General Strategy for Genome Editing Across Nematode Species

Hermaphrodite Species

Male/Female Species

days 1-6

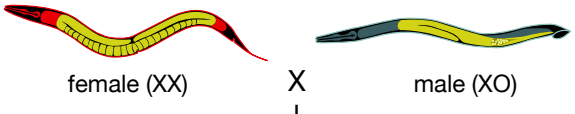
Design, synthesize, validate TALENs.

days 6-8*

Synthesize RNAs for Cas9 and guides.

days 7-8

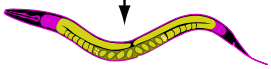
Synthesize TALEN RNAs.



Inject hermaphrodites.

day 9

Inject either TALEN RNAs or Cas9 and guide RNAs with or without ssOligos. Collect progeny in the 8-16 hour window post-injection.



Inject mated females.

day 11

Pick into liquid wells.

Pick individual L3-L4 F1s to single wells for growth.

Pick into agar wells and set up sib matings.

day 13

Pool aliquots from each of 4 single wells to perform CEL-I assay and identify putative mutants from young F2 progeny.

Resuspend worms in 40 µl S medium; pool 5 µl from each of 4 wells for CEL-I assay.

day 14

Screen single wells of pooled CEL-1-positive wells. Transfer animals from positive single wells onto plates with food for overnight recovery.

Screen 5 µl from single wells in pooled positive CEL-1 wells. Put positive single agar well onto plate.

day 15

Pick into liquid wells.

Pick individual animals to individual wells for growth.

Pick into agar wells and set up sib matings.

day 17

PCR region and sequence to identify molecular lesions and recover homozygous mutant worms.

* days 1-5 Cas9 holiday for RNA-mediated delivery

Figure S1 General strategy for genome editing across species. The daily work flow for a complete experiment to delete or insert DNA sequences using TALENs or CRISPR-Cas9 is provided for both hermaphroditic species and male/female species. The center column details the steps common to all species. The right and left columns provide specific details relevant to either hermaphroditic species (left) or male/female species (right).

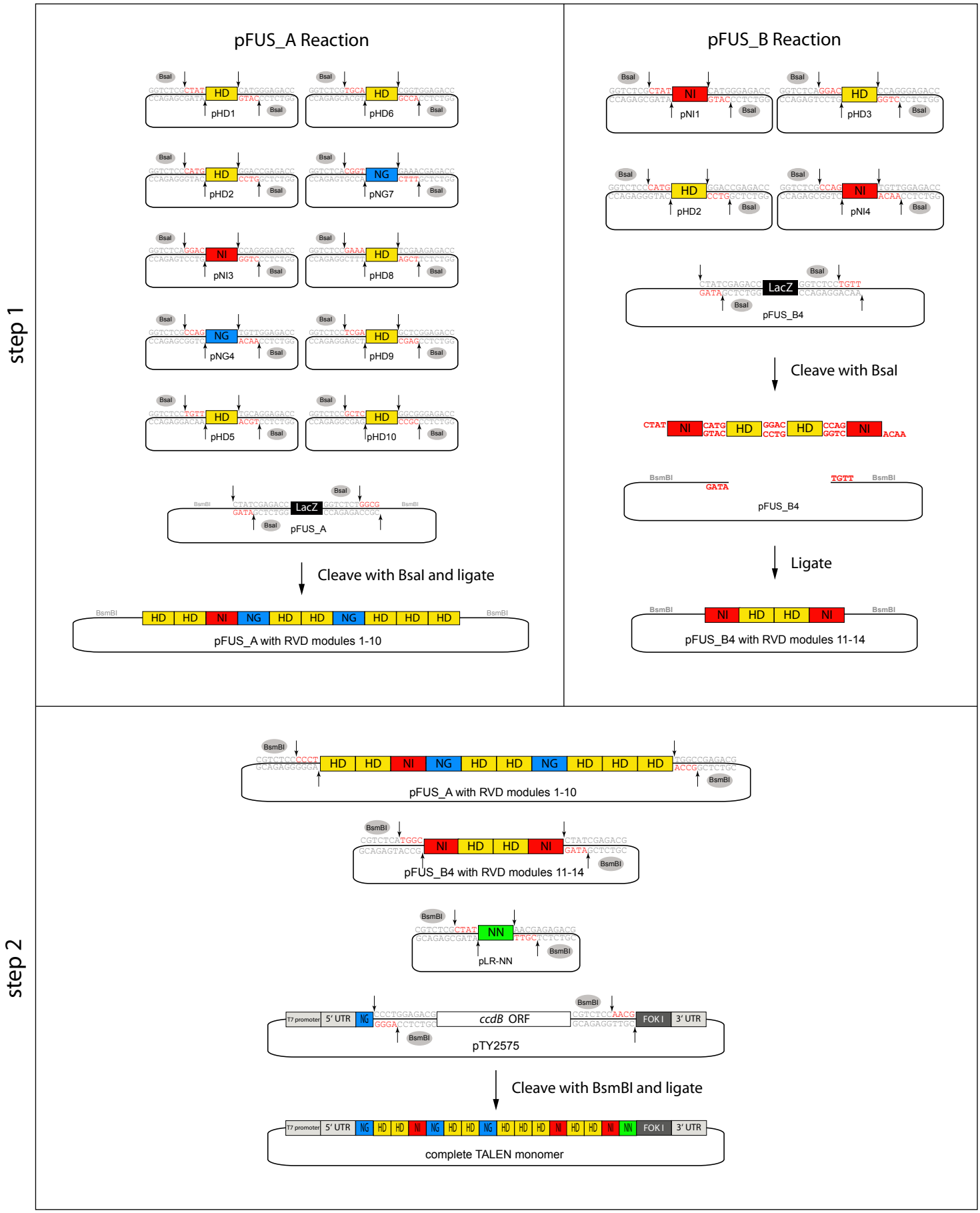
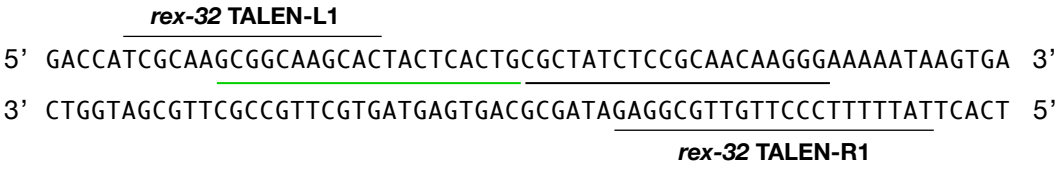

step 1
pFUS_A Reaction
BsaI
HD
pHD1
pHD6
NI
pNI1
pHD3
pHD2
NG
pNG7
pNI4
pNI3
pHD8
pNG4
pHD9
pHD5
pHD10
LacZ
pFUS_A
pFUS_B Reaction
pFUS_B4
Cleave with BsaI
CTAT
CATG
GGAC
CCAG
ACAA
BsmBI
GATA
TGTT
Ligate
pFUS_A with RVD modules 1-10
pFUS_B4 with RVD modules 11-14
step 2
pLR-NN
T7 promoter
5′ UTR
ccdB ORF
FOK I
3′ UTR
pTY2575
Cleave with BsmBI and ligate
complete TALEN monomer

Figure S2 Diagram for the construction of a TALEN. TALENs were constructed using the Golden Gate TALEN Kit. See http://www.addgene.org/TALefector/goldengate/ voytas/. This kit includes numerous RVD-encoding plasmids that permit the efficient assembly of RVD domains in the desired order for DNA recognition. The assembly principle is based on the ability of Type IIS restriction enzymes to cleave outside of their recognition sequence, specifically to one side. The nucleotides between the recognition site and the cleavage site of each RVD has a unique end once cleaved from its plasmid so that the cleaved RVD can only ligate with an RVD having a complementary sequence. Thus, RVDs can assemble in only one order. The kit is organized to permit 10 RVDs to be assembled in one reaction (A reaction) and the next 10 RVDs to be assembled in a second reaction (B reaction) (see step 1). The products of the two reactions are then assembled into the final TALEN (step 2). A more extended explanation for the RVD assembly in the first reaction is the following: For each RVD position in the final assembly, plasmids are provided for all possible RVDs so that after BsaI digestion, any RVD for that position will have the same overhanging sequences. Using the first position as an example, the desired RVD can be retrieved from any one of the plasmids pHD-1, pNN-1, pNG-1 pNI-1, and either pNK-1 or pNH-1. After digestion with BsaI each RVD will have overhangs of 5' CTAT 3' at one end and 3' GTAC 5' at the other end (red bases). Similarly, for the second position, plasmids are provided for all possible RVDs such that after BsaI digestion, all RVDs have a 3' GTAC 5' overhang that uniquely complements the 5' CATG 3' overhang of the first-position RVD.

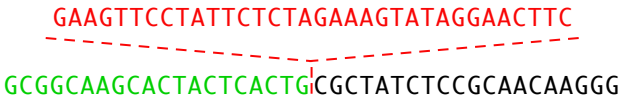
Step 1. The plasmids for the first 10 RVDs (Reaction A) are mixed with the plasmid pFUS_A, T4DNA ligase, and the Type IIS restriction enzyme BsaI. The mixture is treated to 10 cycles of 37°C for BsaI digestion and 16°C for ligation. The plasmids for RVDs 11 - 14 and pFUS_B4 are treated in the same manner. Ligated DNAs from the two reactions are transformed independently into DH5α chemically competent cells and plated on LB agar with spectinomycin, IPTG, and X-gal. For each transformation, white colonies are selected and checked by colony PCR or by restriction digestion of isolated plasmid DNA with ApaI and BamHI. Plasmids can be sequenced to insure that they have the correct RVDs.

Step 2. The two plasmids with the correct RVDs from step 1 are mixed with a plasmid containing the last RVD (pLR-NN in this example), and our customized C. elegans vector (pTY2575) that contains a T7 promoter, a 5' UTR, the initial RVD NG, a ccdB gene for selection, a FokI nuclease monomer, and 3' UTR. The type IIS restriction enzyme BsmBI and T4 ligase are added to this mix and treated to 10 cycles of 37°C and 16°C as above. The resulting ligation is transformed into DH5α cells and plated on LB agar with Ampicillin. The ccdB ORF targets DNA gyrase and kills bacteria that receive a plasmid containing this DNA. In a correctly assembled TALEN clone, the ccdB ORF will have been replaced by TALEN sequences, permitting the bacteria to live. Resulting colonies are screened by colony PCR or by digestion with diagnostic restriction enzymes and sequenced to confirm that the RDVs are correct.

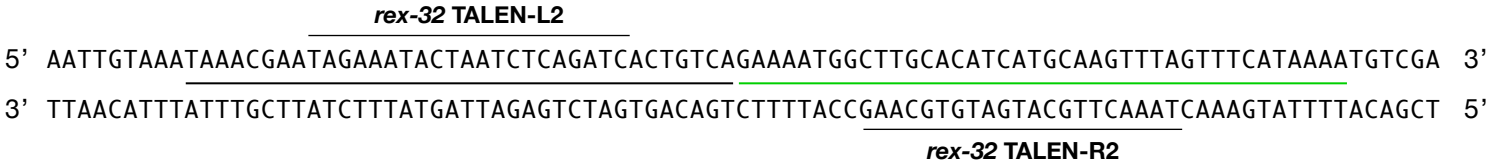
***rex-32* 5' FRT**



5' FRT oligo/final mutant



***rex-32* 3' FRT**



3' FRT oligo/final mutant

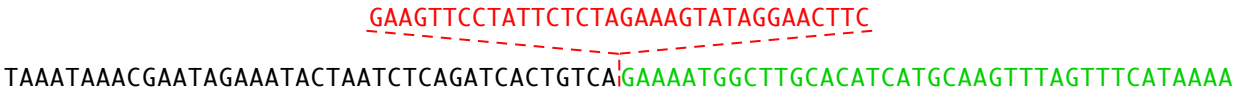


Figure S3 Strategy for inserting FRT sites that flank *rex-32*. Two segments of *rex-32* and the TALE recognition sequences within *rex-32* (black lines) used to target TALEN-induced DSBs for HDR-mediated FRT insertion at the 5' and 3' ends using the ssOligos represented below each DNA segment. The homology arms (green and black) for the 5' FRT insertion were 20 bp, while those for the 3' FRT insertion in the strain carrying the 5' FRT site were 40 bp to direct the ssOligo to the appropriate sequences at the 3' end rather than the 5' FRT sequence.

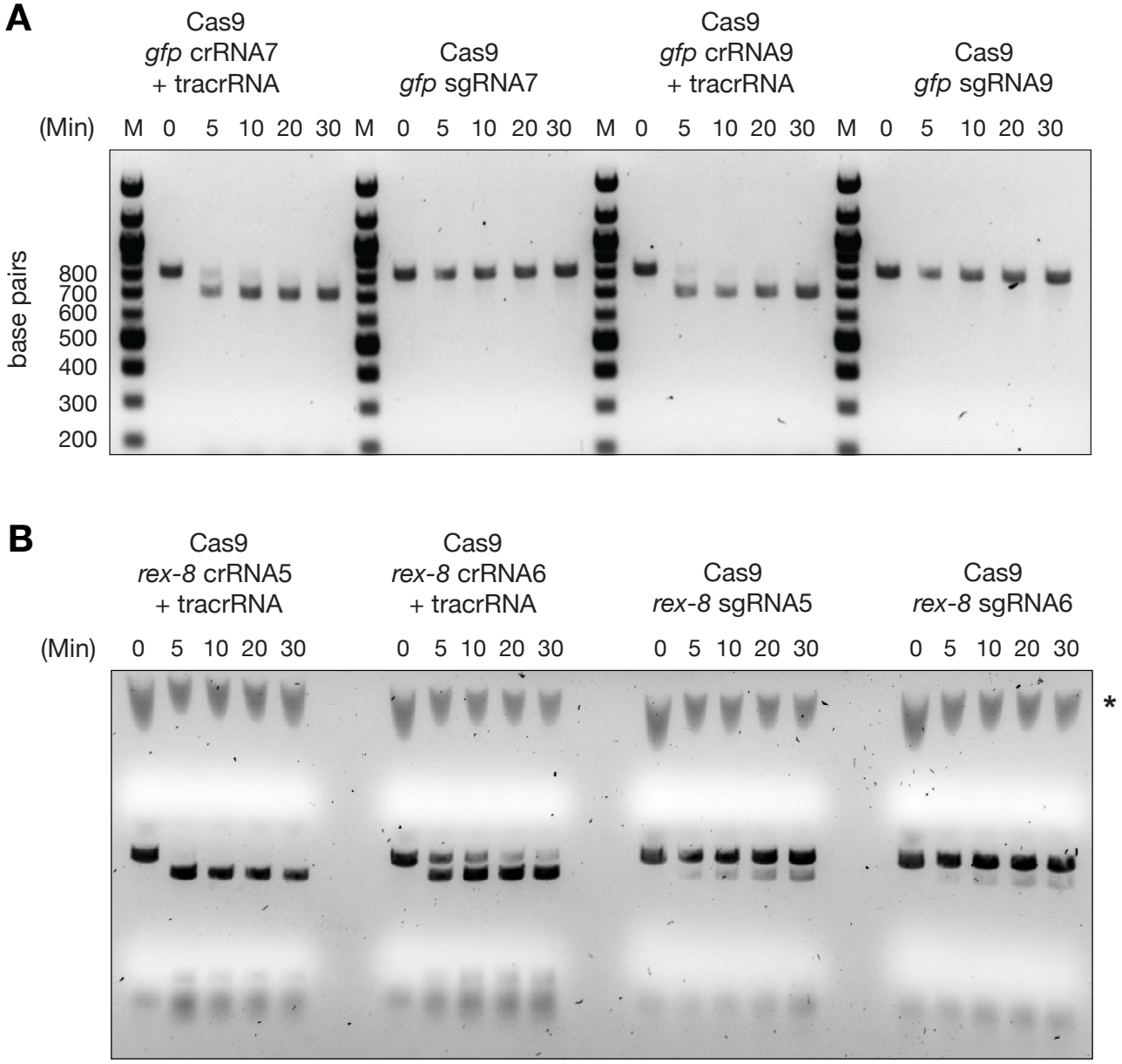


Figure S4 *in vitro* DNA cleavage assays comparing the effectiveness of Cas9-crRNA: tracrRNA and Cas9-sgRNA complexes. Double-stranded DNA cleavage was tested in time course reactions using the molar ratio of Cas9 : guide RNA : target DNA as 0.5 μM : 1 μM : 0.5 μM. Reactions were conducted at 37°C. The *in vitro* assays show that the dual RNA guides are more effective at promoting DNA cleavage than the sgRNAs.

(A) DNA cleavage assays for *gfp.* The target binding sequences in the *gfp* RNA guides are the following: crRNA7, 5' AAAGGGCAGAUUGUGUGGAC 3' and crRNA9, 5' GUGGACAGGUAAUGGUUGUC 3'. M, 100-bp markers.

(B) DNA cleavage assays for *rex-8.* The target binding sequences in the *rex-8* RNA guides are the following: crRNA5, 5' GGUGGAUAAAAUAAUUGAGC 3' and crRNA6, 5' UGGAUAAAAUAAUUGAGCGG 3'. The asterisk shows the location of Cas9-bound DNA in the gel.

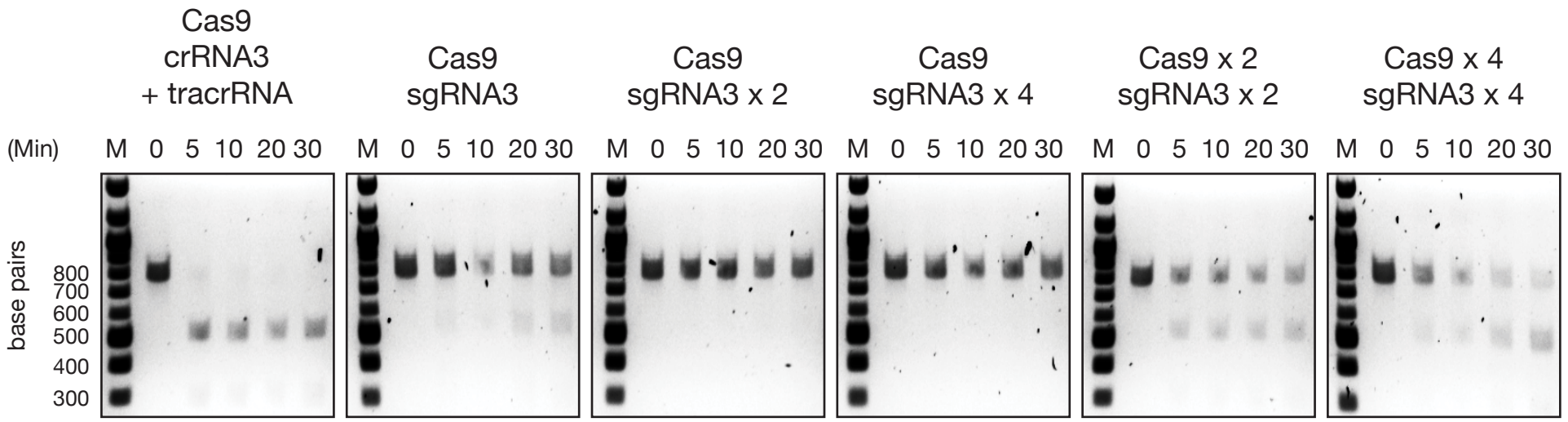


Figure S5 Effect of Cas9 protein and sgRNA concentration on DNA cleavage efficiency *in vitro.* Double-stranded DNA cleavage was tested in timecourse reactions using 250 nM DNA and various concentrations of Cas9 and guide RNAs. Starting (1x) concentration of Cas9 and sgRNA or tracrRNA:sgRNA was 250 nM; x 2 concentration = 500 nM; x 4 concentration = 1 µM. M, 100-bp markers.