ACGT MICROARRAY FACILITY STANDARD OPERATING PR°CEDURE		
TITLE: DNA probe preparation prior to printing on microarray glass slides		**PAGE 1 of 2**
SOP#: MA017	**REVISION LEVEL: 1**	**DATE: 2003**
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PURPOSE:

This SOP describes how to purify PCR products from a cDNA library for spotting on glass microarray slides. Quality control is important to ensure high quality of future hybridisations on manufactured slides.

PR°CEDURE:

Culture of clones:

Aliquot 75 ml LB-medium (with 100 mg/ml ampicilin) per well into 96-well culture plate (Sterilab 612U96)

- Inoculate the LB plate with the cDNA clones using a plate replicator
- Incubate at 37°C overnight shaking at 150 rpm
- Dilute 20 ml overnight grown culture with 20 ml ddH2O and heat for 15min at 99°C
- 1 ml is used as template for the PCR

PCR Amplification:

Using PCR plates AB-0900 (ABgene) and silicon sealing mats Costar 6555 (Sterilab)

One 100 µl reaction contains:

- 1 x Buffer
- 2.5 mM $MgCl_2$
- 0.2 mM each dNTP
- 0.3 µM each primer
- 1 U Taq (Bioline)
- 1 µl of template

Cycling conditions are:

- 96°C for 5 min
- 94°C for 30 s
- 64°C for 30 s
- 72°C for 2 min

A hold of 72°C for 6 min and 4°C for 10 min, then a hold at 25° C for infinity.

PCR product purification:

- Set aside 5 µl of each product to run on gel and 5 µl of 10 random samples for spec readings
- Load the remainder (95 µl) onto Multiscreen purification plate (Millipore MANU03050) on the vacuum manifold with multi-channel pipette
- Apply 24 in Hg (± 800 mbar) vacuum for 10 min
- Remove plate from manifold and blot dry from underneath with paper towels
- Add 50 ml 10mM Tris pH 8.0 to each well and shake vigorously for 5 min taking care not to spill
- Retrieve purified PCR products from each well with multi-channel pipette and transfer to storage plate
- Set aside 5 µl of purified products for gel analysis, 5 µl for DNA quantification and 5 µl of the same 10 random samples for spec readings

Quality Control:

- Run a 1% agarose gel with un-purified and purified samples next to each other.

  The ACGT microarray facility uses the Stretch 204 rig (Abgene AB-0711) using an 8-channel pipette. The pipette aligns with every second well on the gel. You can therefore load un-purified samples in the odd-numbered wells and simply load purified samples in the even-numbered wells. This places the purified and un-purified samples of the same clones next to each other for easy comparison.