# 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.)**

The goal of my project is to make a conditional knockout of My Favorite Gene 2 (Mfg2). My targeting construct contains 2 LoxP sites flanking exons 1 and 2 and has an FRT-flanked PGK-Neo cassette for positive selection in the intron immediately after exon 2. A PGK-DTA is outside the region of homology for negative selection.

## 2. Scientific Information

| | |
|---|---|
| Project Title | $Mfg2^{CKO}$ |
| Name of Construct | Lox-Mfg2-Lox-DTA |
| Name of Gene Modified | Mfg2 |
| Total Size of Linearized Targeting Vector | 9 kb |
| Strain Background for Homology Arms | C57Bl/6 |
| Length of Homology Arms | 3 kb each |
| Positive Selection Marker (ex. PGK-NeoR) | $PGK\text{-}Neo^{R}$ |
| Negative Selection Marker (ex. PGK-DTA) | PGK-DTA |
| Name of ES Cell Line to Be Used | JM8A3.N1 |
| Southern or PCR Genotyping Assay (pick one) | PCR |

**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) | X |
| ES cell targeting (feeder-dependent) | |
| Genomic DNA extraction | X |
| Expansion of positive clones (up to 5 clones) | X |
| Chromosome counts | X |
| Preparation for microinjection | X |

**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.