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CRISPR-Cas9 genome editing by RNP injection

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This protocol describes the steps to make a CRISPR-Cas9 mixture for microinjection and the steps after microinjection to screen for edits. Microinjection is a separate protocol. Please see the **ordering information guide** document for Andersen lab-specific ordering instructions for the reagents below. For a quick video explaining the basic process please follow this link. Note that there are key differences in the video to this protocol and you should follow this protocol explicitly where they may differ.

Reagents:

sgRNA:
Design your guides near your targeted edit site using the instructions below. Order sgRNAs from Synthego and resuspend with provided TE to 100 µM. Store at -20ºC

target repair oligo:
Design your repair oligo using the instructions below. Order as 4 nmol Ultramer DNA oligo from IDT and resuspend to 100 µM in nuclease free water. Store at -20ºC.

dpy-10 sgRNA:
dpy-10 sgRNA sequence: GCUACCAUAGGCACCACGAG
Order from Synthego and resuspend with provided TE to 20 µM. Aliquot and store at -20ºC

dpy-10 repair oligo:
dpy-10 repair oligo sequence:

CACTTGAACTTCAATACGGCAAGATGAGAATGACTGGAAACCGTACCGCATGCGGTGCCTATGGT
AGCGGAGCTTCACATGGCTTCAGACCAACAGCCTAT

Order using the above sequence as a 4 nmol Ultramer DNA oligo from IDT and resuspend to 10 µM in nuclease free water. Aliquot and store at -20ºC.

Cas9:
40 µM Cas9-NSL purified protein obtained from MacroLab Facility at the University of California, Berkeley. This order comes in 10 µL aliquots that are marked with each use and discarded after four freeze/thaws.