ACGT MICROARRAY FACILITY STANDARD OPERATING PROCEDURE		
TITLE: Hybridisation to long oligo arrays		**PAGE: 1 of 2**
SOP#: MA010	**REVISION LEVEL: .1**	**DATE: 24 July 2006**
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1. PURPOSE

This procedure describes hybridisation to arrays containing long oligonucleotides (70mers).

2. SCOPE

This method can be applied to most slide surface chemistries however for some commercially available slides, specific hybridisation buffers are often provided. For more stringent hybridisation, the hybridisation temperature and the wash regime will have to be optimised depending on the result desired. It is recommended that the hybridisation temperature is as close to the Tm of the oligo's as possible for very stringent hybridisation.

3. MATERIALS

Water bath at 55^{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)

Coverslips (24 x 60mm, Marienfeld, Germany)

70% EtOH

MilliQ water (Millipore filtered ddH_2O)

4. PROCEDURE

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).

Mix the following in a microfuge tube:

20 x SSC	6.0ul
10% BSA solution	6.0ul
2% SDS	2.4ul
Labeled Targets	
Water	to 40ul

Denature labelled target mixture by incubating at 95°C for 5min.

Transfer tube immediately to ice.

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 55°C waterbath overnight.

Washing

Wash slides in the following solutions

Wash1: 2 x SSC, 0.5% SDS for 5min at 55°C

Wash 2: 0.5% SDS for 5min at RT

Wash 3: 0.05% SDS for 5min at RT

Dry slides by centrifuging at 1000rpm for 4min.

Scan slides within the hour.