



Figure S1 Schema of gRNA constructs that succeeded or failed to produce the expected phenotype. Dominant coding mutations are shown in red, silent mutations in blue, restriction site in italics. gRNA constructs that worked (green, above locus) from Figure 1 are shown. gRNA constructs that apparently failed (pink, below locus) are shown with expected cleavage site (dotted line and scissors). The failure of gRNAs to produce the expected phenotype may be due to failure at any or a number of steps in CRISPR/Cas9-facilitated HR. All gRNA plasmids were injected at 50ng/ul.

(A) gRNA for *rol-6* bearing a mismatch at the -10 position failed to produce Rol progeny in 20 injected animals. This gRNA may have failed due to the single bp mismatch (bold italics, sequence shown below pink arrow). BbvI site in italics.

(B) The sole gRNA for *unc-43* failed to produce any Unc progeny, with template oligonucleotide at 20ng/ul or 200ng/ul. EciI site in italics. The A/T rich stretch may have caused low expression of the gRNA, as it resembles a PolIII termination signal (GUNNERY *et al.* 1999).

(C) Two additional gRNAs failed for *unc-58*. gRNA#2 was tested with template DNA at 20ng/ul or 200ng/ul, and may have failed because HR from the template oligonucleotide would not prevent further cleavage by Cas9. gRNA#3 may have failed due to inefficient Cas9 activity at an NAG PAM site. DdeI site in italics.

(D) Two additional gRNAs for *sqt-1* failed for unknown reasons. gRNA#3 is one nucleotide longer than gRNA#4. Donor oligonucleotide was AF-JA-113 for gRNA#3. Donor oligonucleotide was AF-JA-91 for gRNA#4. BbvI site in italics.

(E) gRNA used for *lin-14* C-terminus. Successful cleavage was evidenced by HR of a plasmid over the cleavage site.