Miltenyi Biotec

Applications for the MACSQuant® Analyzer*

Determination of ploidy levels in plant cells

Background

Misdivisions during meiosis or mitosis can lead to a multiplication of the entire set of chromosomes. This phenomenon is called pluriploidy and occurs in many plants, in particular flowering plants (angiosperms). Plants can easily adapt to ploidy changes. Due to the changes, altered phenotypes can develop, and as a result the individuals may potentially occupy new ecological niches or outcompete species.[1] Determination of the ploidy level is therefore a central aspect in many plant research fields, including taxonomy, plant development, and plant breeding.

This application note summarizes protocols for the preparation of plant nuclei and subsequent flow cytometric analysis allowing the accurate determination of ploidy levels in plant cells, in particular broccoli (*Brassica oleracea*).

Materials

- Petri dishes
- Ice
- Ice-cold lysis buffer (two options):
  - 45 mM $MgCl_2$, 30 mM sodium citrate, 20 mM 4-morpholinepropane sulfonic acid (MOPS), pH 7.0, 0.1% (w/v) Triton® X-100 in distilled water (Galbraith *et al.*[2])
  - 100 mM citric acid and 0.5% (w/v) Triton X-100 in distilled water (Hanson *et al.*[3])
- Razor blade or scalpel
- Pipettes (10 µL, 1000 µL) and tips
- Sample tubes (e.g. 5 mL tubes or 96-well plate)
- Optional: Pre-Separation Filter, 30 µm (# 130-041-407)
- Propidium Iodide Solution (# 130-093-233)

Methods

Sample preparation

Note: To achieve optimal isolation of nuclei from plant cells, make sure that the lysis buffer stays ice cold during the whole procedure. Working on ice is therefore recommended.

1. Transfer fresh plant material (pieces of ±5 mm in diameter) to Petri dish.
2. Put samples on ice.
3. Add 500 µL of ice-cold lysis buffer to the samples to release the nuclei.
4. Chop the leaves in pieces of ±1 mm with the razor blade or scalpel.
5. Transfer the buffer containing the nuclei to a fresh sample tube: Tilt the dish to move the buffer to the edge of the dish; place the pipette tip in the edge to prevent plant pieces from entering the pipette tip.
6. Optional: Use a Pre-Separation Filter to remove debris from the nuclei suspension.
7. Add 5 µL Propidium Iodide Solution.
8. Analyze the samples on the MACSQuant® Analyzer.

* Applications on the MACSQuant Analyzer, MACSQuant Analyzer 10, and MACSQuant VYB are for research use only.

Flow cytometric data acquisition and analysis with the MACSQuant Analyzer

Note: For detailed information on operating the MACSQuant Instrument, please refer to the MACSQuant Instrument user manual and the MACSQuantify™ Software guide. Figure 2 illustrates the gating strategy for the determination of ploidy levels in broccoli cells.

Instrument setup and data acquisition

1. Open a new analysis window.
2. In the **Channels** tab, click **Advanced**. Activate the checkbox **Height**. Optional: Check **Width**.
3. Define the Experiment settings.

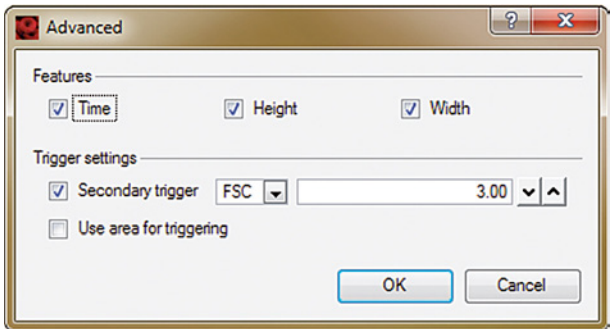


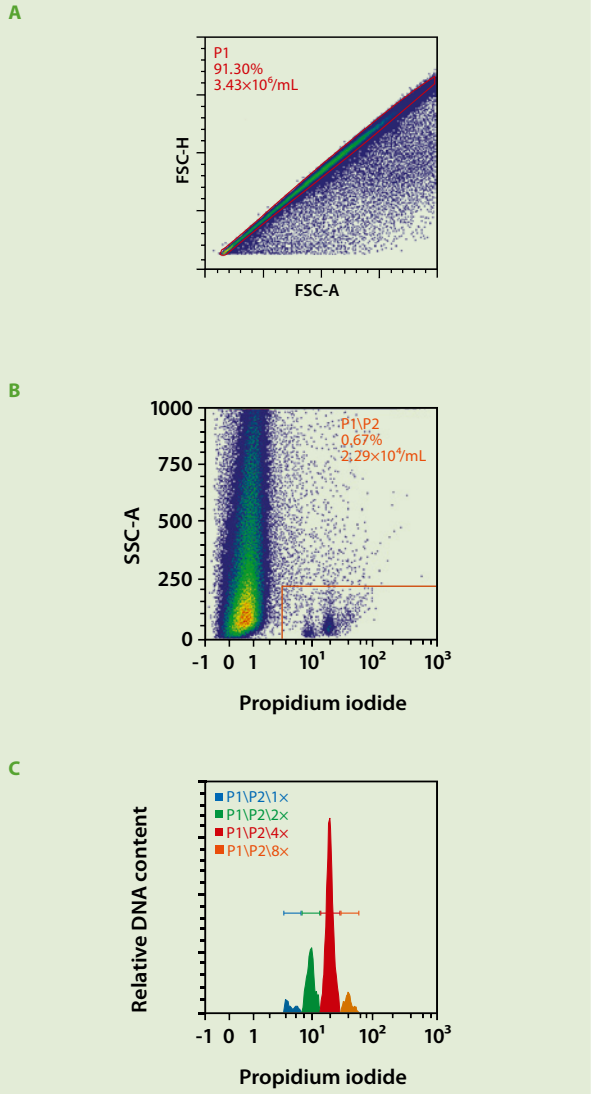
Figure 1: The parameter **Height** must be activated for analysis of ploidy levels. Optionally, **Width** can also be activated.

4. Define the Instrument settings:
   - Set compensation to None (no compensation is required).
   - Set trigger to FSC. Ensure that the fluorescence channel B3 is set to measure using hyperlog scaling.
   - Start acquisition.
   - Adjust the voltage gains so that the peak of diploid (2×; see fig. 2) is around 10.

Note: For optimal results it may be necessary to adjust the FSC and SSC voltage gain settings manually. It is recommended to acquire at least 10,000 events per sample.

Gating strategy

1. Set a gate on singlet events, i.e., the diagonal population in the FSC-A vs. FSC-H plot (P1 in fig. 2A).
2. Set a gate on nuclei, i.e., the propidium iodide (PI)–positive fraction (P2 in fig. 2B).
3. Set a gate at different ploidy levels (1×, 2×, 4×, and 8×) based on the median PI fluorescence. Each ploidy level should have roughly twice the PI fluorescence intensity as the previous level (fig. 2C and table).



Region	Percentage	Count/mL	Median propidium iodide fluorescence
1×	3.50	8.00×10^2	4.45
2×	21.59	4.94×10^3	9.62
4×	62.94	1.44×10^4	19.44
8×	6.77	1.55×10^3	38.19

Figure 2: Gating strategy for the determination of ploidy levels in broccoli cells (see text for details).

References

1. Leitch, A. R. and Leitch I. J. (2008) Science 320: 481–483.
2. Galbraith, D.W. *et al.* (1983) Science 220: 1049–1051.
3. Hanson, L. *et al.* (2005) Ann. Bot. 96: 1315–1320.

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