Bulk Sorting Plant Protoplasts

for Secondary Cell Walls Biosynthesis

Introduction

Secondary cell walls are mainly composed of cellulose, hemicelluloses (xylan and glucomannan) and lignin. These structural elements constitute the bulk of plant biomass and understanding their biosynthesis can potentiate many fields of research ranging from biofuel, agriculture, and plant physiology.[1]

Protoplasts are spherical plant cells lacking cell wall due to digestive enzyme exposure. They can be derived from leaf tissue or cell culture and under defined media they can regenerate a new cell wall.[2] Thus, protoplasts can serve as a unique system for investigation of the regulatory mechanisms of secondary cell wall deposition. To identify re-generating protoplast, fluorescent stains against cell wall components such as cellulose can be applied. However, the expression and staining can be variable and thus sorting can be a useful method to select target populations to accelerate downstream applications. Herein, bulk sorting of protoplasts with fluorescent signal for cellulose stained with AF488 can be performed to select for the regenerating population of protoplasts. However, traditional FACS sorting technology has been challenging for this purpose due to the high pressure applied to the samples that damages protoplasts. Cell sorters and single cell dispensers from Bio-Techne are ideal for this purpose as they can rapidly isolate and dispense the positive protoplasts in bulk by processing 50 protoplasts/second and yielding 80-90% output purity in a single step.

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Methods

Protoplast Preparation

Arabidopsis Thaliana protoplasts were prepared from 3-4 weeks old plants with 1-2cm leaves before flowering. The leaves were cut and exposed to enzyme solution containing 1.5% cellulose and 0.4% macerozyme. The resulting digestion was then strained, pelleted, and resuspended in 0.5 M D+Trehlose dihydrate media.

Sorting of Protoplasts

Samples were stained with AF488 against cellulose and strained using a 40 µm strainer. Samples were diluted to 100,000 protoplasts/mL and visualized on a fluorescent microscope. 600 µL of the cell suspension was loaded into a Bio-Techne Bulk Sorting Cartridge and sorted on a Bio-Techne Cell Sorter and Single Cell Dispenser, gates were drawn to select for positive events and 5,000 protoplasts were dispensed. Subsequently, post-sort protoplasts were visualized on a fluorescent microscope and cryogenically preserved for cry-EM assessment later.

FIGURE // 01

Pre-sort images of AF488 against cellulose stained protoplasts

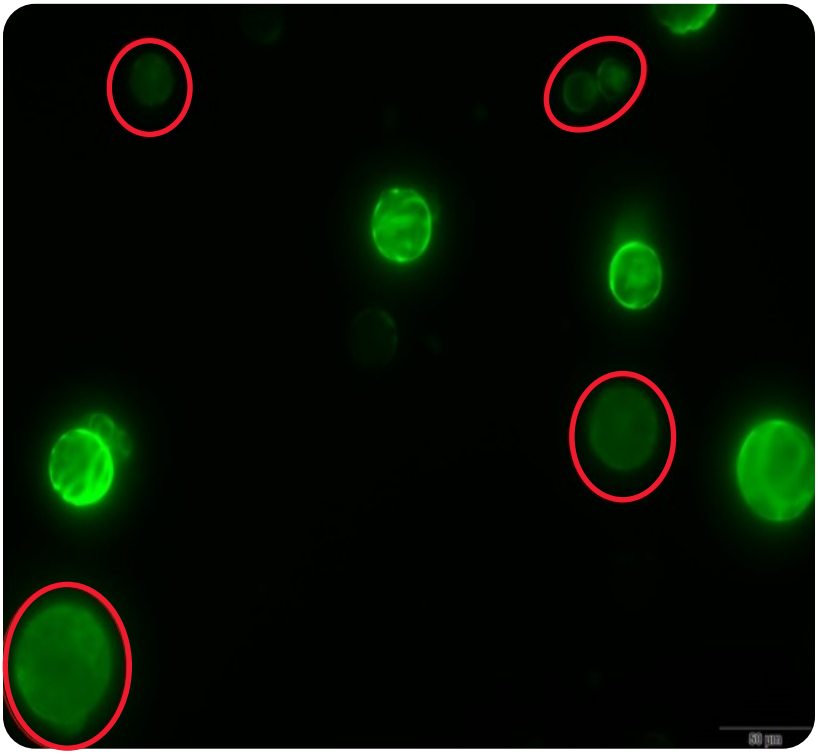

Figure 01. Red circles indicate non-regenerating protoplasts with weak signal diffused throughout the protoplasts. These non-regenerating protoplasts likely contain little to no cellulose and their fluorescent signal is likely background. The target protoplasts are the brightest seen in the image with structural cell wall elements visible.

FIGURE // 02

Bulk sorting of protoplast with AF488 staining of cell wall

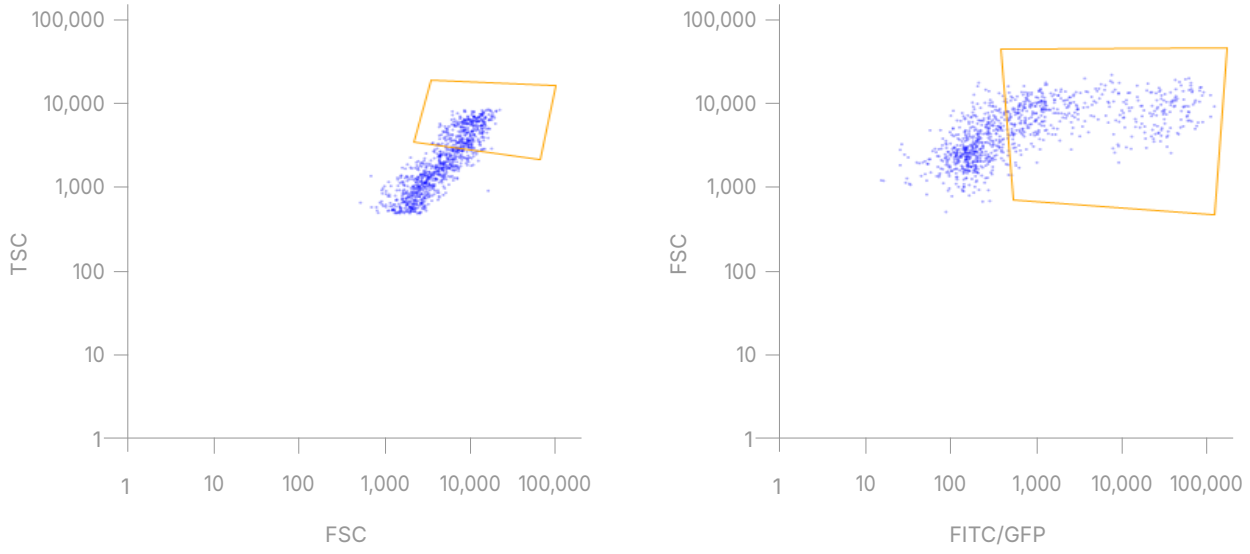

Figure 02. The dot plots above represent the event recognition and gating strategies that were used. On the left, Total Side Scatter (TSC) and Forward Scatter (FSC) were used to identify protoplast and exclude debris by selecting the top right quadrant of the population. On the right, AF488 positive events were selected in tandem with high FSC to dispense only brightly fluorescent events.

FIGURE // 03

Post-sort images of AF488 against cellulose stained protoplasts

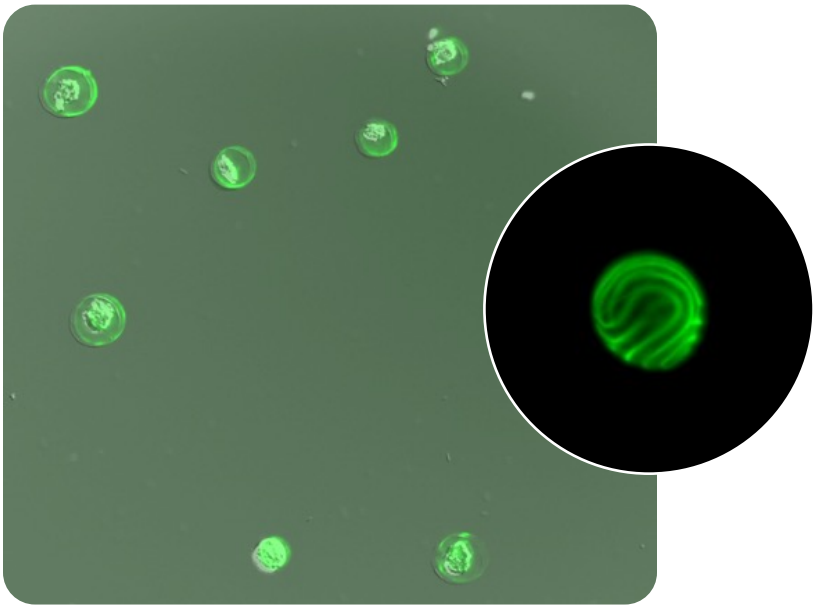

Figure 03. The merged bright field and fluorescence image on the left shows that 100% of observed post-sort protoplasts had AF488 signal and showed structural staining as well as furrowing indicating regenerating cell walls. The right fluorescence image shows a fully regenerated, sorted protoplast.

Summary

Bio-Techne's gentle microfluidic cell sorting technology enables extensive high-throughput screening with ease and gentle sorting by operating at low pressure which allows isolation of intact protoplasts at 50 protoplasts/second sorting speed. When combined with anti-cellulose antibody staining, bulk sorting using the Bio-Techne platform can serve as a critical step to expedite isolation of healthy cell wall regenerating protoplasts, making downstream applications such as cryo-EM or continued culturing more robust. The findings above demonstrate the versability of potential sorting methodologies utilizing the Bio-Techne bulk sorting feature, empowering plant scientists to streamline efficient isolation of cell wall regenerating protoplasts.

REFERENCES

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2. Fowke, L. C., and F. Constabel. "Plant Protoplasts: An Experimental System for Cell Biologists." SpringerLink, Springer US, 1 Jan. 1983, https://link.springer.com/chapter/10.1007/978-1-4684-4379-0_19