Coupling of microarray samples
ASP-version

19th February 2004

Prepering dyes

- Dissolve one original tube of each Dye with 75 μl of DMSO (use vortex).
- Aliquot in amber tubes (10 μl).

Mixing dye and sample

- Resuspended cDNA in every sample tube in 20 μl 0,1M NaHCO3 pH9.
- Mix the two tubes with the same cDNA.
- Add 10 μl of the right cDNA in the right Dye amber tube (20 μl total).
- Coupling during 2 h at RT (2,5 h max.)

Purification using GFX columns

- Add 500μl of CAPTURE BUFFER to every column.
- Transfer the cDNA and mix gently by pipetting up and down 5 times.
- *Leave cDNA in capture buffer longer than 10 min can reduce the probe yield.*
- Centrifuge 13 800 g for 30 s.
- Discard the liquid.
- Add 600 μl of 80% ETHANOL. Centrifuge 13 800 rpm for 30 s.
- Discard the liquid. Repeat the last step twice for a total of 3 washes.
- Discard the liquid. Centrifuge 13 800 rpm for 10 s.
- Transfer each column to a new 1,5 ml tube.
- Add 60 μl of pre-warm (65°C) ELUTION BUFFER. It is crucial that the ELUTION BUFFER completely covers the membrane.
- Incubate the GFX column at room temperature for 1-5 min.
- Centrifuge 13 800 rpm for 1 min.

Measuring the incorporation.

- Use 1 μl of each sample to measure the concentration and incorporation with the NANO DROP.
- Reduce the volume of every sample to 41 μl in speedvac at 42°C for 10 minutes.