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Pretreatment of Chromosome Slides for FISH/SKY

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Reagents

RNase A
Boehringer, Cat 109169, 100 mg
Pepsin
Sigma, P 6887, 5g
1X $MgCl_2$
1X PBS
1M HCl
2X SSC
Formaldehyde (37%)
Ethanol, Absolute

Preparation of Reagents

Pepsin (Stock Solution)
Dissolve pepsin (20 mg/ml sterile water), boil for 15 min
Cool to room temperature
Aliquot and store at -20°C

1X PBS/$MgCl_2$
1M $MgCl_2$ 50 ml
1X PBS 950 ml

RNAase (Stock Solution: 10%)
Dissolve RNase 100 mg/ml, in sterile water
Keep on ice
Make 50 µl aliquots, store at -20°C

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

0.01M HCl
1M HCl 1 ml
dH_20 99 ml
Adjust pH to 2.0
Pre-warm to 37°C in waterbath

Procedure

1. Equilibrate slides in a coplin jar containing 2X SSC for 5 min at RT.

2. Dilute the RNase stock solution (1:200) in 2X SSC.

3. Apply 120 µl to 24 mm x 60 mm coverslip, touch slide to coverslip.

4. Incubate slides in a moist hybridization chamber at 37°C for 45 min.

5. Carefully remove coverslips and wash slides 3 x, 5 min in a coplin jar containing 2X SSC at RT, shaking.

6. Add 2-30 µl Pepsin stock solution (see notes) inside an empty, clean 100 ml glass beaker, then add 100ml pre-warmed 0.01 M HCl; mix well. Pour 50 ml into a coplin jar.

7. Incubate slides in coplin jar for **2-5** min (see notes) at 37°C.

8. Wash 2 x 5 min each in 1X PBS at RT, shaking.

9. Wash 1 x 5 min in 1X PBS/$MgCl_2$.

10. Place slide in 50 ml coplin jar containing 1% Formaldehyde/1X PBS/$MgCl_2$, 10 min at RT.

11. Wash slide 5 min in 1X PBS at RT, shaking.

12. Dehydrate slide in ethanol series: 70%, 90%, 100% ethanol, 3 min each.

13. Air dry slide.

14. Check slides for chromosome morphology, which should be similar to starting material. Select area for hybridization.

Notes

1. The time of pepsin treatment and amount of pepsin stock solution to be used is dependent on (a) the amount of cytoplasm surrounding the metaphase spreads, as observed with a light microscope using phase objectives before slide pre-treatment and (b) the age of the slide. Slides with excess cytoplasm, seen as a gray particulate haze around the chromosomes, or older than six months may require longer treatment with pepsin (3~5 min) and higher concentrations of pepsin ranging from 10-30 µl.

2. After exposure to the pepsin, one can place the slide into a petri dish containing 1X PBS and look at the slide under an inverted microscope to see if longer pepsin treatment is required. If so, place the slide back into the coplin jar containing the pepsin/acid mixture.

3. It is very important that the pepsin be added to the clean beaker first and **not** directly into the acid solution. If the pepsin is added to the acid solution it causes the pepsin to precipitate and it will not dissolve properly into the acid solution.