

POLYTENE *IN SITU*

I. Squash Salivary Glands

- 1 Place 6 ul 1:2:3 lactic acid:water:acetic acid on siliconized cover slip
- 2 Dissect salivary gland in 0.7% NaCl
- 3 Fix gland for 30-60 seconds in 45% acetic acid
- 4 Transfer gland to drop of 1:2:3 on cover slip (18mm square kind)
- 5 Cover with slide and squish
- 6 Dip end of slide with cover slip in liquid nitrogen until bubbling stops; breath on frozen slide to get frost and flip off cover slip with a new razor blade
- 7 Wash 3 x 10 min in 95% EtOH; air dry; can store 4°C for many days

II. Denature Chromosomes

- 1 Incubate slides in 2 x SSC at 65°C for 30 min
- 2 Place them in 2 x SSC at RT for 2-10 min
- 3 Incubate in 70 mM NaOH for 3 min
- 4 Rinse in 2 x SSC
- 5 Soak slides in 70% EtOH 2 x 5 min
- 6 Soak slides in 95% EtOH 2 x 5 min
- 7 Air dry and hybridize the same day

III. Hybridize Probe to Chromosomes

- 1 Make Dig probe 100 ng/ml in Hybe solution
Hybe solution:
 - 2 x SSC
 - 50% deionized formamide
 - 12.3 mM Tris pH 7.5
 - 600 mM NaCl
 - 5 x Denhardt's
 - 1 mM EDTA
 - 30 ug/ml salmon sperm DNA
 - 10% Dextran Sulfate
- 2 Denature probe and add 15 ul to 18mm square coverslip (the same siliconized ones used in part I)
- 3 Invert slide onto coverslip and seal with rubber cement
- 4 Place slides into sealed box with wet paper towel and incubate O/N at 45C

IV. *In Situ* Detection of Dig Probe

- 1 Remove rubber cement by hand and coverslips by moving slides

- gently in a beaker full of 2 x SSC
- 2 Wash slides 3 x 20 min in 2 x SSC at 53°C
 - 3 Rinse in PBS at RT 2 x 5 min
 - 4 Rinse once in PTX for 2 minutes ONLY
 - 5 Rinse again in PBS
 - 6 Dilute anti DIG-HRP 1:50 in PBS + 5% NGS
 - 7 Add 100-200 ul anti DIG solution to slides laying flat and cover with a coverslip to keep from drying; incubate 1 hour RT
 - 8 Wash in PBS 3 x 5 min at RT
 - 9 Rinse in PTX at RT 2 min ONLY
 - 10 Add 100-200 ul DAB solution for at least 5 min; check for signal
 - 11 DAB mix:
 - 50 ul 5 mg/ml DAB
 - 10 ul 1% hydrogen peroxide
 - 10 ul 8% nickel chloride
 - 930 ul PBS

PTX=PBS + 0.1% triton X-100