

Automated High Speed Analysis and Dispensing of Single *Arabidopsis* Seeds into 96 Well Microtiter Plates

Pomeroy, M.
Union Biometrica; Somerville, USA

Scope

Arabidopsis seeds have been sorted into a 96 well microtiter plate with the COPAS *PLUS* (Union Biometrica, Inc.). The selected number of seeds per well was 1.

Introduction

Arabidopsis seeds (approximately 500 μm) have been analyzed and sorted using the COPAS *PLUS* system (figure 1). The main objective of the experiment was to evaluate the process and sort accuracy for dispensing single seeds accurately into 96 well microtiter plates.

Materials

COPAS *PLUS* (Union Biometrica pn 360-5000-000)

COPAS *PLUS* Sheath Fluid (pn 320-5070-000)

Arabidopsis Seeds

750 μm mesh filter

96 well microtiter plates



Figure 1. The COPAS *PLUS* was used to dispense *Arabidopsis* seeds in a microtiter format in less than 2 minutes.

Method

Arabidopsis seeds were diluted to approximately 150 seeds/mL with sheath fluid. The diluted seeds were filtered through a coarse 750 μm mesh filter to remove any gross fibers or particles. The seeds were analyzed on the COPAS *PLUS* system. Two size parameters, Time Of Flight (TOF) and Extinction (EXT), were used to analyze the population.

A region for sorting was defined on a TOF versus EXT dot-plot. Figure 2 shows a dot plot of the *Arabidopsis* seeds and the gate that was set by the operator for sorting. Two 96 well microtiter plates were filled with one seed per well. Fill time per plate and accuracy of the fill were documented.

Results

Plate	Fill Time	Accuracy	Coincidence
1	84 sec	100%	0
2	90 sec	100%	2

Sorting accuracy was confirmed with a visual assessment by microscopy.

Discussion

Fill Time:

According to the specifications, the COPAS PLUS system is capable of dispensing single events in a 96 well microtiter plate within 105 seconds. Both plates demonstrate processing with these specifications.

Accuracy:

Visualization by microscopy confirmed 100% fill accuracy for each well.

Coincidence:

The second plate had two wells with two seeds each, instead of one. The specifications for coincidence are selected by the user and represent a compromise between optimal purity, coincidence and yield. The system specification, which is fewer than four coincidence events per plate, is met.

Reference Articles

Meyerowitz and Somerville, Cold Spring Harbor Press, 1994.

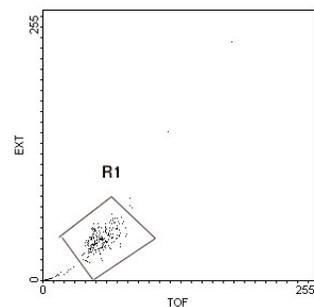


Figure 2. An image of a dot plot showing the seeds and gate that were used to sort into microtiter plates.

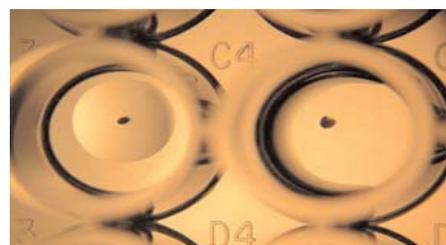


Figure 3. An image showing the single *Arabidopsis* seeds sorted into microtiter plate wells.