ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: Hybridisation		**PAGE: 1 of 3**
SOP#: MA009	**REVISION LEVEL: .1**	**DATE: 31 May 2006**
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1. PURPOSE

This procedure describes pre-treatment and hybridisation to printed Corning GAP II slides containing cDNA elements. The wash protocol for such hybridisations is also included.

2. SCOPE

This method uses formamide, which allows one to hybridise at a lower temperature. For different slide chemistries and for more specific oligonucleotide slides, e.g. diagnostic arrays, hybridisation temperatures, hybridisation buffers and washing regimes have to be further optimised.

3. MATERIALS

Water bath at 60 ^{o}C

Water bath at 42 ^{o}C

Heating block at 95 ^{o}C

Hybridisation chamber

20x SSC (15557-036, Invitrogen)

10% SDS (Sigma L-4390)

Bovine Serum Albumin (BSA) (Roche 735086)

Sigma water (W4502, Sigma)

Formamide (181432, Roche Diagnostics)

Coverslips (24 x 60mm, Marienfeld, Germany)

70% EtOH

4x microarray hybridisation buffer (Amersham)

MilliQ water (Millipore filtered ddH_2O)

4. PROCEDURE

Prepare a 200ml pre-treatment solution and preheat to 60°C in a slide stain rack.
Pre-treatment solution contains:

3.5x SSC (35ml 20x stock)

0.2% SDS (4ml 10% stock)

1% BSA (2g)

Soak slides in stain rack and dish at 60°C for 20min.

During this time practise applying the probe and lowering the cover-slip.

Rinse slides by dipping in dH_2O three times for 2sec each at room temp.

Dip twice in fresh dH_2O

Place slides in centrifuge and dry by centrifuging at 1000rpm for 4min.

Place dry slides into the hybridisation chamber and add 50ul of sigma water to each reservoir on either side of the slide.

Keep the slides free from dust by temporarily covering them with the Perspex cover of the hybridisation chamber.

Hybridisation

Prepare the probe as follows:

Combine the cy3 and cy5 labelled probes into a single tube and dry down in a SpeediVac (55°C, 10min).

Resuspend in 50ul Hybridisation Solution A

Solution A contains:

50% formamide

25% hybridisation buffer (Amersham)

25% Sigma water

Mix briefly by vortex.

Denature at 95°C for 2min in a heating block and place on ice immediately.

Prepare coverslips by spraying with 70% EtOH and wipe them dry.

Place the slides in the Hyb-up chamber and add 20ul mQ per chamber for humidity.

Warm the probe by heating in your hands.

Pipette the probe onto the microarray slide and gently lower the cover-slip.

Close the hyb chamber taking care that the slides are sealed.

Hybridise in a 42°C waterbath overnight.

Washing

Wash slides in the following solutions

Wash1: 1 x SSC, 0.2% SDS for 4min at 42°C

Wash 2: 0.1x SSC, 0.2% SDS for 4min at 42°C

Wash 3: 0.1x SSC, 0.2% SDS for 4min at 42°C

Wash 4: 0.1x SSC 1min at RT

Wash 5: 0.1x SSC 1min at RT

Wash 6: 0.1x SSC 1min at RT

Dip slides in milliQ water a few times

Dry slides by centrifuging at 1000rpm for 4min.

Scan slides within the hour.