Institute of Food Research

Protocol from the *Salmonella* group

Gastrointestinal (GI) tract bacteria detection using a short oligonucleotide microarray

This optimised protocol is designed for 16-21-mer probes.

Slide manufacture and printing

1. Oligonucleotide probes for microarray printing were purchased from Operon (Operon Biotechnologies, Cologne, Germany) and adjusted to 50 pmol μl^{-1} in Quantifoil 1X spotting solution III (Quantifoil Micro Tools GmbH, Jena, Germany).
2. Probes were printed onto epoxysilane coated glass slides (Schott Nexterion, Germany) in duplicate using our Stanford Style microarray spotter (**http://www.ifr.ac.uk/safety/microarrays/default.html#background**). Each probe spot size was 100 µm when printed.

Slide processing for hybridisations

1. Process epoxysilane coated glass slides immediately prior to use.
2. **Note**: Ensure slides are agitated at 250 rpm during all the steps and are not allowed to dry. All solutions are made with Milli-Q water. Washing steps are carried out in black staining troughs (**Photo 2**).
3. Using a diamond tipped pen, mark positions of the microarrays on the glass slides before processing (**Photo 3**). Then put the slides in black slide rack holder (**Photo 4**).
4. Wash slides for 5 mins in 0.1% Triton X-100.

5. Wash slides in 14 N HCl (Sigma-Aldrich, Poole, UK), pH 4.0 for 2 min and then repeat.
6. Place slides in 100 mM KCl for 10 min and then wash twice in Milli-Q water for 1 min.
7. Immerse slides in blocking solution (50 mM ethanolamine (Sigma-Aldrich, Poole, UK) in 0.1 M Tris pH 9.0 at 50°C) for 15 min.
8. Transfer slides to a fresh solution of 0.1% Triton X-100 for 5 min.
9. Wash slides in 2X SSC for 5 min.
10. Wash slides in 0.2X SSC for 5 min.
11. Wash slides in Milli-Q water for 5 min.
12. Spin slides dry at 290 X *g* for 10 min and then store in the dark at room temperature until required. Slides should be used for hybridisation as soon as possible.

Fluorescence labelling of 16S ribosomal gene PCR fragments

1. Extract faecal genomic DNA using the QIAamp DNA stool Mini Kit (Qiagen Ltd., UK) following the manufacturer's instructions.
2. Use 5 ng faecal genomic DNA as template for the PCR amplification of 16S ribosomal genes from faecal genomic DNA. The PCR primers used depend on the individual's needs, but ideally they should amplify the majority of the 16S ribosomal DNA sequence. Clean PCR products using a Qia-quick PCR purification kit (Qiagen Cat: 28104).
3. To label the amplified 16S genes, resuspend 200 ng of purified PCR product in 21 µl water. 200 ng is sufficient for one labelling reaction.

4. Add 20 µl of 2.5 X random primer/reaction buffer mix from the Gibco Bioprime DNA labelling System (Invitrogen, UK).
5. Heat the solution at 100°C for 5 min and then cool on ice for 5 min.
6. Add 5 µl of 10 X dNTP mix (1.2 mM each of dATP, dGTP, dTTP; 0.6 mM dCTP; 10 mM Tris pH 8.0; 1 mM EDTA pH 8.0), 3 µl of 1 mM Cy5 dCTP or Cy3 dCTP (Amersham Biosciences, UK) and 1 µl of Klenow enzyme.
7. Incubate labelling reactions at 37°C for 5 h.
8. Combine Cy3 and Cy5 reactions where appropriate, and use a Qia-quick PCR purification kit (Qiagen Ltd., UK) to remove unincorporated Cy-dyes.

Hybridisation and washing of epoxysilane coated glass slides

1. Concentrate the labelled PCR products to a final volume of 10 µl and add to a pre-prepared mix of 1.5 µl 50X Denhardts solution, 2.25 µl of 20X SSC, 1.125 µl of *E. coli* tRNA (10 µg μl^{-1}) and 0.375 µl of 1 M HEPES, pH 7.0. Then, add 10% SDS (0.375 µl) to the mixture.
2. Incubate at 100°C for 2 min and then leave to stand at room temperature for 5 min.
3. Centrifuge the samples at 9,000 X *g* for 5 min and transfer the supernatant to a fresh tube.
4. Heat the blocked slides on a hot block at 70°C for 30 min.
5. Place a microarray slide in a hybridisation chamber (GeneMachines, CA, USA) and apply the labelling solution over the microarrays. Lower a glass coverslip gently on top of the solution to prevent it drying out and seal the hybridisation chamber.
6. Incubate hybridisations for 15 h at 58°C in a water bath.

7. Place slides in a slide transfer rack for movement between different washes and then directly submerge into 1 litre of wash solution comprising 2X SSC and 0.1% SDS (**Photo 5** and **Photo 6**). Gently stir the wash solution for 5 min at 58°C and ensure the coverslips are removed from the slide.
8. Transfer slides and racks to a solution of 1X SSC and shake at room temperature for 5 min at 250 rpm. Repeat this step.
9. Transfer slides and racks to a solution of 0.2X SSC and shake at room temperature for 5 min at 250 rpm. Repeat this step.
10. Centrifuge dry the slides using a black staining trough (**Photo 2**), the bottom lined with blotting paper, at 290 X *g* for 5 min at room temperature.
11. Scan slides and analyse data.

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