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