ES Cell Targeting Project Summary Form

Please read instructions before completing the form (#4).

1. Project Summary (Please describe the goal of your project. For guidance, please see example project summary form found at http://ESCore.ucsf.edu/forms.)

2. Scientific Information

Project Title	
Name of Construct	
Name of Gene Modified	
Total Size of Linearized Targeting Vector	
Strain Background for Homology Arms	
Length of Homology Arms	
Positive Selection Marker (ex. PGK-NeoR)	
Negative Selection Marker (ex. PGK-DTA)	
Name of ES Cell Line to Be Used	
Southern or PCR Genotyping Assay (pick one)	

IMPORTANT! Please upload a map of your targeting vector and the gel picture to MyCORES with this form

3. Services you need

Services	Requested services
ES cell targeting (feeder-independent)	
ES cell targeting (feeder-dependent)	
Genomic DNA extraction	
Expansion of positive clones (up to 5 clones)	
Chromosome counts	
Preparation for microinjection	

Note: If your targeting vector fails to generate drug-resistant colonies, the Core will charge you a partial fee ($777.64) to compensate the lost reagents and time for each attempt.

4. Requirements for ES Cell Targeting Service

Upon application for services:

- It is required that you have a completed targeting vector. We will not schedule your targeting project until you give us **a map of your targeting vector and a picture of your gel** to show successful preparation of your vector. Your gel will show lanes in the order of marker/uncut/cut/purified DNA. Use 1 µg **λ-HindIII Digest** (NEB cat. #N3012) as your marker and load 500 ng samples of your vector DNA.
- Bring 50 µg of purified targeting construct DNA at 1mg/ml in sterile PBS.
- It is recommended that you have a functional genotyping assay upon application. Long-term storage of the ES cells in the 96-well plates can result in a reduction of cell viability and quality.

After your targeting date:

- Positive colonies will be picked approximately 8-10 days after electroporation.
- Genomic DNA will be available about 3 weeks after the electroporation for your genotyping.
- Please genotype your clones as soon as possible.
- Upon genotyping, let us know which clones are positive and should be expanded (ex, plate 1 well B5). We will revive and expand up to 5 clones. If you want more than 5 clones to be revived, there will be additional charge ($329.80/additional clone).
- After expansion of positive clones, we will provide you with a cell pellet so that you can validate genotyping before injection.
- We strongly recommend getting a chromosome count for your positive colonies and pick the best ones for microinjection.
- Injecting at least two independent clones will increase your chance of getting higher chimeras and allows you to compare phenotypes of your transgenic mice.