Transgene preparation (Black lab)

For DNA injection (linearized plasmid or circular BAC construct)

Purification of DNA

1. For linearized plasmids, we gel purify the insert to be injected with QIAquick Gel Extraction Kit (Qiagen 28704, wash twice with PE Buffer before drying and eluting).

2. For BAC preparation, we had success with the NucleoBond Xtra Midi/Maxi kit (MACHEREY-NAGEL, 740410, 740414)

Preparation of transgene for injection

3. Dilute DNA to desired concentration in 100µl Injection Buffer
(typically 2ng/µl for linearized plasmid but can be up to 4 ng/µl in some cases)
(typically 5ng/µl for circular BAC construct)

4a. Spin at 14,000 rpm for 10min at 4°C.
4b. Take the top 90 µl liquid, transfer to a clean 1.5 ml tube.

5a. Spin again at 14,000 rpm, 10min at 4°C.
5b. Take the top 80µl liquid, transfer to a clean 1.5 ml tube.
Keep on ice until injection.

For CRISPR RNA injection mix

Synthesis and Purification of RNA

1. *In vitro* transcribe guide RNA with MEGAshortscript™ Kit (AM1354M for T7) from Thermo Fisher Scientific.

2. Purify guide RNA with MEGAclear™ Transcription Clean-Up Kit (AM1908) from Thermo Fisher Scientific.

3. We purchase Cas9 mRNA (Cat# CAS500A-1) directly from SBI: (*http://www.systembio.com/*)

Preparation of CRISPR injection mix

4. On the day of injection, dilute *Cas9* RNA and guide RNA(s) to desired concentration in 20µl RNase free Injection Buffer
(typically 50ng/µl for Cas9 mRNA and 10ng/µl for guide RNA)

5. Spin at 14,000 rpm for 10min at 4 °C right before injection.

Injection Buffer
5mM Tris-Cl, pH=7.4
0.01mM EDTA