Livesey lab – Oligo Array Hybridisation Protocol

Questions about this protocol should be directed to James Smith, Livesey lab, Gurdon Institute, University of Cambridge; j.smith@gurdon.cam.ac.uk

We routinely use SMART-amplified double-stranded cDNA for our array hybridisations. That cDNA is generated according to the manufacturer's instructions and used directly in the labelling reaction – we have found little improvement in labelling after cleaning up the PCR-amplified cDNA.

Hybridisation

CY DYES ARE LIGHT SENSITIVE

Labelling

Make up to 35ul DEPC H2O – usually	19ul
Random primers (hex) 1ug/ul	2ul
SMART product – DNA ~400ng/ul	15ul
	36ul per DNA (2 DNA per array)

Denature 95C/ 5min
Snap cool on ice

1 step

Rnd Prm Bf (10x Tris, MgCl2, DTT,BSA)	5ul
3NTP (0.5mM)	5ul
Klenow 10U/ul	3ul
Cy3(pink) or Cy5(purple) dye(cy3 for ref) (1mM)	1ul
	15ul
	50ul sample

Incubate 37C/ 1hour - cover with foil

2 step

Rnd Prm Bf (10x Tris, MgCl2, DTT,BSA)	5ul
3NTP (0.5mM)	5ul
Klenow	1.5ul
Cy3(pink) or Cy5(purple) dye	1ul

Incubate 37C/ 30mins - cover with foil
Denature 95C/ 5min
Snap cool on ice
Add 1.5ul more Klenow
Incubate 37C/ 30mins more - cover with foil

Reaction clean-up

Shake ChromaSpin columns to re-suspend. Number columns and collecting tubes.
Break off bottom first, then lid, put into collection tube, drop in 15ml tube.
Spin at 700g for 5 mins.
Change collecting tube.
Put in the 50ul from each labelled sample to middle of gel. Spin at 700g for 5 mins.
Probes can be stored at 4C - wrap in foil.

Add Cy3 to Cy5 pairwise. Label by both.

For each pair add	20ul Cot-1 DNA (1mg/ml)
	10ul poly Adenylic Acid (10mg/ml)
then 1/10 total volume NaOAc	~13ul
and 3x total volume 100% EtOH	~500ul

Mix well with pipette. Precipitate in foil in freezer for at least 30mins.

Take out, straight in cold room centrifuge at max speed for 15 mins
Pipette off EtOH - care not to disturb pellet
add 1ml 75% EtOH; spin again 5mins.
Decant very carefully, right down to pellet.
Angle tubes down, allow to dry on bench for 15mins
Put hyb buffer in oven to warm to 42C

Add 50ul hybridisation buffer
Heat in 65C bath to resuspend
Vortex
Denature at 95C/ 5mins
Clean slides and lifter slips – barcode if necessary; note barcode numbers
Add probe to slide pipetting at edge of coverslip.
Add 15ul DEPC H2O to wells at each end.
Clip together - don't tip.
Wrap in foil and incubate 42C overnight.

Washing

Make up 1L 2X SSC/ 0.1% SDS at 45C (*make sure solution is at 45C*)
(890ml dH2O, 100ml 20xSSC, 10ml 10% SDS)
1L 0.2x SSC
(990ml dH2O, 10ml 20xSSC)
500ml 0.1x SSC
(497.5ml dH2O, 2.5ml 20xSSC)

Rinse coverslips into beaker of dH2O using squirt bottle with 4xSSC (50ml SSC, 200ml dH2O)

1. Wash slides in 500ml SDS/SSC. Cover and leave for 5mins.
   Remove rack, discard solution. DON'T let slides dry.
2. Cover and leave in fresh SDS/SSC again for 5mins.
   Remove and blot, change rack.
3. Wash in 0.2x SSC for 1 minute
4. Remove and blot, and wash in fresh dish of 0.2x SSC for 1 minute.
5. Remove and blot, and wash in 0.1X SSC for 1 minute.

Spin at 500rpm/ 5min to dry

Rinse cover slips in 96% EtOH individually using forceps, and place in oven to dry between 2 sheets of paper towel.

Hybridisation Buffer (2ml)

1ml	Formamide
0.5ml	20xSSC
0.2ml	50x Denhardt Solution
0.1 ml	10% SDS
50ul	0.2M KPhos (monobasic)
150ul	DEPC
