Microarray Hybridization Protocol

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Introduction:

One microarray set consists of 7 nylon membranes with 2.5 x 7.5 cm dimension. 2304 genes were spotted onto nylon membranes (Schleicher and Schuell GmbH, Dassel, Germany) using a GMS417 Microarrayer (Genetic Microsystems, Woburn, MA) at 665 µm distance between spots.

Preparation of the cDNA probe (one microarray membrane set)

- In tube A prepare: 100µg Total RNA
- DEPC water to 100 µl
- Incubate the tube at 70 °C for 15 minutes (heat block in the hot room).
- Quick chill on ice.
- In tube B combine: 80µl 5X 1st strand buffer
- 40 µl 0.1M DTT
- 10µl RNAse inhibitor 40U/µl (Boehringer 799025)
- 20µl 0.5 mg/ml Oligo(dT)12-18 primer (Pharamacia 27-7858)
- 40µl 10X nucleotide mix without dCTP (Phamacia27-2050,
- 27-2070 and 27-2080)
- 50µl (500 µCi) Redivue [alph-33P]-dCTP (Amersham AH9905)

- DEPC water to 280 µl
- Incubate both tubes at 42 °C for 6 minutes.
- Transfer the contents of tube A to tube B without taking out the tubes from the water bath.
- Add 20µl of Superscript II RT
- Mix well.
- Incubate for 1 hr at 42°C
- Add another 20µl of Superscript II RT
- Incubate for 1 hr at 42°C.
- Add : 50µl 0.5M EDTA pH 8.0
- 100µl 1M NaOH
- Incubate at 65°C for 15 min.
- Cool to R.T
- Add 250µl 1M Tris pH 7.5 to neutralize.
- Purification of probe is done by passing it over micro bio-spin 6 chromatography columns (BioRad 732-6221), as per manufacturer's instructions. Maximal volume per column is 75µl. The draining of the columns can be done outside the hot room. All the spins are done in microcentrifuges.

Prehyb of microarray filters

- Wash filters in 2X SSC at R.T for 10min (Filters are glued to backing paper when DNAs are spotted on it. Therefore, the first thing to do is to wash them.)
- Place bottle of Microhyb solution (Research Genetics HYB250.GF) at 65°C at least 15 minutes before use.
- Denature 1 ml of tRNA (50 µg/µl) (Sigma R-8759) at 95 °C for 10 minutes.

- In 50 ml falcon tube, combine 20 ml heated microhyb solution, 1ml tRNA
- Take 7 polypropylene tubes (15 ml) (Falcon) and make a hole with a needle in their lids.
- Place microarray filters individually into polypropylene tubes. [Use flat forceps to slide the filters into the tubes handling only the edges and never forcing the membrane.] Take care not to crease them.
- Add 3 ml of prehyb solution per tube and incubate for 2-3 hours at 65°C by placing two tubes into large hyb bottle and rotating in hyb oven.
- Place Microhyb solution and dextran sulfate back at 65° C.

Hybridization

PER PROBE:

- Denature 1 ml Cot1 DNA (1mg/ml) (Gibco 18440-016) and 250 µl tRNA (as above) at 95° C for 10 min.
- Place on ice.
- In 50 ml falcon tube combine: 9.5 ml pre-warmed Microhyb solution
- 3 ml 50% Dextran sulfate (Intergen S4030)
- 1 ml Cot1 DNA
- 500 µl polyA
- 250 µl tRNA
- [Note: Pay particular attention to handle Dextran sulfate. 50% Dextran sulfate solution purchased from Intergen should first be aliquoted into 4 ml each after warming the solution and making sure its homogeneity. 50% Dextran sulfate solution is very viscous and may require syringe to measure exact volume.]
- Take into hot room.
- Denature cDNA probe (~ 800 µl) at 95° C for 10 min in water-filled heat block.
- Place on ice.

- Add probe to hyb solution. Mix gently but well.

- Taking one hyb bottle at a time, replace the prehyb solution with the hyb solution. WARNING: MESSY due to needle hole in the lid. If you want to be neat, use large forceps to pull the polyprop tubes out. Use 2 ml of hyb solution per tube.

- Replace hyb bottle and keep rotating at 65° C at least 14 hrs (no harm up to 48 hrs in our hand).

Washing

- Take one bottle at a time and remove tubes with forceps. NEATNESS IS NOT OPTIONAL – THE STUFF IS VERY RADIOACTIVE. GLOVES GET CONTAMINATED ROUTINELY – REPLACE OFTEN.

- Remove membrane from tube using flat forceps. Place 7 membranes (probed with the same probe) into a container with wash solution (see below).

- Washes are as follows: 100 ml of 2X SSC 0.5% SDS RT 20 min

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- Move each membrane into a new polypuropylen tube and do the last 4 washes as follows.

- 3 ml each of 2X SSC 0.5% SDS 65° C 30 min

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- 3 ml each of 0.1X SSC 0.5% SDS 65° C 30 min

- 3 ml each of 0.1X SSC 0.5% SDS 65° C 30 min

- One needs to cut Saran Wrap to a size slightly smaller than the phsophorimager cassette. Fix to the bottom of the cassette with magic tape (this is not trivial).

- Take filters out of wash solution and place on Whatman paper. Blot with another piece of Whatman filter – taking care not to crease the filters (they are curved due to spending time in the 15 ml tubes). Place dried filters onto the Saran wrap. Cut another piece of Saran wrap and try to cover the filters without :

- A. creasing filters

- B: creating bubbles
- C: creasing Saran wrap
- Stick with magic tape.
- Place screen on top of Saran wrap. Place paper towel on top of the screen to make sure there is good contact at all points.
- Expose 7-10 days.

Reference

Tanaka TS, Jaradat SA, Lim MK, Kargul GJ, Wang X, Grahovac MJ, Pantano S, Sano Y, Piao Y, Nagaraja R, Doi H, WoodIII WH, Becker KG, and Ko MSH. Genome-wide expression profiling of mid-gestation placenta and embryo using a 15K mouse developmental cDNA microarray, *Proc. Natl. Acad. Sci. USA*, **97**, 9127-9132, 2000.
