

IN SITU HYBRIDIZATION PROTOCOL
POST-HYBRIDIZATION

1. Make up the solutions for this procedure the day before they're needed. For post-hybridization, you will need:

- ◆ 4X SSC (3 x 300 ml per slide holder)
- ◆ RNase solution (20µg /ml final conc.)
- ◆ 2X SSC
- ◆ 1X SSC
- ◆ 0.5 X SSC
- ◆ 0.1X SSC

2. To make 4X SSC, dilute 1 volume of 20X SSC Stocksolution with 4 volumes of DEPC water. (ie. 200 ml of 20 X SSC + 800 ml double distilled water.)

3. To make 1X SSC, dilute 1 volume of 4X SSC with 3 volumes of water (ie, 100ml : 300 ml).

4. To make 0.5X SSC, dilute 1 volume of 1X SSC with 1 volume of water.

5. To make the RNase solution (make this in a bottle used to make RNase solution only), dilute 0.5ml of the Rnase stock solution (12 mg/ml) in 300 ml solution just before use.

6. All incubations are performed in the waterbath set at 40-42°:

| <u>Solution</u> | <u>Time</u> |
|------------------|-------------|
| ◆ 4X SSC | 20 minutes |
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| ◆ RNase solution | 20 minutes |
| ◆ 2 X SSC | 20 minutes |
| ◆ 1X SSC | 20 minutes |
| ◆ 0.5 X SSC | 20 minutes |
| ◆ 0.1X SSC | 20 minutes |

7. Dehydration of the slides is performed at room temperature with the following solutions:

| <u>Solution</u> | <u>Time</u> |
|--|-------------|
| ◆ 70% ethanol and 0.3 M ammonium acetate | 10 minutes |
| ◆ 90% ethanol and 0.3 ammonium acetate | 10 minutes |
| ◆ 100% ethanol and 0.3 M ammonium acetate | 10 minutes |

8. Dry the slides in fumehood. (For about 1 Hour)

Once the slides are dry, examine the slides to see if the tissue is still there.

Check the slide to see that your labelling (tissue ID #, experiment #, type of probe used, etc) is still legible.

9. Turn on the waterbath in the darkroom and set it for 41°C. Check to make sure that there is no light entering into the darkroom.

10. Remove autoradiography emulsion from the freezer (NOTE: previously used one is wrapped in foil) and open the box in the darkroom with only the red light on.
11. Place the emulsion bottle in the waterbath for around 30 minutes, in order to warm up the emulsion. Also, warm up the emulsion dipping chamber at the same time.
12. With the red light on, pour the warm emulsion into the dipping chamber. Pour only the amount of emulsion that you need, since the excess must be discarded after use.
13. Dip the slides into the dipping chamber and make sure that the tissue is adequately covered with the emulsion solution.
14. When finished, leave the slides standing up on a slide holder in the darkroom .
If any emulsion is left in the bottle, wrap the bottle in aluminum foil and put back in refrigerator.
15. Be sure to turn off the red light before you leave. Lock the darkroom and put the "darkroom in use" notice up.