Microinjection of Mouse or Rat Embryos

The Transgenic Core has successfully created knockout and knock-in mice using the Crispr/Cas9 technology. The standard strains used by our facility are B6D2F1 and C57BL/6. Sprague-Dawley and F344 are common rat strains that we have worked with. Other strains will be considered but the investigator may incur additional costs if costs of materials and time required to complete the project are greater than for our standard strains. Contact Kathy to discuss your specific strain needs.

While each lab has the option of preparing their gRNA, we strongly recommend having Dustin prepare and purify all reagents. This saves labs money and we control some of the quality variation.

Suggested concentrations for microinjection:

- 40-100 ng/µl Cas9 (tested product available from TAF)
- 50 ng/µl sgRNA (each guide)
- 10-20 ng/µl plasmid DNA
- 20-50 ng/µl single oligo DNA (typically ordered from IDT, non-page purified)

Embryo Implantation

Following the microinjections, the embryos are transferred into the oviducts of pseudopregnant recipients. Pups should be born 19 days later and weaned 3 weeks after birth. At the time of weaning, tail tissue samples are collected. Two days after clipping, the samples of founder DNA will be provided to the investigator for genotyping.

Genotyping and F1 Litter Production

From the DNA samples of each potential founder the investigator will have two weeks to complete genotyping the mice. We recommend PCR analysis as the first method of genotyping followed by sequencing. The investigator must provide a copy of the genotyping results to the TAF staff. The TAF then will breed up to three founders when they reach breeding age to wild-type mates to produce one F1 litter from each founder. The founder and litter are transferred to the investigator when the progeny are three weeks old. Any remaining founders will be shipped without breeding to the investigator.