ARABIDOPSIS SORTING BASED ON MORPHOLOGY AND AUTOFLUORESCENCE

Analysis and Sorting of *Arabidopsis* Seeds with Different Ecotypes

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Objective

The objective of this experiment was to analyze different phenotypes of seeds and sort the selected population into 96 well microtiter plates.

Introduction

Different ecotypes of *Arabidopsis* seeds were mixed, analyzed and sorted based on physical parameters and differences in autofluorescence intensity with the COPAS *PLUS* system (Union Biometrica, Inc.). The two ecotypes were compared under a light microscope, which revealed a morphological difference between the two populations. Specifically, one population was slightly larger and had a longer cleft. Analysis under a fluorescence microscope showed the seeds also had slight differences in auto-fluorescence in the green and red emission ranges (See Figures 2 and 3), suggesting that these seeds could be sorted with the COPAS *PLUS.*

Materials

COPAS *PLUS* (Union Biometrica pn 360-5000-000)
COPAS *PLUS* Sheath Fluid (pn 320-5070-000)
Arabidopsis Seeds
96 well microtiter plates

Method

Arabidopsis seeds from two populations were diluted with 25 mL of sheath fluid. The final concentration was 40 seeds per mL (An optimal concentration would be +/- 200/mL). The two groups of seeds were individually analyzed on the COPAS *PLUS* system (See Figures 4 and 5). The size parameter, Time Of Flight (TOF), was used to analyze the population. A region for sorting was defined on an EXT versus FLU dot-plot. EXT is a measure of optical density and FLU is a measure of fluorescence including autofluorescence, fluorescent protein expression, or fluorescent markers. Figure 6 shows a dot plot of the mixed *Arabidopsis* seeds and the sort regions that were set by the operator. Two 96 well microtiter plates were filled with one seed per well, each plate with a different ecotype, high fluorescence-longer cleft-plate 1, low fluorescence-shorter cleft-plate 2. Fill time per plate and accuracy of the fill were documented.

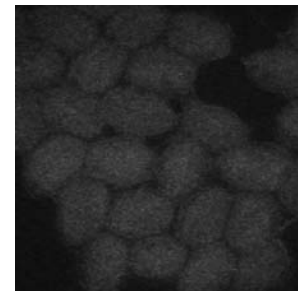**Figure 1.** The COPAS *PLUS* was used to analyze and sort *Arabidopsis* seeds.

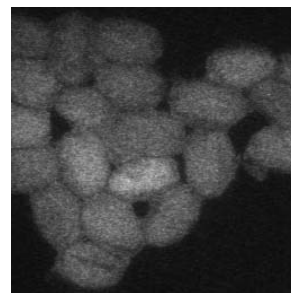**Figure 2.** Phenotype 1 *Arabidopsis* seeds with low autofluorescence.

Figure 3. Phenotype 2 *Arabidopsis* seeds with high autofluorescence.

Results

The COPAS *PLUS* system dispensed 1 seed with high fluorescence per well into Plate 1 in 98 seconds. When viewed using a dissecting microscope, the plate contained 1 seed in each well. Sorting accuracy was 100%. No coincident (extra) seeds were observed. Plate 2 was filled in 98 seconds. That plate contained 95 low fluorescent seeds. Sorting accuracy was 99.4%. Plate 2 contained one coincident (extra) seed. This data is presented in Table 1.

Table 1

Plate	Fill Time	Accuracy	Coincidence
1	98 sec	100%	0
2	98 sec	99.4%	1

Figure 4. An image of a dot plot showing the *Arabidopsis* seeds with high auto-fluorescence.

Discussion

Discrimination of the two ecotypes:
The COPAS *PLUS* was capable of distinguishing between the two phenotypes, based on auto-fluorescence versus extinction. Purity of the sort was 100%.

Fill Time:
According to the specifications, the COPAS *PLUS* system is capable of dispensing single events into a 96 well microtiter plate within 105 seconds. Both plates demonstrated processing with these specifications.

Accuracy:
According to manufacturer's specifications, the COPAS *PLUS* systems dispensing accuracy must be greater than 94%. Both demonstration plates met these specifications.

Coincidence:
According to manufacturer's specifications, the COPAS *PLUS* system can dispense no more than 4 coincident events per plate. Both demonstrations met these specifications. The two plates had only one well out of 192 wells with a coincident (extra) event.

This data demonstrates that the COPAS *PLUS* has a high quality of performance when sorting *Arabidopsis* seeds with different ecotypes.
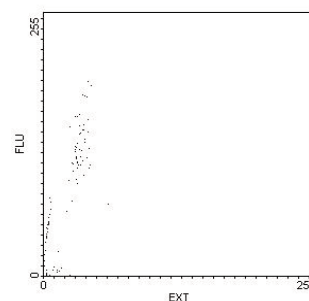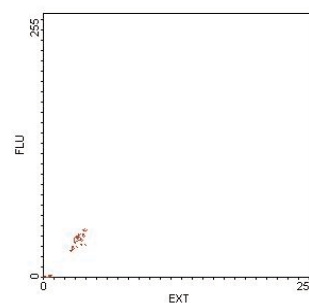

Figure 5. An image of a dot plot showing the *Arabidopsis* seeds with low auto-fluorescence.
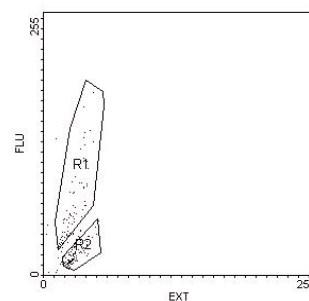

Figure 6. An image of a dot plot showing the mixed *Arabidopsis* seeds and the sort regions.