

## Probe Labeling

### First Strand cDNA synthesis

- Use 10  $\mu\text{g}$  total eukaryotic RNA (which has been DNaseI-treated, Qiagen RNeasy purified)
- Add 2  $\mu\text{l}$  Random Hexamer primers (3mg/ml; LifeTech #48190-011) and RNase-free water to 18.5  $\mu\text{l}$
- Mix well and incubate at 70°C for 10 minutes
- Snap-freeze in dry ice/ethanol bath for 30 seconds
- Microfuge for 1 minute



## Probe Labeling (2)

- Add
  - 5 $\times$  Superscript II buffer 6  $\mu\text{l}$
  - 0.1 M DTT 3  $\mu\text{l}$
  - 50 $\times$  aminoacyl-dNTP mix 0.6  $\mu\text{l}$
  - SuperScript II RT (200U/ $\mu\text{l}$ ) 2  $\mu\text{l}$
- Mix and incubate at 42°C for 3 hours
- To hydrolyze the RNA, add:
  - 1 M NaOH 10  $\mu\text{l}$
  - 0.5 M EDTA 10  $\mu\text{l}$
- Incubate at 65°C for 15 minutes
- Add 10  $\mu\text{l}$  1 M HCl to neutralize



## Probe Labeling (3)

### Cleanup using QiaQuick PCR Columns

- Mix cDNA reaction with 300  $\mu$ l (5 $\times$  reaction volume) buffer PB, transfer to QIAquick column
- Place column in collection tube, centrifuge for 1 min at 14,000 rpm
- Add 750  $\mu$ l Phosphate wash buffer, centrifuge 1 min, 14,000 rpm
- Repeat
- Transfer column to fresh tube, add 30  $\mu$ l 4 mM  $KPO_4$ , pH 8.5, incubate for 1 min, centrifuging 14,000 rpm for 1 min to elute
- Repeat, for total elution volume of 60  $\mu$ l
- Dry in a speed vac



## Probe Labeling (4)

### Secondary Coupling and Cleanup

- Resuspend the aminoallyl-labeled cDNA in 4.5  $\mu$ l 0.1 M carbonate buffer, pH 9.0
- Add 4.5  $\mu$ l of the appropriate NHS-Cy suspended in DMSO
- Incubate the reaction for 1 hour in the dark at room temperature
- To the reaction, add 35  $\mu$ l 100 mM NaOAc pH 5.2
- Add 250  $\mu$ l PB buffer
- Wash and elute as before
- Dry to completion in a SpeedVac



## Hybridization

### Prehybridization

- Place slides to be analyzed into a Coplin jar, fill with prehybridization buffer (5×SSC, 0.1% SDS and 1% BSA), and incubate at 42°C for 45 minutes.
- Wash the slides by dipping five times in room temperature MilliQ water.
- Dip the slides in room temperature isopropanol and air dry.



## Hybridization (2)

### Hybridization

- Resuspend each labeled probe in 12µl of 1× hyb buffer (50% formamide, 5×SSC, and 0.1% SDS)
- Combine 12µl of each of purified Cy3- and Cy5-labeled probes, mix well and add:

COT1-DNA (20µg/µl)	1µl
Poly(A)-DNA (20µg/µl)	1µl
- Heat the probe mixture at 95°C for 3 minutes to denature
- Apply the labeled probe to a prehybridized microarray slide and cover with a 22mm×60mm glass coverslip
- Place the slide in a sealed hybridization chamber, add 20µl H<sub>2</sub>O
- Place sealed chamber in a 42°C water bath, incubate 16-20 hours



## Hybridization (3)

### Washes

- Place the slide in low-stringency wash buffer (1×SSC, 0.2% SDS) at 42°C and gently remove the coverslip by agitating
- Wash the slide at high-stringency in a staining dish (0.1×SSC, 0.2% SDS) at room temperature, agitating for 4 minutes
- Wash the slide in 0.1×SSC, agitating for 4 minutes
- Blow slides dry
- Scan and celebrate