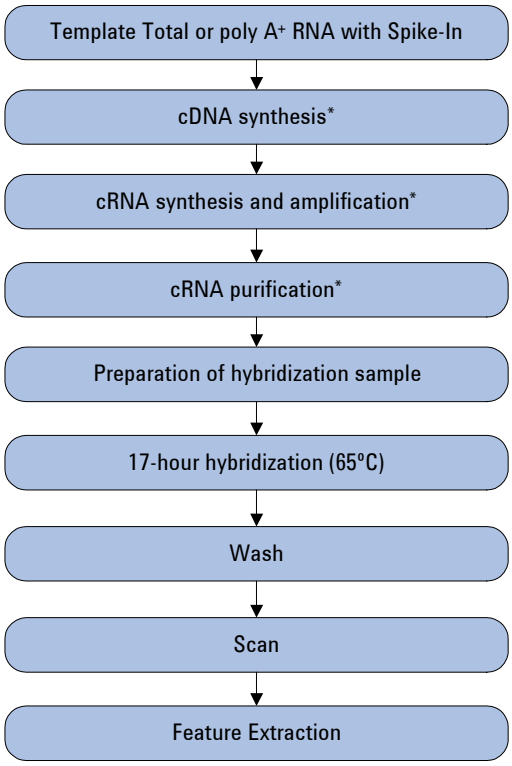
2 Procedures

The Agilent 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.



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

Figure 1 Workflow for sample preparation and array processing.

Sample Preparation

The Low Input Quick Amp Labeling Kit, One-Color generates fluorescent cRNA (complimentary RNA) with a sample input RNA range between 10 ng and 200 ng of total RNA or a minimum of 5 ng of poly A^+ RNA for one-color processing. The method uses T7 RNA Polymerase Blend (red cap), which simultaneously amplifies target material and incorporates Cyanine 3-CTP. Amplification is typically at least a 100-fold from total RNA to cRNA with the use of this kit.

NOTE

For optimal performance, use pure high quality, intact template total or poly A+ 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 73 for general guidance and procedural recommendations on quality assessment of template RNA.

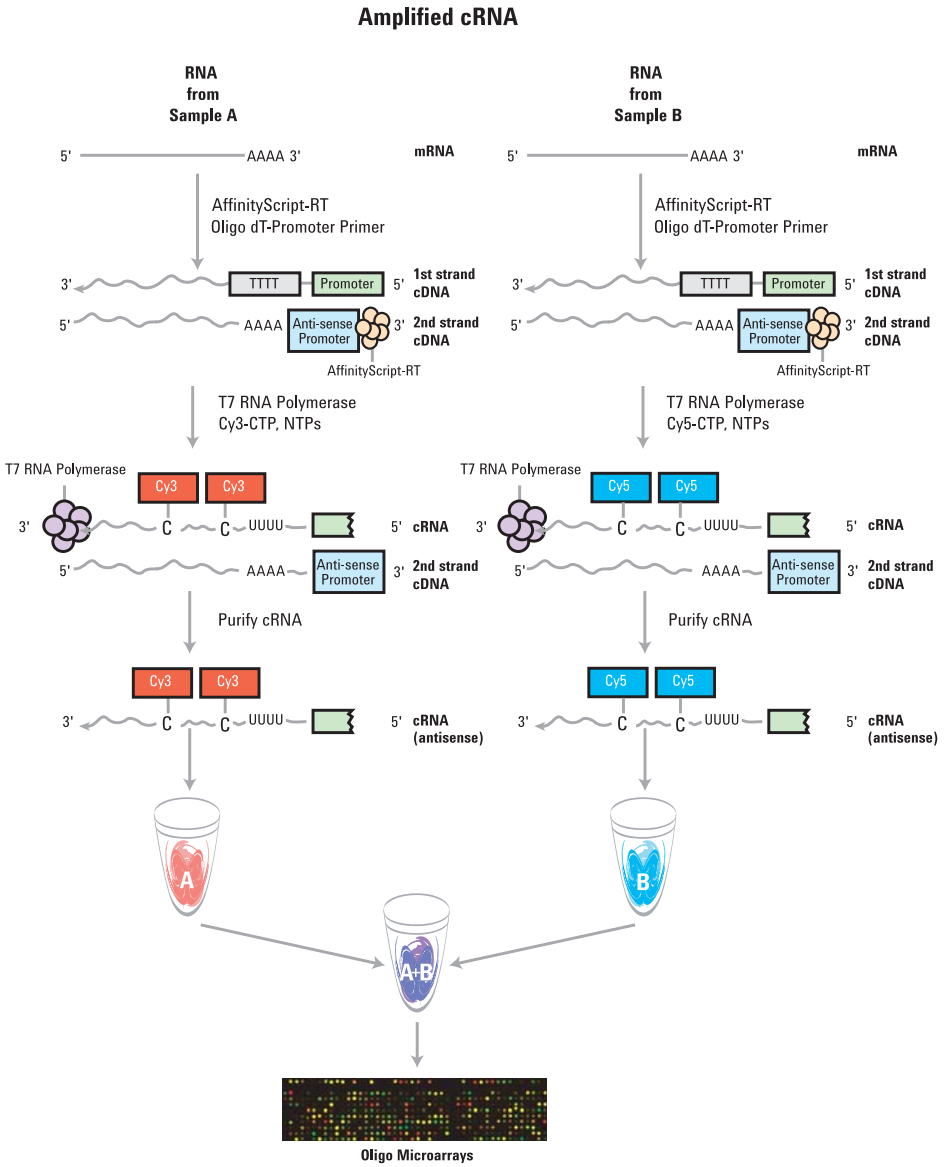


Figure 2 Schematic of amplified cRNA procedure. Generation of cRNA for a two-color microarray experiment is shown.

Step 1. Prepare Spike Mix

(Time required: ~0.5 hours)

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

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

2 Vigorously mix the Spike Mix 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 13 provides the dilutions of Spike Mix for a range of total RNA input amounts. For inputs not shown Table 13, make sure that the amount of spike mix is proportional to the amount of RNA input. If you start with 5 ng mRNA as the input mass, follow the dilution scheme as described in Table 13.

Table 13 Dilutions of 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)	First	Second	Third	Fourth	
10		1:20	1:25	1:20	1:10	2
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
200		1:20	1:25	1:10		2
	5	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 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 drive 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 in a microcentrifuge 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 in a microcentrifuge 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 in a microcentrifuge 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 in a microcentrifuge 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 13 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 26.

Storage of Spike Mix dilutions

Store the 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.

Store the first dilution of the Spike Mix positive controls for up to 2 months in a non-defrosting freezer at -70°C to -80°C. Do not freeze/thaw more than 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 plus diluted RNA spike-in controls does not exceed 3.5 µL. Because the 1× reaction involves volumes of less than 1 µL, prepare components in a master mix and divide into the individual assay tubes in volumes >1 µL. When preparing 4 samples, use the 5× master mix. When preparing 8 samples, use the 10× master mix.

NOTE

The starting input for the Low Input Quick Amp Labeling Kit, One-Color ranges from 10 ng to 200 ng of total RNA. For best results, start with at least 25 ng of total RNA for the 4-pack and 8-pack formats, and 50 ng of total RNA for the 1-pack and 2-pack formats. For the 8-pack microarray format, as little as 10 ng of total RNA can be used to generate high quality data.

1. Add 10 to 200 ng of total RNA to a 1.5-mL microcentrifuge tube in a final volume of 1.5 µL. If samples are concentrated, dilute with water until 10 to 200 ng of total RNA is added in a 1.5 µL volume. Dilute the total RNA just prior to use and store the total RNA at concentrations over 100 ng/µL.
2. Add 2 µL of diluted Spike Mix to each tube. Each tube now contains a total volume of 3.5 µL.

3 Prepare T7 Primer Mix and add to sample:

a Mix T7 Primer (green cap) and water as listed in Table 14.

Table 14 T7 Primer Mix

Component	Volume (µL) per reaction	Volume (µL) per 5 reaction	Volume (µL) per 10 reactions
T7 Primer (green cap)	0.8	4	8
Nuclease-free Water	1	5	10
Total Volume	**1.8**	**9**	**18**

b Add 1.8 µL of T7 Primer Mix into each tube that contain 3.5 µL of total RNA and diluted RNA spike-in controls. 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 Put the reactions on ice and incubate for 5 minutes.

4 Prewarm the 5× First Strand Buffer (green cap) 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.

5 Prepare and add cDNA Master Mix:

a Immediately prior to use, add the components in Table 15 to a 1.5-mL microcentrifuge tube. Use a pipette to gently mix. Keep at room temperature.

The Affinity Script RNase Block Mix (violet cap) is a blend of enzymes. Keep the Affinity Script RNase Block Mix (violet cap) on ice and add to the cDNA Master Mix immediately prior to use.

Table 15 cDNA Master Mix

Component	Volume (µL) per reaction	Volume (µL) per 5 reaction	Volume (µL) per 10 reactions
5× 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
Affinity Script 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.

6 Prepare and add Transcription Master Mix:

a Immediately prior to use, add the components in Table 16 in the order listed into a 1.5 mL microcentrifuge tube. Use a pipette to gently mix. Keep at room temperature.

The T7 RNA Polymerase Blend (red cap) is a blend of enzymes. Keep the T7 RNA Polymerase Blend (red cap) on ice and add to the Transcription Master Mix just before use.

Table 16 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
5× 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)

Use the RNeasy Mini Kit to purify the amplified cRNA samples.

If sample concentration causes difficulty, you can use the Absolutely RNA Nanoprep Kit as an alternative. See "Absolutely RNA Nanoprep Purification" on page 58.

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* spin in a centrifuge.
4. Transfer the 700 µL of the cRNA sample to an RNeasy Mini Spin Column (pink) in a Collection Tube (2 ml). Spin the sample in a centrifuge 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 (2 ml) and add 500 µL of Buffer RPE (containing ethanol) to the column. Spin the sample in a centrifuge 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 Collection Tube (1.5 ml) and spin the sample in a centrifuge 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 Collection Tube (1.5 ml) 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 Collection Tube (1.5 ml). 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

Use the NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.2.1 (or higher) to quantify the cRNA.

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 17 for the recommended cRNA yields and specific activities for hybridization.

CAUTION If the specific activity does not meet the requirements listed in Table 17, do not continue to hybridization. Repeat preparation of cRNA.

Table 17 Recommended Yields and Specific Activity

Microarray format	Yield (µg)	Specific Activity (pmol Cy3 per µg cRNA)
1-pack	5	≥6
2-pack	3.75	≥6
4-pack	1.65	≥6
8-pack	0.825	≥6

NOTE Please refer to "Quality Assessment of Template RNA and Labeled cRNA" on page 73 for general guidance and procedural recommendations on quality assessment of labeled cRNA.

Hybridization

An instructional video that shows hybridization and washing steps can be found at http://genomics.agilent.com. Search for “Running a microarray experiment”.

If you are a first time user, practice the hybridization process before you begin. Use water instead of blocking mix, and use a clean microscope slide and a gasket slide. Make sure you mix and apply the hybridization solution with minimal bubbles. Practice the hyb assembly and the slide disassembly and wash.

CAUTION You must calibrate the hybridization oven regularly for accuracy of the collected data. Refer to *Agilent G2545A Hybridization Calibration Procedure* (p/n G2545-90002, version A1 or higher) for more information.

Step 1. Prepare the 10× Blocking Agent

1 Add 500 µL of nuclease-free water to the vial containing lyophilized 10× Gene Expression Blocking Agent supplied with the Gene Expression Hybridization Kit, *or* add 1250 µL of nuclease-free water to the vial containing lyophilized large volume 10× Gene Expression Blocking Agent.

2 Gently mix on a vortex mixer. 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 that sticks to the tube walls or cap by spinning in a centrifuge for 5 to 10 seconds.

NOTE Divide the 10× Gene Expression Blocking Agent into aliquots small enough to keep the freeze-thaw cycle to 5 times or less. Store at -20°C for up to two months. Before use, repeat step 2 and step 3.

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 18 or Table 19 to a 1.5 mL nuclease-free microfuge tube:

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 18 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
10× Gene Expression Blocking Agent	50 µL	25 µL
Nuclease-free water	bring volume to 240 µL	bring volume to 120 µL
25× Fragmentation Buffer	10 µL	5 µL
Total Volume	250 µL	125 µL

Table 19 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
10× Gene Expression Blocking Agent	11 µL	5 µL
Nuclease-free water	bring volume to 52.8 µL	bring volume to 24 µL
25× 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 2× Hi-RPM Hybridization Buffer stops 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 2× Hi-RPM Hybridization Buffer to stop the fragmentation reaction. See Table 20.

Table 20 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
2× Hi-RPM Hybridization Buffer	250 µL	125 µL	55 µL	25 µL

7 Mix well by careful pipetting part way up and down. Do not introduce bubbles to the mix. The surfactant in the 2× Hi-RPM Hybridization Buffer easily forms 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 Put sample on ice and load onto the array as soon as possible.

Refer to "Microarray Handling Tips" on page 92 for information on how to safely handle microarrays.

Step 3. Prepare the hybridization assembly

Refer to the *Agilent Microarray Hybridization Chamber User Guide* for more details to load slides, and to assemble and disassemble the chambers. This user guide is included with the Agilent Microarray Hybridization Chamber Kit (G2534A) and can also be downloaded from the Agilent web site at www.genomics.agilent.com. Search for **G2534A**.

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. Make sure that the gasket slide is flush with the chamber base and is not ajar.

CAUTION

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

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

2 Slowly dispense the volume of hybridization sample (see Table 21) onto the gasket well in a “drag and dispense” manner.

- Position the slides so that the barcode label is to your left.
- Load the samples left to right. For 8-pack slides, start with the first row. The output files will come out in that same order. Refer to “Array/Sample tracking microarray slides” on page 96 for guidelines on tracking sample position for multipack slide formats.
- Avoid the introduction of air bubbles to the gasket wells. Air bubbles can affect the final sample volume and can cause leakage from the gasket well.

Table 21 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

3 If any wells are unused:

   a Make a 1× solution of the 2× Hi-RPM Hybridization Buffer.

   b Add the volume of 1× Hybridization Buffer equal to the sample volume to each unused well.

   Make sure all wells contain sample or 1× Hybridization Buffer. Empty wells can cause failure in hybridization.

4 Grip the slide on either end and slowly put the slide “active side” down, parallel to the SureHyb gasket slide, so that the “Agilent”-labeled barcode is facing down and the numeric barcode is facing up. Make sure that 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.

5 Put the SureHyb chamber cover onto the sandwiched slides and slide the clamp assembly onto both pieces.

6 Firmly hand-tighten the clamp onto the chamber.

7 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.

Step 4. Hybridize

1 Load each assembled chamber into the oven rotator rack. Start from the center of the rack (position 3 or 4 when counting from the left). Set your hybridization rotator to rotate at 10 rpm when using 2× Hi-RPM Hybridization Buffer.

2 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 (10%) to the Gene Expression wash buffers reduces the possibility of array wash artifacts. Add Triton X-102 (10%) to Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 2 when the cubitainer of wash buffer is first opened.

Do this step to *both* Gene Expression Wash Buffer 1 and Gene Expression Wash Buffer 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 Triton X-102 (10%) 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 (10%) has been added and indicate the date of addition.

Triton X-102 (10%) 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 storage bottle. Repeat until you have enough prewarmed Wash Buffer 2 solution for your experiment.

2 Tightly cap the sterile storage bottle and put in a 37°C water bath the night before washing arrays. Alternatively, remove the plastic cubitainer from the box and put 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.

Solvent wash

Wash staining dishes, racks and stir bars with acetonitrile or isopropyl alcohol to avoid wash artifacts on your slides and images.

- Use acetonitrile for equipment that was exposed to Stabilization and Drying Solution.
- Use isopropyl alcohol for equipment that was not exposed to Stabilization and Drying Solution.

WARNING **Conduct solvent 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 or isopropyl alcohol.

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 solvent 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".

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 the Agilent one-color platform must be done in environments where ozone levels are 50 ppb or less. For Scanner C and Scanner B, if ozone levels exceed 50 ppb in your laboratory, use the Agilent Ozone Barrier Slide Cover (described in this topic). SureScan microarray scanner uses a slide holder with a built-in ozone barrier.

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 22 lists the wash conditions for the wash procedure.

Table 22 Wash conditions

	Dish	Wash Buffer	Temperature	Time
Disassembly	1	Gene Expression Wash Buffer 1	Room temperature	
1st wash	2	Gene Expression Wash Buffer 1	Room temperature	1 minute
2nd wash	3	Gene Expression Wash Buffer 2	Elevated temperature*	1 minute

* 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.

1. Completely fill slide-staining dish #1 with Gene Expression Wash Buffer 1 at room temperature.
2. Put 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. Put this dish on a magnetic stir plate.
3. Put 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 Put 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 Grasp the top corner of the microarray slide, remove the slide, and then put it into the slide rack in the slide-staining dish #2 that contains Gene Expression Wash Buffer 1 at room temperature. Transfer the slide quickly so avoid premature drying of the slides. *Touch only the barcode portion of the microarray slide or its edges!*

   More effort is needed to separate the 4-pack and 8-pack sandwiched slides than the 1-pack and 2-pack sandwiched slides.

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.

10 Transfer slide rack to slide-staining dish #3 that contains Gene Expression Wash Buffer 2 at elevated temperature. Stir using setting 4, or a moderate speed setting, 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. If liquid remains on the bottom edge of the slide, dab it on a cleaning tissue.

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.

For SureScan microarray scanner

- Carefully put the end of the slide without the barcode label onto the slide ledge.
- Gently lower the microarray slide into the slide holder. Make sure that the active microarray surface (with "Agilent"-labeled barcode) faces up, toward the slide cover.
- Close the plastic slide cover, pushing on the tab end until you hear it click.
- For more detailed instruction, refer to the *Agilent G4900DA SureScan Microarray Scanner System User Guide*.

Figure 3 Slide in slide holder for SureScan microarray scanner

For Agilent Scanner B or C only:

- In environments in which the ozone level exceeds 50 ppb, immediately put the slides with active microarray surface (with "Agilent"-labeled barcode) facing up in a slide holder. Make sure that the slide is not caught up on any corner. Put an ozone-barrier slide cover on top of the array as shown in Figure 4. 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 79.

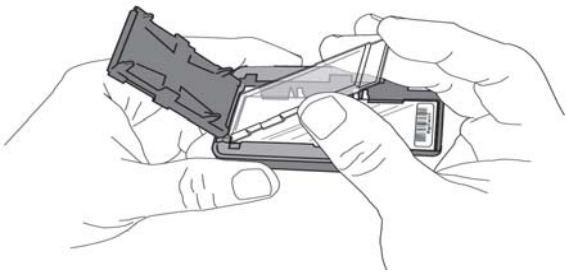


Figure 4 Inserting the ozone-barrier slide cover (shown for Scanner B and Scanner C)

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

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

Agilent provides support for Agilent microarrays scanned on select non-Agilent scanners. Please see “Feature Extraction Compatibility Matrix for Non Agilent scanners” for scanner compatibility and settings (http://www.chem.agilent.com/Library/usermanuals/Public/G1662-90043_ScannerCompatibilityMatrix.pdf).

Agilent can guarantee the quality of data only if the data comes from Agilent microarrays scanned on Agilent scanners.

A SureScan or Agilent C microarray scanner is required for SurePrint G3 formats.

To get scanner profiles from Agilent:

- For Scan Control 9.1.3 or later, go to http://www.genomics.agilent.com/article.jsp?pageId=2610
- For Scan Control 8.x, go to http://www.genomics.agilent.com/article.jsp?pageId=2074

Agilent SureScan Microarray Scanner

1. Put assembled slide holders into the scanner cassette.
2. Select the appropriate scanner protocol:
   - **AgilentG3_HiSen_GX_1color** (for G3 format, high-sensitivity mode)
   - **AgilentG3_GX_1color** (for G3 format, standard mode)
   - **AgilentHD_GX_1color** (for HD format)
3. Verify that the Scanner status in the main window says Scanner Ready.
4. Click **Start Scan**.

Agilent C Scanner Settings

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

2 Select Start Slot *m* End Slot *n* 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.

3 Select **Profile AgilentG3_GX_1color** (for SurePrint G3 formats) or **Profile AgilentHD_GX_1color** (for SurePrint HD formats).

4 Verify scan settings for one-color scans. See Table 23.

CAUTION Do not scan G3 microarrays with HD format settings. The resolution of the resulting image will not be high enough for data analysis.

Table 23 C Scanner Scan Settings

	For HD Microarray Formats	For G3 Microarray Formats
Dye channel	G (*green*)	G (*green*)
Scan region	Agilent HD (61 × 21.6 mm)	Agilent HD (61 × 21.6 mm)
Scan resolution	5 µm	3 µm
Tiff file dynamic range	20 bit	20 bit
Green PMT gain	100%	100%

5 Verify that **Output Path Browse** is set for desired location.

6 Verify that the Scanner status in the main window says Scanner Ready.

7 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.

Agilent B Scanner Settings

Agilent Scan Control software v7.0.03 is recommended for 5 µm scans of SurePrint HD formats. The Agilent B Scanner does not support G3 microarrays. For G3 microarrays, use the Agilent C Scanner or SureScan microarray scanner.

1 Put slide into slide holder, with or without the ozone-barrier slide cover, with Agilent barcode facing up.

2 Put assembled slide holders into scanner carousel.

3 Verify scan settings for one-color scans. See Table 24.

For version 7.X, to change any settings, click **Settings > Modify Default Settings**. A window pops up from which you can change the settings.

Table 24 B Scanner Scan Settings

	For All Formats
Scan region	Scan Area (61 × 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%

4 Select settings for the automatic file naming.
- **Prefix1** is set to **Instrument Serial Number**.
- **Prefix2** 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.

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.

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

1 Open the Agilent Feature Extraction (FE) program.

   To get the most recent Feature Extraction protocols for gene expression, go to 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 a 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 5 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 5 Default settings in FE 10.7.

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 http://earray.chem.agilent.com. 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 from the pull down menu. The appropriate protocol begins with “GE1” for one-color analysis.

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 When the Agilent XDR scanned images are added to Feature Extraction software version 9.1 or later, the High and Low images are automatically combined for data extraction.

NOTE 20-bit single images from the C Scanner are equivalent to 16-bit XDR images from the B Scanner.

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 7 and Figure 8.

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 *Enhanced Quality Assessment Using Agilent Feature Extraction QC Metric Sets, Thresholds, and Charting Tools* (p/n 5989-5952EN) 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. Search for the part number 5989-5952EN.

Automatic Download from eArray

Feature Extraction version 10.7 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 6.

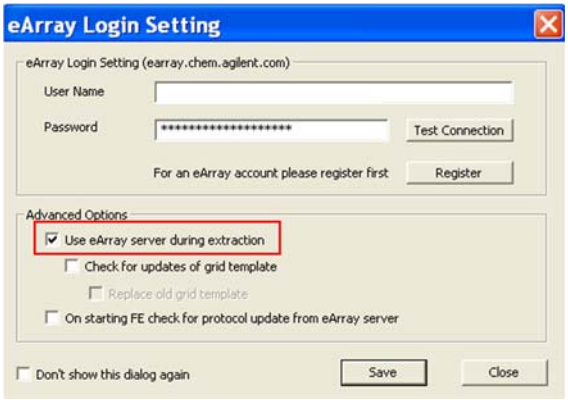
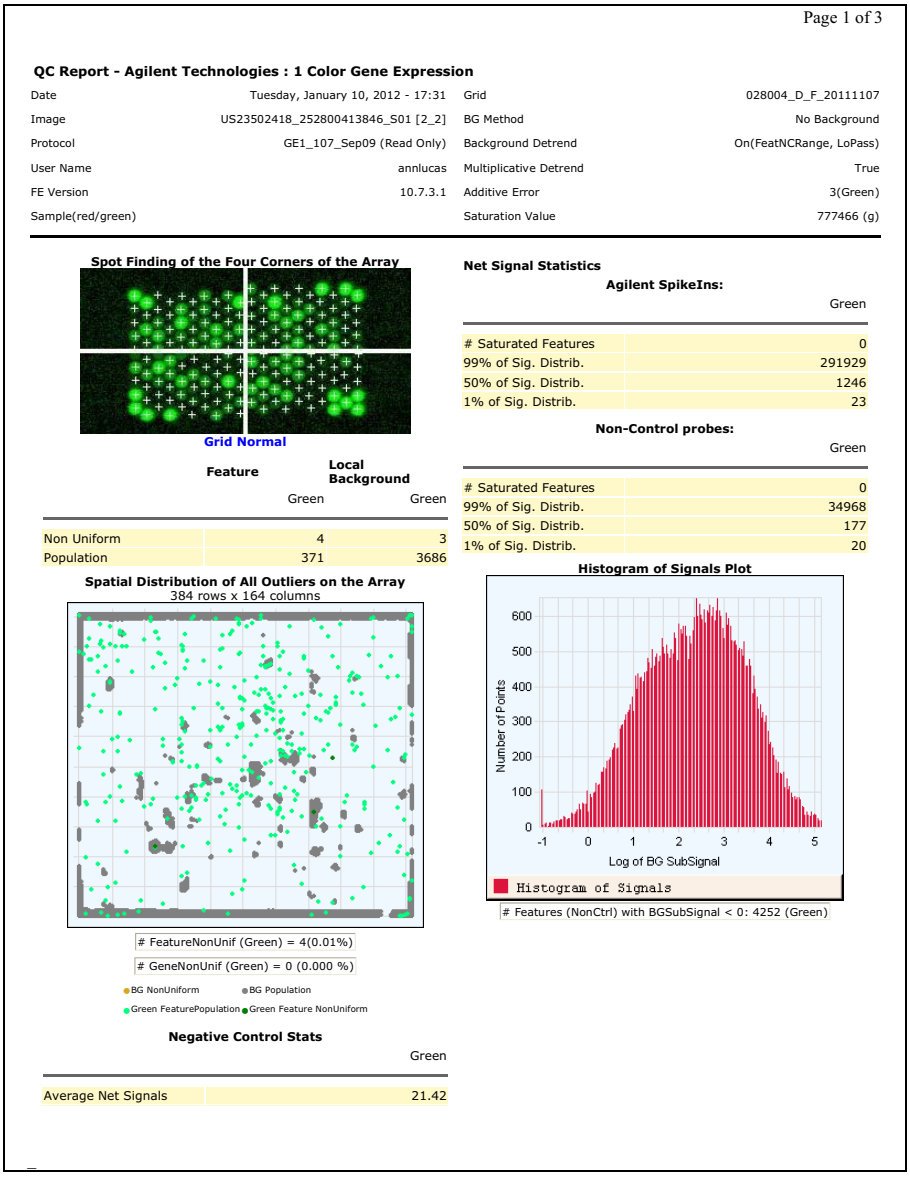


Figure 6 eArray Login Setting



Page 1 of 3

QC Report - Agilent Technologies : 1 Color Gene Expression

Date	Tuesday, January 10, 2012 - 17:31	Grid	028004_D_F_20111107
Image	US23502418_252800413846_S01 [2_2]	BG Method	No Background
Protocol	GE1_107_Sep09 (Read Only)	Background Detrend	On(FeatNCRange, LoPass)
User Name	annlucas	Multiplicative Detrend	True
FE Version	10.7.3.1	Additive Error	3(Green)
Sample(red/green)		Saturation Value	777466 (g)

Spot Finding of the Four Corners of the Array

Grid Normal

Feature	Local Background
Green	Green
Non Uniform 4	3
Population 371	3686

Spatial Distribution of All Outliers on the Array

384 rows x 164 columns

FeatureNonUnif (Green) = 4(0.01%)

GeneNonUnif (Green) = 0 (0.000 %)

BG NonUniform BG Population

Green FeaturePopulation Green Feature NonUniform

Negative Control Stats

	Green
Average Net Signals	21.42

Net Signal Statistics

Agilent SpikeIns:

	Green
# Saturated Features	0
99% of Sig. Distrib.	291929
50% of Sig. Distrib.	1246
1% of Sig. Distrib.	23

Non-Control probes:

	Green
# Saturated Features	0
99% of Sig. Distrib.	34968
50% of Sig. Distrib.	177
1% of Sig. Distrib.	20

Histogram of Signals Plot

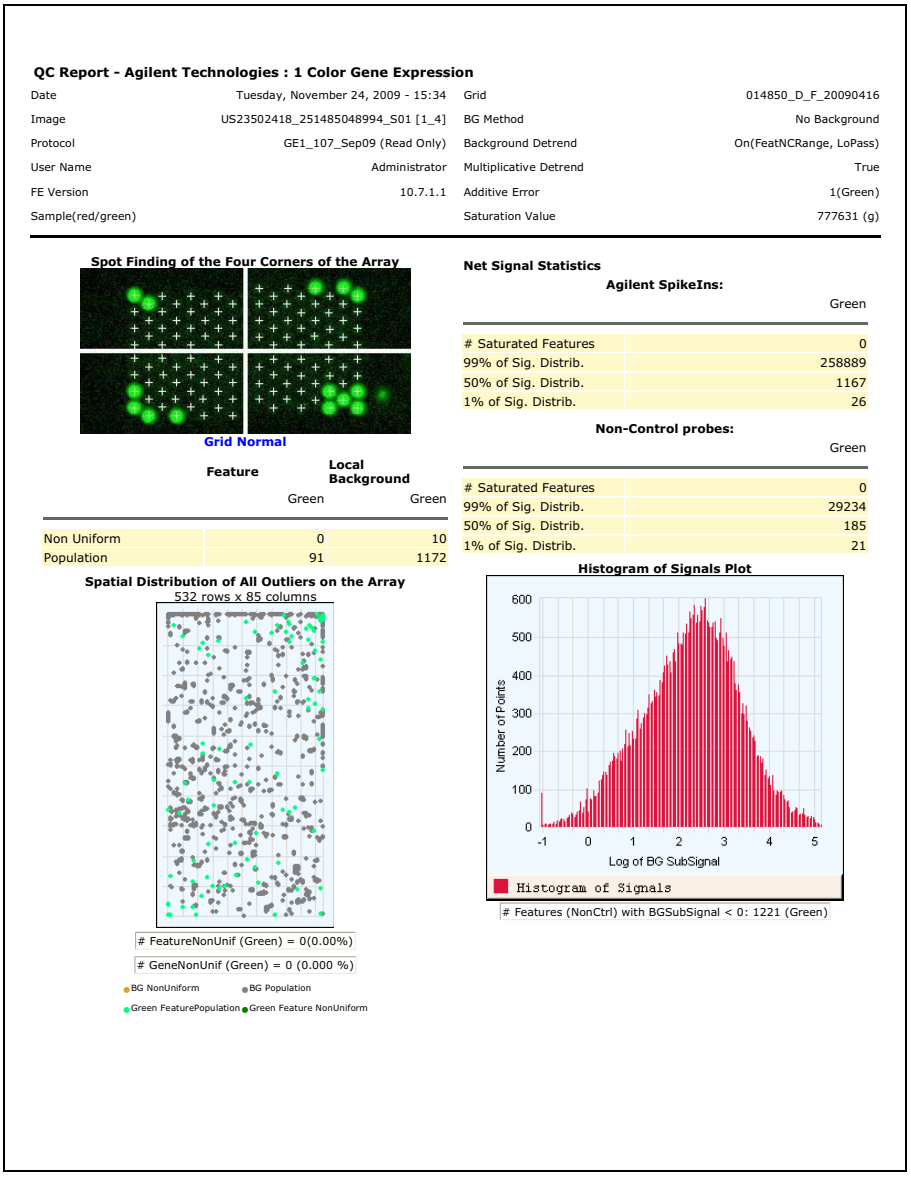
Number of Points

Log of BG SubSignal

Histogram of Signals

Features (NonCtrl) with BGSubSignal < 0: 4252 (Green)

Figure 7 Example of the first page of a QC Report for 8×60K microarray, generated by Feature Extraction Software



QC Report - Agilent Technologies : 1 Color Gene Expression

Date	Tuesday, November 24, 2009 - 15:34	Grid	014850_D_F_20090416
Image	US23502418_251485048994_S01 [1_4]	BG Method	No Background
Protocol	GE1_107_Sep09 (Read Only)	Background Detrend	On(FeatNCRange, LoPass)
User Name	Administrator	Multiplicative Detrend	True
FE Version	10.7.1.1	Additive Error	1(Green)
Sample(red/green)		Saturation Value	777631 (g)

Spot Finding of the Four Corners of the Array

Grid Normal

	Feature	Local Background
	Green	Green
Non Uniform	0	10
Population	91	1172

Spatial Distribution of All Outliers on the Array

532 rows x 85 columns

FeatureNonUnif (Green) = 0(0.00%)

GeneNonUnif (Green) = 0 (0.000 %)

BG NonUniform BG Population

Green FeaturePopulation Green Feature NonUniform

Net Signal Statistics

Agilent SpikeIns:

	Green
# Saturated Features	0
99% of Sig. Distrib.	258889
50% of Sig. Distrib.	1167
1% of Sig. Distrib.	26

Non-Control probes:

	Green
# Saturated Features	0
99% of Sig. Distrib.	29234
50% of Sig. Distrib.	185
1% of Sig. Distrib.	21

Histogram of Signals Plot

Number of Points

Log of BG SubSignal

Histogram of Signals

Features (NonCtrl) with BGSubSignal < 0: 1221 (Green)

Figure 8 Example of the first page of a QC Report for 4×44K microarray, generated by Feature Extraction Software

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3 Supplemental Procedures

The procedures in this chapter are optional but recommended.

Absolutely RNA Nanoprep Purification

As an alternative to the RNeasy Mini Kit, the Absolutely RNA Nanoprep Kit can be used to purify the amplified cRNA after "Step 2. Prepare labeling reaction" on page 26. Use the Absolutely RNA Nanoprep Kit when it is required 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 DNase/RNase-free distilled 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 1× high-salt wash buffer:

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

   b On the 1.67× High Salt Wash Buffer container, mark the check box for **1× (Ethanol Added)**.

   c Tighten the cap on the container of 1.67× High Salt Wash Buffer and store at room temperature.

3 Prepare the 1× low-salt wash buffer:

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

   b On the 5× Low Salt Wash Buffer container, mark the check box for **1× (Ethanol Added)**.

   c Tighten the cap on the container of 5× Low 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 RNA Binding Nano Spin Cup Cap onto the top of the spin cup.
6. Spin the sample in a microcentrifuge at ≥12,000 rpm for 60 seconds.
7. Remove and keep the RNA-binding nano-spin cup. Discard the filtrate. Put the RNA-binding nano-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 **1× High-Salt Wash Buffer** to the RNA-binding nano-spin cup. Cap the RNA-binding nano-spin cup, and spin the sample in a microcentrifuge at ≥12,000 rpm 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 **1× Low-Salt Wash Buffer** to the RNA-binding nano-spin cup. Cap the spin cup, and spin the sample in a microcentrifuge at ≥12,000 rpm 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 RNA-binding nano-spin cup back into the same 2-ml collection tube.
13. Add 300 µL of **1× Low-Salt Wash Buffer** to the RNA-binding nano-spin cup. Cap the RNA-binding nano-spin cup, and spin the sample in a microcentrifuge at ≥12,000 rpm 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 RNA-binding nano-spin cup. Cap the RNA-binding nano-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 rpm 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, One-Color.

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 200 ng of total RNA to a 0.2 mL PCR tube or the well of a 96-well PCR plate in a volume of 1.5 µL. For optimal performance, use at least 25 ng of input total RNA.
2. Add 2 µL of the diluted Spike Mix. Please refer to Table 13 on page 23 for detailed instructions on the preparation and use of Spike-in kits.
3. Prepare the T7 Primer (green cap) Master Mix as described in Table 14 on page 27.
4. Add 1.8 µL of T7 Promoter Primer Mix to the tube that contains 3.5 µL of total RNA and diluted RNA spike-in controls. Each tube now contains a total volume of 5.3 µL.
5. Put the tubes in the thermocycler and run Program 1 to denature the template and anneal the primer.
6. Keep the reaction tubes in the thermocycler at 4°C, or move to bench top rack on ice.
7. Immediately prior to use, gently mix the components in Table 15 on page 28 in the order listed by pipetting, and keep at room temperature.

NOTE Prewarm the 5× First Strand Buffer (green cap) 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 Affinity Script RNase Block Mix (violet cap) on ice. Do not add the Affinity Script RNase Block Mix (violet cap) until just before you start the reactions.

8. 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.
9. 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 16 on page 29 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 (red cap) and Cyanine 3-CTP.

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

NOTE

Do not add the T7 RNA Polymerase Blend (red cap) to Transcription Master Mix until just before you do the reaction.

2 Keep the reaction tubes from step 9 above in the thermocycler at 4°C, or move to bench top 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 61) to synthesize labeled cRNA.

5 Purify the labeled cRNA as described on "Step 3. Purify the labeled/amplified RNA" on page 30.

Quick Amp Labeling Kit Sample Preparation

The Low Input Quick Amp Labeling Kit replaces the Quick Amp Labeling Kit, One-Color. If you have studies that are ongoing, continue to use the Quick Amp Labeling preparation steps described in this section. For new studies, use the Low Input Quick Amp Labeling Kit as described in Chapter 2, “Procedures”.

The Quick Amp Labeling Kit, One-Color 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 Blend (red cap), which simultaneously amplifies target material and incorporates Cyanine 3-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 73 for general guidance and procedural recommendations on quality assessment of template RNA.

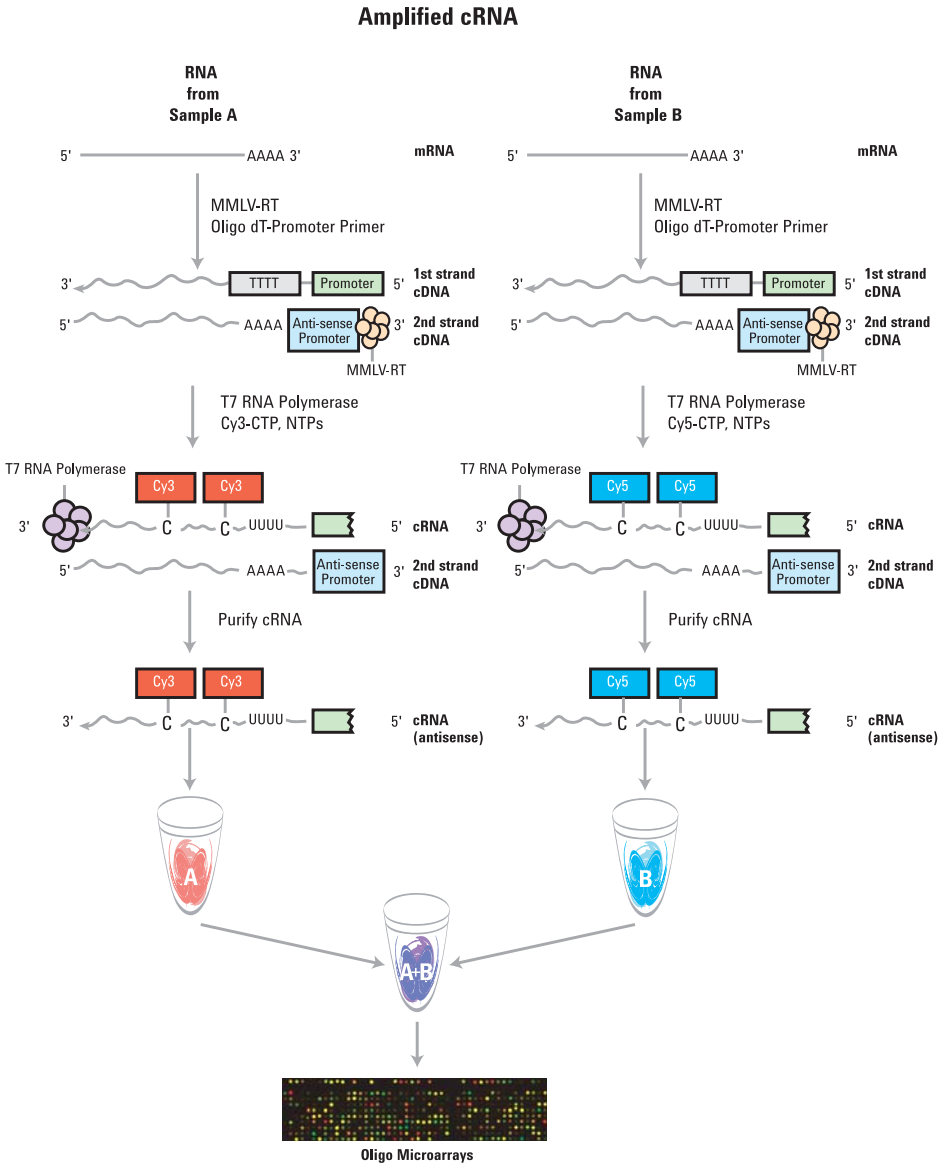

Figure 9 Schematic of amplified cRNA procedure. Generation of cRNA for a two-color microarray experiment is shown. When you generate targets for a one-color microarray experiment, only the Cy3-labeled "A" sample is produced and hybridized.

Step 1. Prepare Spike Mix

Refer to the protocol for the RNA Spike-In Kit, One-Color for in-depth instructions and troubleshooting advice on how to use the spike mix. This protocol is available with the RNA Spike-In Kit, One-Color 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 Spike Mix solution vigorously 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 25 provides the dilutions of Spike Mix for a range of total RNA input amounts. These are diluted such that 1 µL of 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 25 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 Spike Mix dilution appropriate for 200 ng of total RNA starting sample:

1 Make the First Dilution:

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

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

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

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

b Into the First Dilution tube, put 2 µL of the concentrated Spike Mix.

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

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

2 Make 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 from the First Dilution tube.

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

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

3 Make the Third Dilution:

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

   b Into the Third Dilution tube, put 4 µL from the Second Dilution tube.

   c Add 36 µL of the Dilution Buffer (1:10).

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

4 Add 2 µL of Third Dilution to 200 ng of sample total RNA and continue with cyanine 3 labeling using the Quick Amp Labeling Kit as described in “Step 2. Prepare labeling reaction” on page 69.

Storage of Spike Mix dilutions

Store the 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 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 26). Dilute samples so that at least 2 µL of sample is pipetted into the tube.

2 Add 1.2 µL of T7 Promoter Primer (green cap). See Table 26.

3 Add the appropriate volume of diluted Spike Mix as indicated in Table 26.

Table 26 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 Put the reactions on ice and incubate for 5 minutes.

7 Immediately prior to use, gently mix the components listed in Table 27 for the cDNA Master Mix by adding in the order indicated, and keep at room temperature.

8 Prewarm the 5× First Strand Buffer (green cap) 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 (violet cap) and RNase Inhibitor (violet cap) 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 27 cDNA Master Mix

Component	Volume (µL) per reaction	Volume (µL) per 4.5 reactions
5× First Strand Buffer (green cap)	4	18
0.1 M DTT (white cap)	2	9
10 mM dNTP Mix (green cap)	1	4.5
MMLV-RT (violet cap)	1	4.5
RNase Inhibitor (violet cap)	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.

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 28 in the order indicated for the Transcription Master Mix by pipetting at room temperature.

16 Prewarm the 50% PEG (clear cap) 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.

RNase Inhibitor (violet cap), 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 28 Transcription Master Mix

Component	Volume (µL) per reaction	Volume (µL) per 4.5 reactions
Nuclease-free water	15.3	68.9
4× Transcription Buffer (clear cap)	20	90
0.1 M DTT (white cap)	6	27
NTP Mix (blue cap)	8	36
PEG (clear cap)	6.4	28.8
RNase Inhibitor (violet cap)	0.5	2.3
Inorganic Pyrophosphatase (red cap)	0.6	2.7
T7 RNA Polymerase Blend (red cap)	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

Use the RNeasy Mini Kit to purify the amplified cRNA samples.

If sample concentration causes difficulty, you can use the Absolutely RNA Nanoprep Kit as an alternative. See "Absolutely RNA Nanoprep Purification" on page 58.

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 Go to "Step 3. Purify the labeled/amplified RNA" on page 30. Start from step 2 to complete this task.

Step 4. Quantify the cRNA

Use the NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.2.1 to quantify the cRNA.

1 Go to "Step 4. Quantify the cRNA" on page 32 and complete step 2 through step 10

2 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 73 for general guidance and procedural recommendations on quality assessment of labeled cRNA.

3 Continue to the hybridization step at "Hybridization" on page 34.

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 29 and Table 30 to make sure that you have the appropriate analyzer, kits, and compatible assays.

Table 29 Analyzer and Kits

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

Table 30 Compatible Assays

Description	Compatible Assay
RNA 6000 Nano LabChip Kit	Eukaryote Total RNA Nano Assay Qualitative range 5 to 500 ng/µL
RNA 6000 Nano LabChip Kit	mRNA Nano Assay* Qualitative range 25 to 250 ng/µL
RNA 6000 Pico LabChip Kit	Eukaryote Total RNA Pico Assay Qualitative range 50 to 5000 pg/µL in water
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. Start the Agilent 2100 Expert program (version B.02.06 or higher), turn 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 10).

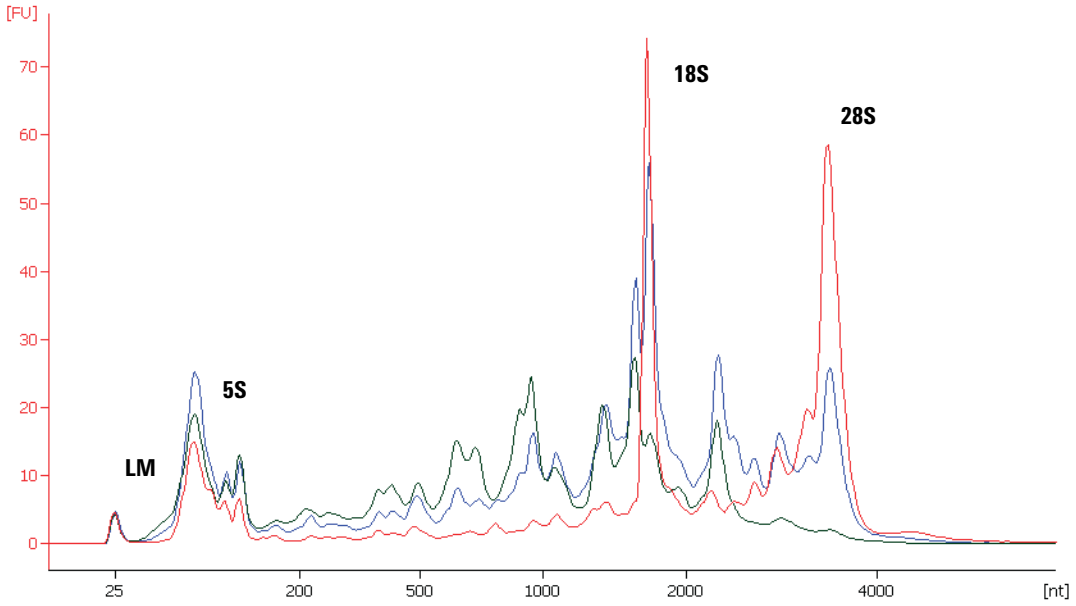

Figure 10 Analysis of (human) total RNA with the Eukaryote total RNA Nano assay using three different samples with decreasing integrity: Red, RIN 8.4; Blue, RIN 5.9; Green, RIN 3,6. Characteristic regions for ribosomal peaks and the lower marker (LM) are displayed.

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 11.

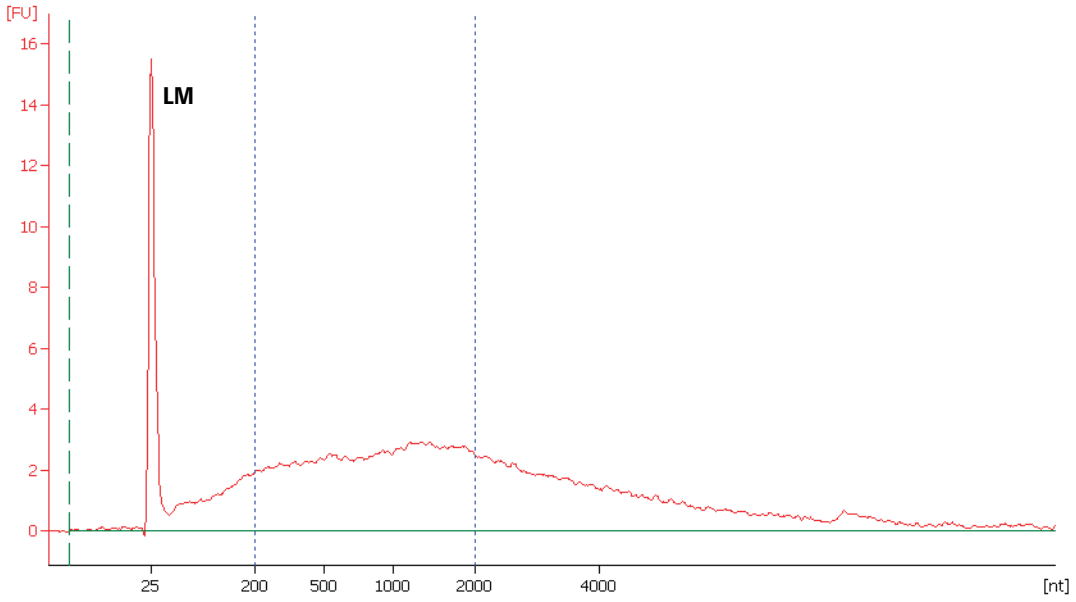

Figure 11 Smear analysis on non-fragmented Cy3 labeled cRNA allows determination of relative concentration of products within size regions. For example, 52 % of overall RNA signal results from fragments with size from 200 to 2000 nt.

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 ND-1000 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

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.

For Scanner C and Scanner B, 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 45.

For the Agilent SureScan scanner, two built-in mechanisms minimize dye signal degradation by ozone and other dye oxidants:

- SureScan slide holder with an integrated ozone barrier in its lid.
- Catalytic ozone decomposition filtering system inside the scanner.

In addition to the ozone barriers, 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. 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* (p/n 5989-0875EN).

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.

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

Use gloves and eye/face protection in every step of the warming procedures.

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 homogeneous 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 and make sure that precipitation does not occur prior to use.*

Step 2. Wash with Stabilization and Drying Solution

NOTE Use fresh Gene Expression Wash Buffer for each wash group (up to eight slides). The acetonitrile and Stabilization and Drying Solution can be reused for washing of up to three groups of slides (for 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 put close to, or preferably in, the same fume hood. Use gloves and eye/face protection in every step of the warming procedures.**

Table 31 lists the wash conditions for the wash procedure with Stabilization and Drying Solution.

Table 31 Wash conditions

	Dish	Wash Buffer	Temperature	Time
Disassembly	1	Gene Expression Wash Buffer 1	Room temperature	
1st wash	2	Gene Expression Wash Buffer 1	Room temperature	1 minute
2nd wash	3	Gene Expression 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

* 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.

1. Completely fill slide-staining dish #1 with Gene Expression Wash Buffer 1 at room temperature.
2. Put 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. Put this dish on a magnetic stir plate.
3. Put 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 put 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 put 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. Put 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 put 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 put 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.

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.

19 Immediately continue at step 14 on page 45.

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 or later.

Note that the default normalization scheme for Agilent one-color data in the GeneSpring GX 9.0 (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.

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4 Reference

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

Kit Contents

The content of the kits used in this protocol (required and optional) are listed here.

Table 32 Low Input Quick Amp Labeling Kit, One-Color

Content
T7 Primer (green cap)
5× First Strand Buffer (green cap)
0.1 M DTT (white cap)
10 mM dNTP Mix (green cap)
Affinity Script RNase Block Mix (violet cap)
5× Transcription Buffer (blue cap)
NTP Mix (blue cap)
T7 RNA Polymerase Blend (red cap)
Nuclease-free Water
Cyanine 3-CTP

Table 33 RNA Spike-In Kit, One-Color

Content
Spike Mix
Dilution Buffer

Table 34 Gene Expression Hybridization Kit

Content
10× Gene Expression Blocking Agent
25× Fragmentation Buffer
2× Hi-RPM Hybridization Buffer

Table 35 Gene Expression Wash Buffer Kit

Content
Gene Expression Wash Buffer 1
Gene Expression Wash Buffer 2
Triton X-102 (10%)

Table 36 RNeasy Mini Kit

Content
RNeasy Mini Spin Column (pink)
Collection Tube (1.5 ml)
Collection Tube (2 ml)
Buffer RLT
Buffer RW1
Buffer RPE
RNase-Free Water

Table 37 Absolutely RNA Nanoprep Kit

Content
Lysis Buffer
1.67× High Salt Wash Buffer
5× Low Salt Wash Buffer
Elution Buffer
DNase Reconstitution Buffer (green cap)
DNase Digestion Buffer (green cap)
Beta-Mercaptoethanol (yellow cap)
RNase-free DNase I
RNA-binding nano-spin cup

Table 37 Absolutely RNA Nanoprep Kit

Content
2-ml collection tube
RNA Binding Nano Spin Cup Cap

Table 38 Quick Amp Labeling Kit, One-Color

Content
T7 Promoter Primer (green cap)
5× First Strand Buffer (green cap)
0.1 M DTT (white cap)
10 mM dNTP Mix (green cap)
RNase Inhibitor (violet cap)
MMLV-RT (violet cap)
4× Transcription Buffer (clear cap)
NTP Mix (blue cap)
Inorganic Pyrophosphatase (red cap)
T7 RNA Polymerase Blend (red cap)
PEG (clear cap)
Cyanine 3-CTP

Supplemental User Guides

First-time users of the Agilent 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.

- Agilent Microarray Hybridization Chamber User Guide
- Hybridization Oven User Manual
- Microarray Scanner System User Guide
- G4900DA SureScan Microarray Scanner User Guide
- Feature Extraction Software Quick Start Guide
- Feature Extraction Software User Guide
- 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 put 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

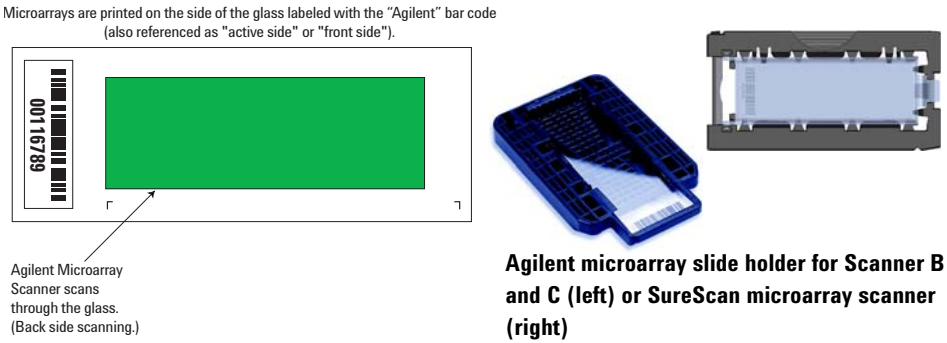

Figure 12 Agilent microarray slide and slide holder. The opposite or "non-active" numerically barcoded side is shown.

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 opening of the slide holder (on SureScan Microarray Scanner G4900DA) or facing the inside of the slide holder (C scanner G2565CA). In this orientation, the "active side" containing the microarrays 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 12 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 13).

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.

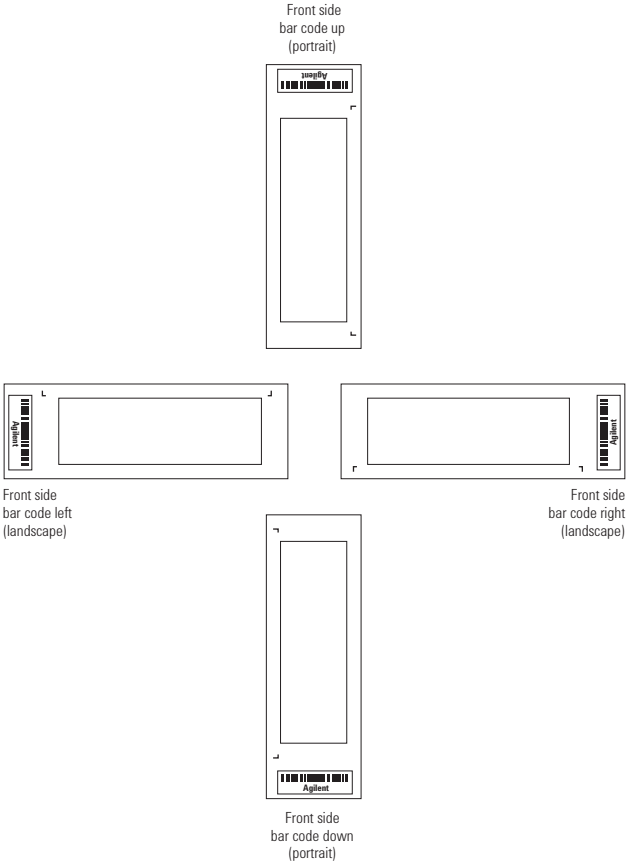

Figure 13 Microarray slide orientation

Array/Sample tracking microarray slides

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

Position the gasket slide in the SureHyb chamber base with the label to the left. Load the samples: top row, left to right, then lower row, left to right. The array suffix assignments from Feature Extraction will be in the order shown.

Arrays

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

Barcode Number _____

Figure 14 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 15 8-pack microarray slide

Related Microarray Reagents

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

www.agilent.com

In This Book

This guide contains information to run the One-Color Microarray-Based Gene Expression Analysis protocol.

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