

## Procedure for pollen tube staining

### *A. Decolorized aniline blue* (Martin, 1959)

1. Fix styles and/or ovaries in FAA at least 24 hours. These may remain fixed indefinitely.
2. Rinse in tap water.
3. Soak 8-24 hours in nearly saturated aqueous NaOH (approx. 8M = 320 g/L). This softens the tissues and permits the dye penetration.
4. Rinse in tap water for 1 hour or more.
5. Stain in decolorized aniline blue for 4 hours.
6. Mount on slide, place a cover slip over it, and tap.
7. Observe under ultraviolet light, using a 356 nm exciter filter and a 520 nm barrier filter.

### *B. Acidic aniline blue* (Motten, 1992)

1. Place styles in a 10 ml beaker.
2. Add 6M NaOH @ 45-55°C, for 10-20 minutes, until style turns a translucent green.
3. Remove the NaOH with a Pasteur pipet.
4. Add distilled H<sub>2</sub>O for about 4 minutes.
5. Replace the H<sub>2</sub>O with 0.5M pH 6 buffer for 5 minutes.
6. Replace with acidic aniline blue for 15-20 minutes.
7. Rinse with distilled H<sub>2</sub>O.
8. Place on a microscope slide, cover with a slip, and squash.
9. Observe under a light microscope.