SKY/FISH of Previously G-Banded Slides

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National Institutes of Health

Reagents

Acetic Acid, glacial
Deionized Formamide
Ambion, Cat. # 9342
dH_2O
Ethanol, absolute
$MgCl_2$, 1M
Methanol, anhydrous
Mallinckrodt AR (ACS), Cat. 3016
Phosphate Buffered Saline (PBS) 1X
Rubber Cement
SSC, 2X
Xylene

Preparation

Fixative (3:1 Methanol:Acetic Acid [Vol:Vol])
Methanol 45 ml
Acetic Acid 15 ml

1X PBS/$MgCl_2$ (f.c. of $MgCl_2$ = 50 mM)
1M $MgCl_2$ 50 ml
1X PBS 950 ml

1% Formaldehyde/1XPBS/$MgCl_2$
Formaldehyde (37%) 2.7 ml
1X PBS 97.3 ml

FA/SSC
Deionized Formamide (FA) 70 ml
2X SSC 30 ml
Adjust pH to 7.0

Procedure: G-Band Slide Pretreatment

1. If required for analysis, first image G-banded slides and record X and Y coordinates from the microscope so one can relocate the identical metaphase spreads that will be subsequently be hybridized with SKY probes (see note 1).

2. Remove immersion oil, if any, from previously G-banded slide by washing the slide in a coplin jar containing xylene for 2 minutes.

3. Rinse slide in a 50 ml coplin jar containing methanol for 2 min.

4. De-stain slide by immersing it into a 50 ml coplin jar containing fixative (Methanol:Acetic Acid).

5. Rinse slide in ddH_2O twice for 5 min each.

6. Rinse slide in 1X PBS twice for 5 min each.

7. Wash slide in 1X PBS/$MgCl_2$ for 5 min.

8. Place slide in 50 ml coplin jar containing

   1% Formaldehyde/1X PBS/1M $MgCl_2$, for 10 min.

9. Wash slide for 5 min in 1X PBS, shaking.

10. Dehydrate slide in ethanol series; 70%, 90%, 100% ethanol, 2 min each

11. Air dry slide.

Procedure: G-Band Slide Denaturation and Hybridization

1. First fill a 50 ml coplin jar with FA/SSC (pH 7, pre-warmed to 70°C) which is then placed inside a waterbath set at 70°C (see note 2).

2. To denature the slide, place it inside the coplin jar for approximately 10-30 sec. Quickly remove slide and place into a new coplin jar containing ice cold ethanol (70%) for 2 min, followed by two more washes in 90%, and 100% ethanol, for 2 min each; air dry slide.

3. Apply probe to dry slide, carefully place a glass coverslip (18 mm^2) over the probe and seal all edges with rubber cement.

4. Hybridize slide for 48-72 hours at 37°C in a moist light tight container (see note 3).

Notes

1. As these slides have been previously G-banded, the slide pre-treatment using pepsin is eliminated as the chromosomes have already been partially digested with trypsin. G-banding enhances the DAPI banding and often results in brighter hybridization signals than the slides pre-treated with pepsin.

2. The time of slide denaturation is dependent on the age of the slide and the extent of digestion by the enzyme (trypsin) used in the G-banding procedure. Older slides (>2 months) require longer denaturation times.

3. Procede with detection of SKY hybridization according to protocols outlined in Detection for SKY located under **SKY Protocols.**