

Preparation of labelled whole chromosome paints by DOP-PCR

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Reagents

- ◆ Flow-sorted chromosomes (approx. concentration 500/ μ l)
- ◆ 2 x PCR buffer: 10 mM MgCl₂, 100 mM KCl, 20 mM Tris-HCl pH 8.4, 0.2 mg/ml gelatin
- ◆ dNTP mix: 2 mM each dATP, dCTP, dGTP, dTTP
- ◆ 6-MW primer: 5' CCGACTCGAGNNNNNNATGTGG 3' (30 μ M)
- ◆ 2.5 U/ μ l *Taq* 1 polymerase (Boehringer Mannheim)
- ◆ 1 mM biotin-16-dUTP or 1 mM digoxigenin-11-dUTP (Boehringer Mannheim)

Method

- 1 Combine in a sterile 0.5 ml microcentrifuge tube:^{a,b,c}
 - x μ l (= 500 flow sorted chromosomes)
 - 50 μ l 2 x PCR buffer
 - 10 μ l dNTP mix
 - 6.6 μ l 30 μ M 6-MW primer
 - 0.5 μ l (= 1.25 U) *Taq* 1 polymerase
 - water to a final volume 100 μ l
- 2 Overlay with 100 μ l mineral oil and run the following program in a DNA thermal cycler:
 - (a) Denature for 3 min at 94 °C.
 - (b) Five cycles of: 1 min at 94 °C, 1.5 min at 30 °C, 3 min at 30–72 °C transition, 3 min at 72 °C.
 - (c) 35 cycles of: 1 min at 94 °C, 1 min at 62 °C, 3 min at 72 °C, with an additional 1 sec/cycle, and final extension time of 10 min.
- 3 Run a 10 μ l aliquot of the amplified products on a 1.2% agarose gel with *PhiX*174 to check the success of the amplification. There should be no amplification in the negative control.
- 4 For second round PCR and labelling, add to a new sterile 0.5 ml microcentrifuge tube:

- 5 μ l of amplified products from first round
 - 25 μ l 2 x PCR buffer
 - 5 μ l nucleotide mix
 - 3.3 μ l 6-MW primer
 - 12 μ l 1 mM biotin-16-dUTP
 - 0.25 μ l *Taq*I polymerase
- 5 Mix well, overlay with 50 μ l mineral oil, and run the following PCR program:
- (d) Denature for 10 min at 93 °C.
 - (e) 25 cycles of: 1 min at 94 °C, 1 min at 62 °C, 3 min at 72 °C, with a final extension time of 10 min.

Remove the mineral oil. Run 10 μ l of labelled products on a 1.2% agarose gel to check the size range. If the labelled fragments are too large, re-cut with 5 μ l DNase I for 30–60 min.

- 6 Purify the labelled DNA through a Select B column (see [Culture and harvesting of human metaphase chromosomes](#)). Measure the DNA concentration of the purified, labelled DNA in a fluorometer (usually 20–50 ng/ml). Ethanol precipitate the labelled DNA with tRNA and salmon sperm DNA as usual, dry, and resuspend in H₂O or TE to a suitable concentration: this is now ready for use as a chromosome paint. Use 100 ng probe plus 6 μ g *Cot-1* DNA per slide.

Notes

- a All of these reagents except chromosomal DNA and *Taq*I polymerase can be sterilized by exposure to short wave UV irradiation (5 min on a transilluminator).
- b All solutions, microcentrifuge tubes, and tips should be autoclaved and kept for PCR only. Use aerosol-resistant tips and add all reagents in laminar flow-hood to minimize contamination.
- c Prepare positive (2.5 pg genomic DNA) and negative (all of the reagents except chromosomes) controls in the same way.