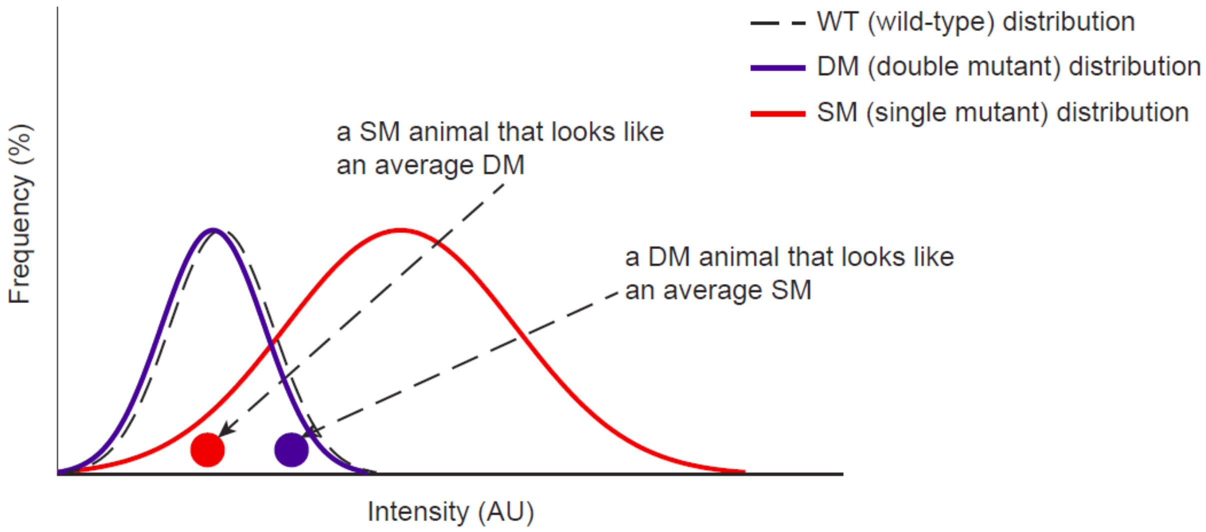
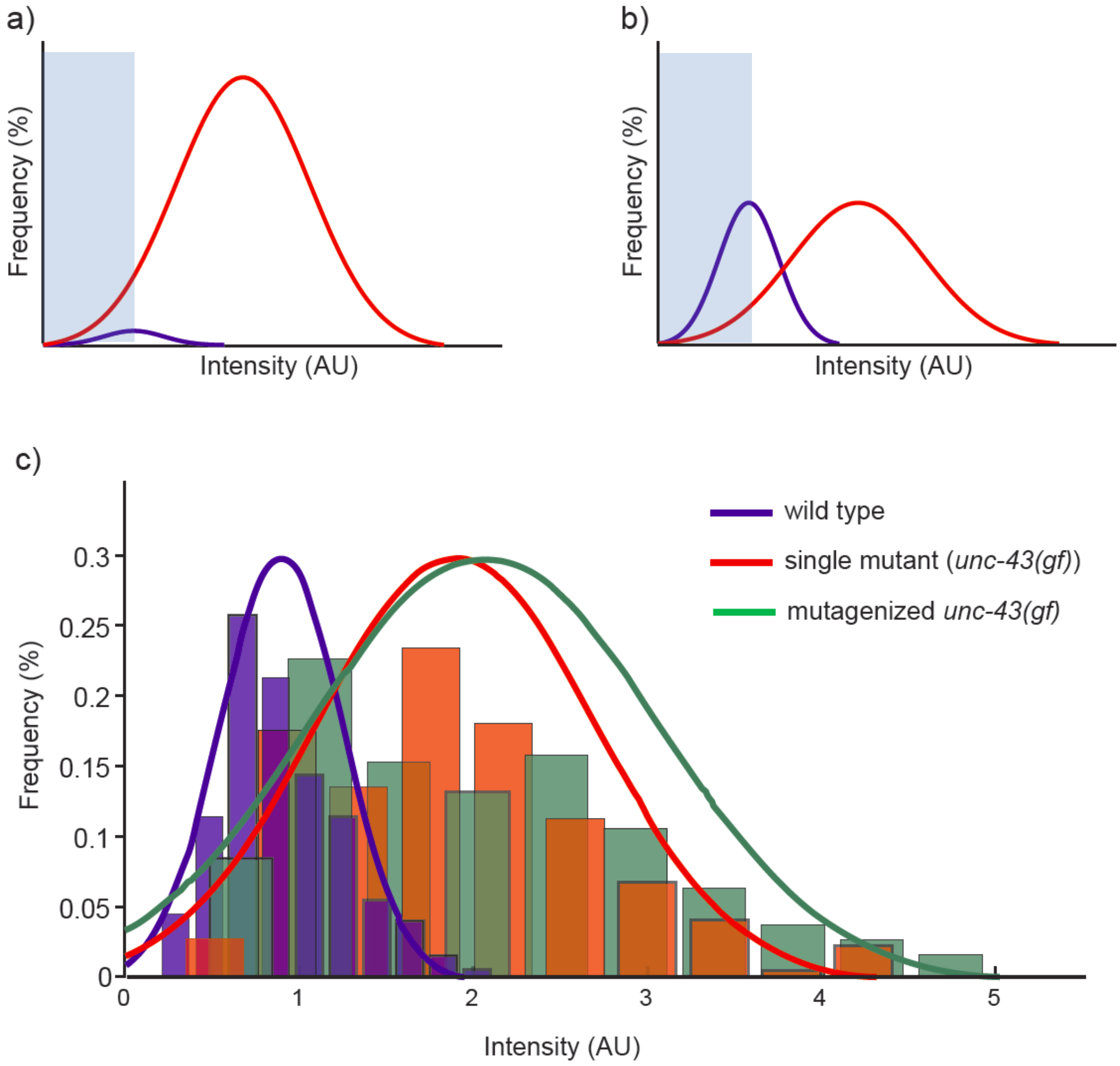
Supplementary Information

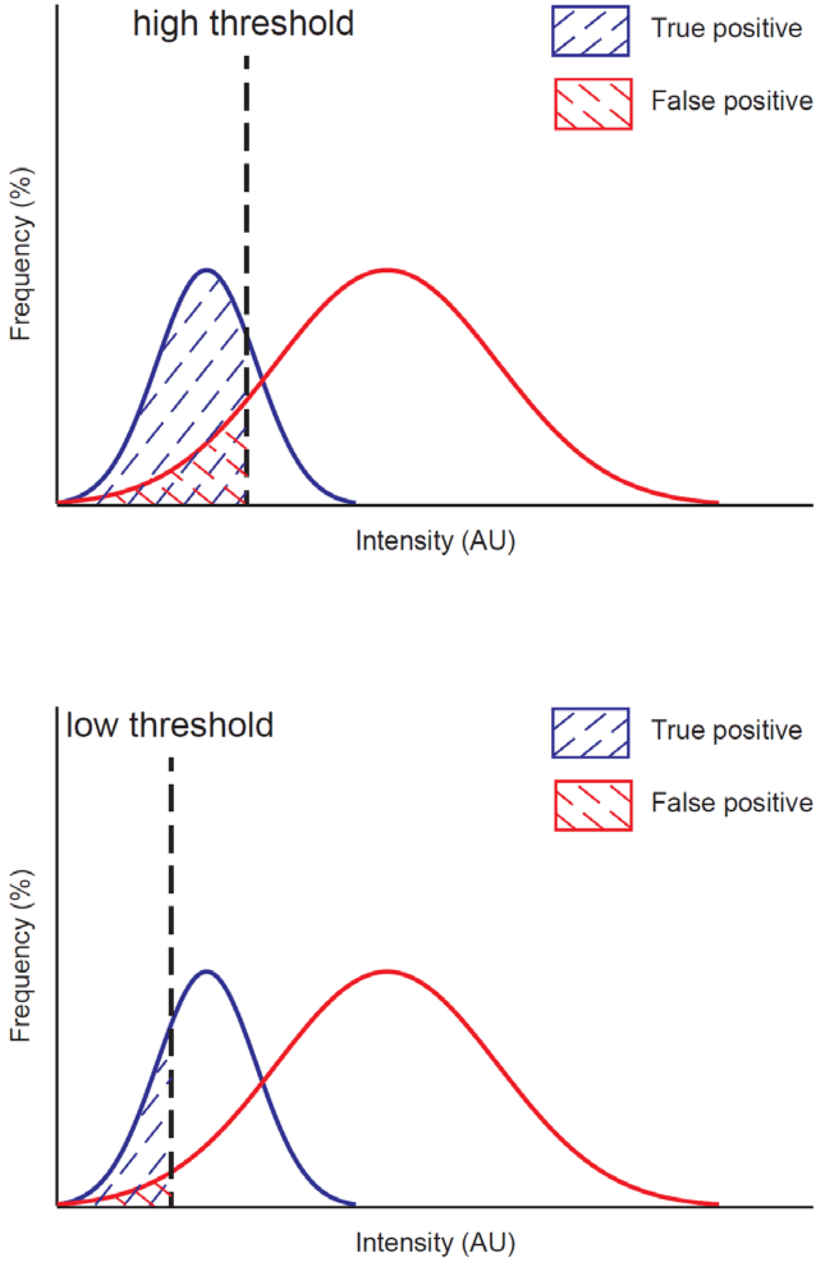
Supplementary Figure 1. Illustration of the stochastic effect of gene expression resulting in overlaps in the expression profiles of isogenic populations. Because of this effect, wild-type or double mutant animals may have a higher expression than single mutant animals, and this effect results in an ultimate limit of enrichment for separations (depending on the size of the subpopulations).



Supplementary Figure 2. (a,b) Occurrences of true positives (blue curve) among all sorted positives (blue shade) depend on the occurrences of the desired population among all animals. (c) Overlaying the WT population, the unc-43 (gf) population, and the mutagenized population expression profiles. This demonstrates that the expression profile of the mutagenized population, which contains desired double mutants, is shifted from the unc-43 (gf) single mutants as expected. This also demonstrates that there are significant overlaps among the three populations such that any sorted mutants will need to be verified in secondary clonal imaging to be sure that they are true double mutants, rather than single mutants that stochastically show low expressions.



Supplementary Figure 3. Applying different thresholds to the same mixed populations will result in either higher chance to find true positives but at a cost of higher false positives, or smaller false positive rates but at a cost of possibility of missing some true positives.



Supplementary Table 1. Characterization of the sorting algorithm using WT (as a surrogate of the double mutant) and *unc-43* (gf) mutants. Mixing different percentages of WT in *unc-43* (gf) and choosing different threshold give different levels of enrichment. The false positives are a result of the stochasticity in gene expressions (as illustrated in previous figures), rather than a result of the algorithm. In the actual screen, it is difficult to know what the true occurrence of double mutants are; we arbitrarily chose 2% as screening threshold, but verified that 43 of all the mutants selected are true mutants with a secondary clonal expression profiling (see text and Figures 7 and 8).

	Mix of 5% WT and 95% *unc-43 (gf)*		Mix of 10% WT and 90% *unc-43 (gf)*		Mix of 50% WT and 50% *unc-43 (gf)*	
	2% selection	5% selection	2% selection	5% selection	2% selection	5% selection
True positives (n)	1	4	4	8	7	13
False positives (n)	3	5	2	4	0	1
Enrichment (%)	530	941	710	710	200	186

Supplementary Figure 4. In primary screening process, the adaptive algorithm removes the effect of day-to-day variations, variations due to animal ages, environmental noise and other systematic biases; however, false positives as a result of stochasticity in gene expressions can only be removed from secondary clonal screens.