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Extraction of nuclei from plant tissue by chopping up prior to cytometry of nuclear DNA

Alternative Extraction buffers

- Brown Nuclear Buffer: 45 mM $MgCl_2$, 60 mM sodium citrate, 20 mM 4-morpholinepropane sulphonate pH7, 0.1% (w/v) Triton X-100, 1% polyvinyl pyrrolidone (~10,000 M_r , Sigma P6755), 5 mM sodium metabisulphite (syn. pyrosulphite, $Na_2S_2O_5$ M_r 190; added every twelve hours from 1 M frozen stock). This is modified from Galbraith *et al.* (1983); stronger buffer (for acidic plants or fruit), protectant against tanning (PVP) and antioxidant (metabisulphite, not toxic like β-mercaptoethanol) are used. Triton may be raised to 0.5% for oily tissues (*Pistacia* sp. etc.) and to lyse chloroplasts which fluoresce red; keep 10% (w/v) Triton X-100 stock, autoclaved and stored at 4°C for this purpose.

- Doležel Nuclear Buffer (Dolezel *et al*., 2007): 20 mM NaCl, 80 mM KCl, 20 mM MgSO4, 2 mM EDTA.Na2, 0.5 mM spermine.HCl, 15 mM Tris pH 7.5, 0.1% (w/v) Triton X-100, 15 mM β -mercaptoethanol (1 µl / ml) added daily – but this is toxic. It is better to use metabisulphite.

- When using intercalating stains such as propidium iodide, add 10 µl RNase/ml from a 1% RNase stock (stored frozen).

Chopping Method

1. Place 1-2 cm^2 leaf (avoiding major vascularisation) or about 150 mg callus, roots, stems, apices, anthers, cells, etc. on a 90 mm plastic or glass Petri dish. Add a smaller quantity of leaf of an internal standard at this stage (e.g. *Arabidopsis thaliana*, tomato, *Petunia*, pea, barley, *Artemisia*, wheat – see "Standards for plant genome size by FC").

2. Cover with 1 mL ice-cold nuclear buffer.

3. Chop up with a fine double-edge razor blade, limiting dispersion or drying. Work quickly: 30 sec should suffice. If mucilage, browning, or instability appears, simply cut less.

4. Transfer this to a 50 µm filter (CellTrics, Partec: reusable) in an ice-cold cytometer sample tube.

5. Add 50 µg/mL propidium iodide and mix promptly. (Increase this concentration to 100 µg/mL for genomes 2C> 20 pg.) Hold samples on ice for at least 5 minutes.

6. Assess by cytometry at room temperature. Gate on Side-Scatter and propidium iodide in order to eliminate debris. Satisfactory samples may be surprisingly dilute compared to preparations of mammalian cells, with only 5-10,000 nuclei/mL and sometimes 10-fold more debris. If possible, record nuclear fluorescence on two scales in parallel, linear and logarithmic. The position of the 2C population (taking the modal peak position) of the unknown relative to the internal standard serves to deduce the 2C DNA value. The logarithmic scale gives a broader view of endoreplication and additional peaks (parasites possibly on tissue, etc.). Note down indicators of quality: Coefficient of Variation of the peaks, stability, symmetry, linearity between 4C and 2C populations, etc.

7. Liquid waste (sheath) containing propidium iodide should be disposed of either as toxic waste or, more conveniently, after absorbing the propidium onto resin overnight (see "Destaining Amresco Imagif").

References

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See note: "**Despatching plant material for cytometric assessment of 2C DNA**".