Protocol G0 Denaturation & Hybridisation Protocol

Combined Whole Chromosome Paints & Pan Centromeric Chromosome Paints

(Protocol G0)

Approx time:

Probe Preparation: 20 min + 16 hrs (Overnight) + 30 min

Solutions to be prepared:	20X SSC

Solution 20XSSC: 87.6g NaCl
44.1g Na Citrate
up to 500ml Deionised Distilled water

Adjust pH to 7.0 using concentrated HCl (before finalising water volume), aliquot and autoclave. Store at 4 °C).

Note: *Ensure all solutions are mixed well.*

Procedure: Slide pretreatment

1. Fix cells in 3:1 ethanol:acetic acid for 30 min, and then let them dry. In order to remove the acid which will interfere with the hybridization the slides are passed through a graded ethanol series 70, 90, 100% for 2 min (each step). Air dry and then the slides can be stored for several days (at RT or 4 °C).
2. In order to ensure the cells stick to the slide the slides are baked at 65 °C for 15 min
3. After baking allow the slides to cool and then transfer slides to acetone for 10 min
4. Air dry slides
5. In order to remove RNA which will cause non-specific binding of the probe and give background, incubate the slides for 1 h at 37 °C in 2X SSC+ RNase (100 µg/ml). Add 200 µl per slide and cover with a parafilm coverslip. Incubate for 1 h at 37 °C in incubator.
6. Wash off in 2X SSC for 5 min
7. Wash in PBS for 5 min
8. Remove excess protein by incubating slides in pepsin. In an Eppendorf tube pipette 1ml of 10mM HCl (stored at 4 °C) and add 0.5 µl of stock pepsin solution (1 mg/ml). Vortex briefly and add 200 µl/slide in humidified slide chamber. Cover with parafilm coverslip and leave at room temperature for 2-3 min

Pepsin Treatment Note: The timing is critical, do not overtreat. Some slides will require different timings depending on the amount of cytoplasm. (NB some cells may require post fixing in formaldehyde after pepsin treatment to retain morphology).

9. Wash slides in PBS for 5 min
10. Dehydrate through ethanol series (2 min each step)
11. Air dry slides
12. Denature cellular DNA by immersing slides in 70% formamide in 2X SSC at 70°C for 2 min(15ml 2X SSC + 35ml formamide).
13. After denaturation place slides in ice cold 70% ethanol for 2 min.
14. Dehydrate cells by passing through ethanol series 2 min each step (70%, 90% and 100%).
15. Air dry slides.

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Procedure: Probe preparation

1. Warm probes to 37°C for 5 min and mix well by vortexing briefly.
2. Add 3 ml of whole chromosome paint to 12ml of hybridisation buffer in a microcentrifuge tube.
3. Denature probe for 10min at 65°C and hold at 37°C for 30-60 min.
4. Add 1ml concentrated centromeric probe + 5ml of hybridisation mix in a separate microcentrifuge tube.
5. Denature probe for 10 min at 85°C. Immediately chill on ice for 1-2 min.
6. Combine whole chromosome probe and centromere probe and apply immediately to denatured cells on slide.
7. Hybridise for approximately 16 hours at 37°C in a humidified chamber.

Procedure: Post Hybridisation Wash

8. Remove cover slip washing for 5 min at 37°C in 2X SSC.
9. Wash slides twice in 50% formamide / 2X SSC 37°C, for 5 min each time.
10. Wash slides in 2X SSC, twice for 5 min each time.

Proceed directly to Detection step in Detection protocol for relevant labels. Do not duplicate Post Hybridisation wash.

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