

General Notes on Hutter Lab CRISPR Strains

This strain was created by the Hutter lab in an effort to obtain null mutations (deletions) in all *C. elegans* genes. The current project (2021-2025) uses CRISPR/Cas9 protocols from the Moerman lab and is funded by the US National Institutes of Health. Please refer to Au *et al.* (G3 9(1): 135-144 2019) for protocol details.

For this project we are using 450 bp homology arms and Homology Directed Repair (HDR) to generate deletions of various sizes, with integration of a 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 DNA sequence and the deletion with the inserted selection cassette are provided for each allele. We annotate various features in the ApE files, including primer binding sites, sgRNAs and homology arms. The feature annotations and DNA sequences are also provided as html and plain text files, which don't require ApE.

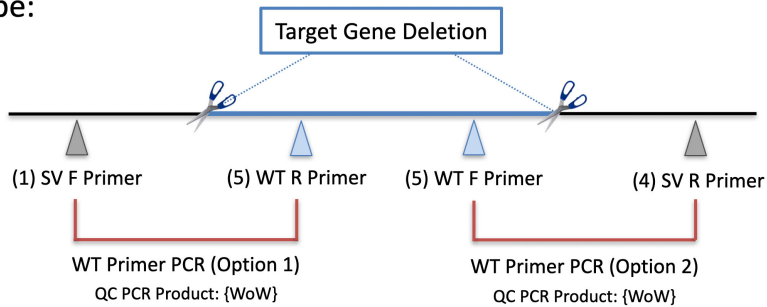
For quality control on each mutant we do four PCRs in duplicate (see Figure on next page):

- One PCR to amplify the upstream selection cassette insertion site,
- One PCR to amplify the downstream selection cassette insertion site,
- One PCR with WT primers on mutant DNA to show that the wild type product is missing from the homozygous mutant, and
- One PCR with WT primers on N2 DNA to show that the wild type product is present in N2.

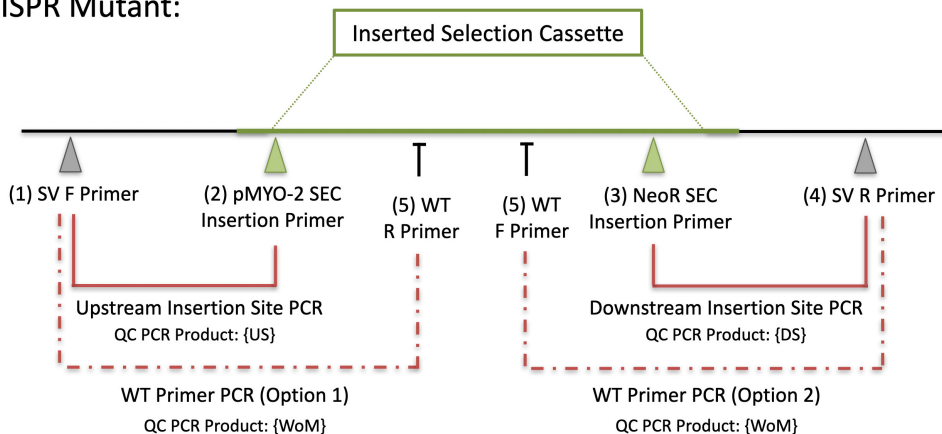
The QC PCR result codes provided in the strain description list these results in the order Upstream Insertion Site {US}, Downstream Insertion Site {DS}, WT PCR on Mutant DNA {WoM} and WT PCR on Wildtype (N2) DNA {WoW}. "P" means pass, "F" means fail, "X" means that the test was not performed. "H" in the 'WT on Mutant' reaction indicates that the test was performed on heterozygous mutants and resulted in a WT PCR product. This is done in cases where homozygous mutants could not be identified with our screening protocol, e.g. when homozygous animals are very slow-growing or lethal. Note: the stocks of heterozygous mutants should be checked for the presence of mutant animals, i.e. animals carrying the *myo-2::GFP* marker. Heterozygous mutant animals can be lost after repeated transfer, when the mutant animals have a growth disadvantage. For all reactions one primer is flanking and the other is in the indel region. We refer to mutations that pass all QC PCRs as "perfect" (i.e., PPPP or PPHP) and those with insertion site PCR failures as "imperfect" (i.e., PFPP or FPPP), 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.

Primer binding sites and quality control PCRs

Wildtype:



CRISPR Mutant:



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.