

Electroporation

Please Note. Investigators must first consult with Andrei Golovko about the design of their targeting vector and they must provide data that single copy 5' and 3' screening probes are in hand. The core will not electroporate constructs unless these conditions are met. ES cell plates stored frozen by the core at -80C are good for a short period of time (a few months) so DNA plates should be screened promptly.

Core Responsibilities

The targeting vector will be linearized and prepped for electroporation by the core. The targeting vector will be introduced into either 129S6 ES cells or C57BL/6 ES cells. The cells will then be grown in the appropriate selection medium and clones picked. Generally the core will pick at least 2 x 96 well plates of clones. The clones will be grown and then split. One set of plates will be frozen at -80°C . Duplicate plates of cells grown on gelatin will be given to the investigator for DNA isolation and screening. Alternatively, TIGM can perform LD PCR-based screening of the clones followed by sequencing confirmation of the targeted locus. In the latter case, the investigator will need to provide a complete sequence of the targeted vector to ensure proper screening strategy design by TIGM staff.

Investigator Responsibilities

- Fill out an Electroporation Service Request Form and send to Andrei Golovko (agolovko@tigm.org, 979-458-5498, fax: 979-458-5559).
- Include IACUC and Institutional Biosafety approval information that is specific to this work
- Deliver to the core 50 ug of targeting plasmid at a concentration of 500 ug/ml. The plasmid should be prepared using the Qiagen mid/maxi prep kit or similar product
- A restriction map indicating the restriction enzyme to be used to linearize the vector.
- The investigator is responsible for screening the ES cell clones to identify targeted clones.

Timeline

Day 1-7: Feeder cells prepared and targeting vector prepped

Day 8: Initiate ES cell culture for electroporation

Day 12: Electroporate targeting vector

Day 13: Start drug selection

Day 21: Pick surviving colonies

Day 25: Split clones to feeder plates and a gelatinized plate

Day 28-29: Freeze clone plates and cell DNA plate. Transfer ES cell DNA plates to investigator for screening or perform screening at TIGM