

Direct delivery CRISPR-HDR editing protocol for *C. elegans*

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Abstract

This protocol is for purification of Cas9::NLSSV40::His6 from:
Paix A, Folkmann A, Rasoloson D, and Seydoux G (2015) [High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR/Cas9 Ribonucleoprotein Complexes](#). Genetics.115.179382; Early online July 17, 2015. doi:10.1534/genetics.115.179382

Please see the [full manuscript](#) for detail.

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Guidelines

Protocol updates will be posted on the [Seydoux lab website](#).

Protocol Overview

- Design crRNA(s) and a repair template for your gene of interest.
- Inject Cas9/crRNA/tracrRNA complexes and repair templates targeting your gene of interest and dpy-10.
- Identify broods with Rollers (first generation after injection).
- Screen Rollers (and their non-Roller siblings, if desired) for edits at your gene of interest
- See Section F for a positive control experiment – tagging gtbp-1 with GFP using dpy-10 co- CRISPR.

A. Preparation of reagents

Cas9

Recombinant Cas9::NLS can be purified from *E. coli* (see attached protocol File S2) or purchased from commercial sources.

tracrRNA

The universal tracrRNA is a structural RNA that links the crRNA to Cas9. The same tracrRNA is used for all experiments.

We order it from Dharmacon #U-002000-05/20/50 (<http://dharmacon.gelifesciences.com/gene-editing/crispr-cas9/edit-r-tracrna/>). The tracrRNA is 74nt long (Jinek et al. 2012):

AACAGCAUAGCAAGUUAUUUUUAAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU
UUU

Upon receipt, briefly spin the tubes and reconstitute at 4µg/µl (0.17nmol/µl): add 29.8µl of Tris pH 7.5 to the 5nmol provided (U-002000-05). Other amounts of tracrRNA are available (U-002000-20/50).

Store
at -80°C.

crRNA

The crRNA consist of a 20nt gene-specific sequence followed by a universal sequence (GUUUUAGAGCUAUGCUGUUUUG) required to interact with the tracrRNA. The 20nt gene-specific sequence must lie upstream of a PAM sequence (NGG) in genomic DNA. DO NOT INCLUDE THE PAM IN THE crRNA!

Remember to look for PAM sites on both DNA strands – crRNAs can target either strand.

Not all crRNAs work well (Table S3). Desired features include (in order of importance) 1) cleavage site as

close as possible to the edit site, 2) good sequence (see below) and 3) few off-target sites (we use the website <http://crispr.mit.edu/> for off-target prediction; Hsu et al. 2013). If there are off-target sites, those sites should have 3 or more mismatches, preferentially close to the PAM.

In our hands, the most predictable determinant of guide RNA efficiency for HDR is distance between the

cleavage site and the edit that you are trying to introduce. Optimal distance is <10 bases. We have obtained edits up to 30 bases away from the cleavage site, but the efficiency of edit incorporation drops

by a factor of ~5-10.

Several recommendations for crRNA sequence have been reported (Farboud et al. 2015; Gagnon et al. 2014; Doench et al. 2014), and we try to follow them when possible. These recommendations are:

- 50 to 75% overall GC content
- GG or G, but no C, for the 3' most residue(s) immediately upstream of the PAM

The Broad Institute website implements these recommendations for guide RNA scoring (<http://www.broadinstitute.org/rnai/public/analysis-tools/sgrna-design>) (Doench et al. 2014).

Order your gene specific crRNA (20nt specific sequence + GUUUUAGAGCUAUGCUGUUUUG) from Dharmacon (<http://dharmacon.gelifesciences.com/gene-editing/crispr-rna-configurator/>).

Also order the crRNA for dpy-10: GCUACCAUAGGCACCACGAG + GUUUUAGAGCUAUGCUGUUUUG

Upon receipt, briefly spin the tubes and reconstitute at 8µg/µl (0.6nmol/µl): add 33.8µl of Tris pH 7.5 to

the 20nmol provided. Store at -80°C.

Repair template design

Repair templates should contain ~35nt homology arms (Paix et al. 2014) (Table S4): sequences at the 5' and 3' end of the repair template that are homologous to sequences flanking the cut and edit in the genomic DNA. Ideally, flanking sequences should terminate with a C or G and contain good sequence diversity at their extremities (no hairpins).

The repair template should also contain mutations that make it resistant to re-cutting by Cas9/crRNA-tracrRNA complex after integration in the genome. These mutations can be 1) insertions that disrupt the crRNA sequence or separate the crRNA sequence from the PAM or 2) mismatches that disrupt the PAM or crRNA sequence (we typically create between 2 and 4 mismatches when disrupting the crRNA sequence, mutations closest to the PAM are the most effective) (Jinek et al. 2012).

Be careful to introduce only silent changes using codons that are used at a frequency similar to the original codon (http://www.genscript.com/cgi-bin/tools/codon_freq_table). If possible, avoid crRNAs that target non-coding sequences since mutations in these sequences could possibly affect regulatory (splicing, promoter) motifs.

For small edits, engineer a restriction site in your repair template to facilitate screening (see Paix et al. 2014). For insertions >20bp, we typically identify the edits by size shift in the PCR product. When inserting a fluorescent protein, it is possible to screen directly by visual examination of F1s or F2s if the pattern is known.

Recommendation for antigenic peptide tag sequences can be found in Paix et al. 2014.

Repair template synthesis

A. Small edits (<100nt):

Use single-stranded oligonucleotides (ssODNs, 200nt maximum size, 4nM ultramer, salt free) ordered from IDT. Reconstitute ssODN at 1µg/µl according to the amount provided by the manufacturer.

Sense-

strand ssODNs have been reported to work better (Katic et al. 2015).

The ssODN repair template for dpy-10 is:

CACTTGAACCTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGGTAGCGGAGC
TT

CACATGGCTTCAGACCAACAGCCTAT - Use a working aliquot at 500ng/µl in H₂O.

B. Large edits (100bp-2kb): Use PCR amplicons.

Note that this type of template may not work efficiently for inserts > 3kb (A. Paix, unpublished).

-Primer design: Design the primers so that they contain the desired homology arms (~35nt), mutations in the crRNA site(s) and sequences complementary to insert. Be sure to have a C or G at the 3'

end of the primers. Where possible, limit the size of the primers to less than 65nt in order to avoid primer contamination after PCR purification (see below). See Table S2 for available plasmids that can be

used as templates to amplify fluorescent proteins and tags.

-PCR: Amplify the PCR template using this reaction mix: Mix 2µl of template plasmid (from a standard miniprep of 1.5ml bacterial culture), 2µl of forward and reverse primers (100µM stock), 194µl of H₂O, 200µl of Phusion Master Mix 2X (NEB, #M0531L). Split the mix in 8 PCR tubes (50µl per tubes) and do a gradient PCR as follow:

98°C, for 2min

98°C, for 30s

60 to 72°C gradient, for 30s

72°C, for 45s

Repeat 29 times

72°C, for 10min

Hold at 10°C

Add 10µl of 6X Orange loading dye (Bioworld, #10570024-1) to each tube and run 8µl of it on an agarose gel.

Pool the positive PCRs in one tube (up to 8) and purify them in one Qiagen minelute column (#28006), elution with 10µl of H₂O. For most templates, the 8 annealing temperatures will give good yield and therefore we routinely pool the 8 reactions together. The expected yield ranges from 1 to 1.5µg/µl.

If the PCR primers are >65nt (typically when the edit is away from the cut), a second (nested) PCR step is necessary because the long primers will be present in the purified PCR pool at a concentration high enough to be toxic for injection. For the nested PCR, use forward and reverse primers of 18-22nt corresponding at the 5' and 3' ends of the template generated in the first PCR. Run a second PCR as before but with the following master mix (for 8 annealing temperatures of PCR): 0.8µl of 1st round of purified PCR, 2µl of forward and reverse primers, 195.2µl of H₂O, 200µl of Phusion Master mix 2X.

B. Preparing injection mixes

I. One locus editing using home-made Cas9

Cas9 prep (10µg/µl): 5µl
tracrRNA (4µg/µl): 5µl
dpy-10 crRNA (8µg/µl): 0.4µl
dpy-10 ssODN (500ng/µl): 0.55µl
Targeted gene crRNA (8µg/µl): 1µl
PCR template (s) (several templates can be mixed): Up to 500ng/µl final in the mix
OR ssODN (s) (several templates can be mixed) (1µg/µl): 2.2µl total
KCl (1M): 0.5µl Hepes pH7.4 (200mM): 0.75µl
H₂O: add if necessary to reach a final volume of 20µl

II. Multi-loci editing using home-made Cas9

Cas9 prep (10µg/µl): 5µl
tracrRNA (4µg/µl): 6.7µl
dpy-10 crRNA (8µg/µl): 0.4µl
dpy-10 ssODN (500ng/µl): 0.55µl
Targeted gene crRNA1 (8µg/µl): 0.75µl
Targeted gene crRNA2 (8µg/µl): 0.75µl
PCR templates (to repair the cuts corresponding to crRNAs 1/2): Up to 500ng/µl final in the mix
OR ssODNs (to repair the cuts corresponding to crRNAs 1/2) (1µg/µl): 2.2µl total
KCl (1M): 0.5µl
Hepes pH7.4 (200mM): 0.75µl
H₂O: add if necessary to reach a volume of 20µl

III. Note for edits on chromosome 2

For loci on LGII, the edits will be linked to the *dpy-10*(Rol) edit. If you prefer to recover edits that are unlinked to the *dpy-10* edit, use 0.28µl of *dpy-10* crRNA to maximize edits in non-Roller animals.

Alternatively, you can use rescue of *pha-1(ts)* mutation (LGIII) as an alternative co-CRISPR strategy as described in Ward, 2015.

IV. Injection mixes processing

Add each components of the injection mix in a 0.5ml tube (add Cas9 last). Place the 0.5ml tube in a 1.5ml eppendorf tube and spin for 2min at 13000rpm. Incubate at 37°C for 10-15min. Immediately load the injection needles and process to injection.

Note that the volume of the injection mixes can be decreased if necessary as long as the molarity of each component is maintained.

C. Injections, worm recovery and handling

Inject both arms of young adult hermaphrodites (with a few embryos). Be sure hermaphrodites are young enough to lay eggs for next two days. See WormBook for injection protocol:

http://www.wormbook.org/chapters/www_transformationmicroinjection/transformationmicroinjection.html

30min to 1h after injection, recover the injected hermaphrodites (P0s) as follows: Every 5-10min, add: 5µl / 5µl / 10µl /10µl /20µl /20µl/ 40µl of 1X M9

Clone out the P0s onto NNGM plates (1 P0 per plate, at 20°C). Use fresh NNGM plates with a thin layer of OP50 bacteria at the center to facilitate screening for Rollers. It is important to avoid that the P0s touch the mineral oil on the injection pad because the oil will kill them. After 20-23 hours, transfer the P0s to second plate (again 1 P0 per plate). For experiments using large PCR repair templates, you may find that edits arise more frequently on the second-day plates (Paix et al. 2014).

Examine the F1s for Rollers 4-5 days after cloning the P0s. You should recover Rollers from ~30-70% of injected hermaphrodites. Determine the number of Roller F1s per P0 and select the 3 P0s giving the most Rollers. These are your “jackpot broods”.

Clone all the Roller F1s (from the jackpot broods or from all broods if you do not have that many). You can also clone non-Rollers (only from jackpot broods) if you prefer to isolate your edit without the *dpy-10* edit (this is useful if your gene is on the same linkage group as *dpy-10*: LGII).

D. Screening for edit of interest

For the insertion of a fluorescent protein, if you know the expected pattern, you can screen the F1s (after you have allowed them to lay eggs) by placing them in a drop of M9 containing levamisole (1mM)

under the 10X objective of a compound microscope. You can also use no coverslip if you want to recover the worms (in that case no need to clone them out first). Use 3 or 12-well microscope slides.

For smaller edits (antigenic tag or mutation), for each F1, pool 10 F2s in 15µl of lysis buffer and PCR the edited locus. Avoid picking bacteria with the F2s. The edits can be detected by a size-change or by

restriction-enzyme digest if a restriction site was included in the rescue template. You can also PCR directly each F1, but we have found in practice that it is easier to PCR cohorts (10 or so) of F2s.

E. Strain establishment

We recommend recovering at least two independent edits (derived from different P0s) for each experiment.

If the edit was identified in a Roller worm, pick ~8 or more non-Roller F2/3s to separate the edit from *dpy-10* and recover homozygous edits.

For fluorescent protein integration, check the segregation of the fluorescent signal in F3 worms derived from singled-out F2s to identify homozygous lines.

Sequence-verify the edits once the homozygous strains are established. Sequence at least the entire sequence that was present in your rescue template. You may also want to sequence possible off-target loci.

Remember that mutants may not be viable when homozygous. We have also isolated edits that cause dominant phenotypes in the F1 generation (dominant sterile or dominant maternal effect lethal/sterile).

Note that some tagged proteins are not fully functional - check the homozygous edited lines for brood size and viability at 20 °C and 25 °C.

F. Special applications of protocol

ORF replacement to obtain null allele and transcriptional reporter

Design crRNAs near the Start and Stop codons. Design repair template with homology arms that reach up to the cleavage sites, and are in frame with the ORF of the gene of interest, if any coding sequence remains after replacement. We recommend recoding any coding sequence remaining between the two cuts to force gene conversion of the entire template.

Make the GFP::H2B repair template as described in **Repair template synthesis**. Use pCM1.35 (Available at Addgene; Merritt et al. 2008) (Table S2) as a PCR plasmid template.

Process as described in **Reagents for replacement of GFP (and eGFP variant) with tagRFP (or other FPs)** for ORF replacement.

Reagents for replacement of GFP (and eGFP variant) with tagRFP (or other FPs)

crRNA GFP Nt (#720 in Table S3): CCAUCUAAUUCAACAAGAAU + GUUUUAGAGCUAUGCUGUUUUG

crRNA GFP Ct (#719 in Table S3): CAAACUCAAGAAGGACCAUG + GUUUUAGAGCUAUGCUGUUUUG

Primers pairs to generate template (from RFP containing-plasmid pAP575-1):

Forward primer (5' to 3', lower case indicating the homology arm sequence):

ggagaagaacttttcactggagttgtcccaattGTGTCTAAGGGCGAAGAGCTG

Reverse primer (5' to 3', lower case indicating the homology arm sequence):

gtaatcccagcagctgttacaaactcaagaaggacATTAAGTTTGTGCCCCAGTTTG

PCR condition: use annealing temperature of 63°C,

elongation step of 45s, pAP575-1 as a plasmid template.

Injection mix:

Cas9 home-made prep (10µg/µl): 5µl

tracrRNA (4µg/µl): 6.7µl

dpy-10 crRNA (8µg/µl): 0.4µl

dpy-10 ssODN (500ng/µl): 0.55µl

crRNA GFP Nt (8µg/µl): 0.75µl

crRNA GFP Ct (8µg/µl): 0.75µl

PCR template for GFP (and eGFP variant) replacement: Up to 500ng/µl final in the mix

KCl (1M): 0.5µl

Hepes pH7.4 (200mM): 0.75µl

H₂O: add if necessary to reach a volume of 20µl

Multi-colors replacement (using the same homology arms than the one specified above - lower case in primer sequences) (Chudakov et al. 2010) can also be performed.

Note that the same injection mix / injection needle can be used on different GFP tagged strains.

Positive control experiment (to test protocol in your hands and/or activity of your home-made Cas9):

Tag *gtbp-1* with eGFP or mCherry using *dpy-10* co-CRISPR.

Prepare the repair PCR template as indicated in **Reagents for *gtbp-1* eGFP and mCherry tagging and Repair template synthesis.**

Make the injection mix as indicated in **injection mixes, part I**. Use the crRNA *gtbp-1* Ct (#728 in Table S3).

Inject 15-20 young adult N2 worms and recover as described in **Worm recovery and handling**. Pool the recovered worms (P0s) on one plate and incubate for 22-23h at 20 °C (day 1). Clone the P0s to individual OP50 plates and incubate at 20 °C for 4-5 days (day 2).

When the F1s reach the adult stage, check for Rollers. At least 3 P0s should give a high number of

Rollers

(>15) (Note that we do not count/examine Dumpy F1s since these are homozygous edits at the *dpy-10*

locus). From those “jackpot broods”, screen the Rollers for fluorescent protein expression as described

in **Screening for edit of interest**. 50% or more of the Rollers (at day 2) should be positive for fluorescence.

Reagents for *gtbp-1* eGFP and mCherry tagging

crRNA *gtbp-1* Ct (#728 in Table S3): CCACGAGGUGGUAUGCGCAG + GUUUUAGAGCUAUGCUGUUUUG

Primers pairs to generate template (from eGFP containing-plasmid pAP682-1):

Forward primer (5' to 3', lower case indicating the homology arm sequence) for eGFP insertion:
ggttcgggtggtgctccacgagtggtatgctgGTGAGTAAAGGAGAAGAAC

Reverse primer (5' to 3', lower case indicating the homology arm sequence) for eGFP insertion:
cttctaatttgtcccgcatTTtgaaaccgctCTTGACAGCTCGTCCATGCC

Primers pairs to generate template (from mCherry containing-plasmid pAP582-1):

Forward primer (5' to 3', lower case indicating the homology arm sequence) for mCherry insertion:
ggttcgggtggtgctccacgagtggtatgctgGTCTCAAAGGGTGAAGAAGATAAC

Reverse primer (5' to 3' lower case indicating the homology arm sequence) for mCherry insertion:
cttctaatttgtcccgcatTTtgaaaccgctCTTATACAATTCATCCATGCC

PCR condition: use annealing temperature of 63 °C, elongation step of 45s, pAP682-1 (eGFP) or pAP582-1 (mCherry) as a plasmid template. Do 8 PCR reactions and pool them (400µl total), purify on minelute column (see **Repair template synthesis**).

Additional references

Chudakov et al., Fluorescent Proteins and Their Applications in Imaging Living Cells and Tissues

Doench et al., Rational Design of Highly Active sgRNAs for CRISPR-Cas9-Mediated Gene Inactivation

Farboud and Meyer, Dramatic Enhancement of Genome Editing by CRISPR/Cas9 through Improved Guide RNA Design

Gagnon et al., Efficient Mutagenesis by Cas9 Protein-Mediated Oligonucleotide Insertion and Large-Scale Assessment of Single-Guide RNAs

Hsu et al., DNA Targeting Specificity of RNA-Guided Cas9 Nucleases

J
inek et al., A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity

Katic, Xu, and Ciosk, CRISPR/Cas9 Genome Editing in *Caenorhabditis Elegans*

Merritt et al., 3' UTRs Are the Primary Regulators of Gene Expression in the C. Elegans Germline

Paix et al., Scalable and Versatile Genome Editing Using Linear DNAs with Microhomology to Cas9 Sites
in Caenorhabditis Elegans

Ward, Rapid and precise engineering of the Caenorhabditis elegans genome with lethal mutation co-conversion and inactivation of NHEJ repair

Protocol

Preparation of reagents: Cas9

Step 1.

Cas9::NLS can be purified from E. coli (see [our protocol](#)) or purchased from commercial sources.

Preparation of reagents: tracrRNA

Step 2.

Order tracrRNA and upon receipt, briefly spin the tubes

Preparation of reagents: tracrRNA

Step 3.

Reconstitute tracrRNA at 4µg/µl (0.17nmol/µl): add 29.8µl of Tris pH 7.5 to the 5nmol provided (U-002000-05).

Preparation of reagents: crRNA

Step 4.

Order your gene specific crRNA (see the guidelines for crRNA notes). Upon receipt, briefly spin the tubes.

Preparation of reagents: crRNA

Step 5.

Reconstitute crRNA at 8µg/µl (0.6nmol/µl): add 33.8µl of Tris pH 7.5 to the 20nmol provided

🔑 NOTES

Alexandre Paix 09 Sep 2015

Store at -80 °C.

Preparation of reagents: repair template

Step 6.

Use single-stranded oligonucleotides (ssODNs, 200nt maximum size, 4nM ultramer, salt free) ordered from IDT. (this is for small edits <100nt; see the guidelines for large edits 100bp-2kb)

Preparation of reagents: repair template

Step 7.

Reconstitute ssODN at 1µg/µl according to the amount provided by the manufacturer. (this is for small edits <100nt; see the guidelines for large edits 100bp-2kb)

Preparing injection mixes

Step 8.

Add each component of the injection mix in a 0.5ml tube (add Cas9 last). [this is for "one locus editing"]

📄 PROTOCOL

. [Seydoux Lab Cas9 injection mix](#)

CONTACT: [Alexandre Paix](#)

📌 NOTES

Alexandre Paix 09 Sep 2015

See the guidelines for multi-locus editing injection mixture

Alexandre Paix 09 Sep 2015

Note for edits on chromosome 2

For loci on LGII, the edits will be linked to the *dpy-10*(Rol) edit. If you prefer to recover edits that are unlinked to the *dpy-10* edit, use 0.28µl of *dpy-10* crRNA to maximize edits in non-Roller animals.

Alternatively, you can use rescue of *pha-1*(ts) mutation (LGIII) as an alternative co-CRISPR strategy as described in Ward, 2015.

Alexandre Paix 09 Sep 2015

Note that the volume of the injection mixes can be decreased if necessary as long as the molarity of each component is maintained.

Step 8.1.

Cas9 prep (10µg/µl): 5µl

Step 8.2.

tracrRNA (4µg/µl): 5µl

Step 8.3.

dpy-10 crRNA (8µg/µl): 0.4µl

Step 8.4.

dpy-10 ssODN (500ng/µl): 0.55µl

Step 8.5.

Targeted gene crRNA (8µg/µl): 1µl

Step 8.6.

PCR template (s) (several templates can be mixed): Up to 500ng/µl final in the mix

Step 8.7.

OR ssODN (s) (several templates can be mixed) (1µg/µl): 2.2µl total

Step 8.8.

KCl (1M): 0.5µl

Step 8.9.

Hepes pH7.4 (200mM): 0.75µl

Step 8.10.

H2O: add if necessary to reach a final volume of 20µl

Preparing injection mixes

Step 9.

Place the 0.5ml tube in a 1.5ml eppendorf tube and spin for 2min at 13000rpm.

🕒 DURATION

00:02:00

Preparing injection mixes

Step 10.

Incubate at 37°C for 10-15min

🕒 DURATION

00:10:00

Preparing injection mixes

Step 11.

Immediately load the injection needles and process to injection.

Injections, worm recovery and handling

Step 12.

Inject both arms of young adult hermaphrodites (with a few embryos).

 **DURATION**

00:30:00

 **NOTES**

Alexandre Paix 09 Sep 2015

Be sure hermaphrodites are young enough to lay eggs for next two days. See [WormBook](#) for injection protocol.

Injections, worm recovery and handling

Step 13.

Recovery A: Add 1X M9: 5µl

(30min to 1h after injection, recover the injected hermaphrodites (P0s) as follows: Every 5-10min, add: 5µl / 5µl / 10µl /10µl /20µl /20µl/ 40µl of 1X M9)

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 14.

Recovery B: Add 1X M9: 5µl

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 15.

Recovery C: Add 1X M9: 10µl

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 16.

Recovery D: Add 1X M9: 10µl

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 17.

Recovery E: Add 1X M9: 20µl

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 18.

Recovery F: Add 1X M9: 20µl

 **DURATION**

00:05:00

Injections, worm recovery and handling

Step 19.

Recovery G: Add 1X M9: 40µl

Injections, worm recovery and handling

Step 20.

Clone out the P0s onto NNGM plates (1 P0 per plate, at 20°C). Use fresh NNGM plates with a thin layer of OP50 bacteria at the center to facilitate screening for Rollers. **It is important to avoid that the P0s touch the mineral oil on the injection pad because the oil will kill them.**

DURATION

20:00:00

Injections, worm recovery and handling

Step 21.

Transfer the P0s to second plate (again 1 P0 per plate).

NOTES

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For experiments using large PCR repair templates, you may find that edits arise more frequently on the second-day plates (Paix et al. 2014).

Injections, worm recovery and handling

Step 22.

Examine the F1s for Rollers 4-5 days after cloning the P0s.

NOTES

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You should recover Rollers from ~30-70% of injected hermaphrodites.

Injections, worm recovery and handling

Step 23.

Determine the number of Roller F1s per P0 and select the 3 P0s giving the most Rollers. These are your “jackpot broods”.

Injections, worm recovery and handling

Step 24.

Clone all the Roller F1s (from the jackpot broods or from all broods if you do not have that many).

NOTES

Alexandre Paix 09 Sep 2015

You can also clone non-Rollers (only from jackpot broods) if you prefer to isolate your edit without the dpy-10 edit (this is useful if your gene is on the same linkage group as dpy-10: LGII).

Injections, worm recovery and handling

Step 25.

See the guidelines for:

- Screening
- Strain establishment
- Special applications of this protocol