

## Isolation of GFP-Expressing *Arabidopsis* Seeds from a Mixed Population

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### Objective

*Arabidopsis thaliana* seeds can be sorted from a large population, based on phenotypic and morphological characteristics, using the COPAS *PLUS* system (Union Biometrica, Inc.).

### Introduction

The use of *Arabidopsis* seeds as a model system for the study of basic genetics has generated the need for a system that can sort these seeds at a high rate based on phenotypic characteristics. The COPAS *PLUS* system has the ability to dispense seeds based on specific physical parameters (size and density) and fluorescent intensity (Figure 1).

In this application, a mixed population of *Arabidopsis* seeds was analyzed using the COPAS *PLUS* system. The population contained wild type seeds and an EMS mutagenized population of a transgenic line driven by a promoter from *Lesquerella fendleri* fused to GFP (Broun et al., 1998). These transgenic seeds are variable in size and fluorescent intensity, due to various mutations segregating in the population.

### Materials

COPAS *PLUS* (Union Biometrica pn 360-5000-000)  
COPAS *PLUS* Sheath Fluid (pn 360-5070-000)  
750 µm Mesh Filter  
*Arabidopsis* Seeds

### Method

Samples were prepared by passing the *Arabidopsis* seeds through a 750 µm mesh filter to remove large particles and germinating seeds. Once the seeds were free of debris, they were diluted with the COPAS *PLUS* sheath. The final concentration of seeds in solution was approximately 150 seeds per mL. The concentration of the sample preparation was checked by extracting a known volume of liquid out of the preparation and by counting the number of seeds present in the aliquot.



**Figure 1.** Picture of the COPAS *Plus* System

The two parameters, Time of Flight (TOF) and Extinction (EXT), are used to initially analyze the population. Time of Flight is a measure of the relative length of each object, and Extinction provides a measurement of its optical density. Figure 2 is a dot plot, with TOF and EXT as the two gating parameters, displaying all of the seeds analyzed during the experiment.

The sorting dot plot in Figure 3 displays a polygonal region positioned around GFP expressing seeds. The sorting region was defined on a dual fluorescence parameter plot, with FLU1 (green fluorescence intensity) and FLU2 (red fluorescence intensity).

## Results

Once the population of interest is identified, the COPAS PLUS will dispense only those seeds that are displayed within the user-selected polygonal region of the sorting dot plot.

### Time of Analysis:

Assuming 40 mL are present in the primary sample cup and a sample concentration of 150 Seeds/mL, 6000 seeds may be analyzed in a single run. If the flow rate of the instrument has been set to 6 seeds/sec, approximately 3000 seeds (50% of the population) can be analyzed within 8 minutes.

### Accuracy:

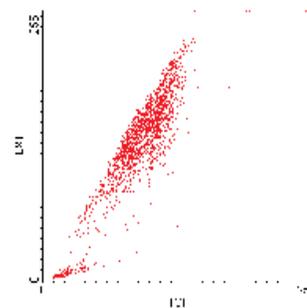
The accuracy of the instrument is directly related to the Sort Width and Sort Delay parameters of the instrument. Once these parameters have been set to the optimal values, a sort accuracy greater than 98% can be achieved.

## Discussion

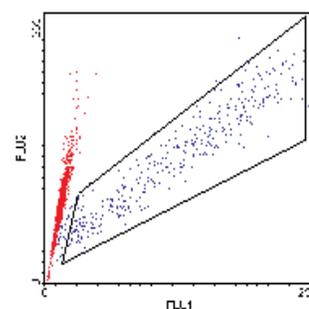
We have sorted *Arabidopsis* seeds expressing varying levels of GFP using the COPAS PLUS technology platform. Two separate trials of the same experiment were performed in order to demonstrate the sensitivity of the instrument for differentiating seeds on the basis of fluorescence intensity. In the first experiment, we were able to selectively sort out seeds that were expressing GFP. The second experiment consisted of dispensing seeds that were not expressing GFP. Once the sorting region was set around a population of interest, the instrument selected and dispensed only seeds within that population with purity greater than 98%. Based on these data, the results obtained were within the manufacturer's specifications of the instrument.

## References

Broun, P., Sekhar, B. and Somerville, C. A biunctional oleate 12-hydroxylase: desaturase from *Lesquerella fendleri*. *The Plant Journal*, 13(2), 201-210, 1998



**Figure 2.** Dot plot showing the entire population of seeds analyzed by the COPAS PLUS. TOF represents the physical parameter Time of Flight and EXT represents the optical characteristic Extinction.



**Figure 3.** Dot plot showing a polygonal region around the seeds to be extracted from the total population. FLU1 is green fluorescence intensity and FLU2 is red fluorescence intensity.

### Note:

**The sort parameters are set on the user interface screen**