

**A. H974A**

<i>rol-6</i> gRNA plasmid	<i>rde-1</i> gRNA plasmid	Injected	F1 Rol	F1 Rol with BbvI site	F1 Rol with BbvI site			F1 Rol without BbvI site		
					sequenced	<i>rol-6</i> locus	<i>rde-1</i> locus	sequenced	<i>rol-6</i> locus	<i>rde-1</i> locus
50ng/ul	50ng/ul	34	23	14	11	5 HR/wt 6 other	7 HR/wt 4 other	8	7 HR/wt 1 other	ND

**B. D801A**

<i>rol-6</i> gRNA plasmid	<i>rde-1</i> gRNA plasmid	<i>rde-1</i> donor DNA	Injected	F1 Rol	F1 Rol with NaeI site	sequenced	<i>rol-6</i> locus	<i>rde-1</i> locus
25ng/ul	50ng/ul	200ng/ul	38	14	9	9	7 HR/wt 2 other	2 HR/wt 6 other 1 NHEJ*
50ng/ul	25ng/ul	20ng/ul	20	29	16	6	4 HR/wt 2 other	5 HR/wt 1 other
25ng/ul	25ng/ul	20ng/ul	38	14	5	5	5 HR/wt	5 HR/wt

**C. D718A**

<i>rol-6</i> gRNA plasmid	<i>rde-1</i> gRNA plasmid	Injected	F1 Rol	F1 Rol with SnaBI site	sequenced	<i>rol-6</i> locus	<i>rde-1</i> locus
25ng/ul	25ng/ul	19	36	22	22	16 HR/wt 6 other	17** HR/wt 5*** other

**Figure S2** Observations of CRISPR/Cas9 effects on the selected and nonselected alleles

(A) Co-convertants between *rol-6*(*su1006*) and *rde-1*(*H974A*) tended to have mutations on both copies of the targeted loci. HR/wt indicates HR off the donor DNA at one allele, and the other allele was wt. "Other" indicates HR off the donor DNA, as well as additional mutations. The most common event in the other category was HR of one copy and NHEJ of the second copy. Two additional F1 Rol animals failed single worm PCR.

(B) Extent of HR and additional mutations for a range of gRNA plasmid concentrations at *rde-1*(*D801A*). \*This event was NHEJ creating a NaeI restriction site. An additional F1 Rol animal in the 25ng/ul/25ng/ul set of injections failed single worm PCR.

(C) Extent of HR and additional mutations observed among co-convertants for the *rde-1*(*D718A*) mutation. An additional F1 Rol animal failed single worm PCR. \*\*Of these, 14 were partial HR at only the SnaBI site. \*\*\*Of these, 4 were partial HR at only the SnaBI site.