PRETREATMENT OF SLIDES FOR IN SITU

Pretreat slides immediately before hybridization. Solutions do not need to be made RNAase-free.

1. Deparaffinize sections by incubating in histoclear for 2 X 10 minutes in a fumehood.

2. Rehydrate through an ethanol series – 2' each. (100% x 2, 95%, 80%, 60%, 30%, distilled H_2O x 2).

3. Incubate in 0.2 M HCl (1.7 ml conc HCl/100ml) for 20 minutes.

4. Rinse in distilled H_2O and transfer to 2 X SSC (300 mM NaCl, 30 mM Na citrate) for 30 minutes.

5. Rinse in distilled H_2O, blot excess H_2O with a paper towel, and incubate in **proteinase K** for 30 minutes at 37° C (See Note 2).

6. Rinse briefly in PBS and then block protease with 2 $mgml^{-1}$ glycine in PBS (2 minutes).

7. Rinse in PBS for 2 X 30 seconds.

8. Fix for 20 minutes in freshly prepared 4% formaldehyde in PBS. (See Note 3.)

9. Rinse in PBS for 2 x 5 minutes.

10. Put 500 ml 0.1 M triethanolamine buffer (6.5 ml triethanolamine + 2 ml conc. HCl in 500 ml) in a glass dish. Put an empty slide rack and stir bar in the bottom. Place on stirrer and stir vigorously. Add 500 µl acetic anhydride and quickly place slide rack containing samples in the dish. (See Note 4.) Stir for 10 minutes.

11. Rinse in PBS for 2 x 1 minute and then dehydrate back through the ethanol series used in step 2.

12. Air-dry slides wrapped in paper towels. Slides are now ready to hybridize.

Notes

1. With the exception of step 5, everything is done at room temperature.

2. Proteinase digestion can be varied via proteinase concentration or time in order to obtain good hybridization signals without loss of morphology. These parameters must be optimized empirically for different tissues and different proteinases.

   Maize leaf sections - use 1 μg ml^{-1} proteinase K.

   Incubate in 100 mM Tris pH 8, 50 mM EDTA. (i.e. 5 ml 2 M Tris and 10 ml 0.5 M EDTA in 100 ml).

3. Steps 8-10 should be carried out in a fumehood. To make paraformaldehyde solution, add solid to PBS (0.15 M NaCl, 10 mM sodium phosphate pH 7.5). Stir in fumehood to dissolve. Will need to add NaOH pellets to get solid into solution and then re-pH with HCl.

4. Acetic anhydride is very unstable ($T_{1/2}$ is under 2 minutes) in aqueous solution, and therefore slides really do have to be added immediately.