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Automated sorting of live *C. elegans* using laFACS

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To the Editor: A recent paper in *Nature Methods* describes the use of fluorescence-activated cell sorting (FACS) to sort *Caenorhabditis elegans* embryos¹. Here we report FACS-based method to sort live *C. elegans* larvae, which permits us to rapidly collect large quantities of live genotyped worms from a mixed population. Using GFP-marked balancer chromosomes (Fig. 1a), and live-animal FACS laFACS, we routinely collected >100,000 genotyped animals in less than one hour. To test laFACS, we combined it with large-scale RNA interference (RNAi) screening and identified genetic interactors of *mel-28*, an important regulator of nuclear envelope and chromatin functions^{2–5}.

Although a FACS machine is designed to sort single cells, a few modifications enabled sorting of ~0.25 mm long L1 *C. elegans*. First, we used a reduced drop-drive frequency of ~16.4 kHz to keep the larvae undamaged. We also used a 100-μm nozzle and set a gate to capture events with a high forward-scatter signal (Fig. 1b), confining our collections to larger objects (Supplementary Methods). After worm sorting, the same FACS machine could be used for several other applications, including sorting of yeast, *Drosophila melanogaster* cells, mammalian cells, and plant protoplasts. The worm sort had no impact on these applications, and the worm-specific FACS modifications were easily reversed to accommodate single-cell applications.

We used laFACS to collect *mel-28* (*maternal-effect-lethal-28*) homozygous worms from a mixed population. *mel-28* homozygous hermaphrodites derived from heterozygous mothers appear phenotypically indistinguishable from wild-type animals until they mature and produce only inviable progeny. This selection is usually performed manually and thus is not amenable to large-scale applications. We first generated a GFP-marked strain in which the *mel-28* (*t1684*) mutation is balanced over a chromosome bearing *lag-2::GFP*⁶ (Fig. 1a). We sorted L1 worms, collecting GFP-negative *mel-28(t1684)* homozygotes. We selected GFP-negative larvae on the basis of the ratio of green (GFP fluorescence; 530/30 nm) to red (red-spectrum autofluorescence; 610/20 nm) signal (Fig. 1c,d). We separated GFP-positive and GFP-negative worms (Supplementary Figure 1). Typically, from a population of 600,000 larvae we retrieved ~130,000 healthy animals after one sort. This first-pass population was ~95–98% homozygous ($n > 1,000$, verified by microscopy). A second sort recovered an essentially 100% pure population of about 100,000 homozygous larvae ($n > 20,000$, verified by microscopy and by genetic analysis). This scheme can be easily adapted for the majority of *C. elegans* genes using available balancers.

We used the collected homozygous *mel-28* animals to perform an RNAi-based synthetic interaction screen using clones representing chromosome I genes⁷. The ability to easily collect large amounts of *mel-28* homozygous animals allowed us to perform the RNAi repeatedly (up to 16 times). From over 2,000 genes tested, 12 showed synthetic phenotypes with *mel-28*: *npp-2*, *npp-4*, *npp-12*, *npp-14*, *npp-17*, *his-67*, *his-68*, *exos-3*, *pas-5*, *phi-56*, *rpa-0*, and

rpl-30 (Supplementary Fig. 2 and Supplementary Table 1). These results agreed well with *mel-28*'s role in coordinating chromatin and nuclear envelope functions^{2,3}. Obtaining large quantities of pure mutant populations could also be useful for chemical screens, microarrays, or biochemical assays, expanding the arsenal of high-throughput tools available in *C. elegans*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

laFACS of GFP-negative L1 larvae. **(a)** Genetic scheme. A *mel-28* mutant allele is kept over a balancer chromosome containing a GFP marker and a recessive lethal allele. GFP-negative progeny (F1) are *mel-28* homozygotes and grow up to produce only dead embryos. **(b)** A dot-plot of forward scatter (FSC) versus side scatter (SSC) signals. The gate on FSC separates the larvae (blue) from debris (black). **(c)** A dot-plot of red-spectrum autofluorescence (RSA) versus GFP signals. GFP-positive *mel-28* heterozygotes (green) are defined and used to set a gate for the GFP-negative larvae (red) to be sorted. **(d)** A dot-plot of the RSA versus GFP signals post-sort.