**Preparation OF CRISPR/Cas9 mRNA For Microinjection**

*(TRANSGENIC RESOURCES PROGRAMM, University of Washington (http://www.uwtransgenics.org/)*

The following protocol was adapted from the Transgenic Core at the University of Michigan.

**Materials:**

* *in vitro* transcribed mRNA
* Oligonucleotide resuspended in RNAse free water
* DNA donor plasmid prepared with an endotoxin free plasmid kit
* 0.02 um Anotop 10 Syringe Filters (Whatman Cat. No. 6809-1002)
* *Do not filter nucleic acid solutions through the Anotop filters, the 0.02 um pores
will trap nucleic acids..*
* 1 M Tris-HCl, pH 7.4 (Sigma Cat. no. T2663)
* 0.5 M EDTA (Sigma Cat. no. E7889)
* Nuclease Free Water (Sigma Cat. no. W4502)

**RNase-Free Microinjection Buffer:**

\*All ingredients and supplies, should be RNase-free.

**RNase Free Microinjection Buffer (10 mM Tris-HCl, pH 7.4, 0.25 mM EDTA)**

|  |  |
| --- | --- |
| **For 10 ml ( mix together)** | **For 20 ml ( mix together)** |
| - 9,9 ml Water | - 19,8 ml Water |
| - 100 µl Tris-HCL, pH 7,4 | - 200µl Tris-HCL, pH 7,4 |
| - 5 µl EDTA | - 10 µl EDTA |

- Wash 0.02 um filter with 1 ml buffer – discard wash

- Filter remaining buffer through filter for use as dialysis buffer or for use as microinjection buffer.

**Preparation:**mRNA and sgRNA are transcribed *in vitro* with kits and procedures as described (Geurts et al, 2009, Wefers et al., 2013, Yang et al., 2013).

To avoid clogging microinjection needles, wash buffers and elution buffers used in mRNA and sgRNA purification should be pre-filtered through 0.02 um filters. Do not pass nucleic acid solutions through the filters as the pore size on the filters is small enough to trap nucleic acids.

Plasmid DNA donors should be purified with endotoxin free kits. Pre-filter all buffers through 0.02 um filters. Do not pass nucleic acid solutions through the filters.

Mix together nucleic acids in 0.02 um filtered RNAse-Free Microinjection Buffer at the desired concentrations. Prepare several aliquots of 50ul in 1.5ml microtubes. Store at -80°C.

To prevent cannula clogging the injection mix was filtered shortly before the microinjection using a Corning® Costar® Spin-X®centrifuge tube-filter 0.22µm (Sigma Aldrich, CLS8160).

Pipette the mix onto a 0.22 µm Costa Spin filter and centrifuge for 10 min at full speed and 4 ° C.a

**Cave:** If Cas9 protein is used, it must be checked after filtering that it has not got stuck in the filter.

**Concentration: 100ng/ul Cas9 mRNA + 50ng/ul gRNA (+ 200ng/ul oligo)**