Co-Crispr injection

Preparing injection mixture:

Add components of the injection mixture in the following sequence:

1.Cas9 0,5 µl of 10 µg/µl stock
2.Add tracr-5 µl of 0,4 µg/µl stock
3.Add crRNA-1,12 µl of 1, µg/µl stock
4.Incubate this mixture at 37°c for 10 minutes before adding any DNA. Adding any double strand DNA before RNP complex formation reduces HDR efficiency
5.Add 1,6 µl prl-3 roller plasmid of 500 ng/µl stock
6.Add 1XTE buffer to bring the volume to 20 µl
7.To avoid needle clogging, centrifuge the mixture at max speed for 2 minutes, transfer about 17 µl of the mixture to a fresh Eppendorf tube and proceed to loading the needle

Micro injection and screening:

1.Inject 20-25 animals and transfer them onto individual plates (first time window). After 18-20h,

transfer the injected animals to the new plates (second time window)
2.After 2-3 days screen the Dpy, Dpy like, Roller phenotype from the first time window, pick the plates that segregate F1 Dpy-Roller and from these plates, pick about 8 F1 non Dpy-Roller and perform the lysis and genotyping after these animals have produced the F2 progeny