Co-CRISPR Screening Kit with unc-18

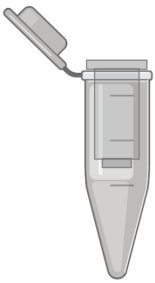
Quick Reference Card

Components and Protocol

Read full protocol before initial kit usage

Component	Description	Quantity
A	Co-CRISPR injection mix (red capped 0.5 ML vial)	3 Vials
B	Filter	3 Filters
C	Empty Vials	3 Vials
D	NGM plate with worms to be used as injection strain	1 Plate
E	Screening primers (blue capped 0.5 ML vial)	1 Vial
F	Quick reference card	1
G	Kit protocol	1

A



B+C

D

E

QuikCRISPR Co-CRISPR Kit - unc-18

Read full protocol before initial kit usage

Note: Store injection mix tubes (red-capped tubes) at -20ºC until ready to use

Preparing the mix - final volume of 20µL:

1. Prepare a 10µL volume of your injection mix components (Cas9, sgRNA(s), donor homology template, nuclease-free H2O).
2. Spin down component A (red cap) using a benchtop mini-centrifuge.
3. Resuspend component A with 10µl of nuclease-free H2O. Mix gently by pipetting.
4. Add your injection mix (step 1) to the Component A tube for a final mix volume of 20 uL. Mix gently by pipetting.
5. Incubate at room temperature for 10 minutes.
6. Your injection mix is now complete.

Injecting your *C.elegans*:

1. To filter your injection mix, place 1x Component B into 1x Component C. Pipette injection mix from component A vial onto the filter pad of Component B.
2. Centrifuge for 15 seconds at 17,000 x g with filter attached
3. Remove filter (Component B) and discard filter. The injection mix should be in the bottom of the tube (Component C).
4. Load injection needle with filtered injection mix.
5. Inject about 24 *C.elegans* (Component D) with filtered injection mix.
6. Place injected *C. elegans* onto individual, seeded NGM plates.

Screening your injected *C.elegans*:

1. After 4 days, look for *C. elegans* that are crawling like N2.
2. Move up to 40 crawling *C. elegans* to individual plates
3. After 24 hours, the *C. elegans* should have laid numerous eggs. Recover the animal isolated in step 1 and screen by PCR for your CRISPR edit.
4. From plates that screen positive, continue to isolate and screen *C. elegans* from subsequent generations until you obtain homozygous animals.
5. When you have obtained a homozygous line, use Component E (blue cap) to screen the unc-18 locus to ensure recovery to wildtype. Wildtype band is 403bp, Mutant band (Component D) is 458bp. If your line is homozygous for the wildtype band, you are done.