Microarray-Based Gene Expression Analysis with Tecan HS Pro

Hybridization Supplement

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

This Hybridization Supplement provides the steps to use the Tecan HS 4800 Pro or Tecan 400 Pro Hybridization Station to hybridize Gene Expression microarrays.

Agilent Technologies

Please refer to the full Agilent Gene Expression protocols listed in Table 1 for sample preparation, sample labeling, scanning, and feature extraction steps as indicated in this supplement.

Table 1 Agilent Gene Expression protocols

Standard protocol	Pub Number
Two-Color Microarray-Based Gene Expression Analysis Protocol	G4140-90050
One-Color Microarray-Based Gene Expression Analysis Protocol	G4140-90040

See Figure 1 and Figure 2 for an overview of the sample prep, hybridization, wash, and analysis workflow.

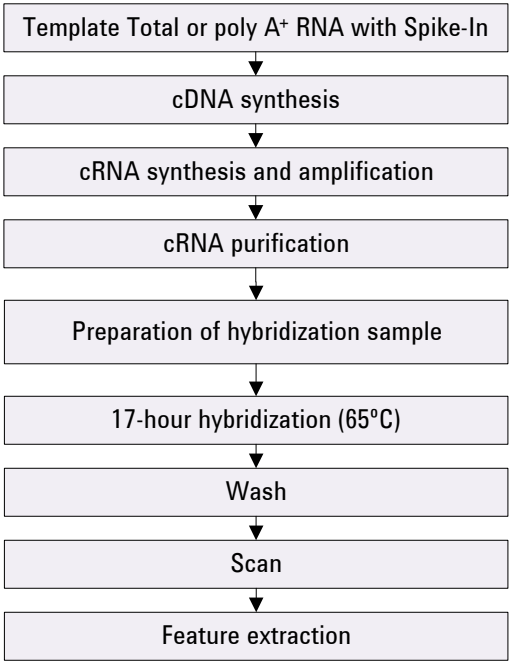


Figure 1 Workflow for sample preparation and array processing.

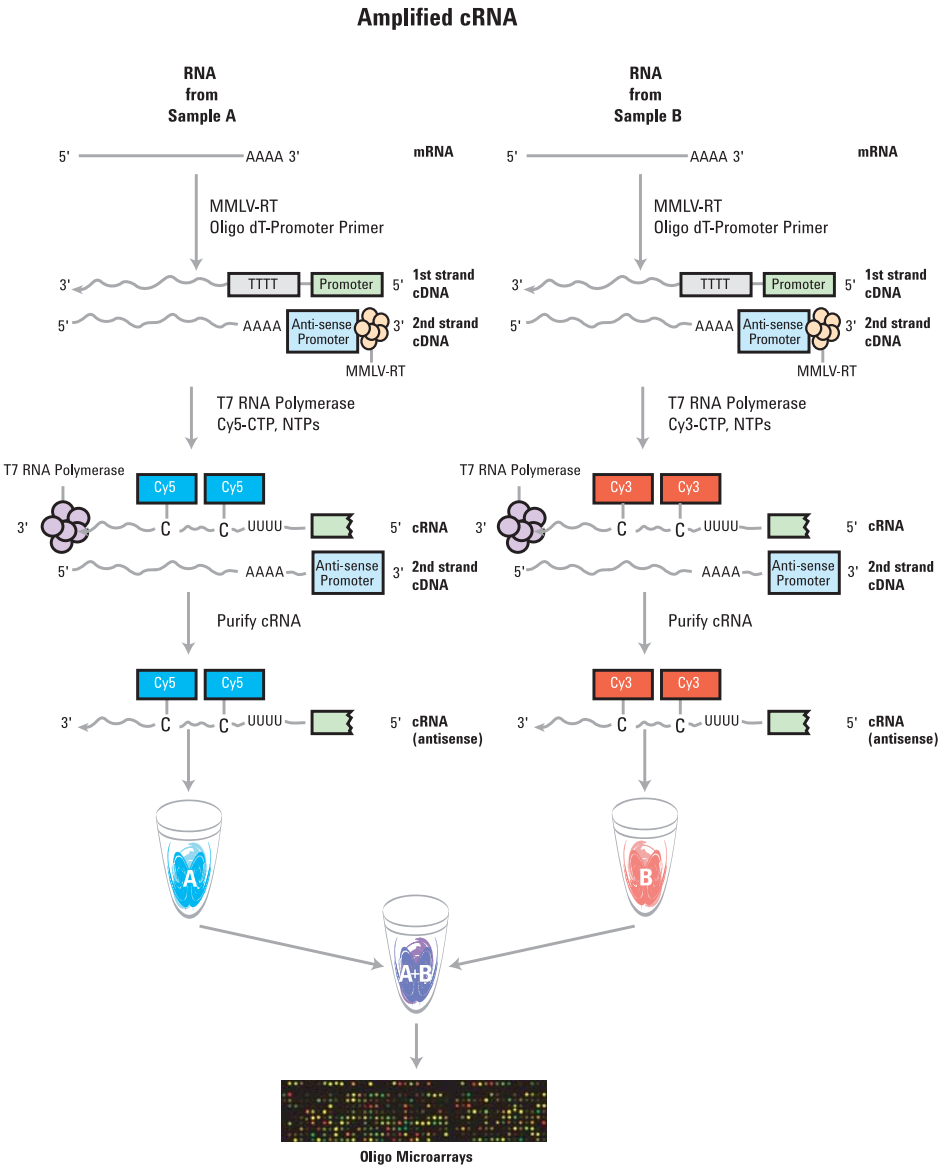


Figure 2 Schematic of amplified cRNA procedure. Generation of cRNA for a two-color microarray experiment is shown. When you generate targets for a one-color microarray experiment, only the Cy3-labeled "B" sample is produced and hybridized.

Before you begin

Step 1. Prepare for the analysis

1 Make sure you have a copy of the full protocol that contains the sample preparation procedures for your analysis. Download the latest protocol from http://www.genomics.agilent.com. Search on the publication number.

2 Read and understand the chapter “Before You Begin” from the full protocol. (See Table 1 on page 2 for the publication numbers.)

- Make sure you understand the procedural notes and safety information.
- Make sure that you have the required equipment and reagents to do the analysis. Refer to Table 2 and Table 3 instead of the full protocol for the required and optional hybridization and wash equipment and reagents.

Table 2 Required equipment and reagents

Description	Vendor and part number
Gene Expression Hybridization Kit	Agilent p/n 5188-5242
Gene Expression Wash Buffer Kit	Agilent p/n 5188-5327
Oligo aCGH Wash Buffer Additive	Agilent p/n 5190-0401
Oligo aCGH Prehybridization Buffer	Agilent p/n 5190-0402
nuclease-free 1.5 mL microfuge tubes	Ambion p/n 12400 or equivalent
DNase/RNase-free distilled water	Invitrogen p/n 10977-015
Tecan HS Pro 400 or 4800	
HS Pro Control Manager Software 3.0 or later	

Table 3 Optional reagents

Description	Vendor and part number
ethanol (95% to 100% molecular biology grade)	Sigma-Aldrich p/n E7023-6×500ML
*Stabilization and Drying Solution	Agilent p/n 5185-5979
acetonitrile	Sigma p/n 271004-1L

* Recommended when processing microarrays in high ozone environment.

Step 2. Test and prepare the equipment for first use

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

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

   To prepare the Cleaning and Conditioning Solution, in the Tecan wash bottle, add the components in Table 4. Shake to mix thoroughly.

Table 4 Cleaning and Conditioning Solution

Component	Volume
Oligo aCGH Wash Buffer Additive	1 mL
Gene Expression Wash Buffer 2	1 L

NOTE

Use the Cleaning and Conditioning Solution to rinse the instrument at regular intervals of approximately every 2 weeks to coincide with the replacement of the hybridization chamber O-rings.

8 Run a rinsing procedure with distilled water.

9 For conditioning the hybridization chambers, start a hybridization run (for example 12 hours) at the temperature used in the protocol (65°C) with a 1:2 dilution of 2× Hi-RPM Hybridization Buffer and dummy glass.

10 Run a rinsing procedure with Final System Drying to dry the chambers.

This step is described in the Tecan operating guide.

Step 3. Set up the reagent bottles

- Prepare the six Wash Bottles according to Table 5.

  Note that the Gene Expression microarray analysis with Tecan HS Pro uses the same Oligo aCGH Prehybridization Buffer and Oligo aCGH Wash Buffer Additive that the CGH microarray analysis uses.

Table 5 Reagent bottle configuration

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

* Spike the Oligo aCGH Wash Buffer Additive into the Gene Expression Wash Buffer 2 at the ratio of 100 µL per 1 liter of wash buffer

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

Step 4. Prepare and label samples

Refer to the full Agilent Gene Expression protocols ("Sample Preparation" section) to do these steps.

Hybridization

Step 1. Prepare the 10× Blocking Agent

Refer to the full Agilent Gene Expression protocols.

Step 2. Prepare hybridization samples

1 Equilibrate water bath to 60°C.

2 For each microarray, add each of the components as indicated in Table 6 (for 1-color analysis) or Table 7 (for 2-color analysis) to a nuclease-free 1.5 mL microfuge tubes.

3 Mix well but gently on a vortex mixer.

Table 6 Fragmentation mix for 1-color analysis

Components	Volume/Mass
cyanine 3-labeled, linearly amplified cRNA	1.65 µg
10× Gene Expression Blocking Agent	6 µL
DNase/RNase-free distilled water	bring volume to 28.8 µL
25× Fragmentation Buffer	1.2 µL
Total Volume	30 µL

Table 7 Fragmentation mix for 2-color analysis

Components	Volume/Mass
cyanine 3-labeled, linearly amplified cRNA	825 ng
cyanine 5-labeled, linearly amplified cRNA	825 ng
10× Gene Expression Blocking Agent	6 µL
DNase/RNase-free distilled water	bring volume to 28.8 µL
25× Fragmentation Buffer	1.2 µL
Total Volume	30 µL

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

CAUTION Do not exceed 30 minutes. Adding the 2× Hi-RPM Hybridization Buffer will stop the fragmentation reaction.

5 Add 2× Hi-RPM Hybridization Buffer to the fragmentation mix at the appropriate volume to stop the fragmentation reaction. See Table 8.

Table 8 Hybridization mix

Components	Volumes per hybridization
cRNA from Fragmentation Mix	30 µL
2× Hi-RPM Hybridization Buffer	30 µL

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

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

Use immediately. Do not store.

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

Refer to “Microarray Handling Tips” in the full Agilent Gene Expression protocols for information on how to safely handle microarrays.

Step 3. Tecan Hybridization

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

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

- Set up the reagent bottles (see Table 5 on page 7).
- Load slides.
- Inject samples.

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

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

You can download the Agilent Gene Expression script from http://www.chem.agilent.com/Library/software/Public/Agilent 44K - GE.zip.

The Gene Expression script contains the steps in Table 9. See “Agilent Gene Expression Tecan Script” on page 17 for more information.

3 Turn on the reagent bottle heating.

4 Click **Go**.

Table 9 Gene Expression script

Step Number	Program Step	Step Action
1	Wash	Oligo aCGH Prehybridization Buffer at 65°C
2	Sample Injection	Sample Loading (55 µL with A4X44k)
3	Hybridization	65°C for 17 hours
4	Wash	Gene Expression Wash Buffer 1 at Room Temp

Table 9 Gene Expression script

Step Number	Program Step	Step Action
5	Wash	Gene Expression Wash Buffer 1at 37°C
6	Drying	2 minutes at 30°C

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

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

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

At this point, you can process the slides with the Agilent Stabilization and Drying Solution to protect the dyes against ozone degradation. See "Step 2. Wash with Stabilization and Drying Solution" on page 14.

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

7 If your analysis is prone to ozone-related problems as described in the full Agilent Gene Expression protocols, follow these steps to wash your microarray slides in "Preventing Ozone-Related Problems" on page 12.

Step 4. Scan the slides and extract features

Refer to the full Agilent Gene Expression protocols to:

- Scan the microarray slides.
- Use Agilent Feature Extraction software to extract data.

Preventing Ozone-Related Problems

If your analysis is prone to ozone-related problems as described in the full Agilent Gene Expression protocols, follow these steps to wash your microarray slides in Stabilization and Drying Solution.

Step 1. Prepare the Stabilization and Drying Solution

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

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

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

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

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

2 If needed, gently mix to obtain a 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 prior to use*.

Step 2. Wash with Stabilization and Drying Solution

For more information, visit www.agilent.com to download our technical note on **Improving Microarray Results by Preventing Ozone-Mediated Fluorescent Signal Degradation** (publication 5989-0875EN).

NOTE The acetonitrile and Stabilization and Drying Solution can be reused for washing of up to three groups of slides (that is, a total of 24 slides).

WARNING **The Stabilization and Drying Solution must be set-up in a fume hood. Gloves and eye/face protection should be used in every step of the warming procedures.**

Table 10 lists the wash conditions for the wash procedure with Stabilization and Drying Solution.

Table 10 Wash conditions

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

1. In the fume hood, fill slide-staining dish #1 approximately three-fourths full with acetonitrile. Add a magnetic stir bar and place this dish on a magnetic stir plate.
2. In the fume hood, fill slide-staining dish #2 approximately three-fourths full with Stabilization and Drying Solution. Add a magnetic stir bar and place this dish on a magnetic stir plate.
3. If you haven't already done so, remove the MTP slide adapter from the Tecan HS Pro hybridization station.
4. Remove the slides from the MTP slide adapter and put them in a slide rack.
5. Immediately transfer the slide rack to slide-staining dish #1 containing acetonitrile, and stir using setting 4 for 10 seconds.
6. Transfer slide rack to slide-staining dish #2 filled with Stabilization and Drying Solution, and stir using setting 4 for 30 seconds.

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

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

9 Dispose of acetonitrile and Stabilization and Drying Solution as flammable solvents.

Reference

Kit Contents

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

Table 11 Gene Expression Hybridization Kit

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

Table 12 Gene Expression Wash Buffer Kit

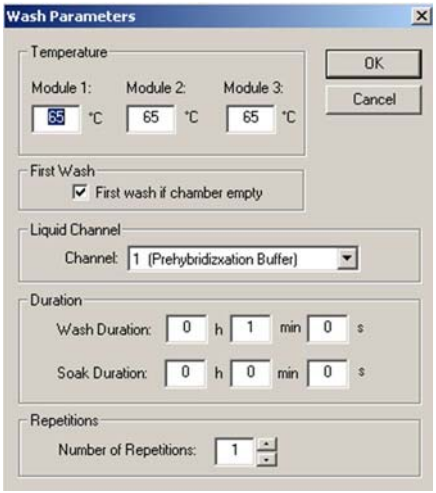
Gene Expression Wash Buffer 1
Gene Expression Wash Buffer 2
Triton X-102 (10%)

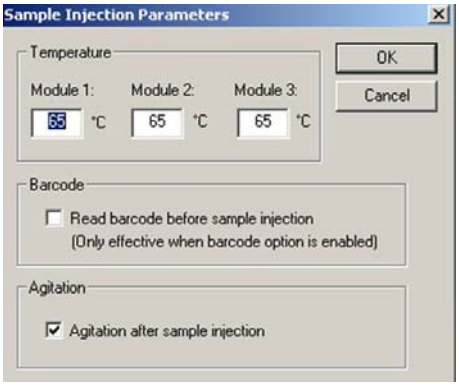
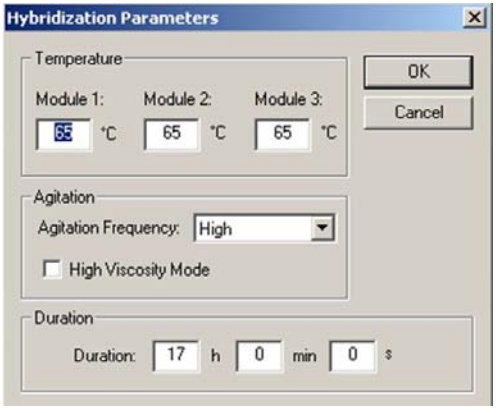
Agilent Gene Expression Tecan Script

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

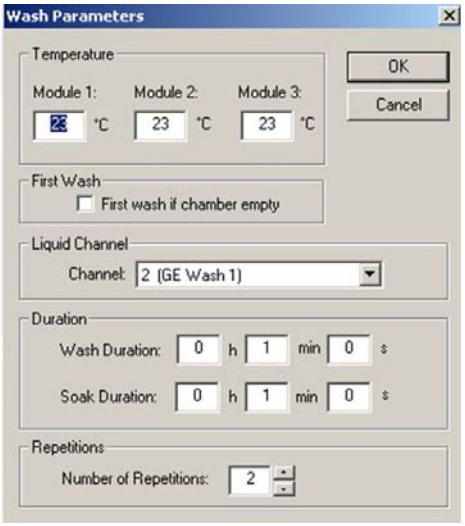
You can download the Agilent Gene Expression script at http://www.chem.agilent.com/Library/software/Public/Agilent 44K - GE.zip.

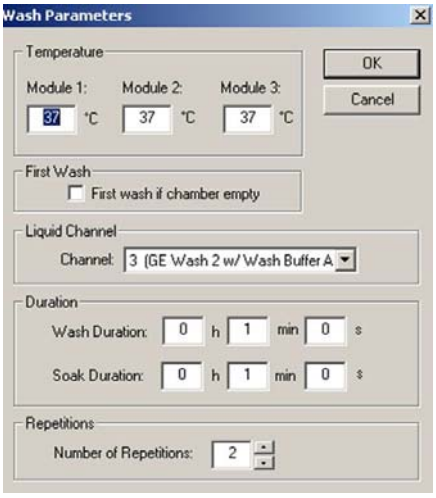
The steps in the script are described here. The graphics in the table contain the specific parameters that are contained in the script.

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

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

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



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

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In This Book

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

Version A0, August 2015

G4140-90061

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