

# ES Cell Targeting Core: Important Information about your Project

## **Prior to Targeting Date:**

- It is **REQUIRED** that you have a functional PCR assay. We will not schedule your targeting project until you have a functional PCR assay.
- Please inform us if you would like the electroporation to be in a strain other than 129 SvEv Tac.
- Please inform us if you require screening with a drug other than G418.

## **On your targeting date, please bring:**

- 30 ug of purified DNA at 1ug/ul in sterile PBS
- Completed ES Cell Production Request Form

If you have to change your electroporation date please let us know at least 2 days prior to the scheduled date.

## **After your Targeting Date:**

- Positive colonies will be picked approximately 10 days after electroporation.
- Dehydrated DNA will be available 3-4 weeks after electroporation.
- PCR results from the 96 well DNA must be returned to the Core within 4 months. Long term storage of the ES Cells in 96 wells can result in a degradation of the cell quality.
- After PCR positive results are received we will grow your cells in 24 wells, and provide you with a cell pellet to use for Southern Blotting and any other testing you may require. Up to 24 colonies can be expanded at this stage.
- Up to 6 positive colonies from Southern screening will be karyotyped and expanded.
- Up to 4 positive, karyotypically normal clones will be injected into blastocysts.

Targeting Core Project #: \_\_\_\_\_

Cell Lines to Inject: \_\_\_\_\_

Microinjection Project # \_\_\_\_\_

## ES Cell Production Request Form

Investigator: \_\_\_\_\_ Building & Room No: \_\_\_\_\_

Phone: \_\_\_\_\_ E-mail: \_\_\_\_\_

Contact Person: \_\_\_\_\_ E-mail: \_\_\_\_\_

Animal Protocol #: \_\_\_\_\_ Mouse Room Location: \_\_\_\_\_

Budget #: \_\_\_\_\_

Construct Name: \_\_\_\_\_ Drug selection required: \_\_\_\_\_

Strain of Arms of Homology: \_\_\_\_\_ Size of arms: \_\_\_\_\_

Targeted ES Cell Production Service	Check Service	Services
Electroporation		Electroporation of one 10cm plate of ES cells. Cells will be grown in selective media for 10 days and up to 200 colonies will be picked and grown on 96 well plates. Samples will be sent to the investigator for evaluation, and the colonies will be frozen in the 96 well plate.
DNA isolation		DNA from cells in the sample 96 well plate will be isolated and given to the investigators for evaluation via PCR.
Expansion of Positive Colonies		Up to 24 clones selected by the investigator will be expanded into one well of a 24 well plate. One vial of cells will be frozen from each plate, and a sample will be sent to the investigator.
Further Expansion & Karyotype Analysis		Cells chosen for injection will be expanded. 5 vials will be frozen per line. Cell lines will be karyotyped prior to injection.
Karyotyping		Karyotyping of additional cell lines.

Approved by Lab PI: \_\_\_\_\_ Date: \_\_\_\_\_

ES Cell Targeting Core Facility  
Core Director: Paul Worley, MD  
Core Director: Alex Kolodkin, Ph.D.,  
Contact Personnel: Holly Wellington,  
Chip Hawkins

Tel: 5-4050 E-mail: pworley@jhmi.edu  
Tel: 4-9499 E-mail: kolodkin@jhmi.edu  
Tel: 4-3858 E-mail: hwellington@jhmi.edu  
Tel: 4-3858 E-mail: chawkins@jhmi.edu