Fluorochromes:

- **Propidium iodide (PI):** Prepare a stock solution of 1 mg/ml and filter through a 0.22 µm filter. Store in 1 ml aliquots at -20°C. The working concentration of PI is usually 50 µg/ml.
- **4',6-diamidino-2-phenylindole (DAPI):** Prepare stock solution of 0.1 mg/ml and filter through a 0.22 µm filter. Store in 1 ml aliquots at -20°C. The working concentration of DAPI is usually 4 µg/ml.

Isolation buffers:

Isolation buffers must be prepared using either single- or double-distilled water, filtered through a 0.22 µm filter to remove suspended particles, and stored as specified. The pH of the buffers is adjusted either with 1M NaOH or with 1N HCl.

- **Lysis buffer LB01 (Doležel *et al.*, 1989):** 15 mM Tris, 2 mM Na_2EDTA, 0.5 mM spermine.4HCl, 80 mM KCl, 20 mM NaCl, 0.1 % (v/v) Triton X-100. Adjust to pH 7.5. Add β-mercaptoethanol to give a final concentration of 15 mM. Store the buffer either at 4°C if used regularly or at -20°C in 10 ml aliquots. Concentrations of 0.5%, 4% and 8% of Triton X-100 can be used in recalcitrant materials.

- **Tris $MgCl_2$ buffer (Pfosser *et al.*, 1995):** 200 mM Tris, 4 mM $MgCl_2$, 0.5 % (v/v) Triton X-100. Adjust pH to 7.5 and store at 4°C.

- **Galbraith's buffer (Galbraith *et al.*, 1983):** 45 mM $MgCl_2$, 20 mM MOPS, 30 mM sodium citrate, 0.1 % (v/v) Triton X-100. Adjust pH to 7.0. Store the buffer either at 4°C if used regularly or at -20°C in 10 ml aliquots.

- **General purpose buffer (Loureiro *et al.*, 2007):** 0.5 mM spermine.4HCl, 30 mM sodium citrate, 20 mM MOPS, 80 mM KCl, 20 mM NaCl, 0.5 % (v/v) Triton X-100. Adjust to pH 7.0. Store the buffer either at 4°C if used regularly or at -20°C in 10 ml aliquots.

- **Woody plant buffer (Loureiro *et al.*, 2007):** 200 mM Tris, 4 mM $MgCl_2$, 2 mM Na_2EDTA, 86 mM NaCl, 10 mM sodium metabisulphite, 1 % PVP-10, 1 % (v/v) Triton X-100. Adjust to pH 7.5. Store the buffer either at 4°C if used regularly or at -20°C in 10 ml aliquots.

- **$MgSO_4$ buffer (Arumuganathan and Earle, 1991):** 9.53 mM $MgSO_4$, 47.67 mM KCl, 4.77 mM HEPES, 6.48 mM DTT (*see* **note 1**), 0.25 % (v/v) Triton X-100. Adjust to pH 8.0. Store the buffer either at 4°C if used regularly or at -20°C in 10 ml aliquots.

- **De Laat's buffer (de Laat and Blaas, 1984):** 15 mM HEPES, 1mM Na_2EDTA, 0.2 % (v/v) Triton X-100, 80 mM KCl, 20 mM NaCl, 15 mM DTT, 0.5 mM spermine.4HCl, 300 mM sucrose. Adjust to pH 7.0 and store at 4°C.

- **Ebihara's buffer (Ebihara *et al.*, 2005)**: 50 mM Na_2SO_3, 50 mM Tris, 40 mg/mL PVP-40 (*see* **Note 7**), 140 mM β-mercaptoethanol. Adjust to pH 7.5 and store at 4°C.

- **Seed buffer (Matzk *et al.*, 2001)**: 5 mM $MgCl_2$, 85 mM NaCl, 100 mM Tris, 0.1 % Triton X-100. Adjust to pH 7.0 and store at 4°C.

- **Otto's buffers (Otto, 1992)**:
  Otto I: 100 mM citric acid monohydrate, 0.5 % (v/v) Tween 20 (cell culture tested grade of Tween 20 from Sigma-Aldrich (cat. no. P2287) is used. Tween 20 for molecular biology (Sigma cat. no. P9416) is not suitable for FCM. Store at 4°C.

  Otto II: 400 mM Na_2HPO_4. Store at room temperature. The fluorochrome (DAPI or PI, see above) can be added to Otto II before adjusting the final volume of the stock solution. If this is done the buffer should be stored in the dark at room temperature.

- **Baranyi's buffer (Baranyi and Greilhuber, 1995)**: Baranyi's solution I: 100 mM citric acid monohydrate, 0.5 % (v/v) Triton X-100. Store at 4°C.

  Baranyi's solution II: 400 mM Na_2HPO_4, 10 mM sodium citrate, 25 mM sodium sulphate. Store at room temperature.

- **Mishiba's buffer (Mishib*a et al.*, 2000)**: Solution A: (see recipe for Galbraith buffer, i.e. buffer 3 above). Solution B: 10 mM Tris, 50 mM sodium citrate, 2 mM $MgCl_2$, 1 % PVP-40 (original recipe used PVP K-30), 0.1 % Triton X-100, 18 mM β-mercaptoethanol. Adjust to pH 7.5. Store at 4°C.

- **Brown's Nuclear Buffer:** 45 mM $MgCl_2$, 60 mM sodium citrate, 20 mM 4-morpholinepropane sulfonate 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.S

- **Doležel's 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.

References

Arumuganathan K, Earle ED. 1991. Nuclear DNA content of some important plant species. *Plant Molecular Biology Reporter*, 9: 208–218.

Baranyi M, Greilhuber J. 1995. Flow cytometric analysis of genome size variation in cultivated and wild *Pisum sativum* (Fabaceae). *Plant Systematics and Evolution*, 194: 231–239.

de Laat AMM, Blaas J. 1984. Flow cytometric characterization and sorting of plant chromosomes. *Theoretical and Applied Genetics*, 67: 463–467.

Doležel J, Binarova P, Lucretti S. 1989. Analysis of nuclear DNA content in plant cells by flow cytometry. *Biologia Plantarum*, 31: 113–120.

Doležel J, Greilhuber J, Suda J. 2007. Flow Cytometry with Plant Cells Analysis of Genes, Chromosomes and Genomes. Weinheim: *Wiley-VCH Verlag GmbH & Co. KGaA*.

Ebihara A, Ishikawa H, Matsumoto S *et al.* 2005. Nuclear DNA, chloroplast DNA, and ploidy analysis clarified biological complexity of the *Vandenboschia radicans* complex (Hymenophyllaceae) in Japan and adjacent areas. *American Journal of Botany*, 92: 1535–1547.

Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E. 1983. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science*, 220: 1049–1051.

Loureiro J, Rodriguez E, Doležel J, Santos C. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany*, 100: 875–888.

Matzk F, Meister A, Brutovská R, Schubert I. 2001. Reconstruction of reproductive diversity in *Hypericum perforatum* L. opens novel strategies to manage apomixis. *The Plant Journal*, 26: 275–282.

Mishiba KI, Ando T, Mii M *et al.* 2000. Nuclear DNA content as an index character discriminating taxa in the genus *Petunia* sensu Jussieu (Solanaceae). *Annals of Botany*, 85: 665–673.

Otto F. 1992. Preparation and staining of cells for high-resolution DNA analysis. In: Radbruch A ed. Flow cytometry and cell sorting. Berlin: *Springer-Verlag*, 101–104.

Pfosser M, Amon A, Lelley T, Heberlebors E. 1995. Evaluation of sensitivity of flow-cytometry in detecting aneuploidy in wheat using disomic and ditelosomic wheat-rye addition lines. *Cytometry*, 21: 387–393.