Hybridization and Washing of Oligo Arrays

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Array Preparation with Hybex and Lifter Slips

Tools needed:

- Hybex Microarray Incubation System from SciGene (Cat. #1057-36-1) http://www.scigene.com/products/hybex_arrays.html
- M-Series Lifter slips from Erie Scientific (22x Cat. #60I-M-5522) http://www.eriemicroarray.com/coverglass/lifterslips-m.aspx

1. Thoroughly clean LifterSlips with clean water followed by 100% EtOH rinse. Dry LifterSlips with clean dry air or lens paper. Dust off arrays as well.
2. Wet the pads in the tops of two Hybex chambers.
3. Place up to 16 arrays on top of Hybex slide racks or a tip box. Lay the LifterSlips on each slide, with the white edge legs down.
4. One at a time, slowly pipette 55µl probe onto the array, by pipetting the solution onto the slide, adjacent to one corner of the LifterSlip. Just touch the pipette tip to the edge of the lifter slip and watch to ensure the probe slowly wicks under, evenly and smoothly, without bubbles.
5. Holding the arrays at the ends, insert up to 4 arrays into each of two Hybex racks.
6. Place two racks onto a Hybex chamber base, close the cover, and then screw the chamber together. Repeat with a second chamber, place both into a Hybex heating base and set to 65°C.
7. Hybridize at 65°C for 14 to 18 hours.

Day 2

Washing with the Little Dipper Microarray Processor in an Ozone-free Enclosure

8. Use an ozone free environment http://www.scigene.com/products/britespot.html or a room with appropriately controlled atmospheric conditions (ozone at less than 5 ppb during the washing step).
9. 20 minutes before washing, turn on ozone scrubber and decrease ambient ozone to less than 5 ppb.
10. Prepare wash solutions as indicated in the table below. Heat the first wash solution in the microwave for 2-3 minutes, then bring up to temperature with the Little Dipper bath.

Note: Wash 1A is performed at 55 to 60°C – this is critical – not at room temperature!!

Step	Wash	Description	Wash Buffer	Vol (ml)	SSC	SDS (10%)	Temp	Robot Bath	Time	Agitation
1	1A	2x SSC, 0.03% SDS	1	500	500 ml 2x	1.5 ml	55-60°C	1	60 sec	350 cpm
2	1B	2x SSC	1	500	500 ml 2x	—	RT	2	30 sec	400 cpm
3	1C	2x SSC	1	500	500 ml 2x	—	RT	3	30 sec	300 cpm
4	2	1x SSC	2	500	500 ml 1x	—	RT	4	90 sec	350 cpm
5	3	0.2x SSC	3	500	500 ml 0.2x	—	RT	5	90 sec	350 cpm
6	C	Centrifuge	—	—	—	—	RT	C	300 sec	—

11. Unscrew Hybex chamber, take out racks, keeping the arrays horizontal and place into separate hot bath or directly into wash 1A for coverslip removal.
12. Once submerged, quickly flip each rack so the arrays are vertical and the coverslips fall off. Align racks together and insert handle. Lift the racks so that coverslips fall to the bottom. Place rack assembly into the positioning unit of bath 1 and start wash program.

Array Preparation with 5-Hyb Chambers, Waterbath and Regular Coverslips

Note: Lifter slips are recommended to avoid loss of spot signal in the middle of the array and Hybex units so the Lifter slips do not stick to the top of the Hyb chamber and for ease of converting the hyb rack to a wash rack)

1. Clean coverslips with an air blast and keep free of dust. Place 5 arrays in 5-Hyb chamber.
2. One at a time, pipette probe onto the array, and slowly drop coverslip on top of array, using tweezers and bringing coverslip down diagonally to avoid bubbles.
3. Once all 5 arrays are prepared, pipette a total of 300µl of 3x SSC in drops of 10µl. Place 4 drops in between each array and 2 drops on the array at either end.
4. Place 5-Hyb cover on top and tighten screws with electric drill. Place in waterbath gently and evenly with a glove and hybridize at 65°C for 14 to 18 hours.

Day 2

Manual Washing with 5-Hyb Chambers and Regular Coverslips

5. Ready washes in chambers to volume as indicated in the table below. Avoid adding excess SDS. The Wash 1B chamber and the Wash 2 chambers should each have a slide rack ready.

Note: Washes 1A and 1B are performed at 55 to 60°C – this is critical – not at room temperature!!

6. Microwave the wash solution for 2 to 3 minutes, checking every 30 seconds with a thermometer.
7. Put the wash chamber in a waterbath, with the water in the waterbath at the same level as the wash solution – monitor the temperature during the washing steps.) Washes 1C, 2, and 3 are performed at room temperature.

Wash	Description	Wash buffer	Vol (ml)	SSC	SDS (10%)
1A	2x SSC, 0.03% SDS	1	500	500 ml 2x	1.5 ml
1B	2x SSC, 0.03% SDS	1	400	400 ml 2x	1.2 ml
1C	2x SSC	1	~500	~500 ml 2x	--
2	1x SSC	2	~500	~500 ml 1x	--
3	0.2x SSC	3	~500	~500 ml 0.2x	--

8. Blot dry chamber exterior with towels. Unscrew chamber with electric drill.
9. Using tweezers put the array into Wash 1A. Gently allow coverslip to fall off and immediately after coverslip is off transfer to the slide rack in Wash 1B. Repeat for up to 5 arrays. Agitate in Wash 1B for 1 minute.
10. Remove with tweezers, dip in Wash 1C chamber and swirl around *without* a rack to get rid of SDS, Transfer to the rack in Wash 2 chamber.
11. Wash arrays by submersion and agitation for 1.5 minutes in Wash 2 chamber.
12. Transfer the entire rack to the Wash 3 chamber and agitate for 1.5 minutes. Carry the entire Wash 3 chamber and rack over to the centrifuge.

Transfer the slide rack quickly and evenly to a bucket in the centrifuge. Spin dry by centrifugation in a slide rack in a Beckman GS-6 tabletop centrifuge at 600 RPM for 2 to 5 minutes.

Note: Reducing ozone is critical during the centrifuge step to avoid loss of Cy5 signal and position-dependant Cy5 loss (left to right red gradient).

Scan Arrays

1. Scan arrays immediately on an Axon 4000B or 4200 Autoloading scanner http://www.moleculardevices.com/pages/instruments/microarray_main.html, or store in an ozone-free environment. Scan within 12 hours of washing or as soon as possible.

2. Adjust the PMTs so that the spots in the corner of each sector saturate (those are the "landing light" spots for gridding purposes), but the rest of the array is just slightly below saturation (less than 1-2 non landing-light spots per sector saturated). Adjust the red PMTs first and match the green PMTs by the histogram and eyeball.