Chromosome painting

The chromosome painting protocol follows this general outline:

(a) Preparation of the hybridization probe (steps1-10).

(b) In situ denaturation of the target DNA and hybridization (steps 11-16).

(c) Post-hybridization washing and detection (steps 17-31).

Equipment and reagents

- Labeled probe (400-600 ng per hybridization)
- Human Cot-1 DNA^{TM} 1mg/ml or mouse Cot-1 DNA 1mg/ml (Gibco BRL)
- Salmon Testes DNA 9.7mg/ml (Sigma)
- Sodium acetate 3M pH 5.2
- Formamide Molecular Biology Grade (Fisher Scientific)
- Formamide 70% v/v in 2XSSC, pH7.5
- Dextran Sulfate (Sigma). Stock solution of dextran sulfate 20 % w/v: dissolve 20 g in 100 ml of 2XSSC, adjust pH to 7.0, autoclave, store at -20 °C. Before using heat, vortex and spin down 14,000 g for a few sec
- 20XSSC
- Absolute ethanol
- Albumin Bovine (Sigma). Blocking solution: Bovine albumin 3 % w/v in 4XSSC/Tween 20
- Polyoxyethilene-sorbitan monolaurate (Tween 20) (Sigma)
- Fluorescin-steptavidin 1mg/ml (Amersham Pharmacia Biotech)
- DAPI (Sigma)
- Mounting medium (Molecular Probes)
- Slide warmer
- Watherbath or heat block
- 60 mm^2 cover glasses
- Coplin jars
- 1.5 ml microcentrifuge tubes

- Microcentrifuge

Method

1. Put in a 1.5 ml microcentrifuge tube 400-600 ηg (≅ 4 μl) of probe DNA. Add 10 μg of the appropriate Cot-1 DNA (10 μl) and 10 μg (1 μl) of salmon testes DNA.
2. Add to the total volume 1/10 volume of 3M sodium acetate pH 5.2 and 3 volumes of ice-cold absolute ethanol.
3. Put the tubes -70 °C for 30 min.
4. Spin down the samples at 14,000 g in 4 °C microcentrifuge for 30 min.
5. Remove carefully the supernatant and dry the DNA probe under vacuum for 2 min.
6. Resuspend the DNA probe in 5μl deionized formamide (pH 7.5) and incubate at 37°C for 30 min. Vortex and spin down at 14,000 g for a few sec every 10 min.
7. Add 5 μl of dextran sulfate 20% w/v in 2xSSC.
8. Denature the DNA probe by heating at 80 °C for 5 min.
9. Spin down a few sec and incubate the DNA probe at 37 °C for 30 min to pre-anneal.
10. Keep on ice until used in step 15.
11. For chromosome slides denaturation place a 60 mm^2 cover glass on a slide warmer at 70 °C and apply 110 μl of formamide 70% v/v solution[a].
12. Touch the denaturing solution with the preparation (sample side) and incubate onto the hot plate for 1-2 min at 70 °C[b].
13. Remove the cover glass and immerse immediately in ice-cold ethanol 70 % v/v for 2 min to stop denaturation.
14. Dehydrate the slide trough an ethanol series of 85% v/v, and 100 % v/v for 2 min each at room temperature and then air dry.
15. Apply the previously denatured and pre-annealed probe. Cover with a 22 mm cover glass and seal the edges with rubber cement.
16. Incubate protected from light in a moist chamber at 37 °C overnight.
17. For post hybridization washes and signal detection. Prepare Coplin jars containing the following solutions:

(a) Formamide 50% v/v in 2XSSC, pH7.5.

(b) 1X SSC.

(c) 4XSSC/ Tween 20 0.1 % v/v (2 jars).

(d) DAPI staining solution, 80ng/ml in 2XSCC (Stock solution: 2 mg DAPI/10 ml sterile H_2O). Cover with aluminum foil.

(e) Distilled H_2O.

18. Warm the washing solutions a, b and c (1 jar) at 46 °C for 30-40 min.
19. Remove rubber cement and coverslip from the hybridized slide. Immerse the slide in jar a; agitate for a few sec and leave it in the jar for 5 min[c].
20. Transfer the slide to jar b; agitate for a few sec and leave it in the jar for 5 min.
21. Transfer the slide to jar c; agitate for a few sec and leave it in the jar for 5 min.
22. Dip the slide in 4 x SSC/Tween 20 at room temperature.
23. Apply 100 µl of blocking solution (Bovine albumin 3 % w/v in 4XSSC/Tween 20) to a 60 mm^2 coverslip, touch the slides to coverslip. Incubate in moist chamber at 37 °C, protected from light for 30 min[d,e].
24. Rinse the blocking solution off by dipping the slide in 4XSSC/Tween 20 for 3 min.
25. Apply to a 60 mm^2 coverslip 100 µl of fluorescin-streptavidin (dilution 1:100 in 4XSSC/Tween 20/Bovine albumin 1 % w/v). Touch the slide to coverslip. Incubate in moist chamber at 37°C, protected from light for 30 min[f].
26. Rinse the slide in 4 x SSC/Tween 20 at room temperature 2 x 5 min shaking gently.
27. Transfer the slide to jar d. Counterstain with DAPI for 5 min at room temperature and protected from light.
28. Rinse the in H_20, jar e, for 5 min at room temperature.
29. Air dry and dehydrate the slide trough an ethanol series of 70 % v/v, 85% v/v, and 100 % v/v for 2 min each at room temperature.
30. Air dry. Apply 30-35 µl antifade mounting media and cover with 60 mm^2 coverslip. Store at 4 °C in the dark until analysis.
31. Visualize by eye using a fluorescence microscope

[a]Make the chromosome spreads as described. Let them dry at room temperature for 1-2 days.

[b]Check the chromosome and nuclear morphology under the phase contrast microscope at low magnification before and after the denaturation procedure to optimize the time for each batch of chromosome preparations. Usually chromosomes acquire a fuzzy aspect due to overdenaturation. If so, reduce the time or the temperature by 3-5 °C. Otherwise increase the denaturation time. For interphase FISH the denaturation time usually is slightly longer. Sometimes it is necessary to remove remnants of cytoplasm.

[c]Do not let the slides dry during the washing and detection procedure.

[d]For probes directly labeled with a fluorescent nucleotide, i.e., Spectrum red-dUTP steps 22-24 are avoided and the procedure continues to step 10.

[e]Spin down all of the solution at 14,000 g for a few sec. before applying to the coverslips. In particular all fluorescent dyes.

[f]Multiple chromosomes can be detected simultaneously provided that distinct detection systems such as biotin/streptavidin and digoxygenin/anti-digoxigenin are used. Probes labeled with Digoxigenin-UTP are detected with Anti-digoxigenin-fluorescein (1:100 dilution).