General Notes on Moerman Lab CRISPR Strains

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This strain was created by the Moerman 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 program ApE (<http://jorgensen.biology.utah.edu/wayned/ape/>), and ApE files for both the WT context and the deletion/selection cassette insertion mutation are provided for each allele. We annotate various features in the ApE files, including primer binding sites, sgRNA guides and homology arms. Colored highlighting for overlapping features is necessarily stacked, so sometimes the full extent of a given feature is not immediately clear. Double-clicking any displayed part of a feature will highlight it no matter where it is in the stack.

For quality control on each mutant we do eight PCRs (four primer pairs, in duplicate): two specific reactions each to amplify the upstream and downstream cassette insertion sites, and then two 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, WT on Mutant and WT on N2. "P" means pass, "F" means fail, and "x" means either that the test was not performed or that the test was performed on heterozygous mutants (in cases where homozygous mutants cannot be identified reliably). In some cases the WT assays use flanking primers, and in most cases one primer is flanking and the other in the deleted region. We refer to mutations that pass all QC PCRs as "perfect" and those with insertion site PCR failures as "imperfect," although in the absence of a higher-resolution assay it is impossible to know the exact structure. In the case of an insertion site reaction that fails, generally either the product is not made or the product is the wrong size.

**IMPORTANT NOTES ABOUT DELETION EXTENT:** The deletion flanking sequences and the deletion size referred to in the strain description are **those expected** if the CRISPR event is perfect. **Whole-genome sequencing of a number of strains with imperfect QC has shown that insertion site PCR failure can indicate local rearrangement at the site. This can result in a deletion that is a different size than expected, and it could extend into nearby genes (see Au *et al.* paper for examples)**. This in turn can mean that the selection cassette cannot be excised with Cre recombinase. For all our homozygous viable CRISPR deletion strains in the CGC collection, WT primer assays show that the gene is disrupted even if the event tests as imperfect.

We strive to generate QC-perfect deletions for as many gene targets as possible. However, the constraints of the projects demand that we prioritize the number of targets and not spend excessive time trying to obtain perfect alleles at the expense of additional genes. In each case, we submit for the CGC collection the allele closest to perfect among the candidates we obtained.