**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 10-20 minutes at 37oC 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 oC 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
* When using two crRNA for making double DSBs and inserting large fragments, it is better that each one will be on a different strand. The most efficient orientation is OUT-OUT (after the cut the PAM sites remain on the strand), the slightly less efficient orientation is IN-IN (after the cut most of the recognition sites remain on the strand). The least efficient orientations are the IN-OUT or OUT-IN that arise from crRNAs that are on the same strand.