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Hybridizing Cy-labeled DNA to homemade PLL oligo microarrays

Protocol History: I adapted this protocol from that used by Joseph DeRisi's Lab at U.C. San Francisco.

Materials

Supplies:

0.5ml tubes
Lifterslips, Erie#22x40I-M-5516, available as VWR #48382-242
Heat block with 0.5ml-tube block
Hyb ovens with accurate and precise temperate
Monitoring digital thermometer
Hyb chambers (e.g. from Genetix)
Microcentrifuge
Centrifuge with plate-spinning rotor
Slide racks

Reagents:

20X SSC	Applied Biosystems/Ambion	# AM9770
HEPES, make to 1M, pH7	Sigma	#H4034-25G
Ambion H20	Applied Biosystems/Ambion	# AM9937
polyA, make 10mg/ml	Sigma-Aldrich	#P9403-25MG
10% SDS	Applied Biosystems/Ambion	# AM9822

Protocol

The total volume of your hybridization reaction will depend on the size of your lifterslip. I am currently using mid-sized lifterslips with a recommended volume of 29µl; I use 30µl. For the prototype array, I used smaller lifterslips for which my hybridization volume was 25µl.

For one reaction:	For 3.1 reactions:	For all reactions:
	Multiply column 1 values by 3.1 if you are hybridizing triplicate arrays for each sample.	*Multiply column 1 or 2 values (depending on if doing triplicate or single arrays) by ~110% of the number of samples you've got.*
DNA:	*DNA:*	
19.83µl Cy3-DNA	61.47µl Cy3-DNA	
1µl Cy5-cospot-complement	3.1µl Cy5-cospot-complement	
Mix H1:	*Mix H1:*	*Mix H1:*
4.49µl 20X SSC	13.92µl 20X SSC	20X SSC
0.62µl 1M HEPES, pH 7.0	1.92µl 1M HEPES, pH 7.0	1M HEPES, pH 7.0
2.24µl Ambion H2O	6.94µl Ambion H2O	Ambion H2O
Σ = 7.34µl	Σ = 22.78µl	
		For H2, multiply values by ~130% of the # of samples.
Mix H2:	*Mix H2:*	*Mix H2:*
1.22µl 10mg/ml polyA	3.78µl 10mg/ml polyA	10mg/ml polyA
0.62µl 10% SDS	1.92µl 10% SDS	10% SDS
Σ = 1.84µl	Σ = 5.7µl	
Σ = 30µl	Σ = 93µl	

- Make up Mix H1 and H2, mix each well

- Aliquot Mix H1 into 0.5ml tubes, one for each sample. Thus, if hybing 3 arrays per sample, make hyb mix for all three slides in same tube.
- Add Cy5-DNAs, if relevant, and add Cy3-DNAs. Mix well.
- Add H2 into each tube, and mix thoroughly by pipetting.

- Heat at 100 deg. C for 2" if 30μl, 4" if 93μl
- Spin max speed 1"

- Load samples onto arrays, quickly, and load arrays into pre-heated hyb chamber (with water in base).

Note: If doing many hybridizations at once, I will heat, spin and load tubes 1 chamber at a time, so 9 or 10 slides at a time. You don't want your DNAs to cool off too much between when you heat them and when you load them on the array and get them into the warm chamber. So how many you do at once partly depends on how fast your technique is.

- Hyb arrays overnight, >= 12 hours.

Washing Arrays:

Prepare in bowls:

Wash Solution I:
- 18ml 20X SSC
- 1.8ml 10% SDS
- 580.2ml MilliQ H2O

Wash Solution II:
- 1.8ml 20X SSC
- 598.2ml MilliQ H2O

- Remove 1 hyb chamber at a time from hyb oven. Quickly, transfer slides from hyb chamber to a slide rack submerged in Wash Solution I.

For doing many slides at once, I have two bowls of Wash Sltn I set up, and use the first for gently swooshing off the coverslip and have the slide rack in the second (gentle coverslip removal can be tricky with the slide rack in the same bowl). To remove the coverslip, I hold the slide horizontally and submerge it into the solution, moving it down while tilting it forwards and moving it back, all at once with a swoop of the wrist. This allows the coverslip to float off cleanly with minimal chance of it scratching or touching the array as it's coming off. In theory. Experiment and find your own best way to do it – sometimes the PLL coating can be very delicate and you really want to be as gentle as possible.

- Rinse slides in Wash Sltn I vigorously for 30 sec by plunging slide rack up and down.
I use a plastic tub around the bowl for this because I always splash a lot.

- Transfer the slide rack to Wash Sltn II, blotting base of slide rack on kimwipes to remove excess SDS.
- Rinse slides in Wash Sltn II vigorously for 30 sec
- Cover the bowl with foil so it is dark, and transfer bowl to rotator. Rotate max speed allowable (keeps slides covered still and doesn't splash) for 5".

Note: If I'm doing a lot of slides at once, certainly if doing half the rack or more, I transfer the slides to a clean bowl of Wash Sltn II after 2.5".

- Quickly transfer the slide rack to a plate-rotor in the centrifuge, into a rotor-cup lined with large kimwipes. You may blot the slide rack on other kimwipes as you transfer it. Make sure you have set up balance slides during the previous step – you want the slides to spin ASAP once they are out of the liquid. Spin the slides 90 x g for 5", or until dry. Spin the slides with the array face facing into the direction of spin.

Note: Make sure the centrifuge is very clean before you do this. Dust is your enemy – it autofluoresces.