See the document "Reagents for plant genome size by flow cytometry" for the instructions on how to prepare the solutions used in this protocol.

1. Healthy leaf tissue* from the species to be studied and a calibration standard are placed together in a plastic Petri dish in around 1000 µl of nuclei isolation buffer, chopped up with a razor blade and supplemented with 100 µg/ml ribonuclease A.

2. The amount of target species leaf (about 25 mm^2) is approximately twice that of the internal standard.

3. 600 µl of the suspension of nuclei in isolation buffer (usually LB01) is filtered through a nylon mesh with a pore size of 70 µm and stained for 20 min with 36 µl of propidium iodide (1 mg/ml) to a final concentration of 60 µg/ml; tubes are kept on ice during staining and until measurement. Amounts of isolation buffer and propidium iodide can be proportionally increased up to 1200 µl and 72 µl respectively, depending on the specific requirements of the target species tissue (as different tissues may absorb more or less reagents).

4. For each population, five individuals are analysed; two samples from each individual are extracted and measured independently.

5. The total nuclear DNA content is calculated by multiplying the known DNA content of the internal standard** by the quotient between the 2C peak positions of the target species and the chosen internal standard in the histogram of fluorescence intensities for the 10 runs, based on the assumption that there is a linear correlation between the fluorescence signals from stained nuclei of the unknown specimen and the known internal standard and the DNA amount.

6. Mean values and standard deviations are calculated based on the results for the five individuals (ten samples). We also calculate the mean half peak coefficient of variation (HPCV) corresponding to ten samples for the target plant and for the internal standard is also calculated.

Optional: to ascertain that the instrument gives a linear response across the range of genome sizes studied, we can perform several assays that include both internal standards and one of the populations with the highest genome size at the same time. If the difference between the obtained results with respect to each standard is negligible, this verifies the linearity of the flow cytometer for this interval and the use of the chosen internal standards.

(*) Although this protocol is usually performed on fresh leaf tissue, it has also been successfully tested on seeds.

(**) We typically use as internal standard species: *Solanum lycopersicum* L. 'Montfavet 63/5', (2C=1.99 pg), *Petunia hybrida* Vilm. 'PxPc6' (2C=2.85 pg), *Pisum sativum* L. 'Express Long' (2C=8.37 pg) and *Triticum aestivum* L. 'Chinese Spring' (2C=30.9 pg). For more details on appropriate internal standards, see the document "Standards for plant genome size by flow cytometry".

Our flow cytometer

We regularly perform our measurements at the Centres Científics i Tecnològics of the Universitat de Barcelona using an Epics XL flow cytometer (Coulter Corporation, Hialeah, Fla.). The instrument is set up in the standard configuration: excitation of the sample is done using a standard 488-nm air-cooled argon-ion laser at 15 mW power. Forward scatter (FSC), side scatter (SSC), and red (620 nm) fluorescence for propidium iodide are acquired. Optical alignment is based on optimised signal from 10- nm fluorescent beads (Immunocheck, Epics Division, Coulter Corporation). Time is used as a control of the stability of the instrument. Red fluorescence is projected on 1024 monoparametrical histograms. Gating single cells by their area versus peak fluorescence signal excluded aggregates. Acquisition is automatically stopped at 8000 nuclei.

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