

## **Blastocyst Injection**

### Core Responsibilities

ES cells will be injected into C57BL/6 albino blastocysts. If no or low percentage chimeras are obtained from the first injection, a second injection will be performed. The core typically injects approximately 30 - 40 blastocysts at a sitting. If no or poor chimeras are produced after the second injection the core will consult with the investigator. The core does not guarantee chimeras or germ line transmission from the submitted clone(s). The likelihood of an ES cell clone contributing to the germ-line is highly dependent on the quality of the starting ES cell line, the passage number of the clone and on how the clone was handled in culture prior to delivery to the core. For ES cell clones that originate from outside the core (i.e. the MMRRRC, IKMC, your lab or a collaborators lab, etc.) and that haven't been checked for pathogens, the core will first submit one vial of cells to the Research Animal Diagnostic Laboratory (RADIL) at the University of Missouri for mycoplasma and mouse pathogen testing (IMPACT IV test, ~ \$200 per clone). IMPACT IV is the base assay. If the clone is free of mouse pathogens the core will thaw the second submitted vial and expand the clone and prep the cells for microinjection. If the ES cells are differentiated or fail to grow properly the core will notify the investigator. At weaning the core will notify the investigator of the number of chimeras produced and their percentage chimerism. Following serology testing the chimeras will be transferred to the investigator.

### Investigator Responsibilities

- Complete a Blastocyst Injection Service Request form and forward to Andrei Golovko ([agolovko@tigm.org](mailto:agolovko@tigm.org), 979-458-5498, fax: 979-458-5559).
- List IACUC and Biosafety approval numbers
- Submit two vials of the frozen clone on dry ice. One vial will be used for pathogen testing and the second for expansion and preparation of cells for injection.
- For its records the core requests that the investigator report back on whether the chimeras generated were germline transmitters.

### Timeline

Day 1: Microinject ES cell clone into blastocysts

Day 17: Pups born

Day 38: Pups weaned and investigator notified on the number of chimeras and the percent chimerism

Day 45 - 48: Chimeric mice are transferred to investigator following serology results