imagif La plateforme du vivant	Protocol n° : **CY-06**	Version English	
	DESPATCHING PLANT MATERIAL FOR CYTOMETRIC ASSESSMENT OF 2C DNA		

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www.imagif.cnrs.fr/pole-1-Biologie_cellulaire.html

Purpose

Colleagues often need to send plant material by post for cytometric assessment of its 2C DNA value. The aim of this note is to avoid sample deterioration, to ensure reliability and rigour, with adequate sampling and communication.

Materials

Pertinent plant tissue

Thin blotting paper

Plastic bags (14x8 or 9x5 cm), e.g. small food-freezer bags

Permanent marker

A summary file of samples

Methods

1. Despatching

The package is to be despatched with all costs paid using either a delivery service (DHL, etc.) or routine mail services but avoiding weekends. Proper customs and plant health documentation must be provided where necessary. Our cytometry lab needs to be reserved in advance and informed of despatch, and we will confirm reception. We aim to retain material until results have been accepted by the client; it is then destroyed. Files are maintained for 10 years.

Use the address:

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2. Labelling samples

Identify each sample clearly in a manner readable without having to open each bag. The marker (or pencil) must be water-resistant. Group samples together by theme or sector, by taxon, population, etc. If several tissues are in one bag, it must be clear as to whether these are simply additional tissues from the same individual or replicates to be analysed separately (e.g. individuals from a population site). Test preservation well prior to the experiment, or maybe make a simple trial despatch.

Provide a summary of samples, both on paper (enclosed) and as a file (Word or Excel) sent by electronic mail. We disapprove of unintelligible codes. If full randomisation is necessary for statistical design, we must be consulted, as it makes the task more difficult.

Indicate:

- whether several leaves in a bag represent different individuals to be analysed as replicates or simply additional material of the same individual(s)/population.
- priorities or difficulties to be expected.
- possible or expected ploidy difference (giving details).
- if possible, the expected 2C value (in pg or Mbp) as found in databases (e.g. http://www.asteraceaegenomesize.com or http://data.kew.org/cvalues/), in our or your publications, or from cytogenetic information.
- the internal sample used previously or suggested in publications
- the statistical design of the experiment: e.g. 5 analyses per population, several populations per taxon, etc.
- whether the aim is to establish a reliable 2C value or more simply to distinguish ploidy groupings or population uniformity.

The aim is to be able to return to the field (or accessions), after analysis or after publication, in order to identify the same specimen or an atypical population. In certain situations herbarium vouchers should be maintained.

Do not hesitate to include several individuals as routine reference samples throughout different campaigns, to be used year after year, covering your accessions or those in the scientific literature.

3. Preparing tissues for despatch

To assess the 2C DNA value, several cm^2 of leaf is adequate; however, to allow for errors in the lab (adjusting the protocol for interfering compounds, mucilage, etc., and to adapt the internal standards), a more generous quantity of material is desirable.

To maintain tissue in good condition during transport, it is recommended to place each sample in slightly humid absorbent paper (type blotting or filter paper: **not** Kleenex which tears and is fluorescent). This is then to be enclosed in folded aluminium foil or a plastic bag (typically 14x8 cm or 9x5 cm). Avoid excess humidity or humid tissue in contact with plastic as decay will set in. If necessary, wipe field samples clean.

For some species, it is far easier to analyse stems and petioles than the leaves themselves.

Analysing *in vitro* samples is usually easy. However, recently-treated material (e.g. from colchicine) is generally chimeric and gives histograms that are highly ambiguous: such experiments require proper consultation for design.

4. Precautions

Be careful not to damage samples by extreme cold! This has occurred with too cold compartments in refrigerators (e.g. with tropical plants) or freezer blocks that are too strong or too close to the samples! The cytometric task then becomes laborious or impossible.

Exceptional field conditions and despatch may necessitate tissue lyophilisation or, less satisfactorily, immediate storage with silica-gel. Precision will suffer and a systematic deviation relative to fresh material might be observed, justifying application of a correction factor (Razafinarivo *et al.*, 2012). Such strategies are thus less favourable, and in any case must be initially validated and routinely checked.

Material frozen in liquid nitrogen can be analysed directly. However, the internal standards may need the same treatment.

Useful references

Reviews:

- Catrice O, Coba de la Peña T, Brown SC. 2006. Applications en biologie végétale: contraintes, succès, espoirs. Chapitre 12 in "La cytométrie en flux", Ronot X, Grunwald D, Mayol JF, Boutonnat J (eds). Tec & Doc - Lavoisier, Paris, pp 235-253.
- 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*

Buffers:

- Coba de la Peña T, Brown SC. 2001. Flow cytometry. In: "Plant Cell Biology: A practical approach". Hawes C, Satiat-Jeunemaître B (eds). Oxford: *Oxford university press*, chpt. 3.

Teaching:

- Marie D, Brown SC. 1993. A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell,* 78: 41–51.

Example:

- Siljak-Yakovlev S, Pustahija F, Solic EM, Bogunic F, Muratovic E, Basic N, Catrice O, Brown SC. 2010. Towards a genome size and chromosome number database of Balkan flora: C-values in 343 taxa with novel values for 242. *Advanced Science Letters,* 3: 190–213.

Lyophilisation:

- Razafinarivo NJ, Rakotomalala JJ, Brown SC, Bourge M, Hamon S, de Kochko A, Rakotondravao A, Poncet V, Tranchant-Dubreuil C, Couturon E, Guyot R, Hamon P. 2012. Geographical gradients in the genome size variation of wild coffee trees (*Coffea*) native to Africa and Indian Ocean islands. *Tree Genetics and Genomes,* 8: 1345–1358.