

2017/11/13, written by Daehan (modified from Shannon's protocol Cas9 Protein Microinjection)
2018/11/23, updated by Daehan to fix dpy-10 repair template sequence

Abby Derberg's protocol (from IDT) with 1:12 dpy-10 ratio

- Total volume : 10ul
- 17.5 uM Cas9, crRNA:tracrRNA duplexes in final volume (including dpy-10)
- 6uM ssDNA repair for target
- 0.5uM ssDNA repair for dpy-10

1. In PCR strip tube (AB-0266, Thermo Scientific), combine the following reagents:

Step 1. tracrRNA + crRNA

Reagents	Stock concentration (uM)	Volume (ul)
tracrRNA	200	0.88
target crRNA	100	1.64
dpy-10 crRNA	100	0.12

Note) if you use multiple target crRNA, combine them with (1.64ul / n of target) volume for each crRNA.

2. Incubate in thermocycler at 95°C for five minutes.

3. Combine the following reagents:

Step 2. Add Cas9

Reagents	Stock concentration (uM)	Volume (ul)
Cas9	(11 mg/ml, 69uM)	2.52

Note. Add Cas9 (Catalog #1074181 from IDT at 61 μ M, or 100 μ g) to the PCR strip tube with RNAs. We use the Cas9 directly from IDT and do not dilute with HEPES/KCl as suggested in their protocol because high concentrations of Cas9 are needed for microinjection in *C. elegans*.

4. Incubate at room temperature for five minutes

5. Combine the following reagents:

Step 3. Add others

Reagents	Stock concentration (uM)	Volume (ul)
target repair oligo	100	0.6
dpy-10 repair oligo	10	0.5
nuclease free D.W		3.74

6. Incubate at room temperature for 60 minutes (or at 37°C for 30 minutes).

7. Load the pulled capillary needle (1B100F-4, World Precision Instruments) with injection mix using a pulled 10 μ L mouth pipet capillary (2-000-010, Drummond). Load about 1 μ L of liquid into each injection needle. The injection mix will stay near the top of the needle but will move to the tip once the needle has been broken (the glycerol makes the injection solution more viscous than typical injection mixtures). Avoid loading bubbles into the needle as these tend to result in generation of Cas9-dependent aggregates/crystals and needle clogging.

8. Inject using "Microinjection" protocol.

Reagents	Stock concentration (uM)	Volume (ul)
tracrRNA	200	0.88
target crRNA	100	1.64
dpy-10 crRNA	100	0.12
Incubate 5 min at 95C, 5min at 10C		
Cas9	(10 mg/ml, 60uM)	2.87
Incubate 5 min at RT		
target repair oligo	100	0.6
dpy-10 repair oligo	10	0.5
nuclease free D.W		3.39
Total		10

Notes:

tracrRNA

Order from IDT (Catalog #1072532) and dilute to 200 μ M in Duplex Buffer (Catalog #11-01-03-01, IDT).

Add 25 μ L duplex buffer to 5 nmol tracrRNA to make 200 μ M solution.

Store at -20°C.

crRNAs

Order from IDT (Alt-R CRISPR crRNA, 2nmol) and dilute to 100 μ M in Duplex Buffer (Catalog #11-01-03-01, IDT).

Add 20 μ L duplex buffer to 2 nmol crRNA to make 100 μ M solution.

Store at -20°C.

dpy-10 crRNA sequence: GCUACCAUAGGCACCACGAG

Look for crRNA as close as possible to the edit site (ideally, <20 bp). We use Benchling to find guides and look for the highest possible on/off target scores.

Repair oligos

Order 4 nmol Ultramar DNA oligos from IDT and dilute to 100 μ M in water.

Store at -20°C.

dpy-10 repair oligo:

CACTTGAACCTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGGTAGCGGA
GCTTCACATGGCTTCAGACCAACAGCCTAT

Repair oligos should have: (i) opposite stranded-ness as the crRNA, and (ii) ~36 bp of homology on the PAM-distal side of the cut (cut occurs 3 bp from the PAM site into the guide) and ~91 bp homology on the PAM-proximal side. The repair oligo should have a synonymous change in the PAM site or two synonymous changes within the crRNA region if PAM editing is not an option.

