This protocol follows the technique of Geber and Schweizer (1988) with minor modifications of Siljak-Yakovlev *et al.* (in press) and some adaptations of our own.

Previously: thaw and preheat (in the incubator at 37°C) the enzyme mixture (see "Reagents"). Prepare a preheated glass Petri dish (incubate at 37°C) with wet filter paper and two matches with their heads cut on it.

1. Wash the roots (3-4 if they are large, 6-7 if they are small) for 15 minutes in 0.05M citrate buffer in a vial with gentle agitation in 3D-shaker.

2. Cut root tip(s) under the magnifying glass, over a clean slide, with a little bit of 0.05M citrate buffer to avoid desiccation.

3. Add enzyme mixture (either undiluted or 1:1 diluted in 0.05M citrate buffer). Use approximately a 20 µl volume. This volume is adjustable and will depend on the size of the root tip. Carefully, move the slide containing the enzyme mixture and root tip(s) in the preheated Petri dish, leaning on both matches.

4. Incubate (37°C) for variable time. This depends on the species, the hardness and size of the material, its age, etc. It can vary from 10 to 60 minutes. Less concentrated enzyme mixtures will need more digestion time.

5. Look under the magnifying glass to see if the tissue is properly digested (if the material disaggregates easily). Carefully remove the excess of enzyme with a pipette. Wash with 0.05M citrate buffer and slightly dry it again.

6. Put a drop of freshly prepared 45% acetic acid (pipette) in a clean slide (or treated with poly-L-lysine).

7. Carefully, crush the material with needles, tweezers or scalpel.

8. Very gently, apply a coverslip, avoiding the formation of air bubbles. Press carefully, with the help of some filter paper or by tapping with needles or tweezers.

If the material has not been sufficiently dispersed, you can try to heat it with a Bunsen, but the slide should always be kept far away from the flame.

9. View with a phase contrast microscope at x20 and x40 or with a normal microscope with a tiny drop of acetic carmine 1%. *If England Finder is available, record the position of the good metaphases.*

10. **Coverslip removal:** following Conger and Fairchild (1953) and Siljak-Yakovlev *et al.* (in press):
- freeze preparations to below -70°C either using liquid nitrogen, CO_2, dry ice or on a metal plate in a -80°C freezer (in the last case, it should be there for at least 24 hours)
- quickly remove coverslips with a razor blade, rinsing briefly with absolute EtOH. Air dry and store at RT until next used (for at least2 days).

Literature

Conger AD & Fairchild LM. 1953. A quick freeze method for making smear slides. Stain Technol 28: 281-283.

Geber G & Schweizer D. 1988. Cytochemical heterochromatin differentiation in *Sinapis alba* (Cruciferae) using a simple air-drying technique for producing chromosome spreads. Plant Syst Evol 158: 97-106.

Siljak-Yakovlev S, Pustahija F, Vicic V & Robin O. Molecular cytogenetics (FISH and fluorochrome banding): resolving species relationships & genome organization. In: Methods in Molecular Biology, Series Ed.: Walker, John M. Springer-Verlag (in press).

(Non-mandatory steps in italics)