



Glass slide DNA microarray transcript profiling protocol

1. Subject

This protocol describes how to prepare labelled cDNA from total RNA and hybridise the labelled cDNA with glass slide microarrays for transcript-profiling experiments. This method allows you to measure the abundance of every transcript in the cell at a given time and to compare transcript levels in for example, 2 different growth conditions or different genetic backgrounds.

2. Principle

cDNA is made from mRNA and labelled with a fluorescent dye. The labelled cDNA is then hybridized to oligonucleotides or PCR products spotted onto glass microarray slides. Microarrays normally cover the majority of genes within a sequence genome. "Control" cDNA is labelled with one dye and "treatment or sample" cDNA with another. Co-hybridization of both samples with the DNA spotted on the microarray is then performed. Slides are scanned with a laser and spot intensities measured allowing the quantification of transcript levels in both control and samples. Fold changes in expression levels between the two samples can then be calculated. Experiments are performed in triplicate to obtain statistically significant data.

3. Materials and Reagents

- 3.1 Total RNA sufficiently concentrated (greater than $4.2 \mu\text{g } \mu\text{l}^{-1}$)
- 3.2 Ice bucket with wet ice
- 3.3 Oligo dT 15 primer (Promega C110A)
- 3.4 DEPC H₂O
- 3.5 Cy3-dUTP (GE Healthcare PA53022)
- 3.6 Cy5-dUTP (GE Healthcare PA55022)
- 3.7 Superscript II Reverse transcriptase provided with 5x First Strand buffer (Invitrogen 18064-022; $400 \text{ U } \mu\text{l}^{-1}$)
- 3.8 5 mM DTT (Invitrogen)
- 3.9 100 mM dATP, dCTP, dGTP, dTTP (GE Healthcare)
- 3.10 RNase-free microcentrifuge tubes
- 3.11 Waterbath at 42°C
- 3.12 Heating block at 70°C
- 3.13 20 μM EDTA
- 3.14 500 mM NaOH [4.5]
- 3.15 500 mM HCl [4.6]
- 3.16 GFX purification columns (1 per sample), Capture and Wash Buffers provided (GE Healthcare 28-9034-70)
- 3.17 Pipettes 0.5-20, 20-100, 50-200, 200-100
- 3.18 Sterile RNase-free pipette tips white, yellow and blue
- 3.19 Sterile RNase-free microcentrifuge tubes 1.5 ml
- 3.20 Microcentrifuge
- 3.21 BSA

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- 3.22 Formamide
- 3.23 isopropanol
- 3.24 Heating block at 100 °C
- 3.25 SSC/SDS wash solutions [4.9-4.13]
- 3.26 Glass microarray slides (commercial or custom-designed) and lifter-slips (Erie Scientific Company 441-2-775)
- 3.27 Glass array hybridisation cassette (Applied Biosystems AM10040)
- 3.28 Hybridisation oven/incubator at 42°C

4. Solutions

4.1 Labelling master mix:

Prepare 300 µl labelling master mix as follows (n=20). Store unused @ -20°C:

110.4 µl	DEPC H ₂ O	
120 µl	5x RT buffer	(1x)
60 µl	5 mM DTT	(1 mM)
3 µl	100 mM dATP	(500 µM)
3 µl	100 mM dCTP	(500 µM)
3 µl	100 mM dGTP	(500 µM)
0.6 µl	100 mM dTTP	(100 µM)

4.2 DEPC Treated H₂O

dH ₂ O	200 ml	500 ml	1000 ml
Diethyl polycarbonate(0.1%)	0.2 ml	0.5 ml	1 ml

Leave overnight at room temperature. Autoclave.

4.3 0.5 M Na₂EDTA

dH ₂ O	100 ml	200 ml	500 ml
Na ₂ EDTA (MW: 372.08)	18.6 g	37.2 g	93.05 g

pH to 8.0 to ensure solubility

4.4 20 mM EDTA

	20 ml	50 ml	100 ml
0.5 M Na ₂ EDTA	0.8 ml	2 ml	4 ml
DEPC treated H ₂ O	19.2 ml	48 ml	96 ml

4.5 500 mM NaOH

DEPC H ₂ O	0.5 ml	10 ml	20 ml
NaOH (MW = 40)	0.1 g	0.2 g	0.4 g



4.6 500 mM HCl

	5 ml	10 ml	20 ml
11.6 M HCl (from supplier)	215.5 µl	431 µl	862 µl
DEPC treated H ₂ O	4.785 ml	9.569 ml	19.138 ml

4.7 Make 400 ml Prehybridisation Mix containing:

100 ml	20x SSC	(5x)
4 ml	10% SDS	(1%)
4 g	BSA	(1%)

4.8 Make 1.02 ml 2x Hybridisation Mix containing:

0.5 ml	formamide	(50%)
0.5 ml	20x SSC	(10x)
20 µl	10% SDS	(0.2%)

4.9 20x SSC

DEPC H ₂ O	200 ml	500 ml	1000 ml
NaCl (3 M) (MW = 58.4)	35.04 g	87.6 g	175.2 g
Na ₃ Citrate.2H ₂ O (0.3M) (MW = 294.1)	17.65 g	44.12 g	88.23 g

4.10 10% SDS

DEPC H ₂ O	100 ml	200 ml	500 ml
SDS (MW = 288.4)	10 g	20 g	50 g

4.11 Wash Buffer I

	50 ml	100 ml	200 ml
20x SSC (2x)	5 ml	10 ml	20 ml
10% SDS (1x)	5 ml	10 ml	20 ml
dd H ₂ O	40 ml	80 ml	160 ml

4.12 Wash Buffer II

	50 ml	100 ml	200 ml
20x SSC (1x)	2.5 ml	5 ml	10 ml
10% SDS (0.2x)	1 ml	2 ml	4 ml
dd H ₂ O	46.5 ml	93 ml	186 ml

4.13 Wash Buffer III

	50 ml	100 ml	200 ml
20x SSC (0.1x)	0.25 ml	0.5 ml	1 ml
10% SDS (0.2x)	1 ml	2 ml	4 ml
dd H ₂ O	48.75 ml	97.5 ml	195 ml

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4.14 Wash Buffer IV

	50 ml	100 ml	200 ml
20x SSC (0.1x)	0.25 ml	0.5 ml	1 ml

5. Protocol

Preparation & Purification of labelled cDNA

- 5.1 Set up the following reactions in RNase-free microcentrifuge tubes for both “control” and “sample” RNA. To 25 µg RNA add 4 µl oligo dT (2 µg @ 0.5 µg µl⁻¹). Make up to 10 µl with DEPC H₂O.
- 5.2 Incubate for 10 min @ 70°C.
- 5.3 Incubate for 1 min on ice.
- 5.4 Add 15 µl of the labelling master mix [4.1].
- 5.5 Add 3 µl Cy3 dUTP (GE Healthcare) to the control and 3 µl Cy5 dUTP (GE Healthcare) to the sample (to which the control is to be compared).
From now on keep the samples hidden from light as much as possible.
- 5.5 Add 1 µl Superscript II reverse transcriptase (Invitrogen; 400 U).
- 5.6 Mix thoroughly. Briefly centrifuge to collect the contents at the bottom of the tube. Incubate for 1 h @ 42°C.
- 5.7 Add a further 1 µl Superscript II reverse transcriptase (Invitrogen; 400 U). Incubate for 1 h @ 42°C. Pulse to collect the contents at the bottom of the tube.
- 5.8 Add 1.5 µl 20 µM EDTA to stop the reaction.
- 5.9 Add 1.5 µl 500 mM NaOH. Incubate for 10 min @ 70°C to degrade the RNA.
- 5.10 Add 1.5 µl 500 mM HCl to neutralise the reaction.
- 5.11 For each paired samples mix the Cy3 and Cy5 samples together (i.e. co-purify samples which will be co-hybridised on the same micorarray slide).
- 5.12 Add 500 µl of GFX Capture Buffer to the mixed Cy3+Cy5 sample, mix and load onto the GFX purification column.
- 5.13 Centrifuge for 30 s @ max. Discard contents of collection tube.
- 5.14 Add 500 µl GFX Wash Buffer (with ethanol added).
- 5.15 Centrifuge for 30 s @ max. Discard collection tube.
- 5.16 Transfer column into a fresh microcentrifuge tube.
- 5.17 Add 27 µl sterile H₂O. Leave for 1 min @ room temperature.
- 5.18 Centrifuge for 1 min @ max. Discard column. Keep sample in the dark.

Hybridisation of Glass Slide Microarrays

Prehybridisation:

- 5.19 Fill a Coplin jar with Prehybridisation Mix [4.2] and insert slides into the mix ensuring that they are not touching
- 5.20 Prehybridise for 45 min @ 42°C.
- 5.21 Wash by running under dH₂O tap. Tip out and refill 5 times.
- 5.22 Fill jar with isopropanol.
- 5.23 Air dry slides.

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Hybridisation:

- 5.24 Add 25 μ l of the hybridization mix [4.3] to 25 μ l of the purified Cy3- and Cy5-labelled cDNA mix eluted from GFX column.
- 5.25 Boil for 3 min to denature. Pulse centrifuge to collect contents at the bottom of the tube.
- 5.26 Carefully add the mixture to the middle of the printed part of the microarray slide.
- 5.27 Place a 22x50 mm lifter-slip over the mixture. Bubbles should resolve themselves.
- 5.28 Place in a Glass array hybridization cassette.
- 5.29 Incubate overnight @ 42°C.

Washing:

- 5.30 Wash slides in 2x SSC, 1% SDS [4.11] for 15 min @ room temperature with agitation.
- 5.31 Wash slides in 1x SSC, 0.2% SDS [4.12] for 8 min @ room temperature with agitation.
- 5.32 Wash slides in 0.1x SSC, 0.2% SDS [4.13] for 5 min @ room temperature with agitation.
- 5.33 Wash slides in 0.1 x SSC [4.14] for 5 min @ room temperature with agitation.
- 5.34 Place slide in a 50 ml Falcon tube. Centrifuge for 4 min @ 800 rpm to dry.
- 5.35 Scan slide.

6. Literature

- 6.1 Hauser, N.C., Vingron, M., Scheideler, M., Krems, B., Hellmuth, K., Entian, K-D, and Hoheisel, J.D. (1998) Transcriptional profiling on all open reading frames of *Saccharomyces cerevisiae*. *Yeast* **14**: 1209–1221.
- 6.2 Enjalbert, B., Smith, D.A., Cornell, M.J., Alam, I., Nicholls, S., Brown, A.J., Quinn, J. (2006) Role of the Hog1 stress-activated protein kinase in the global transcriptional response to stress in the fungal pathogen *Candida albicans*. *Mol Biol Cell* **17**: 1018-1032.

