Single guide with repair injection mix (for swapping alleles of a target gene):

1. In PCR strip tube (AB-0266, Thermo Scientific), combine the following reagents:
   0.34 μL tracrRNA (200 μM stock in Duplex Buffer)
   0.48 μL target crRNA (100 μM stock in Duplex Buffer)
   0.20 μL *dpy-10* crRNA (100 μM stock in Duplex Buffer)

Sequence for *dpy-10* crRNA and notes on these reagents are at the end of the protocol.

2. Incubate in thermocycler at 95ºC for five minutes.

3. Add 1.95 μL Cas9 (Catalog #1074181 from IDT at 61 μM, or 100 μg) 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 five minutes

5. Add the following reagents to the Cas9 and RNA PCR strip tube:
   0.20 μL target repair oligo (100 μM stock in water)
   0.168 μL *dpy-10* repair oligo (40 μM stock in water)
   1.622 μL water

Sequence for *dpy-10* repair oligo is at the end of the protocol.

6. Separate the injection mix tube from the other strip tubes and place injection mix tube inside a 1.7mL microfuge tube with a trimmed 1000 μL pipet tip to make the mix tube fit properly inside the microfuge tube. (If you do not add the pipet tip, the cap of the PCR tube will get stuck on the lip of the microfuge tube and the cap will pop off during the spin.) Spin at maximum speed for five minutes.

7. Load the pulled capillary needle (1B100F-4, World Precision Instruments) with injection mix using a pulled 10 μL mouth pipet capillary (2-000-010, Drummond) . Be careful to take from the surface of the liquid as to avoid any pellet that formed during the spin. Load about 1 μL 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.

Two guides without repair injection mix (for insertion/deletion events without repair construct):

1. In PCR strip tube (AB-0266, Thermo Scientific), combine the following reagents:
   0.34 μL tracrRNA (200 μM stock in Duplex Buffer)
   0.48 μL target crRNA 1 (100 μM stock in Duplex Buffer)
   0.48 μL target crRNA 2 (100 μM stock in Duplex Buffer)
   0.20 μL *dpy-10* crRNA (100 μM stock in Duplex Buffer)

Sequence for *dpy-10* crRNA and notes on these reagents are at the end of the protocol.

2. Incubate in thermocycler at 95ºC for five minutes.

3. Add 1.95 μL Cas9 (Catalog #1074181 from IDT at 61 μM, or 100 μg) 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 five minutes.

5. Add the following reagents to the Cas9 and RNA PCR strip tube:
    0.168 μL *dpy-10* repair oligo (40 μM stock in water)
    1.382 μL water
Sequence for *dpy-10* repair oligo is at the end of the protocol.

6. Separate the injection mix tube from the other strip tubes and place injection mix tube inside a 1.7mL microfuge tube with a trimmed 1000 μL pipet tip to make the mix tube fit properly inside the microfuge tube. (If you do not add the pipet tip, the cap of the PCR tube will get stuck on the lip of the microfuge tube and the cap will pop off during the spin.) Spin at maximum speed for five minutes.

7. Load the pulled capillary needle (1B100F-4, World Precision Instruments) with injection mix using a pulled 10 μL mouth pipet capillary (2-000-010, Drummond) . Be careful to take from the surface of the liquid as to avoid any pellet that formed during the spin. Load about 1 μL of liquid into each injection needle. The injection mix will stay toward 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.

Notes:

tracrRNA
    Order from IDT (Catalog #1072532) and dilute to 200 μM in Duplex Buffer (Catalog #11-01-03-01, IDT).
    Add 25 μL duplex buffer to 5 nmol tracrRNA to make 200 μM solution.
    Store at -20ºC.

crRNAs
    Order from IDT (Alt-R CRISPR crRNA, 2nmol) and dilute to 100 μM in Duplex Buffer (Catalog #11-01-03-01, IDT).
    Add 20 μL duplex buffer to 2 nmol crRNA to make 100 μM solution.
    Store at -20ºC.

    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 μM in water.
    Store at -20ºC.

    dpy-10 repair oligo:
CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTA
GGTAGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT

    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.