Supplementary file S4
Protocol for preparation and preservation of nuclear suspensions in 30% glycerol


Step I (field laboratory)
- **Otto I isolation buffer** (0.1 M citric acid, 0.5% Tween-20), ice-cold
- 60% glycerol solution
- reference standard(s): living plant(s) with appropriate genome size (optimally to be transported in seeds and grown at the site)
- approximately 5-cm plastic Petri dishes
- razor blades (double-edged)
- 30- to 50-μm nylon mesh
- approximately 2-ml sample tubes (lockable) and appropriate tube holder
- 0.1 to 1ml micropipette with appropriate tips
- Freezer

1. Using a sharp razor blade, chop an appropriate amount of intact vegetative tissue from the plant to be analysed together with tissue of the internal reference standard into fine pieces in a plastic Petri dish containing 500 μl of ice-cold Otto I isolation buffer.

2. Mix the homogenate and filter the nuclear suspension through a 30- to 50-μm nylon mesh (to remove large debris) into lockable sample tubes.

3. Add 500 μl of 60% glycerol and shake gently.

4. Store in a freezer at approximately -18°C.

Step II (FCM laboratory)
- **Otto I isolation buffer**, ice cold
- **Otto II staining solution** (Otto II buffer (0.4 M Na₂HPO₄.12 H₂O) supplemented with β-mercaptoethanol (final concentration of 2 μL mL⁻¹) and a fluorochrome (PI plus RNase IIA, both at final concentrations of 50 μg mL⁻¹ or DAPI at a final concentration of 4 μg mL⁻¹)
- flow cytometer with appropriate excitation source (e.g., mercury arc lamp or UV diode for DAPI staining, or 488-/532-nm laser for PI staining) and appropriate software for histogram evaluation
- bench-top centrifuge
- 3.5-ml flow cytometer sample tubes and appropriate tube holder
- set of micropipettes with appropriate tips

5. Centrifuge the sample for three minutes at 3,200 RPM. After centrifugation, remove the supernatant, resuspend the pellet in 100 μl of fresh ice-cold Otto I isolation buffer and incubate for 15 min at room temperature.

6. Add 1 ml of fresh Otto II staining solution and shake well. Let the sample stain for 5 to 15 min at room temperature and then analyse on a flow cytometer.