

Random prime labelling of sonicated salmon sperm DNA with Cy3/Cy5

Overview

Sonicated salmon sperm DNA is labelled with Cy3 or Cy5 dCTP and then mixed to produce a Cy3- and Cy5-labelled pool of probe DNA that can be used as a landmark for cDNA or gDNA arrays. The landmark is printed within every sub-grid of a microarray and is used to assist with the spot-finding, principally sub-grid recognition.

Equipment and reagents

- Sonicated salmon sperm DNA (Amersham; Cat. No 27-4565-01)
- dATP, dCTP, dTTP and dGTP (Sigma; Cat. No dNTP-100A)
- Cy3 dCTP (Amersham; Cat. No PA 53021)
- Cy5 dCTP (Amersham; Cat. No PA 55021)
- MilliQ water
- BioPrime DNA labelling kit (GibcoBRL; Cat. No. 18094-011)
 - ◆ 2.5 X Random Primer Reaction Buffer: 125 mM Tris-HCL (pH 6.8), 12.5 mM MgCl₂, 25 mM 2-mercaptoethanol, 750 µg/ml oligodeoxyribonucleotide primers
 - ◆ Klenow Fragment: 40 U/µl Klenow in 50 mM Potassium Phosphate (pH 7.0), 100 mM KCL, 1 mM DTT, 50% Glycerol
 - ◆ Stop buffer: 0.5 M Na₂EDTA (pH 8.0)
- Hot-block, Grant QBT2
- Micro 20 centrifuge, Hettich
- AutoSeq G-50 column (Amersham; Cat. No. 27-5340-01)

Procedure

1. Denature the sonicated salmon sperm DNA (sssDNA):
 - ◆ 2 µl 1µg/µl sssDNA
 - ◆ 19 µl MilliQ water
 - ◆ 20 µl 2.5 X Random Primer Reaction Buffer
 - ◆ Incubate at 100 °C for 5 minutes in a hotblock, then place on ice
2. Prepare a concentrated stock of 10 X low-C dNTP mix:
 - ◆ 25 µl of 100 mM dATP
 - ◆ 25 µl of 100 mM dGTP
 - ◆ 25 µl of 100 mM dTTP
 - ◆ 10 µl of 100 mM dCTP
 - ◆ Make to 500 µl with DEPC treated MilliQ water
 - ◆ Store in small aliquots at -20 °C
3. Perform the random prime labelling:
 - ◆ 5 µl 10 X low-C dNTP mix
 - ◆ 3 µl Cy3/Cy5 dCTP
 - ◆ 1 µl Klenow
 - ◆ Incubate at 37 °C for 2 hours
 - ◆ Stop the reaction by adding 5 µl stop buffer
4. Purify the Cy3/Cy5 labelled sssDNA:
 - ◆ Reduce volume of probe to approximately 25 µl, by placing in a speed vac with medium heat. With our machine, this takes about 30 mins.
 - ◆ Resuspend the resin in the G-50 column by vortexing gently.

- ◆ Loosen the cap a quarter turn and snap off the bottom closure.
 - ◆ Place the column in a 1.5 ml tube.
 - ◆ Pre-spin column at 5,000 rpm for 1 min to remove the buffer (see information supplied with the columns for calculating centrifugation speed). Blot the tip of the column dry using a clean paper towel.
 - ◆ Remove the top cap and place column in a new 1.5 ml tube. Pipette the sample onto the centre of the angled surface of the compacted resin bed being careful not to disturb the resin. Do not allow any of the sample to flow around the sides of the bed.
 - ◆ Spin for 1 min at 5,000 rpm. The unincorporated dye and nucleotides should be retained by the column and the purified labelled probe should pass through into the support tube. Discard the column.
5. Make up to 100 μ l with MilliQ water and then store in aliquotes at -20 °C in the dark
 6. Before use, make dilutions and empirically determine which dilution to use for printing

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