Heritable Custom Genomic Modifications in *Caenorhabditis elegans* via a CRISPR–Cas9 System

Yonatan B. Tzur,* Ari E. Friedland,* Saravanapriah Nadarajan,* George M. Church,*,† John A. Calarco,‡,1 and Monica P. Colaiácovo*,1

*Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, †Wyss Institute for Biologically Inspired Engineering, and ‡FAS Center for Systems Biology, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT We adapted the CRISPR–Cas9 system for template-mediated repair of targeted double-strand breaks via homologous recombination in *Caenorhabditis elegans*, enabling customized and efficient genome editing. This system can be used to create specific insertions, deletions, and base pair changes in the germline of *C. elegans*.

GENOME engineering has proven to be a useful tool in biological research. Specific and accurate insertions, deletions, and replacements allow for numerous invaluable ways to examine gene function. Yet, the accurate and efficient insertion or deletion of large, specific sequences has been challenging in most metazoans (Gaj *et al.* 2013). In the nematode *Caenorhabditis elegans*, creating heritable mutations had until recently involved random mutagenesis, and the insertion of genes into the genome had been limited to random loci, often resulting in the silencing of the inserted transgene in the germline due to repetitive insertions (Kelly *et al.* 1997; Jackstadt *et al.* 1999; Wilm *et al.* 1999). In the past few years new methods for genome editing have become available for this model system, all of which involve the creation of a specific double-strand break in the genome. The most widely applied method uses the Mos1 transposon and transposase (Robert and Bessereau 2007; Frokjaer-Jensen *et al.* 2008, 2010, 2012). Although this method enables precise genome editing, it is limited by the availability of a transposon at the desired locus.

In bacteria and archaea, CRISPRs (clustered regularly interspaced short palindromic repeats) and Cas (CRISPR-associated) proteins are used to protect the cell against invading viruses and exogenous DNA (Terns and Terns 2011; Sorek *et al.* 2013; Wiedenheft *et al.* 2012). In the type II CRISPR systems, the Cas9 endonuclease specifically cleaves the exogenous DNA by interacting with two types of RNAs, tracrRNA (*trans*-activating CRISPR RNA), and crRNA (CRISPR RNA), which contain sequences complementary to the invading element and are generated from the CRISPR loci. Given the potential for RNA-mediated programmable DNA cleavage, this system was pared down to two components by using Cas9 from *Streptococcus pyogenes* and an engineered single-guide RNA (sgRNA) (Jinek *et al.* 2012). Recently, this simplified system was transferred to several eukaryotic cell cultures and was shown to actively create specific double-strand breaks, enabling changes in the genomes of those cells (Cho *et al.* 2013a; Cong *et al.* 2013; Dicarlo *et al.* 2013; Jinek *et al.* 2013; Mali *et al.* 2013). Moreover, this system can now be used for genome editing in metazoan organisms such as mice, flies, and fish (Gratz *et al.* 2013; Hwang *et al.* 2013; Shen *et al.* 2013; Yu *et al.* 2013).

We have recently reported the successful use of the CRISPR–Cas9 system in the nematode *C. elegans*, establishing a robust strategy for creating random insertion and deletion (indel) mutations in the germline of the worm by expressing Cas9 and sgRNA targeted to the desired site (Friedland *et al.* 2013). Although this method provides an efficient and facile strategy to create loss-of-function gene mutations, it involves nonhomologous end joining and therefore does not allow for user-specified changes, the complete removal of genes, or the introduction of tags. Here we report the use of this method for template-mediated repair of targeted double-strand breaks via homologous

doi: 10.1534/genetics.113.156075
Manuscript received August 4, 2013; accepted for publication August 16, 2013
Supporting information is available online at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156075/-/DC1.
[1]Corresponding authors: 52 Oxford St., Northwest Lab Room 365.10, Cambridge, MA 02138. E-mail: jcalarco@fas.harvard.edu; 77 Ave. Louis Pasteur, NRB, Room 334, Boston, MA 02115. E-mail: mcolaiacovo@genetics.med.harvard.edu

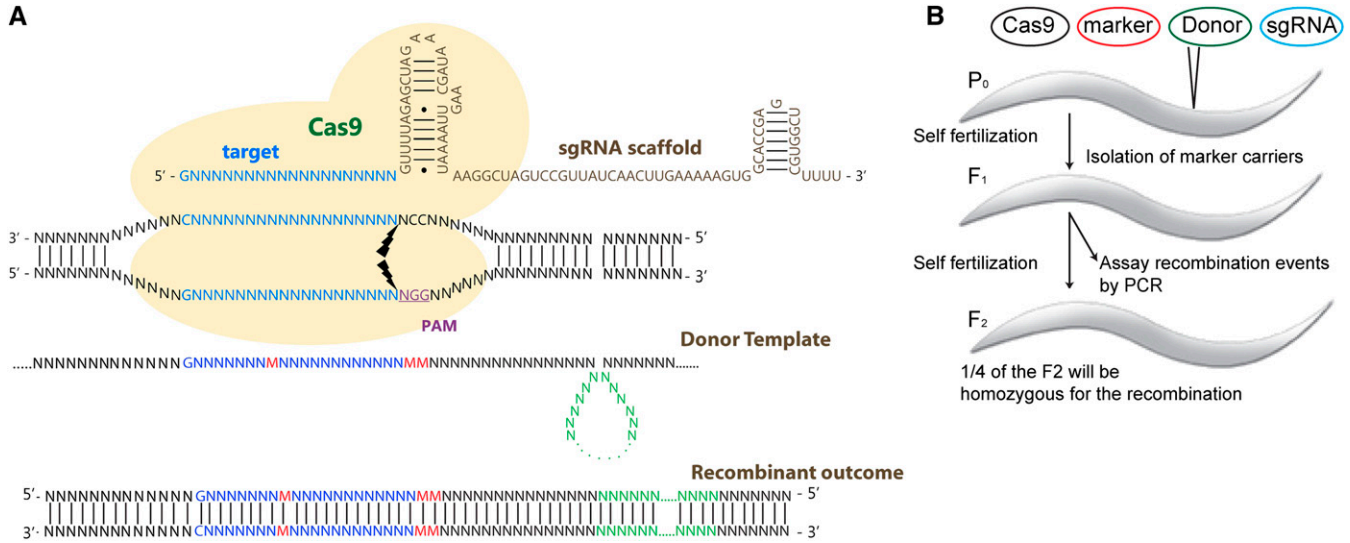

Figure 1 Rationale and design applied in adapting the CRISPR–Cas9 system for homologous recombination-mediated genome engineering in *C. elegans*. (A) Schematic representation of the elements generating the engineered product. Cas9 (yellow) interacts with the sgRNA carrying a G/A $(N)_{19}$NGG sequence, where G/A$(N)_{19}$ corresponds to 20 nucleotides complementary to the homologous genomic target, cleaving the target double-stranded DNA (bolts) at the 3′ *N*GG sequence that corresponds to the essential protospacer-associated motif (PAM). A donor vector that shares homology with the genomic locus provides a template for repairing the break, thus inserting custom mutations (red), deletions, and insertions (green) to the recombinant outcome. (B) Experimental design to generate and screen for worms carrying engineered genomes. Adult worms were injected with CRISPR–Cas9 expression vectors containing Cas9, a mCherry marker, donor template sequence, and the targeting sgRNA.

recombination, enabling customized and efficient genome editing. This article is one of six companion articles (Chiu *et al.* 2013; Cho *et al.* 2013b; Katic and Grosshans 2013; Lo *et al.* 2013; Waaijers *et al.* 2013) that present different approaches to and features of CRISPR–Cas9 genome editing in *C. elegans*.

Results

Site-directed gene insertion using the CRISPR–Cas9 system

We hypothesized that targeted DNA double-strand breaks created by the CRISPR–Cas9 system can also be repaired by the homologous recombination pathway and that specific insertions and/or deletions can be engineered into the germline using a donor vector (Figure 1A). To test this hypothesis, we injected young adult worms with a mixture of four plasmids (see Supporting Information, File S1) containing our codon-optimized Cas9 driven by the *eft-3* promoter, a *klp-12* targeting sgRNA driven by a U6 snRNA promoter (Friedland *et al.* 2013), an mCherry reporter driven by a body wall muscle promoter to label F_1 progeny and serve as a marker for extrachromosomal array formation, and a donor vector containing an *eft-3* promoter-driven GFP transgene flanked on either side by 1.5 kb of sequence homologous to regions upstream and downstream of the Cas9-induced cleavage site at the *klp-12* locus (Figure 1B and Figure 2A). We isolated F_1 progeny expressing GFP and mCherry and then screened for F_2 animals maintaining broad GFP but not mCherry expression, because these animals may have lost the extrachromosomal array and integrated the GFP transgene into the *klp-12* locus. We additionally surveyed these GFP-expressing F_2 animals by amplifying regions of genomic DNA specific for the recombinant product (see Supporting Information Table S1, Figure 2B). Using this combined screening approach, 1/72 (1.3%) isolated F_1 animals generated progeny producing the expected PCR amplicon, indicating that homologous recombination occurred (Table 1). Consistent with the heritable nature of this recombination event, we obtained homozygous lines by isolating F_2 worms producing 100% GFP-expressing F_3 and subsequent progeny (Figure 2C). We further verified that the transgene was inserted seamlessly by sequencing our recombinant-specific PCR amplicon (Figure S1).

A one-step CRISPR–Cas9-mediated gene replacement strategy

Next, we tested whether we could use our expression vector system to knock out a complete gene and replace it with a GFP transgene. We generated an sgRNA targeting the *lab-1* gene and verified its effectiveness in generating indel mutations at the appropriate locus by direct sequencing when injected with Cas9 (data not shown; Friedland *et al.* 2013). We then injected this sgRNA expression vector, our Cas9 expression vector, the mCherry reporter vector, and a donor vector containing a GFP transgene driven by the *baf-1* promoter, flanked by 1020 and 1029 bp upstream and downstream of the *lab-1* coding region, respectively

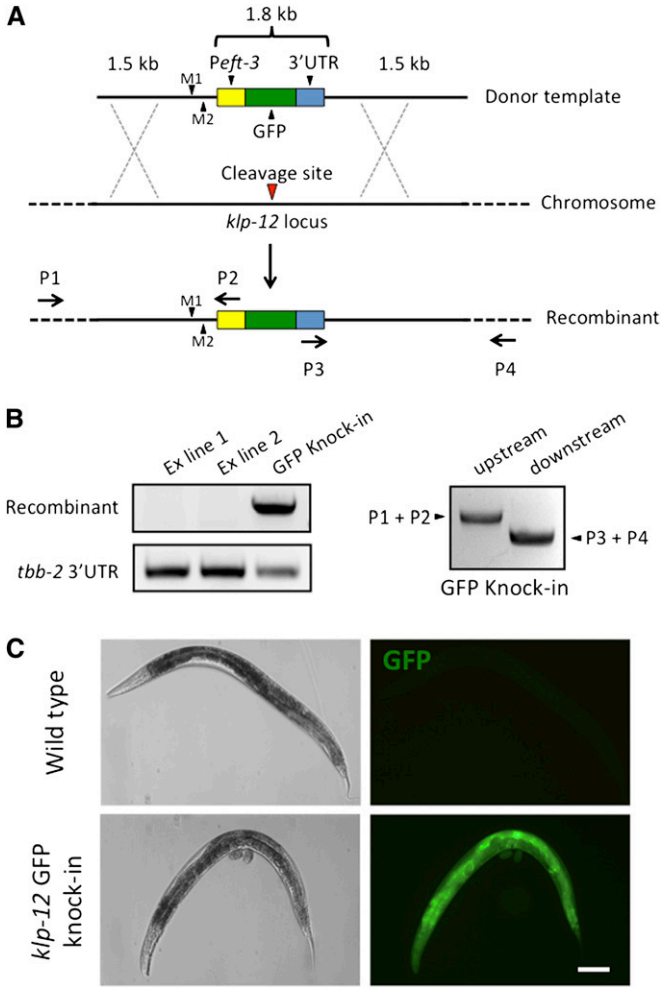

Figure 2 Insertion of the *Peft-3*::GFP::*tbb-2* 3′-UTR transgene into the *klp-12* locus by CRISPR–Cas9 mediated homologous recombination. (A) Design of donor repair template and diagram of expected recombinant product. The donor template (top image) contains 1.5 kb of homology (solid black lines) flanking the Cas9 cleavage site at the *klp-12* locus (middle image, red triangle). The donor also carries a GFP transgene (top and bottom images, green box) flanked by the *eft*-3 promoter (yellow box), and the *tbb*-2 3′-UTR (blue box), and mutations to introduce a *Hin*dIII recognition sequence (M1) and to destroy the protospacer-associated motif (PAM) of the sgRNA target sequence (M2). Any recombination events (bottom image) can be screened using PCR primers complementary to the transgene (arrows P2 or P3) and complementary to genomic regions outside the homology arms of the donor (arrows P1 or P4; dashed lines mark outer genomic regions). (B) PCR confirmation of recombinant animals. Left top: PCR assay using primers specific for a recombination event (P3 and P4 from A) sampling lysates of pooled F_2 animals from two different lines carrying the transgene in an extrachromosomal array along with our body wall muscle mCherry marker (lanes 1 and 2) and a candidate recombinant line (lane 3) identified by fluorescence microscopy. Left bottom: PCR assay using primers complementary to the *tbb*-2 3′-UTR to serve as a loading control. Right: PCR assays testing the recombinant line from the left, amplifying recombinant-specific PCR products spanning upstream (lane 1, primers P1 and P2) and downstream (lane 2, primers P3 and P4) of the cleavage site. (C) GFP expression in a strain in which a GFP transgene was inserted into the *klp-12* locus. Bar, 100 μm.

(File S1 and Figure 3A). This donor vector was designed to completely remove the 652 bp of *lab-1* and replace it with *Pbaf-1*::GFP. We screened for successfully engineered genomes in two independent experiments by using a similar PCR assay as described above, and we isolated F_1 worms that had recombinant progeny in 1/40 (2.5%) and 4/24 (16.7%) of the worms (Table 1 and Figure 3B). Again, isolated from the original insertion strain were homozygous recombinant progeny that express GFP through multiple generations, both in somatic and germline tissues (Figure 3C and Figure S2). Immunostaining using LAB-1-specific antibodies confirmed that the protein was absent in our recombinant line (Figure 3D). Sequencing of a PCR product spanning the recombinant locus confirmed the excision of the *lab-1* gene and its replacement with the GFP transgene (Figure S3). Consistent with the proposed functions for *lab-1* in promoting sister chromatid cohesion and accurate meiotic chromosome segregation (de Carvalho *et al.* 2008), we observed increased embryonic lethality (45%, $n = 651$; Emb) and a high incidence of males (11%, $n = 361$; Him) in our *lab-1* knock-out GFP knock-in line. These levels are similar to those reported for the *lab-1* RNAi-depleted worms (57% Emb and 6% Him), and higher than those observed for the *lab-1(tm1791)* hypomorph mutant (22% Emb and 4% Him) (de Carvalho *et al.* 2008), underscoring the importance of generating full knock-outs for assaying the null phenotypes of genes. We did not observe any new or unexpected phenotypes for either of the strains reported here.

Finally, to test whether the inserted promoter might regionally perturb gene expression, we assessed by RT–qPCR the expression of *asfl-1* and *T05F1.11*, which flank *lab-1* (Figure S4). We found no significant change in the expression of *T05F1.11* ($P = 0.153$ by the two-tailed Mann–Whitney test, 95% C.I.), but the expression of *asfl-1*, which lies upstream of *lab-1*, exhibited a small yet significant change ($P = 0.046$ by the two-tailed Mann-Whitney test, 95% C.I.). Further experiments will be required to determine whether this increase is due to a polar position effect exerted by the inserted promoter-*gfp* fusion or a secondary effect of the *lab*-1 knock-out. Altogether, these results demonstrate that CRISPR–Cas9 is a useful system for the seamless replacement of genes in the *C. elegans* genome.

Discussion

The results presented here show the powerful use of the CRISPR–Cas9 system to accurately engineer the *C. elegans* genome, thereby enabling the creation of almost any custom mutation desired. We were able to insert and delete genes, as well as do both in one step. The transgenes were seamlessly inserted and were expressed both in the soma and the germline, indicating the functionality and specificity of this method. Although we did not observe any off-target effects, it would be a good practice to outcross any mutation created using the CRISPR–Cas9 system. This method (also see accompanying *C. elegans* studies by Chiu *et al.* 2013; Cho *et al.*

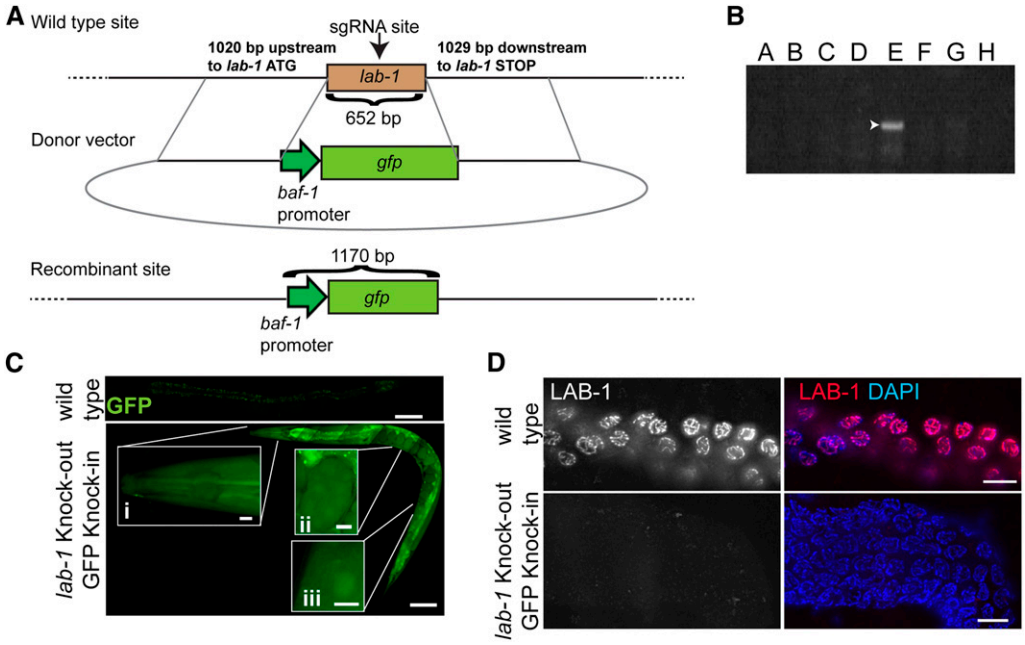

Figure 3 Insertion of the *Pbaf-1*::GFP transgene into the *lab-1* locus and excision of the *lab-1* gene by CRISPR–Cas9 mediated homologous recombination. (A) Design of donor repair template and diagram of expected recombinant product. The donor template (middle image) contains 1020 bases upstream to the *lab-1* ATG and 1029 downstream of the *lab-1* STOP codon regions of homology (solid black lines). The Cas9 cleavage site at the *lab-1* locus (652 bp) is located 310 base downstream of the ATG (arrow, top image). The donor also possesses a GFP transgene (884 bp, green box) flanked by the *baf-1* promoter (286 bp, green arrow). (B) PCR detection of recombinant specific species. PCR amplicon generated by using primers annealing 1167 bp upstream of the *lab-1* ATG and to the *baf-1* promoter. A–D and F–H, negative clones. E, a positive clone. Arrowhead, ~1200-bp fragment. (C) GFP expression in a strain in which a GFP transgene was inserted and *lab-1* was deleted. Bars, 100 μm. Insets depict GFP expression in the head (i), embryo (ii) and oocyte (iii). Bars, 10 μm. (D) Anti-LAB-1 (red) and DAPI (blue) costaining of late pachytene nuclei in the germlines of (top)wild-type, (bottom) *lab-1*-deleted, and *gfp*-inserted worms. Bars, 10 μm.

2013b; Katic *et al.* 2013; Lo *et al.* 2013; Waaijers *et al.* 2013, in this issue of *GENETICS*) now paves the way to various *in vivo* applications, including the replication of mutations identified in other organisms and the assessment of protein–protein binding sites as well as the amino acids predicted to undergo post-translational modifications. Tagging of genes in their own natural sites will become easier, thus enabling scientists to monitor their expression more accurately and facilitating immunoprecipitation experiments. Different gene mutation projects have provided the worm community with a growing collection of invaluable base change and deletion mutants. The CRISPR–Cas9 system now provides the community the ability to expand this collection and obtain precise gene deletions or targeted changes in a simple and rapid manner.

Other methods have been previously implemented in *C. elegans* to create similar engineering capabilities like ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases), MosSCI (*Mos1*-mediated single copy insertion), and MosDel (*Mos1*-mediated deletion) (Frokjaer-Jensen *et al.* 2008, 2010, 2012; Wood *et al.* 2011). The CRISPR–Cas9 method offers comparable and sometimes superior efficiency, along with three additional advantages: (1) It does not require the complex engineering of a special nuclease for every project, (2) it does not require any positive genetic selection marker, and (3) it can be applied to practically any locus given that the only requirement for the targeting site is the presence of G/A$(N)_{19}$NGG. These advantages, combined with the fact that this method calls for no specific genetic background, should now make it possible to sequentially create lines harboring multiple mutations in tightly linked genes that would otherwise require difficult genetic crosses. Finally, our results not only more generally demonstrate the versatility of the CRISPR–Cas9 system for customized engineering, now reported for several metazoan species, but also more specifically represent a significant step forward in facilitating a wide range of gene function and regulatory studies in *C. elegans*.

Acknowledgments

We thank Yoav Mayshar, John Aach and Elisabeth Altendorfer for valuable suggestions. pAD010 was a kind gift from Yosef Gruenbaum. Some strains were provided by the Caenorhabditis Genetics Center (CGC), which is funded by U.S. National Institutes of Health (NIH) Office of Research

Table 1 Summary of experiments creating homologous recombination-mediated mutations

Experiment	Gene	Injected worms	F1 worms	Recombinant worms	Frequency (%)
A	*klp-12*	13	72	1	1/72 (1.3)
B	*lab-1*	9	40	1	1/40 (2.5)
C	*lab-1*	7	24	4	4/24 (16.7)

Infrastructure Programs (P40 OD010440). This work was supported by NIH grant R01GM072551 to M.P.C., by an NIH Early Independence Award (1DP5OD009153) and additional support from Harvard University to J.A.C., and by an NIH National Human Genome Research Institute Center of Excellence in Genome Sciences grant (P50 HG005550) to G.M.C. A.E.F. is supported by a Ralph Ellison/American Federation for Aging Research postdoctoral fellowship.

Literature Cited

Chiu, H., H. T. Schwartz, I. Antoshechkin, and P. W. Sternberg, 2013 Transgene-free genome editing in *Caenorhabditis elegans* using CRISPR-Cas. Genetics 195: 1167–1171.

Cho, S. W., S. Kim, J. M. Kim, and J. S. Kim, 2013a Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. Nat. Biotechnol. 31: 230–232.

Cho, S. W., J. Lee, D. Carroll, J.-S. Kim, and J. Lee, 2013b Heritable gene knockout in *C. elegans* by direct injection of Cas9-sgRNA ribonucleoproteins. Genetics 195: 1177–1180.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto *et al.*, 2013 Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819–823.

de Carvalho, C. E., S. Zaaijer, S. Smolikov, Y. Gu, J. M. Schumacher *et al.*, 2008 LAB-1 antagonizes the Aurora B kinase in *C. elegans*. Genes Dev. 22: 2869–2885.

DiCarlo, J. E., J. E. Norville, P. Mali, X. Rios, J. Aach *et al.*, 2013 Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. Nucleic Acids Res. 41: 4336–4343.

Friedland, A. E., Y. B. Tzur, K. M. Esvelt, M. P. Colaiacovo, G. M. Church *et al.*, 2013 Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. Nat. Methods 10: 741–743.

Frokjaer-Jensen, C., M. W. Davis, C. E. Hopkins, B. J. Newman, J. M. Thummel *et al.*, 2008 Single-copy insertion of transgenes in *Caenorhabditis elegans*. Nat. Genet. 40: 1375–1383.

Frokjaer-Jensen, C., M. W. Davis, G. Hollopeter, J. Taylor, T. W. Harris *et al.*, 2010 Targeted gene deletions in *C. elegans* using transposon excision. Nat. Methods 7: 451–453.

Frokjaer-Jensen, C., M. W. Davis, M. Ailion, and E. M. Jorgensen, 2012 Improved Mos1-mediated transgenesis in *C. elegans*. Nat. Methods 9: 117–118.

Gaj, T., C. A. Gersbach, and C. F. Barbas, 3rd, 2013 ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol. 31: 397–405.

Gratz, S. J., A. M. Cummings, J. N. Nguyen, D. C. Hamm, L. K. Donohue *et al.*, 2013 Genome engineering of *Drosophila* with the CRISPR RNA-guided Cas9 nuclease. Genetics 194: 1029–1035.

Hwang, W. Y., Y. Fu, D. Reyon, M. L. Maeder, P. Kaini *et al.*, 2013 Heritable and precise zebrafish genome editing using a CRISPR-Cas system. PLoS ONE 8: e68708.

Jackstadt, P., T. P. Wilm, H. Zahner, and G. Hobom, 1999 Transformation of nematodes via ballistic DNA transfer. Mol. Biochem. Parasitol. 103: 261–266.

Jinek, M., K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna *et al.*, 2012 A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816–821.

Jinek, M., A. East, A. Cheng, S. Lin, E. Ma *et al.*, 2013 RNA-programmed genome editing in human cells. Elife 2: e00471.

Katic, I., and H. Grosshans, 2013 Targeted heritable mutation and gene conversion by Cas9-CRISPR in *Caenorhabditis elegans*. Genetics 195: 1173–1176.

Kelly, W. G., S. Xu, M. K. Montgomery, and A. Fire, 1997 Distinct requirements for somatic and germline expression of a generally expressed *Caernorhabditis elegans* gene. Genetics 146: 227–238.

Lo, T., C. S. Pickle, S. Lin, E. J. Ralston, M. Gurling *et al.*, 2013 Heritable genome editing using TALENs and CRISPR/Cas9 to engineer precise insertions and deletions in evolutionarily diverse nematode species. Genetics 195: 331–348.

Mali, P., L. Yang, K. M. Esvelt, J. Aach, M. Guell *et al.*, 2013 RNA-guided human genome engineering via Cas9. Science 339: 823–826.

Robert, V., and J. L. Bessereau, 2007 Targeted engineering of the *Caenorhabditis elegans* genome following Mos1-triggered chromosomal breaks. EMBO J. 26: 170–183.

Shen, B., J. Zhang, H. Wu, J. Wang, K. Ma *et al.*, 2013 Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell Res. 23: 720–723.

Sorek, R., C. M. Lawrence, and B. Wiedenheft, 2013 CRISPR-mediated adaptive immune systems in bacteria and archaea. Annu. Rev. Biochem. 82: 237–266.

Terns, M. P., and R. M. Terns, 2011 CRISPR-based adaptive immune systems. Curr. Opin. Microbiol. 14: 321–327.

Waaijers, S., V. Portegijs, J. Kerver, B.B.C.G. Lemmens, and M. Tijsterman *et al.*, 2013 Crispr/Cas9-targeted mutagenesis in *C. elegans*. Genetics 195: 1187–1191.

Wiedenheft, B., S. H. Sternberg, and J. A. Doudna, 2012 RNA-guided genetic silencing systems in bacteria and archaea. Nature 482: 331–338.

Wilm, T., P. Demel, H. U. Koop, H. Schnabel, and R. Schnabel, 1999 Ballistic transformation of *Caenorhabditis elegans*. Gene 229: 31–35.

Wood, A. J., T. W. Lo, B. Zeitler, C. S. Pickle, E. J. Ralston *et al.*, 2011 Targeted genome editing across species using ZFNs and TALENs. Science 333: 307.

Yu, Z., M. Ren, Z. Wang, B. Zhang, Y. S. Rong *et al.*, 2013 Highly efficient genome modifications mediated by CRISPR/Cas9 in *Drosophila*. Genetics (in press).

Communicating editor: O. Hobert

GENETICS

Supporting Information
http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.156075/-/DC1

Heritable Custom Genomic Modifications in *Caenorhabditis elegans* via a CRISPR–Cas9 System

Yonatan B. Tzur, Ari E. Friedland, Saravanapriah Nadarajan, George M. Church, John A. Calarco, and Monica P. Colaiácovo

DOI: 10.1534/genetics.113.156075

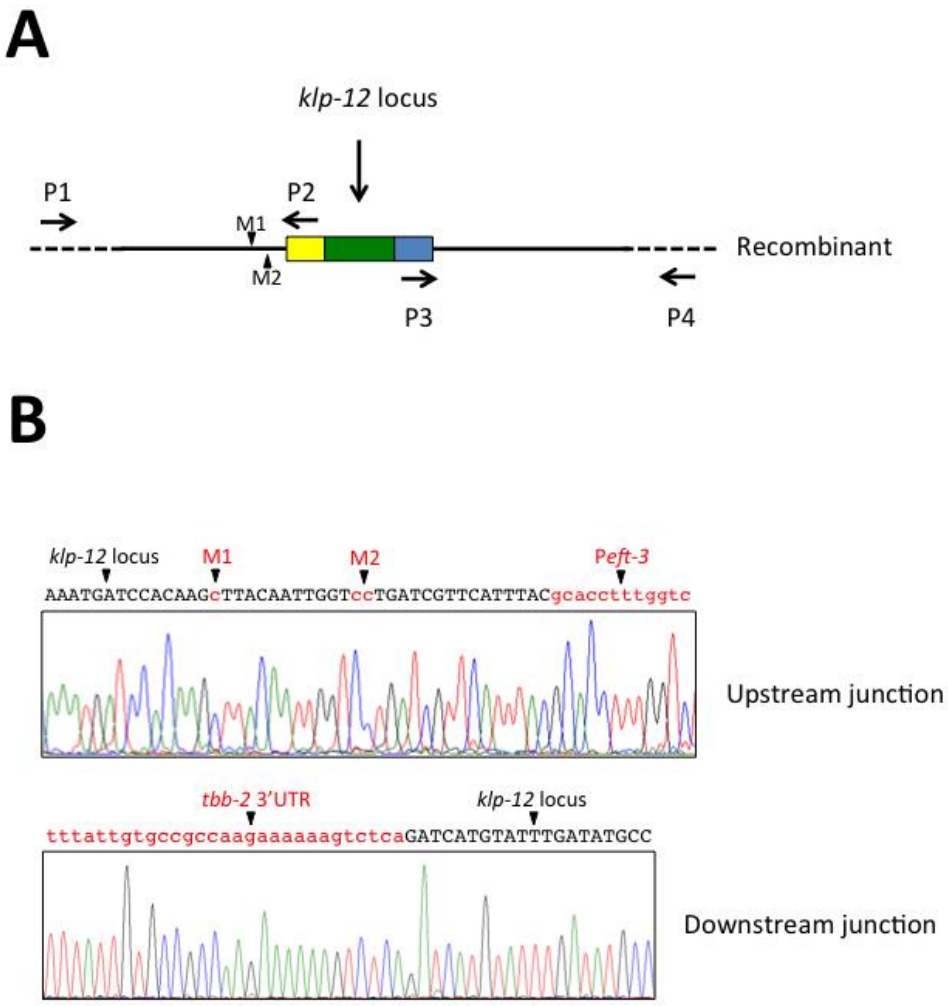

Figure S1 Confirmation of seamless recombination at the *klp-12* locus. Upper panel shows a sequence trace of the upstream recombinant junction. Endogenous upstream *klp-12* sequence is flanked by the M1 and M2 mutations described in Figure 2A, and the beginning of the *eft-3* promoter sequence. Bottom panel shows a sequence trace of the downstream recombinant junction, marking the end of the *tbb-2* 3'UTR followed again by endogenous *klp-12* sequence. All introduced sequences are labeled in red lower case letters.

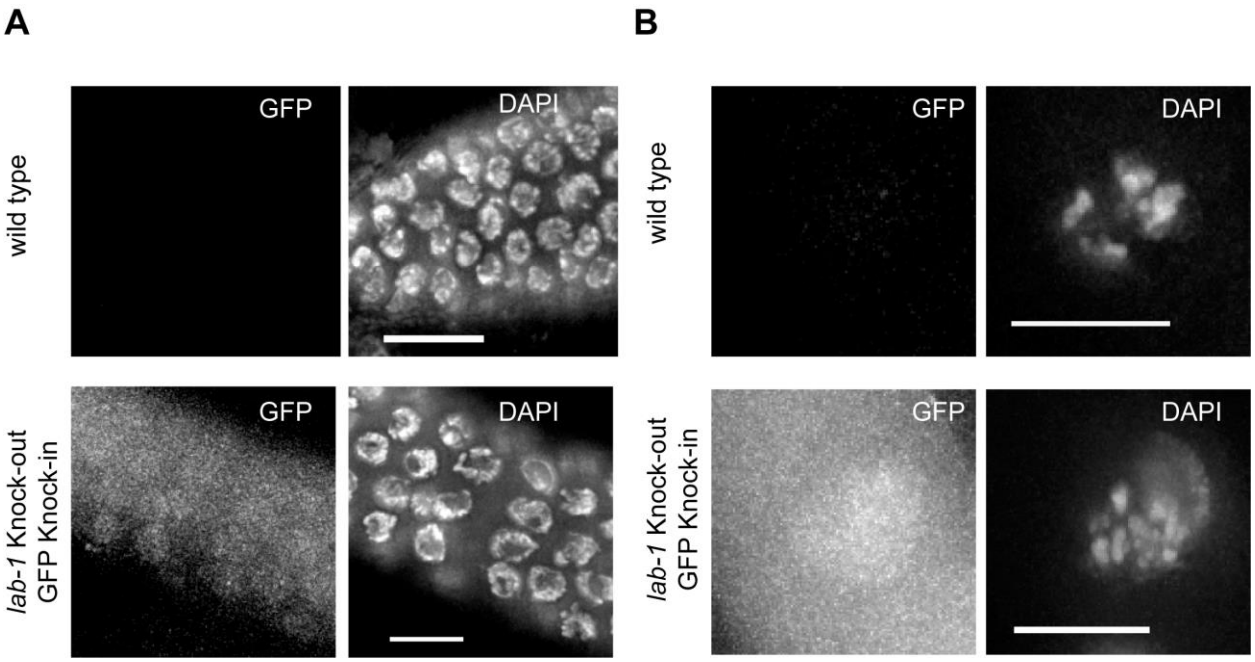

Figure S2 Immunostaining of germline nuclei with an anti-GFP antibody to examine the expression of the *Pbaf-1*::GFP transgene inserted into the *lab-1* locus. Shown are meiotic nuclei in early prophase I (transition zone and early pachytene) (A), and diakinesis (B), from whole mounted gonads of wild type and *lab-1* knockout GFP knock-in worms, co-stained with DAPI and anti-GFP. Bars, 10mm.

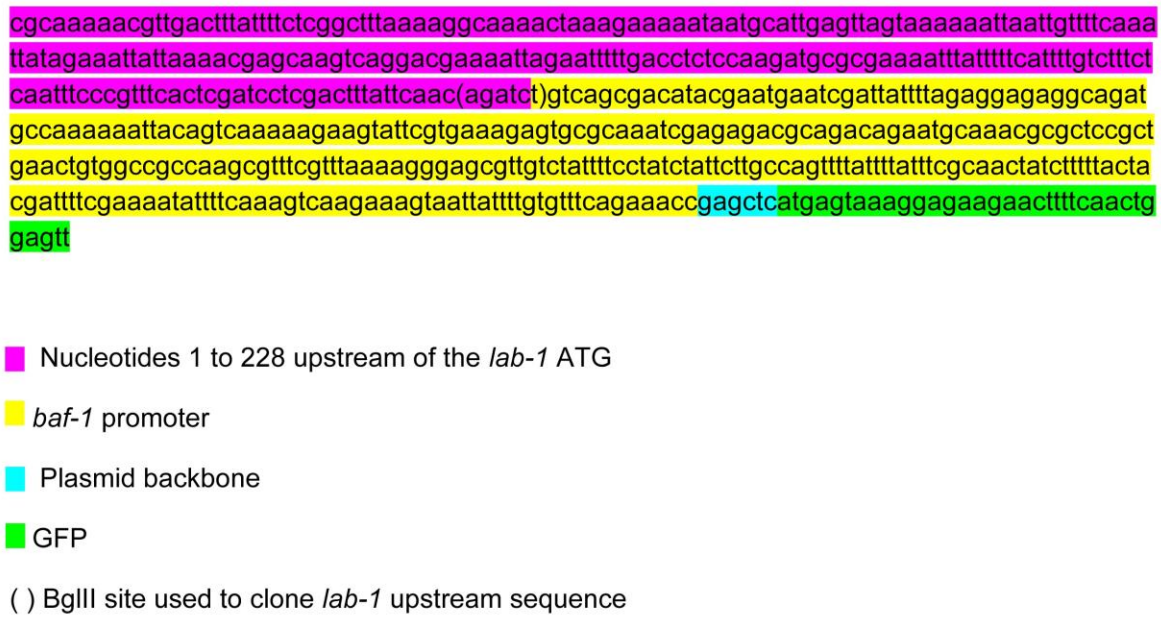

Figure S3 Confirmation of seamless recombination at the *lab-1* locus. Sequence resulting from the PCR product generated by using primers 1100 base pairs upstream and downstream of *lab-1.* Sequences from the *lab-1* upstream region (violet), *baf-1* promoter (yellow), plasmid backbone (light blue), and *gfp* (green) are marked. The site by which the upstream fragment was cloned is marked with brackets.

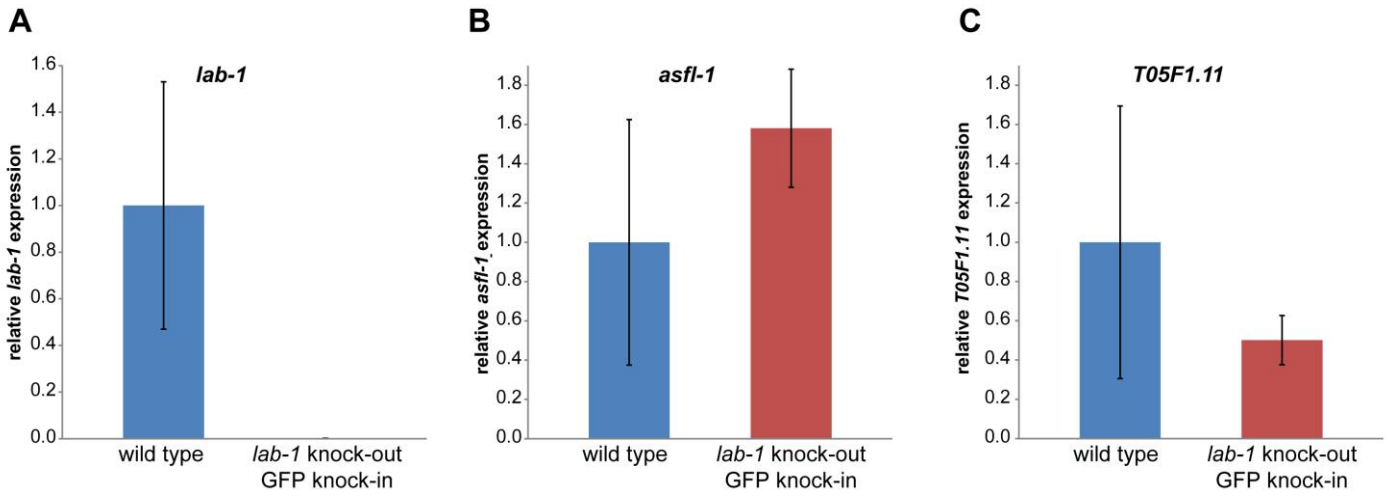

Figure S4 *lab-1* knock-out and *GFP* knock-in effect on the expression of flanking genes. RT-qPCR expression analysis of *lab-1* (A), *asfl-1* (B), and *T05F1.11* (C). Relative ± SEM values are presented. Sample values were normalized to *gpd-1* (GAPDH).

File S1

Supplementary Materials and Methods

Strains and maintenance. The N2 Bristol strain was used as the wild type background for all experiments. Worms were cultured at 20 and 25 °C under standard conditions as described in (Brenner 1974). The following strains were generated in this study: CV370 *lab-1(rj10[baf-1p::gfp])I,* JAC338 *klp-12(csb19[eft-3p::gfp::tbb-2 3'UTR]) IV.*

Plasmids. The pUC57 *klp-12* sgRNA and *Peft-3::*cas9 plasmids were described in (Friedland *et al.* 2013). The *lab-1* sgRNA plasmid was constructed by replacing the *unc-119* sgRNA sequence with a sequence corresponding to 310-330 bp downstream of the *lab-1* ATG genomic sequence as described (Friedland *et al.* 2013). To create the *klp-12 Peft-3*::GFP donor template repair vector, upstream and downstream 1.5 kb *klp-12* homology arms were amplified from N2 genomic DNA using the primers klp-12 F and klp-12 PAM mut HIII R and primers klp-12 ds F and klp-12 R, respectively (see supplementary Table 1 for a complete list of primers). We amplified the *eft-3* promoter using our *Peft-3*::Cas9-SV40 NLS vector (Friedland *et al.* 2013) as a template with primers klp-12 PAM *P*eft-3 F and *P*eft-3 GFP R. The *tbb-2* 3'UTR was also amplified from our *Peft-3*::Cas9-SV40 NLS vector using the primers *tbb-2* UTR F and *tbb-2* UTR klp-12 R. The GFP transgene was amplified from the pBALU1 vector (a kind gift from Oliver Hobert) in two fragments using the primers GFP F and intron R and intron GFP F and GFP *tbb-2* UTR R. The PCR products corresponding to the *eft-3* promoter, both GFP amplicons, and the *tbb-2* 3'UTR, were sequentially stitched together by overlapping PCR to create one seamless product. Finally, the upstream and downstream *klp-12* homology arms, and the GFP transgene fragment, were all cloned into the pUC57 vector using Gibson Assembly (Gibson *et al.* 2009).

The pAD010 vector was a kind gift of Yosef Gruenbaum and was described in (Bank *et al.* 2011). In brief, the pEGFP1 vector (Clontech Laboratories) was used to clone the *baf-1* promoter (286 bp upstream to the ATG) between the BglII and SacI sites, the *unc-119* rescue fragment between the NotI and HpaI sites, and GFP between the SacI and SacII sites. The *lab-1* donor vector was constructed by cloning 1020 bp upstream and 1029 bp downstream of the *lab-1* coding sequence into the pAD010 vector by using the BglII and SacII sites, respectively.

RT-qPCR. RNA was extracted from 20 worms in triplicate using TRIzol (Invitrogen). RT-PCR was performed using the SuperScript III First Strand Synthesis System (Invitrogen). Quantitative PCR was done using the SsoFast EvaGreen Supermix (Biorad). Values were normalized to *gpd-1* expression.

DNA microinjection. Plasmid DNA was microinjected into the germline as described in (Kadandale *et al.* 2009). Injection solutions in experiment A (Table 1) were prepared to contain a final concentration of 150 ng/µl with pCFJ104 (P*myo-3*::mCherry, obtained through Addgene through the kind gift of E. Jorgensen) as a co-injection marker at 5 ng/µl, the donor vector at 50 ng/µl, the sgRNA vector at 45 ng/µl, and the *Peft-3*Cas9-SV40 NLS*tbb-2* 3'UTR at 50 ng/µl. In experiment B and C (Table 1) a total of 500 ng/µl solution was used with pCFJ104 and pCFJ90 (P*myo-2*::mCherry, also obtained from Addgene through E. Jorgensen) as co-injection markers at 25 ng/µl and 2.5 ng/µl, respectively, the donor vector at 92.5 ng/µl, the sgRNA vector at 180 ng/µl, and the *Peft-3*Cas9-SV40 NLS*tbb-2* 3'UTR at 200 ng/µl.

Screening for recombinant worms. F_1 animals were screened for fluorescence using a Zeiss Axiozoom microscope, and candidates were isolated, allowed to lay eggs, and then lysed in 5 µl of single worm lysis buffer (10 mM Tris pH 8.0, 50 mM KCl, 2.5 mM MgCl2, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin and 100 µg/ml proteinase K), placed in -80 °C for 10 minutes, followed by a 1 h incubation at 60 °C, and then 15 minutes at 95 °C to inactivate the proteinase K. The recombinant fragment was amplified by using one primer within the insertion and one on the genomic sequence flanking the donor vector sequence.

Phusion high fidelity polymerase (Thermo Scientific) was used as recommended by the manufacturer, using all 5 µl of worm lysate as a template (see Supplementary Table 1 for a list of all primers used for PCR amplification and genotyping).

To monitor inheritance of the homologous recombination engineered mutation, we repeated the PCR reaction for the F2 progeny of the F1s that showed the recombinant-specific PCR fragment, and then sequenced a PCR amplicon that was generated by primers annealing to sites outside the donor vector.

Immunostaining, Imaging and Microscopy. Whole mount preparation of dissected gonads, DAPI staining and LAB-1 immunostaining, were carried out as in (Colaiacovo *et al.* 2003; Saito *et al.* 2009; Tzur *et al.* 2012). Primary antibodies were used at the following dilutions: rabbit α-LAB-1, 1:500; and chicken α-GFP, 1:500 (Abcam). Secondary antibodies used were Cy3 anti-rabbit and FITC anti-chicken (both from Jackson Immunoresearch) each at 1:1000. Immunofluorescence and GFP images were collected at 0.2 µm and 1 µm increments, respectively, with an IX-70 microscope (Olympus) and a cooled CCD camera (model CH350; Roper Scientific) controlled by the DeltaVision system (Applied Precision). Images were subjected to deconvolution analysis using the SoftWorx 3.0 program (Applied Precision) as in (Nabeshima *et al.* 2005).

Supplementary References

Bank, E. M., K. Ben-Harush, N. Wiesel-Motiuk, R. Barkan, N. Feinstein *et al.*, 2011 A laminopathic mutation disrupting lamin filament assembly causes disease-like phenotypes in *Caenorhabditis elegans*. Mol Biol Cell **22:** 2716-2728.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71-94.

Colaiacovo, M. P., A. J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo *et al.*, 2003 Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. Dev Cell **5:** 463-474.

Friedland, A. E., Y. B. Tzur, K. M. Esvelt, M. P. Colaiacovo, G. M. Church *et al.*, 2013 Heritable genome editing in *C. elegans* via a CRISPR-Cas9 system. Nat Methods **10**: 741-743.

Gibson, D. G., L. Young, R. Y. Chuang, J. C. Venter, C. A. Hutchison, 3rd *et al.*, 2009 Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods **6:** 343-345.

Kadandale, P., I. Chatterjee and A. Singson, 2009 Germline transformation of *Caenorhabditis elegans* by injection. Methods Mol Biol **518:** 123-133.

Nabeshima, K., A. M. Villeneuve and M. P. Colaiacovo, 2005 Crossing over is coupled to late meiotic prophase bivalent differentiation through asymmetric disassembly of the SC. J Cell Biol **168:** 683-689.

Saito, T. T., J. L. Youds, S. J. Boulton and M. P. Colaiacovo, 2009 *Caenorhabditis elegans* HIM-18/SLX-4 interacts with SLX-1 and XPF-1 and maintains genomic integrity in the germline by processing recombination intermediates. PLoS Genet **5:** e1000735.

Tzur, Y. B., C. Egydio de Carvalho, S. Nadarajan, I. Van Bostelen, Y. Gu *et al.*, 2012 LAB-1 targets PP1 and restricts Aurora B Kinase upon entrance into meiosis to promote sister chromatid cohesion. PLoS Biol **10:** e1001378.

Table S1 List of oligonucleotide primers used in this study

klp-12 **GFP transgene donor vector cloning and recombinant screening primers**	
klp-12 F	CGACGTTGTAAAACGACGGCCAGTGAATTCGATTCACACAACTTGCGAAAATTCTTG
klp-12 PAM mut HIII R	GTAAATGAACGATCAGGACCAATTGTAAGCTTGTGGATCA
klp-12 PAM Peft-3 F	CAATTGGTCCTGATCGTTCATTTACGCACCTTTGGTCTTTTATTGTCAACT
Peft-3 GFP R (P2)	GTGAAAAGTTCTTCTCCTTTACTCATTAAGCCTGCTTTTTTGTACAAACTTGTGAG
GFP F	ATGAGTAAAGGAGAAGAACTTTTCAC
intron R	GTACCGAACTGTTTAAACTTACGTG
intron GFP F	CACGTAAGTTTAAACAGTTCGGTACTAACTAACCATACATATTTAAATTTTCAGGTGCTG
GFP tbb-2 UTR R	GGGAATGCTTGAAAGGATTTTGCATTTATCCTATTTGTATAGTTCATCCATGCCATG
tbb-2 UTR F (P3)	GATAAATGCAAAATCCTTTCAAGCATTCC
tbb-2 UTR klp-12 R	GAGTAGGCATATCAAATACATGATCTGAGACTTTTTTCTTGGCGGCACA
klp-12 ds F	GATCATGTATTTGATATGCCTACTC
klp-12 R	AACAGCTATGACCATGATTACGCCAAGCTTGAAGACGTGTCAATTTCGAATCAC
klp-12 us outside HR F (P1)	GAGCGAAAAGTGTCGGTTATTTACG
klp-12 ds outside HR R (P4)	CATCAGTGTTCGGCTGAAATGTGATAG
Cloning ***lab-1*** **upstream region**	
lab-1-974UPSF-bglII	TTCTGAAGATCTGGAATGGACTGTCATTAGAC
lab1-2-UPSR-bglII	TGGCTCAGATCTGTTGAATAAAGTCGAGGATC
Cloning ***lab-1*** **downstream region**	
lab-1-24F-sacII	TAATCGCCGCGGTCAAACTCAAAAACGCTGTG
lab-1-840DWSR-SacII	CGCCGACCGCGGCAAGCTACTTGGTGACAATG
Creating ***lab-1*** **sgRNA**	
lab-1 mgRNA-F-Cor	GATCTGGGTGCCCGATGAGTGTTTTAGAGCTAGAGCTAGAAATAGC
lab-1 mgRNA-R	ACTCATCGGGCACCCAGATCAAACATTTAGATTTGCAATTCA
Detecting ***lab-1*** **knock-out and** ***gfp*** **knock-in**	
CC01F	CTGCAGCGCAAAATAATTCA

bafP-R	TTTGGCATCTGCCTCTCCTC
Amplifying and sequencing *lab-1* locus outside the donor vector homology region	
lab1+1100-R	GCATTGGTTAATCACTGGAA
CC01F	CTGCAGCGCAAAATAATTCA
gfp-N-R	GTGCCCATTAACATCACCAT

RT-qPCR

gpd-1F	ACTCGTCCATTTTCGATGCT
gpd-1R	TCGACAACACGGTTCGAGTA
lab-Ex34-RT-F	CCAACCTCAGGAATCTGTGTCTT
lab-405-RT-R	CCTCGGATGTATCGGAATCC
asfl-ex34-RT-R	TCATCATCGTCCTCTTCCTCC
asfl-532-RT-F	CCATCATCATGCAATGGCAT
T05F-Ex34-R	TCCCAAGTTGCAATTTCAATAATC
T05F-454-F	GCTCATGATGAAATTCGCTACAA