**Our injection mix and other tips**

Please note: We mostly use smaller concentrations of injection materials than what is written in Paix's paper. In addition, we use a commercial CAS9 enzyme from IDT (Alt-R® S.p. Cas9 Nuclease V3), I suppose that CAS9 from a different source will require calibration.

**Injection mix (20µl)**

CAS9 enzyme (provided conc. 10µg/µl): 1.6µl

tracrRNA (0.4µg/µl): 5µl

dpy-10 crRNA (0.4µg/µl): 1µl

dpy-10 ssODN (1µg/µl): 0.8µl

Targeted gene crRNA (0.6mM): 2µl

Targeted gene ssODN (1µg/µl): 4.5µl

KCl (1M): 0.5µl

DDW: 4.6µl (to reach a final volume of 20µl)

Incubate the mix for 20 minutes at 37°C and then centrifuge with maximum speed for 5 minutes before loading the needle.

tracrRNA preparation: IDT provides 5nmol of tracrRNA which are 111µg and a duplex buffer. For 0.4 µg/µl add 277µl of the buffer provided. Before adding spin-down the tracrRNA tube.

crRNA preparation: we buy 10nmol of crRNA of the targeted gene. After a quick spin down, we add 16µl of TE buffer (pH 7.5) and warm the solution at 40 °C until the powder dissolves (a few minutes). Do not mix actively (no pipetting or vortexing). Store the crRNA in the freezer (-20 or below).

TE buffer preparation: in final volume of 100ml put 1ml of Tris 1M pH 7.5 and 0.2ml of EDTA 0.5M

I don't think there is much importance to the order in which we make the injection mix. I usually put the water, KCl and tracrRNA together first to get some volume and then add the dpy-10 and targeted gene crRNAs, ssODNs and lastly I add the enzyme. I mix the solution by pipetting while I add each of the components.

Other tips:

- When you design the homology arms: one of them should start after the edits and the other should start **immediately after** the CAS9 cut site (they write that in the paper – but it's important for successful recombination so I wrote it here also)
- Whenever possible it's better to mutate the PAM site rather than the 20nt sequence to ensure that the enzyme will not recut
- I like to use the IDT website to check crRNAs efficiency and possible off targets (https://eu.idtdna.com/site/order/designtool/index/CRISPR_SEQUENCE)

- If your edit is downstream to the PAM site, your template (ssODN) should be designed according to the **opposite** strand, if its upstream, the template should be designed according to the **same** strand.
- The dpy-10 phenotype is much more visible in adult worms. Give the F1 progeny time to grow before determining if the injection was successful or not