General Notes on Rougvie Lab CRISPR Strains

This strain was created by the Rougvie lab in an effort to obtain null mutations (deletions) in all *C. elegans* genes. The current project (2017-2021) uses CRISPR/Cas9 technology and is funded by the Canadian Institute for Health Research and the US National Institutes of Health. Please refer to our paper (Au *et al.*, G3 9(1): 135-144 2019) for protocol details.

For this project we are using 450-bp homology arms and HDR to generate deletions of various sizes, with integration of a Calarco/Colaiacovo selection cassette that confers *myo-2* GFP (or rarely *myo-*3 GFP) and G418 resistance (Norris *et al.*, Genetics 201: 449-458 2015). The selection cassette can be excised, subject to some caveats (see end of penultimate paragraph), by injecting Cre recombinase and selecting for non-GFP animals.

We annotate guide RNAs, expected mutation structure and the primers used for quality control assays with the browser-based program Benchling (http://www.benchling.com), and Genbank files for the WT context, deletion/selection cassette insertion mutation, repair template plasmid, and, if used, guide RNA plasmid are provided for each allele.

In some cases we do quality control on each mutant by doing three PCRs (four primer pairs): two specific reactions each to amplify the upstream and downstream cassette insertion sites, and then one each on mutant and N2 templates with WT primers to show that the predicted product is missing from the mutant and present in N2. The QC PCR result codes provided in the strain description list these results in the order Upstream Insertion Site, Downstream Insertion Site, and WT on N2 and WT failure on mutant. "P" means pass, "p" that the test was performed on heterozygous mutants (only applicable when assessing WT PCR products), "F" means fail, and “x” means that the test was not performed. In other cases, we do quality control on each mutant by doing four PCRs (6 primer pairs): two specific reactions each to amplify the upstream and downstream cassette insertion sites, then two each on mutant and N2 templates with upstream and downstream flanking primers and corresponding primers in the deleted region. The QC PCR result codes provided in the strain description list these results in the order Upstream Insertion Site, Downstream Insertion Site, WT Upstream region on N2 and failure in mutant, and WT downstream region on N2 and failure on mutant. "P" means pass, "p" that the test was performed on heterozygous mutants (only applicable when assessing WT PCR products), "F" means fail, and “x” means that the test was not performed.

We submit for the CGC collection the alleles that pass quality control assays. In the case the allele is homozygous lethal or sterile, we balance the allele before submitting.