Protocol for hybridization with MAUI hybridization and wash system
V1.3

Important: Up to **12** slides can be hybridized in one run. If you hybridize 12 slides on one run, it is recommended that you separate your slides into 2 groups and wash **one by one**

A) Pre-heat hybridization system

Turn on the machine (Heat on/off) and set at 42 C at least **6 h** before sample injection.

Note: Getting to set temperature and being stable are very **slow** for MAUI. You may need to turn it on **6 h** before your hybridization. If you run samples serially, you **do not need to turn the heating off** until there is no one running samples the next day.

B) Pre-hybridization

1. prepare pre-hybridization buffer (the same as Tecan)
   100 ml recipe: 74 ml DI water, 25 ml 20 X SSC, 1ml 10% SDS and 0.1 g BSA
2. Add about 50 ml pre-hybridization buffer to a coplin jar and put the slides in with the barcode at the top. Each jar can hold up to **5** slides. Cover the jar with foil, put it in a water bath and incubate for **45 min at 42 C**.

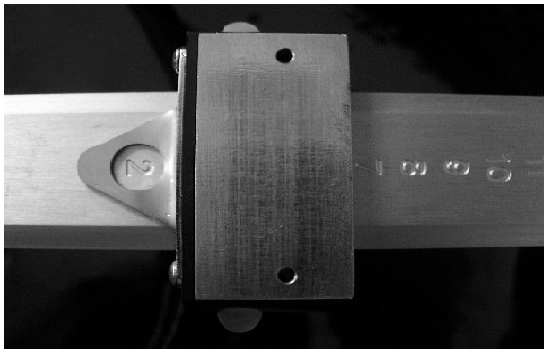
C) Pre-heat items

During pre-hybridization, pre-heat the following items:

1. Turn on hyb oven to heat **Gilson CP 100** tips at 65 C
2. Turn on 3 heatblocks: 98 C for denaturing, 65 C to hold denatured samples until injection and 65 C as a heat plate for sample injection

D) Washing after pre-hybridization

1. Take the slide out from the pre-hybridization buffer and wash **twice** with **wash buffer III** for **5 min each**. Use the jars and slide carrier for manual washing.
2. While the slides are soaking, prepare the MAUI wash system for spin dry.
   i) Fill the **carrier** of the MAUI wash system with **DI water**.
   ii) Adjust the balance. Set to the number which reflects the number of slides in the Slide Carrier.



iii) Press **fluidics engagement level handle down** and make sure that

fluidics interface comes out.

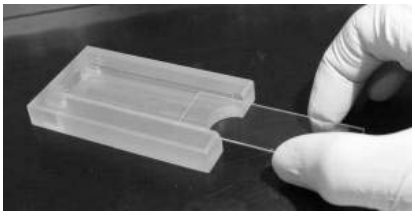
3. After the wash buffer III rinses are finished (step D 1), put slides into the carrier (full of DI water) and place the carrier in the machine. Hold on for 10 sec, close the lid and start *Spin Dry* (80 sec, 500g, set already)

E) MAUI mixer-slide assembly

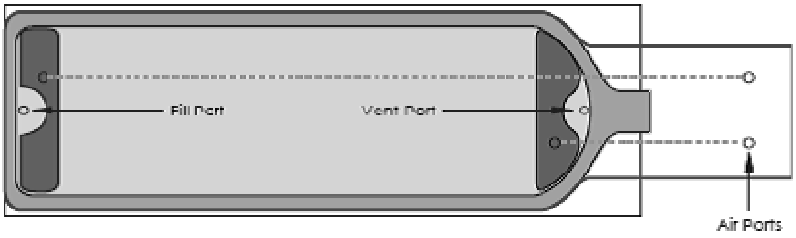
Make sure the slide and the mixer is clean. If they have dust or water droplets, use the Whoosh-Duster to clean them.

For AO mixer

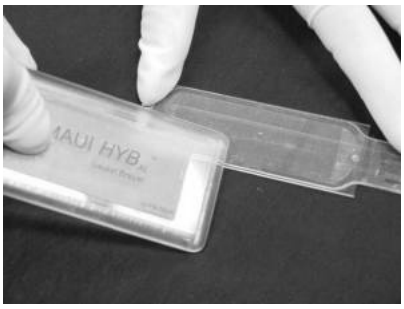
1. Insert the microarray slide into A/D jig with barcode end out.



2. Remove the mixer from the package.
3. Remove the adhesive gasket release liner using your fingers or forceps. DO NOT touch the side which will attach to the slide.
4. Hold the mixer adhesive side down. Align as shown in the following figure. First make sure the mixer is positioned correctly on the non-barcode end and then slowly lower the mixer to adhere it to the slide.



5. Take the mixer-slide assembly out and rub the gasket brayer over the top of the MAUI mixer to ensure a good seal. Make sure you don't press on the center of the mixer.



6. Put the assembly in the hybridization system to pre-heat the slides.
   Important: Record the barcode of the microarray, position in the hybridization system and sample name in your notebook. DO NOT label on the slide.

F) Prepare hybridization solution

Hybridization solution contains 45% of formamide, 5 X SSC, 0.1% SDS and 0.1 mg/ml Salmon sperm DNA. **Depending on samples, you may need to adjust formamide concentration to get satisfying spots number.**

For each slide, add **50** ul of hybridization solution and **2** ul of universal standard (0.1 pmol/ul) to your dried labeled sample. **The volume is already adjusted for the volume-lose during denaturation and extra volume needed for sample injection, so there is no need to increase the volume.**

The following recipe is based on **45%** of formamide (volumes in µL)

	Stock	Final conc.	1 X	2 X	4 X	7 X	10 X	13 X
Formamide	100%	45%	22.5	45	90	157.5	225	292.5
SSC	20 X	5 X	12.5	25	50	87.5	125	162.5
SDS	10%	0.1%	0.5	1	2	3.5	5	6.5
Sperm DNA	10 mg/ml	0.1 mg/ml	0.5	1	2	3.5	5	6.5
water			14	28	56	98	140	182
Final volume			50	100	200	350	500	650

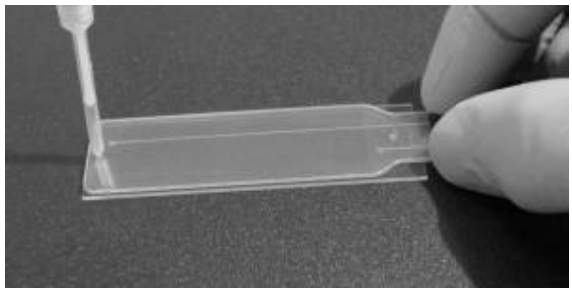
Alternatively, you can add bromephenol blue (BPB) in hybridization buffer for better visualization of buffer injection (volumes in µL)

	Stock	Final conc.	1 X	2 X	4 X	7 X	10 X	13 X
Formamide	100%	45%	22.5	45	90	157.5	225	292.5
SSC	20 X	5 X	12.5	25	50	87.5	125	162.5
SDS	10%	0.1%	0.5	1	2	3.5	5	6.5
Sperm DNA	10 mg/ml	0.1 mg/ml	0.5	1	2	3.5	5	6.5
Bromephenol blue	0.5%	0.1%	10	20	40	70	100	130
water			4	8	16	28	40	52
Final volume			50	100	200	350	500	650

Make sure the sample is well dissolved.

G) Sample injection

1. Put the samples (in 1.5 ml tube) in the heating block set at 98 C for 3 min
2. Spin the sample tubes briefly (a few seconds). Put the samples in the heat block set to 65 C for sample injection.
3. Sample injection
   - i) Take the mixer-slide assembly out of the hybridization station and put it on the back of the heating block set at 65 C.
   - ii) Fill the pipette tip. **MAKE SURE that no bubbles in the tip (any bubbles should be all above the liquid).**
   - iii) Insert the end of the pipette tip into the fill port on the mixer. The AO mixer **must be filled from the non-tab end.** Apply a slight downward force to the tip to ensure a tight seal. With a continuous motion, inject the sample into the fill port. It should be quick (but not too quick) and should be finished within several seconds. Leave the tip in place, still applying force until the hybridization solution emerges from the vent port.



   - iv) **Gently** use a Kimwipe to wipe off excess liquid that remains around the fill or vent ports. **DO NOT press too much** or the hybridization buffer will wick out of the mixer.
   - v) **Lightly** place an adhesive port seal over the fill and vent ports. Use your fingers to press both port seals firmly **AT THE SAME TIME** to assure the seals remain in place.
   - vi) Put the assembly back on to the hybridization system and close the bay clamp.

   Note: Keep the lid of MAUI closed as much as possible to keep the temperature stable.

H) Start hybridization

After sample injection, close the cover. Turn on mixing, and press B. The system will check the seal of the assembly (station seal test). A Green light indicates a good assembly.

The hybridization should be performed overnight (over 10 h)

If possible (you are able to see), make sure the bladders of the mixer move at the beginning and the end of your hybridization.

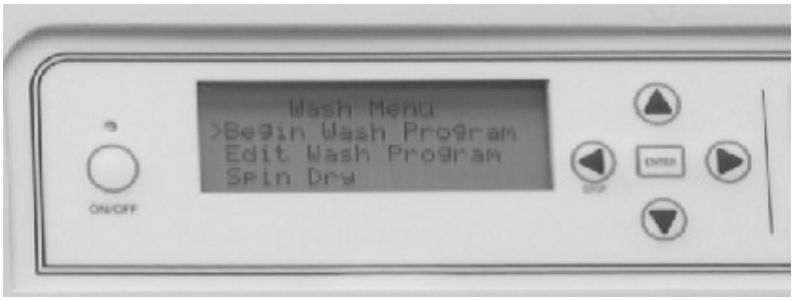
Note: Keep the lid of MAUI closed as much as possible to keep the temperature.

I) Prepare wash buffer I and pre-warm it in a water bath at 42 C

2 bottles of wash buffer I are needed. One is for the wash system, at least 500 ml for each run. Another is for removing the MAUI mixer.

NEXT MORNING

J) MAUI wash system setting (before each washing)



Buttons on the wash system:

ON/OFF: turn on/off the system.

Up/Down: move the cursor up/down

Left: move the cursor to the left, go to upper level of the menu, STOP button during washing

Right: move the cursor to the right, open the lid (hold)

Enter: perform selection

1. Make sure that all the lines have enough buffer and the tubes are in the right buffer (Bottles 4 and 5 contain nano-pure water).
2. Turn on **ZerOzone.** The ozone concentration in Oklahoma is high and ozone will damage Cy5. ZerOzone reduces ozone at 1000 ppb to less than 5 ppb inside of the MAUI wash system within 2 minutes.
3. Select *Utility Menu*/ *Manual Prime*
4. Adjust balance. Set the number which reflects the number of slides in the Slide Carrier. The carrier can hold up to **11** slides. The track near the agitation rack is too narrow to insert a slide.
5. Press **fluidics engagement level handle down** and make sure that **fluidics interface comes out**.
6. Washing program

FGA 06

Cycle	1	2	3	
spin-dry (seconds)				90 sec
Draw(seconds)	36	20	20	
Heat (C)	42	Bypass	Bypass	
Fill (seconds)	10	20	20	

Agitation (seconds)	40	30	50	
Bottle	Buffer 1	Buffer 2	Buffer 3	
Bottle 1	Wash buffer I			
Bottle 2	Wash buffer II			
Bottle 3	Wash buffer III			

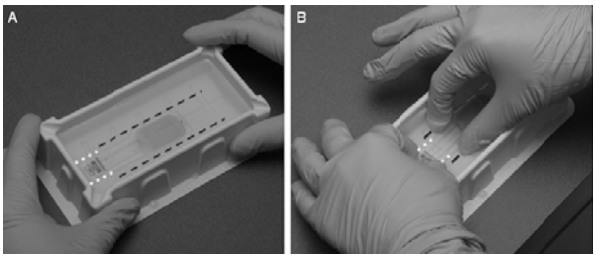
Recipes for wash buffer I, II and III are the same as those used with the TECAN hybridization station.

Note: It seems that increasing the filling time reduces the signal intensity, and that increasing agitation time helps to get slides clean. If necessary, you may adjust washing program for your sample.

7. Place the carrier (no slides) in the **right position**. Make sure the injection and the agitation will be good.

K) Remove the mixer from the slides

1. Turn off mixing of the MAUI hybridization system
2. Add wash buffer I (42 C) in a pipettor basin (pictured below), which serve as a disassembly basin.



3. Take the assembly from the hybridization system. Immerse it in wash buffer I. Grasp the tab and slowly peel off the mixer from the slide. Put the slide in a coplin jar in which there is wash buffer I (42 C). **Avoid drying the slide during this process**. Repeat until all the slides are ready for washing. Turn off the MAUI hybridization system.

L) Washing

1. Take all of the slides to the wash system. Fill the carrier with pre-warmed wash buffer I. Place slides into slots of the carrier.
   Important: Barcode should be at the bottom of the carrier.
2. Close the lid and start the washing program (**FGA**)
   During washing, you can open the lid to make sure everything is working fine, e.g. **(1) agitation**, **(2) buffer injection and (3) drainage.** You can open the lid at any time except **when it is heating**. Also the lid must be **closed** before it starts **spin dry.**
   Important: make sure the drainage container is not too full and you may lift the drainage tube a little to help get waste buffer down if necessary.

3. Remove the slides from the carrier after washing and scan.

M) Cleaning

1. **After washing, put all the lines in nano-pure water and start *Flush System*.**
2. **Clean all the containers, buffer leaks or spills that occurred during the post hybridization processing, hybridization and washing. The pre-hybridization buffer and wash buffer I contain a high concentration of salt and it will cause problems if left to dry.**
3. **Clean up the o-rings and the underside of the clamps on the hybridization system using a Kimwipe dampened with methanol. This may be done once a week.**

For details of MAUI hybridization & wash system, please refer to User's Guide provided by the company.