Simultaneous Analysis of GFP-Transgene Expression and Plant Cell Ploidy with the BD Accuri™ C6 Flow Cytometer

(Data courtesy of Dr. David Galbraith, Department of Plant Sciences, University of Arizona, Tucson)

Technical Bulletin

Contents

Introduction

The amount of DNA contained within a haploid nucleus of eukaryotic organisms is termed the C (constant) value. Monosomatic tissues containing mitotically active cells are characterized by cells with nuclear DNA contents ranging from 2C to 4C, depending on the position of the cells within the cell cycle. In polysomatic tissues, endoreduplication can occur, producing uninucleate cells having multiplicative DNA contents (2nC, where n = 1,2 3…).

Here, Galbraith et al use the BD Accuri™ C6 flow cytometer to test a flow cytometric method developed to analyze nuclear C-values in combination with transgenic expression of a nuclear-targeted version of the Green Fluorescent Protein (GFP) placed under the regulation of cell type specific promoters. This method can precisely define the C-value within any specific cell type in complex organs and tissues of plants.

Arabidopsis thaliana root was used to illustrate and demonstrate this method.

Materials and Methods

GFP Transgenic *A. thaliana* Production

For nuclear labeling, a fusion of GFP with the coding region of a histone 2A gene (HTA6; At5g59870), under the transcriptional control of the Cauliflower Mosaic Virus (CaMV) 35S promoter, was used. *A. thaliana* transformation and crossbreeding were performed using standard methods.[1]

Plant Cell Preparation

Root homogenates of wild-type (WT) and transgenic *A. thaliana* plants expressing HTA6-GFP were prepared, and nuclei isolated, according to previously described procedures.[2] The isolated nuclei were stained with propidium iodide (PI) for the determination of DNA content according to established procedures.

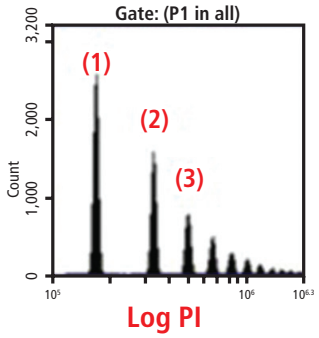
BD Accuri C6 DNA Measurement Validation

BD Biosciences DNA QC Particles (Cat. No. 349523) were used to verify the BD Accuri C6 flow cytometer for performance of DNA analysis. Chicken erythrocyte nuclei (CEN) from the kit were stained with PI according to package instructions.

Flow Cytometry

The BD Accuri C6 flow cytometer with BD Accuri™ C6 software was used for the analysis. Single-cell suspensions of *A. thaliana* root nuclei were passed through a 488-nm laser for excitation, and fluorescence emissions were collected at 530 nm (+/-15 nm) for GFP and 585 nm (+/-20 nm) for PI. Dual parameter plots of DNA content (PI) vs GFP expression were first gated on plots of PI vs side scatter (SSC) to exclude debris. Samples were analyzed using a fluidics rate of Slow (core size = 10 µm, flow rate = 14 µL/min), and at least 15,000 gated events were collected per sample.

Results



Linearity of PI-FL2-A Channel:
Mean Peak 2/ Mean Peak 1 = 1.96

CV of PI+ Peaks:
Peak 1: 2.5%
Peak 2: 2.3%
Peak 3: 2.5%

Figure 1. BD Accuri C6 flow cytometer performance using the CEN standard.

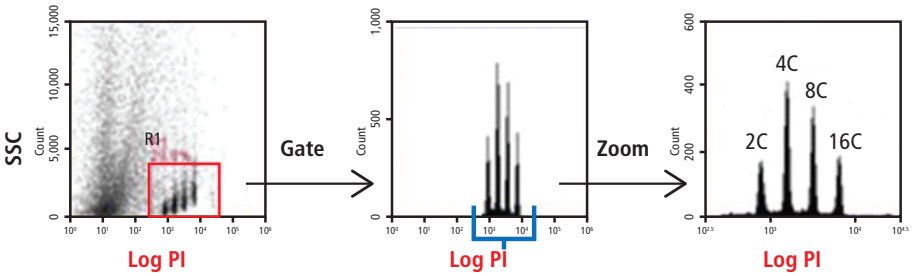


Figure 2. Analysis of WT *A. thaliana* root nuclei, showing initial PI vs SSC gate and polyploid populations (2C through 16C).

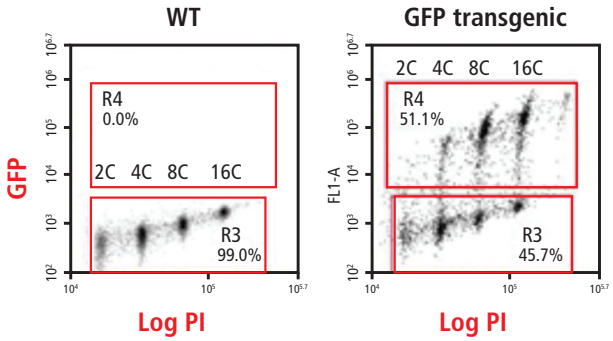


Figure 3. Analysis of WT and GFP transgenic *A. thaliana* root nuclei. GFP-transgene expression is concentrated in nuclei with >4C DNA content (Region R4 vs R3, right 2D plot above).

References

1. Zhang C, Gong FC, Lambert GM, Galbraith DW. Cell type-specific characterization of nuclear DNA contents within complex tissues and organs *Plant Methods.* 2005;1:7.
2. Galbraith DW, Harkins KR, Maddox JM, Ayres NM, Sharma DP, Firoozabady E. Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science.* 1983;220:1049-1051.

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