

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.

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.

Guide to using the gRNA expression cassette (pRB1017) vector

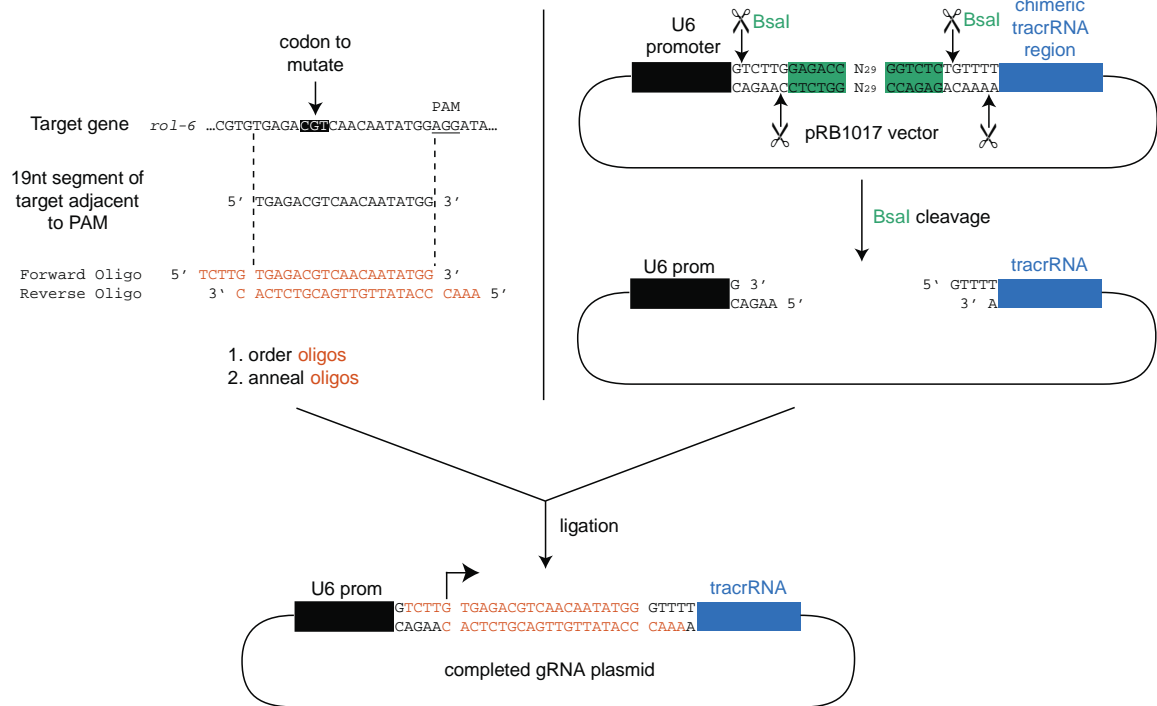


Figure S3 Guide RNA cloning strategy

First, the base to mutate and a nearby PAM are identified. 19 nucleotides upstream of the NGG PAM constitutes the guide RNA target sequence, and forward and reverse oligonucleotides with additional 5' and/or 3' nucleotides ends are ordered (left). The recipient guide RNA vector (pRB1017) is cut with Bsal (sites boxed in green), generating 4 nt overhangs complimentary to the oligonucleotide ends. Oligonucleotides are annealed, then ligated into the Bsal-cut vector. The guanosine where U6 initiates transcription is indicated with an arrow.

File S1

Supplement

gRNA constructs

Plasmid pRB1017, containing a *C. elegans* U6 promoter from *R07E5.16*, was used to drive expression of all gRNAs.

pRB1017 was made by cloning a gBlock (sequence:

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GGGAAGCTTCAAAAAAAGTAGCAATAAAGGAATAAAAAACTGTACACCTTAAAGGCGCACACTCTGTTTTGCAAATTTTATTTTT  
AGTTGTGAATTTCTGCTGAGACTGAAAATAGCAACTTTAGTACTACTATAATTTGTCAACCTTTTCAAAAAAGCATGCAATTTT  
GAGAACTCTTATAAAAGCTATTATTAATAAAAAACACCTTTTTTCCAAAATTATTCCACAAAAATATGTTATGAAATGCCTACACCT  
CTCACACACTCTTATACTACTCTGTCAAACCTCACGAGATGTCTGCCGCCTCTGTGTgCCCCTATATAAACACCTCTATTGCGA  
GATGTCTTggagaccggaaccatggctcgagaaaccggtactcggtctctGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGCCGTT  
ATCAACTGAAAAAGTGGCACCGAGTCGGTCTTTTTGTGAAATTTGCTAGCGG, BsaI-excised insert for gRNA cloning in  
lower case, see below) between the NheI and HindIII sites of pDR274 (HWANG et al. 2013).
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gRNA plasmids were designed and cloned as follows (Figure S3): pRB1017 contains two opposing non-palindromic BsaI sites, i.e. 5'-...GTCTTGGAGACC N₂₉ GGTCTCTGTTTT...-3', BsaI sites underlined. As BsaI cuts outside of its recognition site and leaves a four nucleotide 5' overhang, BsaI digestion of pRB1017 leaves the vector backbone without BsaI sites, but with 3'-AGAA-5' and 5'-GTTT-3' overhangs. For a given target mutation, a nearby PAM site (NGG) was located, ideally so that the gRNA overlapped the position of the mutation of interest (site is N₁₉NGG). Forward and reverse oligonucleotides bearing the gRNA site and complementary overhangs were ordered (forward: 5'-TCTTGN₁₉-3' and reverse 5'-AAACN*₁₉C-3', where N*₁₉ denotes the reverse complement of N₁₉). The additional G is required for transcription from the U6 promoter, and in our experiments a mismatch with the target site at this position does not confer adverse effects on gRNA functionality. Forward and reverse oligonucleotides were annealed, and then ligated into the cut vector. All plasmids were confirmed by restriction digest as well as sequenced with the M13 forward primer. We noted a tendency for single gRNA vectors to concatamerize in *E. coli* (even in *recA1* strains) and avoided gRNA plasmid preps where this was observed to be the case.

Microinjection

Plasmid DNA was prepared using Cetyl Trimethyl Ammonium Bromide (CTAB) precipitation (MELLO and FIRE 1995), and quantified using the Qubit Broad Range assay (Life Technologies). In all injections, the Cas9 plasmid (pDD162 (DICKINSON *et al.* 2013)) was present at 50ng/ul, and unless otherwise indicated, donor DNA was present at 20ng/ul (~600nM for ~100nt ssDNA). DNA mixtures were made up in injection buffer (20mM potassium phosphate, 3mM potassium citrate, 2% PEG, pH 7.5) and spun for >10' at >13,000 rcf. Attempts were made to microinject the distal arms of both gonads of young adult animals. Injected animals were rehydrated in recovery buffer (1mg/ml salmon sperm DNA, 4% glucose, 2.4mM KCl, 66mM NaCl, 3mM MgCl₂, 3mM CaCl₂, 3mM HEPES pH 7.2) prior to being placed on a small NGM plate seeded with OP50.

Throughout the paper, the number of injected animals includes animals that died yielding few progeny. We estimate on average ~20-40% of injected animals died before giving rise to sufficient progeny to accurately screen activity of the CRISPR/Cas9 system.

Screening

The F1 was screened for Rol, Dpy, and/or Unc phenotypes ~3-4 days after injection. Because some of the Unc phenotypes cause a developmental delay, and the Rol phenotypes do not manifest until ≥L3 stage, we found it helpful to move the injected parent to a new plate every ~12 hours. We found the majority of HR animals were born between 12 and 48 hours after injection.

Where long dsDNA (and not a ssDNA oligonucleotide) is used as a donor, transgenic animals provide a second possible source of PCR signals. Among the F1 progeny screened in Figure 4, we observed several transgenic animals, which lost *rde-1(AAA)* sequences within a generation or displayed non-mendelian inheritance patterns. The rate of such events was only slightly decreased when PCR screening primers outside of the donor dsDNA sequence were used.

Mitigating Additional Mutation Events Recovered During Co-Conversion

The observation that only one copy of a target locus is subject to efficient oligonucleotide-templated conversion may prove informative for optimizing conversion by CRISPR/Cas9. Because only a single genomic copy is receptive to templated mutation, increasing Cas9 and/or gRNAs above a certain level may increase the incidence of additional cleavage events, most prominently of deletions that break the non-selected allele, without an increase in conversion frequency. Excessive Cas9 activity may be undesirable for two reasons: (1) more Cas9 activity could be accompanied by an increased propensity for off-target cleavage and (2) untemplated mutations at the unselected copy of marker

locus limit the utility of that marker locus (and strain) for immediate experimentation, including further rounds of CRISPR/Cas9 co-conversion. Under this logic, avoiding excessive Cas9 activity may prove optimal for recovery of desired HR events in the absence of additional mutagenic events.

We carried out a series of experiments at different gRNA plasmid concentrations to assess whether these might affect the balance between wanted and unwanted events. Examining both *rol-6* loci or both *rde-1* loci for several F1 Rol animals from a high gRNA plasmid concentration (50ng/ul of each guide plasmid and 20ng/ul of each template DNA), we found a considerable degree of unwanted deletions and other mutations in the second, unselected allele. Six of 11 co-convertants had additional mutations resembling NHEJ at the non-converted allele at the *rol-6* locus, and 4 of 11 for the *rde-1* locus (Figure S2).

A two-fold reduction of the *rde-1* gRNA alone, or both *rol-6* and *rde-1* gRNAs together resulted in a higher number of animals where the templated mutations were recovered over a wild type copy at both *rol-6* and *rde-1* loci. Under all injection conditions the frequency of co-conversion remained high (35-60%), as did the total number of F1 Rol animals. At D718 in *rde-1*, with 25ng/ul of each gRNA plasmid, we observed 16 out of 22 animals were *rol-6(su1006)/+* and 17 out of 22 animals were *rde-1(D718A)/+*. Out of 5 co-convertant animals sequenced for D801A, all were *rol-6(su1006)/+*; *rde-1(D801A)/+*. While further optimization remains to be done (and will possibly be locus and/or gRNA-specific), simply lowering the concentration of gRNA plasmids provides one strategy to reduce the production of additional mutations with no loss in co-conversion frequency or yield of mutants.

Sequence of PCR product used for integration at *rde-1*:

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GCCAAATGAAAAACAGAGGAGCGCAATCTATTGTACGACGCGACGAAAAATGAATATGCCGTAAGTTTCAGAAAATTGAAAGTTT
TTAAATATCATATTTACAGTTCTACAAAAATTGTACACTAAATACCGGAATCGGTAGATTTGAAATAGCCGCAACAGAAAGCGAAGA
ATATGTTTGAACGCTCTCCGATAAAGAACAAAAAGTCTTAATGTTTCATTATCATTCCAAACGACAACGAATGCTTACGGTTTTG
TGAAACATTATTGCGATCACACCATCGGTGTAGCTAATCAGCATATTACTTCTGAAACAGTCACAAAAGCTTTGGCATCACTAAGG
CACGAGAAAGGATCAAAACGAATTTTCTATCAAATGTCATTGAAAATCAACGCGAAATTAGGAGGTATCAACCAAGAGCTCGACT
GGTCAGAAATTGCAGAAATATCACCAGAAGAAAAAGAAAGACGGAAAAACAATGCCATTAATATGTATGTTGGAATTGCTGTAAC
TCATCCAACCTCTACAGTGAATGATTATTCTATAGCGGCTGTAGTAGCGAGTATCAATCCAGGTGGAACACTATATCGAAATA
TGATTGTGACTCAAGAAGAATGTCGTCCCGGTGAGCGTGCAGTGGCTCATGGACGGGAAAGAACAGATATTTTGGAAAGCAAAGT
TCGTGAAATTGCTCAGAGAATTCGCAGAAGTGAGTTGCTTGTAGTATTTAAAAGATCTCTGGGATTTTTAATTTTTTTGTAACCTT
CAGAACAACGACAATCGAGCACCAGCGCATATTGTAGTCTATCGAGCTGGAGTTAGCGATTTCGGAGATGCTACGTGTTAGTCATG
ATGAGCTTCGATCTTTAAAAAGCGAAGTAAAACAATTCATGTCCGGAACGGGATGGAGAAGATCCAGAGCCGAAGTACACGTTTCAT
TGTGATTCAGAAAAGACACAATACACGATTGCTTCGAAGAATGGAAAAAGATAAGCCAGTGGTCAATAAAGATCTTACTCCTGCT
GAAACAGATGTCGCTGTTGCTGTTAAACAATGGGAGGAGGATATGAAAGAAAGCAAAGAAACTGGAATTGTGAACCCATCA
TCCGGAACAACAGTGGATAAACTTATCGTTTCGAAATACAAATTCGATTTTTTCTGGCATCTCATCATGGTGTCTTGGTACATCTC
GTCCAGGACATTACACTGTTATGTATGACGATAAAGGAATGAGCCAAGATGAAGTCTATGTAAGCGTTTTGAATAGCAGTTAGCG
ATTTTAGGATTTTGAATCCGCATATAGTTATTATAAAAAATGTTTCAGAAAATGACCTACGGACTTGCTTTTCTCTCTGCTAGATG
TCGAAAACCCATCTCGTTGCCTGTTCCGGTTCATTATGCTGCTTTATCATGTGAAAAAGCGAAAGAGCTTTATCGAACTTACAAGGA
ACATTACATCGGTGACTATGCACAGCCACGGACTCGACACGAAATGGAACATTTTCTCCAACTAACGTGAAGTACCCTGGAATGT
CGTTCGCATAACATTTTGCAAAAGTGTCCCGTTTTCAATCAAATTTTCAATTTGTAGATATTGACTTACTTTTTTTAAAGCCCGG
TTTCAAAAATTCATCCATGACTAACGTTTTTCATAAATTAATTTGAAATTTATCTGTGTTTATTATTACTCTAAATTTGTTTTG
AACGTGAGCATCATATCTTAAACTACTTATTGATAACGGTTTTCATAAAGATGTTT
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Table S1 Oligonucleotides used in this study

A: Oligonucleotides used for gRNA construction

corres p. plasmid	name	sequence	locus	strand	use with	HR or NHEJ observed?
pJA45	AF-JA-79	TCTTGTGCCATTAAGTATGTATGT	<i>rde-1(D718)</i>	forward	AF-JA-80	yes
	AF-JA-80	AAACACATACATAGTTAATGGCAC	<i>rde-1(D718)</i>	reverse	AF-JA-79	
pJA46	AF-JA-84	TCTTGATATTGTAGTCTATCGAGA	<i>rde-1(D801)</i>	forward	AF-JA-85	yes
	AF-JA-85	AAACTCTCGATAGACTACAATATC	<i>rde-1(D801)</i>	reverse	AF-JA-84	
pJA14	AF-JA-6	TCTTGATAAATGAGCATAATGAAC	<i>rde-1(H974)</i>	forward	AF-JA-7	yes
	AF-JA-7	AAACGTTCAATTATGCTCATTATC	<i>rde-1(H974)</i>	reverse	AF-JA-6	
pJA42	AF-JA-56	TCTTGTGAGACGTCAACAATATGG	<i>rol-6(su1006)</i>	forward	AF-JA-57	yes
	AF-JA-57	AAACCCATATTGTTGACGTCTCAC	<i>rol-6(su1006)</i>	reverse	AF-JA-56	
pJA52	AF-JA-105	TCTTGTGAGACGTCCACAATATGG	<i>rol-6(su1006)</i>	forward	AF-JA-106	no
	AF-JA-106	AAACCCATATTGTTGACGTCTCAC	<i>rol-6(su1006)</i>	reverse	AF-JA-105	
pJA43	AF-JA-67	TCTTGCGCGAGTTTTATTAGAAG	<i>unc-43(n498)</i>	forward	AF-JA-68	no
	AF-JA-68	AAACCTTCTGAATAAACTCGCGC	<i>unc-43(n498)</i>	reverse	AF-JA-67	
pJA50	AF-JA-101	TCTTGTCACGCACATGGTCACTA	<i>unc-58(e665)</i>	forward	AF-JA-102	yes
	AF-JA-102	AAACTAGTGACCATGTGCGTGGAC	<i>unc-58(e665)</i>	reverse	AF-JA-101	
pJA44	AF-JA-72	TCTTGGGAAACAAATTTTCTTTC	<i>unc-58(e665)</i>	forward	AF-JA-73	no
	AF-JA-73	AAACGAAAGAAAAATTTGTTTCCC	<i>unc-58(e665)</i>	reverse	AF-JA-72	
pJA48	AF-JA-94	TCTTGTTTTCTTTCAGGTCTTCCG	<i>unc-58(e665)</i>	forward	AF-JA-95	no
	AF-JA-95	AAACCGGAAAGACCTGAAAGAAAAC	<i>unc-58(e665)</i>	reverse	AF-JA-94	
pJA59	AF-ZF-821	TCTTGGAACCTCGTGCAAAACAAC	<i>unc-109(n499)</i>	forward	AF-ZF-822	yes
	AF-ZF-822	AAACGTTGTTTGACACGAGTTCC	<i>unc-109(n499)</i>	reverse	AF-ZF-821	
pJA58	AF-ZF-825	TCTTGCTACCATAGGCACCACGAG	<i>dpy-10(cn64)</i>	forward	AF-ZF-826	yes
	AF-ZF-826	AAACCTCGTGGTGCCTATGGTAGC	<i>dpy-10(cn64)</i>	reverse	AF-ZF-825	
pJA54	AF-JA-109	TCTTGTGTGGAGTTGGGGTAGCGT	<i>sqt-1(e1350)</i>	forward	AF-JA-110	yes
	AF-JA-110	AAACACGCTACCCAACTCCACA C	<i>sqt-1(e1350)</i>	reverse	AF-JA-109	
pJA55	AF-JA-111	TCTTGGGAAGGACATAGTTGTCAT	<i>sqt-1(e1350)</i>	forward	AF-JA-112	yes
	AF-JA-112	AAACATGACAACATATGTCCTTCCC	<i>sqt-1(e1350)</i>	reverse	AF-JA-111	
L8191	AF-KLA-164	TCTTGATTGTGGACCTGAAGAGG	<i>lin-14 C-term</i>	forward	AF-KLA-171	yes
	AF-KLA-171	AAACCTCTTCAAGGTCCACAATC	<i>lin-14 C-term</i>	reverse	AF-KLA-164	

B: Donor oligonucleotides for HR

name	sequence	locus
AF-JA-53	TGTGGGTTGATATGGTTAAACTTGGAGCAGGAACCGCTTCCAACCGTGTGCGGTGCCAACAATA TGGAGGATATGGAGCCACTGGTGTTCAGCCACCAGCACCAAC	<i>rol-6(su1006)</i>
AF-JA-65	TAAGTTCGATAAAGCTCTTTCGCTTTTTCACATGATAAAGCAGCATAATGAACCGGAACAGGCAA	<i>rde-</i>

	CGAGATGGGTTTTTCGACATCTAGCAGAGAGAAAAAG	1(H974)
AF-JA-69	TTTACTTTTAAATTTACAATTTTCTATCCGAAATACTCACCTTGCATCCGCCTTTGAATAAAACTCGC GAGCAACAATGTCCTCAAACAGTTCTCCTCCGGTAAC	unc-43(n498)
AF-JA-76	ATTTTGTGGTATAAAAATAGCCGAGTTAGGAAACAAATTTTCTTTTCAGGTTTCTCAGTAGTGACCA TGTGCGTGGATCTTGCCTCCACACATCTCAAGGCGTACTT	unc-58(e665)
AF-JA-81	ATATCACCAGAAGAAAAAGAAAGACGGAAACAATGCCATTAACATGTACGTAGGAATTGCTG TAACTCATCCAACCTCCTACAGTGAATTGATTATTCTATAGCGGCTG	rde-1(D718)
AF-JA-86	CTTTCAGAACAACGACAATCGAGCACCAGCGCATATTGTAGTCTATCGAG CCGGC GTTAGCGATTCGGAGATGCTACGTGTTAGTCATGATGAGCTTCGATCTTT	rde-1(D801)
AF-JA-91	GGGGATCCATCAGCATGTGGAGTTGGGGTAGCGTGTGCTCTTCATATTGGCAGCGGACACGCT TGCTAGATCTTCCGATGACAACATGTCCCTCCACAATCC	sqt-1(e1350)
AF-JA-113	TTGACTGGTGGAGCAGATGGGGATCCATCAGCATGTGGAGTTGGGGTAGCGTTCGTCTCTTCA TATTGGCAGCGGACACGCTTGTAGATCTTCCGATAAACCCTATGTCCTTCCACAATCCATTGGTA GATTGCTAGAAAATTAATAA	sqt-1(e1350)
AF-JA-116	CAGCCGCTATAGAATAATCAATTCCTACTGTAGGAGTTGGATGAGTTACAGCAATTCCTACGTAC ATAGTTAATGGCATTGTTTTCCGCTTTCTTTTCTTCTGGTGATAT	rde-1(D718)
AF-ZF-827	CACTTGAACCTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGG TAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT	dpy-10(cn64)
AF-ZF-820	GAAAGGTTAGGAGAGGCAATATATCAACCAACTGAGCAAGATATTCTCGAACATGTGTAATAA CTACTGGTATTGTTGAAGTTCACCTCACATTCAAAAATCTCAATTTCAAGTGAG	unc-109(n499)

C: Oligonucleotides for single worm PCR

name	sequence	locus	use with
AF-JA-54	GCCATTGTATTTTCTGGAGCCAC	<i>rol-6(su1006)</i>	AF-JA-55
AF-JA-55	CTCCACGTGGTCCTCCTCCATTC	<i>rol-6(su1006)</i>	AF-JA-54
AF-JA-77	TGCTGTAAACAATGGGAGGAGG	<i>rde-1(H974)</i>	AF-JA-78
AF-JA-78	AGATATGATGCTCACGTTCAAAACG	<i>rde-1(H974)</i>	AF-JA-77
AF-JA-82	GCCAAATGAAAACAGAGGAGCGCA	<i>rde-1(D718)</i>	AF-JA-83
AF-JA-83	ATCTTTATTGACCACTGGCTTATC	<i>rde-1(D718)</i>	AF-JA-82
AF-JA-87	AGTCACAAAAGCTTTGGCATCAC	<i>rde-1(D801)</i>	AF-JA-88
AF-JA-88	ATCTAAAATCGCTAACTGCTATTC	<i>rde-1(D801)</i>	AF-JA-87
AF-JA-96	CGGAGATATCGTTGTGACTGATTAC	<i>unc-58(e665)</i>	AF-JA-75
AF-JA-75	CTGACTGGAAGGAATTGTGACGGA	<i>unc-58(e665)</i>	AF-JA-96
AF-JA-70	CAAACAAGTGACAAACCTTCAAGTC	<i>unc-43(n498)</i>	AF-JA-71
AF-JA-71	GAAGCTCAAAAGTTTCAAACACG	<i>unc-43(n498)</i>	AF-JA-70
AF-ZF-823	GATGTGGTGGCAGCAATGGAGGACAC	<i>unc-109(n499)</i>	AF-ZF-824
AF-ZF-824	AATTTCTACTGTTGTCTCATCTTC	<i>unc-109(n499)</i>	AF-ZF-823
AF-ZF-831	GTCAGATGATCTACCGGTGTGTCAC	<i>dpy-10(cn64)</i>	AF-ZF-832
AF-ZF-832	GTCTCTCCTGGTGCTCCGTCTTAC	<i>dpy-10(cn64)</i>	AF-ZF-831
AF-JA-92	GCGTCGCGTCCCTTCTCTCCTG	<i>sqt-1(e1350)</i>	AF-ZF-93
AF-JA-93	ACATCCGACTCCTTATCTCCCG	<i>sqt-1(e1350)</i>	AF-ZF-92
AF-KLA-175	ACTCGAACTATGCAAATCTTC	<i>lin-14 C-term</i>	AF-KLA-176
AF-KLA-176	GAGATACTACAATGTGCGAA	<i>lin-14 C-term</i>	AF-KLA-175

D: Oligonucleotides for *rde-1* PCR donor construct and screening

name	sequence	raison d'etre
AF-JA-21	AAACATCTTTATGAAACCGTTATC	reverse PCR primer for <i>rde-1</i> donor PCR product, with AF-JA-82
AF-ZF-438	GTGTAAGTGTTTTCTACGTAGATT	with AF-JA-82, forward PCR primer for screening for integration
AF-JA-36	GTGAGTACCAATGAGCGATGTCATC	with AF-JA-77, reverse PCR primer for screening for integration

Supplemental References

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