DNA Microinjection

Core Responsibilities

The core will inject at least 125 fertilized FVB embryos with a DNA construct provided by the investigator. The surviving embryos will be transferred to recipient females. All resulting live pups will be transferred to the investigator after weaning and serology testing. We normally expect between 15 and 30 pups born with 20-30% being transgenic. If less than 15 pups are produced, the core will perform a second round of injection at no cost. The core does not guarantee transgenic mice but if you fail to obtain transgenic mice the core will repeat the injection at no charge if you can document that your screening procedure (PCR or Southern) is able to detect the transgene at a single copy level and you have an internal control. Please forward this information, a figure legend and a short description of the method to Andrei Golovko at agolovko@tigm.org for review.

Injections using C57BL/6 embryos are also possible but please note that efficiencies are lower. Contact Andrei Golovko for C57BL/6 injection pricing.

BAC clones can also be injected. Please contact the core to obtain protocols for preparing BAC DNA and polyamine storage buffer. You are advised to first check your BAC clone prep on a pulse-field/FIGE gel to verify the integrity of the BAC clone before delivery to the core.

Investigator Responsibilities

- Complete a Pronuclear Injection Service Request form.
- Provide information that the investigator has the necessary institutional animal and biosafety approvals for the project.
- Provide at least 5 ug of the DNA fragment at a concentration of 50 ug/ml; Plasmid DNA should be prepared using the Qiagen EndoFree Plasmid Midi (Maxi) Kit or similar product; DNA fragment should be purified using QIAquick Gel Extraction Kit (Qiagen Cat. No. 28704).
- **Provide documentation that a PCR or Southern detection strategy is in place that can detect the transgene at single copy levels when mixed with mouse tail DNA (see http://www.med.umich.edu/tamc/spike.html).**
- Report back to the core the number of transgenic mice generated.

Time Line for Pronuclear Injection

Day 1-7: Transgene fragment prepared for microinjection
Day 8: Microinject DNA and culture surviving embryos overnight
Day 9: Embryos transferred to recipient mice
Day 28: Pups born
Day 49: Pups weaned
Day 56-59: Mice transferred to the investigator after serology testing completed