A

3.0 kb
1.0 kb
gfp insert (4.2 kb)
native locus (1017 bp)

B

native unc-32: CGTGAAGCTGAGGAGAATCTCTTAAGtcatcagccacttcaaaaggtgt
gRNA binding site
PAM

Strain 1
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

Strain 2
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------gcacttcaaaaggtgt
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------gcacttcaaaaggtgt

Strain 3
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------ccacttcaaaaggtgt
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------ccacttcaaaaggtgt

Strain 4
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

Strain 5
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

Strain 6
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

Strain 7
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

Strain 8
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg
CGTGAAGCTGAGGAGAATCTCTTCTTACTCTCT---------cgccacccttaaacaagttg

C

i

targeting plasmid

origin ampR

sgRNA GFP unc-119 GFP

array markers

Prab-3:mCherry
Pmyo-2:mCherry
Pmyo-3:mCherry

ii

ampR origin sgRNA GFP unc-119 GFP

 TTGTACTACACACAGGACAAGCGGA | INSERT agttttacagtt TTTTACAGTTTAAAAAGCTGAAAT
 rict-1 chr. II duplication

iii

unc-119 GFP

 ACGTTCAACATGTTTTTCGAAAAAT | INSERT aagca..tggGT TTCTAAAACAAAAAAAATTTCATCAA
 multiple potential locations unknown origin
S3 Fig Unintended CRISPR outcomes: dead-end targets and dark inserts.

(A and B) Dead-end target sites: 20 EG9881 animals were injected with a guide RNA and repair template for tagging unc-32 with gfp. At starvation of the primary plates, array+, gfp- animals were singled to generate secondary plates, and the plates were incubated until starvation. Some lines failed to generate GFP+ animals (8/20 lines) by the time the secondary plate starved. These lines were analyzed by PCR amplification of the targeted insertion site (A) and then followed by sequencing (B). (A) PCR amplification of the unc-32 target locus. Expected amplicon sizes for the native locus and for a complete gfp+ Cbr-unc-119 insert are indicated. (B) Sequencing results for the two unc-32 loci in each strain. The native unc-32 target site is shown above with the guide-RNA binding site and Cas9 cut site highlighted. For each strain, the sequences for both chromosomes are shown. Unique alleles are individually color coded to highlight sibling relationships. Strains 1, 2, 4, 6 and 7 likely originated from a single parental, and strains 3 and 8 likely originated from a single parent. These sequences document six different healing events: three chromosomes healed by NHEJ (red, blue, purple), one chromosome (green) incorporated a truncated GFP, one chromosome (brown) inserted 4 nucleotides at the cut site, and one chromosome (pink) inserted a novel segment with sequence variations (underlined). ‘X’ indicates that two nucleotides were present at this position. It is possible this sequence represents a de novo event segregating in this strain.

(C) Location of two ‘dark inserts’. Two strains with off target insertions of unc-119(+) were located by whole genome sequencing (Illumina). After aligning all reads to the reference C. elegans genome, reads that failed to align to the genome were aligned to the sequences of all injected plasmid species. From this mapping, we determined which array sequences were present in the genome. To locate the junctions between the plasmid and the genome, we identified regions by inspection where the plasmid reads contained unmapped tails. BLAST analysis of the unmapped tails was used to determine the insertion site. The insertion site of one of the off-target events is in a central intron of the rict-1 gene. The sequences flanking the second off-target insertion are present at multiple loci in the genome, preventing exact mapping. (i) Schematic representation of the CRISPR targeting injection mix containing a targeting plasmid and multiple array marker plasmids. (ii) Schematic representation of rict-1(ox773), an off-target insertion of the entire gfp::snb-1 targeting plasmid into intron #9 of rict-1a on chromosome II. (iii) Schematic representation of the off-target insertion of a portion of the unc-32::gfp targeting plasmid into a non-unique genomic location. The insertions were not at sites obviously similar to the predicted guide RNA binding sites.