Flow Cytometry Protocol
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REAGENTS:
Woody Plant Buffer* (WPB; Loureiro et al., 2007)
- 0.2M Tris, pH 7.5
- 4mM MgCl2
- 2mM EDTA
- 86 mM NaCl
- 10 mM Sodium Metabisulfite
- 1% (w/v) PVP-10
- 1% (v/v) Triton X-100

*Final concentrations are given. Buffers should be stored in aliquots at 4-8°C and remain stable for multiple months.

For a final volume of 1000 mL of WPB:

Reagent	Volume/Mass	Final Concentration
1M Tris-HCl, pH 7.5	200mL	0.2M
$MgCl_2$ $6H_2O$	0.81g	4mM
0.5 M EDTA $Na_2 2H_2O$	4mL	2mM
NaCl	5.03g	86mM
Sodium metabisulfite	1.901g	10mM
PVP-10	10g	1%
Triton X-100, pH 7.5	10mL	1% (v/v)
H_2O	Top up to 1000mL (~700-746mL)	

P.I. Stock Solution
- 1 mg propidium iodide in 1 mL of DI water
  Note: Store as 1 mL aliquots at -20°C. Once tawed, stable at room temperature but should be protected from light.

Other supplies
- RNase A (optional, for fresh material)
- BD Falcon tubes with 5uL mesh cell strainger cap, 5 mL polystyrene round-bottom
  - Ref 352253, 25/pack: pore size 15-50 um
- Petri dishes on ice
- Single-edged razor blades
- Standards

Standard	2C Genome Size (pg)	Reference
Raphanus sativus cv. Saxa	1.11	Doležel et al. 1992
Solanum lycopersicum cv. Stupicke	1.96	Doležel et al. 1992
Glycine max cv. Polanka	2.50	Doležel et al. 1994
Zea mays CE-777	5.43	Lysák et al. 1998
Pisum sativum cv. Citrad	9.09	Doležel et al. 1998
Secale cereale cv. Dankovske	16.19	Doležel et al. 1998
Vicia faba cv. Inovec	26.90	Doležel et al. 1992
Allium cepa cv. Alice	34.98	Doležel et al. 1998

METHODS

Note: In advance, book the flow cytometry machine via iLab and prepare the buffer and staining solutions (above).

1. Place the petri dish with the samples on ice.
2. Place leaf material in half of a petri dish. The amount of tissue is group-specific and can be rather subjective. Estimate by eyeballing the size of a pinky nail – aim for 250 mg fresh weight. Include a standard tissue to be co-chopped. A smaller amount of the standard relative to the standard may be used to prevent the standard signal from swamping out the sample.
3. Add 1000 uL of ice-cold WPB to the petri dish and chop tissue with a razor blade for 30-60 seconds. Retain the pipette tip from te step to mix the sample later. Note: the amount of chopping may be adjusted depending on the tissue. Finer chopping may result in more noise (more degraded nuclei). Use a new razor blade for each sample. Make sure to not over-chop!
4. Swirl the chopped matieral in the WPB for 20-30 seconds until a green tint appears in the liquid.
5. Remove the end of the retained pipette tip with the razor blade and use the larger opening to mix the material by pipetting up and down a couple of times (optional).
6. Filter the suspension through the filtered tubes and keep on ice. Transfer 500 uL of the filtrate to a new 1.5 mL Eppendorf tube.
7. Repeat steps 2-6 for each sample.
8. After finishing all samples, add 2.5uL of RNaseA (1mg/mL to 10mg/mL stock) and incubate for 10 minutes. This step is optional.
9. Add 5 uL of P.I. to each tube containing filtrate. Cover tubes with tin foil to protect them from light. Incubate on ice for at least 10 minutes. Dry tissue may require longer incubation.
10. Run on Accuri C6 flow cytometer (see Galbraith, 2009). Note: the BD Accuri C6 cytometer uses a nozzle of 150-200 um. Speed: start slow. If it looks good, can increase to medium or fast. Count to at least 2,000 events (or many more).