The Use of Bead Beating to Prepare Suspensions of Nuclei for Flow Cytometry from Fresh Leaves, Herbarium Leaves, Petals and Pollen.

Leaves, Petals and Pollen

CASE STUDY

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Overview

Keywords: Bead beating, fresh leaves, herbarium leaves, petals, pollen, *Allium cepa, Nicotiana tabacum, Petroselinum crispum, Rosa canina, Rosa rugosa*

Aim of the study: Use of a bead beating method to prepare suspensions of plant nuclei for measurement of DNA amounts by flow cytometry

Application: Nuclei staining and DNA measurement by flow cytometry

Sample: *Rosa canina, Rosa rugosa, Nicotiana tabacum*

Sample type: Leaves, petals and pollen

Material: FastPrep-24™ instrument, 2 mL Lysing Matrix tubes containing 1 mm silica beads or 2.5 mm Zirconia/silica beads

Buffer: Lysis buffer contained 0.1 M citric acid and 0.5% Triton X-100 in deionized distilled water

Protocol and Parameters

A two-stage procedure was used to prepare suspensions of pollen nuclei together with leaf nuclei of the calibration standard.

1. First, 7 mg of fresh leaf tissue was placed in a 2 mL screw capped tube along with 10 zirconia/silica beads (2.5 mm diameter) and 1 mL of lysis buffer and homogenized at speed of 5 m/s for 45 s with the FastPrep-24™ instrument.
2. The suspension was then filtered and filtrate added to 4 mg of pollen in a fresh tube, along with glass beads of 1.0 mm diameter.
3. Lysis buffer was added to bring the total volume to 1 mL.
4. The mixture of leaf and pollen nuclei was then homogenized at 4 m/s for 30 s with the FastPrep instrument.
5. The final suspension was then filtered and the filtrate was incubated with RNase at 37°C for 30 min.

Conclusion

Bead beating with the FastPrep-24™ instrument enabled suspensions of nuclei to be prepared simultaneously from 12 samples by agitating for 45 s. In a normal working day, the author was able to prepare, stain and analyze suspensions of up to 36 leaf samples and estimate DNA amounts in three replicates per sample by flow cytometry.

In the standard method, the gloved hand of the experimenter is in contact with harmful substances in the nuclei isolation buffer. The minimal handling of the isolation buffer in the bead beating method reduces this potential hazard.

The versatility of the bead beating method was demonstrated with suspensions of nuclei prepared from fresh leaves of species that range in DNA amounts from 1.13 pg (*R. rugosa*) to 33.5 pg (*A. cepa*), suspensions of nuclei from fresh petals and dry herbarium leaves of *R. canina*, and pollen of *R. rugosa*.