MI PathArray™ Tecan HS Pro Hybridization Station™ Protocol

Note: This protocol was developed for use on the Tecan HS 400 Pro Hybridization Station. Parameters may need adjustment when used on other models. A ready-to-use "MI PathArray.hpr" file of this program may be downloaded from the MI website at www.microarrays.com/MIPathArrayinfo

Required Components (available from MI)

MI PathArray™ slide
MI Hybridization Kit (MIHK001)

Other materials

Prepared, labeled cDNA
Amber 1.5ml microcentrifuge tubes
Dry Bath or Heat Block, set to 98˚C
Containers for mixing wash buffers
Pipettes and tips
Tecan HS Pro Hybridization Station™
Spectrophotometer
Microcentrifuge

Program Parameters for use on the Tecan HS Pro Hybridization Station™

Enter the following parameters into the HS Pro Software or download and import the "MI PathArray.hpr" program from the Microarrays, Inc. website listed above.

Program Start

Wash:
Temp: 42˚C
First: Yes
Ch 1
Runs 1
Wash 1:00
Soak 0:00
Sample Injection:
Temp: 42˚C
Agitation: Yes
BCR: No

Note: If on-board denaturation is preferred, enter those parameters here.

Denaturation:

Temp: 98˚C

Time: 3:00

Agitation: Yes

Hybridization:

Temp: 42˚C

Agitation Frequency: High

High Viscosity: No

Time 16:00:00

Wash:

Temp: 42˚C

First: No

Ch 1

Runs 2

Wash 1:00

Soak 1:00

Wash:

Temp: 23˚C

First: No

Ch 2

Runs 2

Wash 1:00

Soak 0:30

Wash:

Temp: 23˚C

First: No

Ch 3

Runs 2

Wash 1:00

Soak 0:30

Slide Drying:

Temp: 23˚C

Time 3:00

Final Manifold Cleaning: No

Ch: No

Program End

Prepare wash solutions for use on the Tecan HS Pro Hybridization Station

Note: Prepare all wash solutions for set-up of the HS Pro Hybridization Station. Volumes may be proportionally adjusted based upon number of arrays to be processed.

Wash Solution 1:	447.5ml deionized water
	50ml Wash Reagent A
	2.5ml Wash Reagent B
Wash Solution 2:	1425ml deionized water
	75ml Wash Reagent A
Wash Solution 3:	300ml Wash Solution 2
	1200ml deionized water

Prepare Hybridization Solution

1) Dissolve 25-50ng pre-labeled cDNA test sample in 55µl of MI Hybridization Buffer in a 1.5ml amber microcentrifuge tube.

2) Mix gently by tapping the tube and microfuge tube briefly to collect contents.

3) Heat hybridization solution in a dry bath or heat block at 98˚C for 3 minutes.
Note: Sample denaturation can be done on the Tecan HS Pro if preferred. Additional denaturation program parameters must be added into the program just prior to the sample hybridization step.

4) Microfuge the tube briefly.

Basic preparation of the Tecan HS Pro Hybridization Station

Note: For detailed instructions on instrument preparation, please refer to the Tecan HS 4800 Pro/HS 400 Pro Hybridization Station Instruction Manual, Document 30033333 2007-09, Revision 4.0, Software Revision Level 4.0.

5) Turn on the power to the Tecan HS Pro Hybridization Station and open the HS Pro Control Manager Software. Enter and save or load the hybridization program to be used (see recommended program parameters above).

6) Fill wash bottles with prepared wash solutions and ensure correct liquid line connections are made per the programmed wash solution locations in the Tecan HS Pro Control Manager software.

7) Insert the QuadChambers into the chamber frame. Insert the injection inlet plugs into the injection ports.

8) Apply the MTP slide adapter to the heat plate module of the instrument and fill the adapter with slides in all 4 locations. When using the QuadChambers, test array locations are sites 2 & 4. “Demo” slides should be inserted in all non-testing locations in the adapter to ensure proper sealing of the system.

9) The microarray is printed on the surface of the slide where the barcode numbering is legible. Place the microarray slide into position on the slide adapter so that the printed face (barcode-legible side) of the array is face-up.

*Note that one end of the MI PathArray slide contains a barcode. This end of the array should be located in the slide adapter closest to the front face of the instrument. Be certain to avoid placement of the quad chamber gasket wells over the barcode. Improper placement of the array will lead to misalignment of the gaskets with the printed array areas.

10) Slowly close the chamber frame.

11) Ensure the nitrogen gas is flowing to the instrument.

12) Ensure that the liquid waste collection container is not full. Empty if required.

13) Initiate Program to begin first wash of the arrays.

14) Inject prepared, denatured hybridization solution when prompted by the instrument.

Injection of the Sample

Note: Ensure that no air bubbles are injected into the system as this may cause high background on the array.

15) Slowly, pipette the contents of each tube into the proper injection port as prompted by the instrument. Seal the injection inlet with the injection plug.

Handling of Hybridized Arrays

16) At completion of the Tecan Hybridization Station program, slowly release the chamber frame and allow the gaskets to separate from the hybridized arrays.

17) Remove the arrays from the MTP slide adapter. Take measures to avoid introducing dust or abrading the surface of the slide in handling.

18) Processed arrays should be scanned immediately or stored in a lightproof slide holder until ready to scan. For best results, arrays should be scanned within 2 hours of processing.

References

Tecan HS 4800 Pro/ HS 400 Pro Hybridization Station Instruction Manual, Document 30033333 2007-09, Revision 4.0, Software Revision Level 4.0.