

## Timetable for Gene Targeting

1. DNA (and paperwork) received by the Core from the investigator.
  - Mitotically-inactivated feeder cells plated.
  - ES cells thawed and expanded for electroporation.**TIME = 7 days**
2. Targeting construct electroporated into ES cells (*investigator will receive e-mail*).
  - begin positive ( and negative) drug selection.
  - individual cells allowed to expand into colonies.**TIME = 13-18 days, depending upon drug marker.**
3. Pick colonies and array into 96-well format (*investigator will receive e-mail*).
  - expand cells to fill 96 well plates.**TIME = 8 days**
4. Split clones onto gelatin plates , freeze down master plates.
  - expand cells to fill 96 well gel plates
  - prepare genomic DNA in 96-well format.
  - freeze down DNA plates. (*investigator will receive e-mail*).**TIME = 10-12 days**

Time from delivery of targeting vector to Core to delivery of DNA plates to the investigator for analysis is approximately 5-6 weeks, assuming no queue exists for targeting.

5. Once putative positives identified by investigator and relayed to the Core.
  - master plates thawed and select clones expanded to 6 cm plate
  - half of well frozen back, other half plated onto 10 cm gel plate.
  - DNA prep from confluent 10 cm gel plate, given to investigator. (*investigator will receive e-mail*).**TIME = 20-25 days**

Total time from delivery of targeting vector to Core to delivery of targeted clone(s) either to investigator or to Transgenic Core is approximately 8-9 weeks, plus the time it takes investigator to analyze clones in 96-well format (typically 2-3 weeks) and to confirm clone is correctly targeted at end of procedure (typically 2 weeks). **Thus, the total time it takes to generate a targeted ES clone, assuming no queue exists for targeting and all goes well, is 12-14 weeks.** Please note there is little the Gene Targeting Core can do to hasten this time frame- as it is mostly a function of how long it takes cells to undergo drug selection and how long it takes an individual clone to grow sufficiently for analysis or freeze down.

Additional modifications (Cre addition and reselection, etc) will require additional time, and may require a third screening of clones by the investigator to confirm proper targeting of the allele.

**TIME = 15-30 days, depending upon the procedure.**

Regarding the queue: The targeting Core is able to initiate 3 new targeting experiments each week. Typically, the queue is 0-1 week, and has never been more than 3 weeks. While the Core operates on a "first come- first served" basis, we do reserve the right to stagger individual investigator's targeting experiments so that no single lab dominates use of the Core to the exclusion of other groups (example: a lab turning in 6 constructs simultaneously).