REQUEST FOR ES CELL ELECTROPORATION AND SELECTION

PI ____________________________           Charge Account (nick name) ______________
Tel:____________________            Date__________

Contact person: _____________________________________ Tel: _________________________________
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For detailed discussion of ES cell services and gene targeting strategies contact Tobias D. Raabe, Ph.D. Tel.: 215-898-2795; e-mail: raabe@mail.med.upenn.edu

ES cell electroporation and selection are part of gene targeting, a procedure involving construction of a targeting vector, electroporation of ES cells, selection of homologous recombinant ES clones, injection of ES cells into blastocysts, generation of chimera and finally germ line transmission. Germine transmission depends on the availability of high percentage chimera. Injections are done at the Transgenic Mouse Facility at Department of Genetics, School of Medicine.

Prices:

Electroporation and selection (R1, TL1 or V6.5 ES cells)...............................$4,000
Electroporation and selection (C57BL/6 ES cells)...............................$4,900

If after an initial round of electroporation and selection no correct recombinant clone was found, a second round of electroporation and selection will be performed for an additional $2,000.

IMPORTANT: Gene targeting requires **smooth interaction** between Penn Gene Targeting and the client lab over a prolonged period of time. The following pages are designed to facilitate the information transfer necessary for success. All information regarding the targeting vector must be completed by the client lab before Penn Gene Targeting can start to grow ES cells.
Summary of Services: We will grow up mouse ES cells, electroporate them with the customers targeting vector and select antibiotic resistant clones. Four cultures would be grown from each resistant clone, out of which two would be frozen for later injection and two would be provided for the purpose of DNA extraction.

IMPORTANT: We reserve the right to reject targeting vectors for electroporation that appear unlikely to yield homologous recombinants. In this case, we will propose necessary alterations to the vector and/or the targeting strategy.

IMPORTANT: Frozen recombinant ES clones should not be stored longer than about two months at –80 °C. Otherwise we cannot guarantee the survival of ES clones after thawing. To provide an incentive for the customer to work on the Southern and PCR strategy for detection of recombinant clones before electroporation and selection, we will give preferential treatment to customers who can provide evidence of a working Southern and/or PCR strategy. Thus, a targeting vector from a customer with evidence of a working Southern/PCR assay will be electroporated earlier than that of a customer without such evidence if it is not possible to do both electroporations at the same time.

A WORKING ASSAY MUST FEATURE THE FOLLOWING:

1. The assay must be done with actual ES cell DNA isolated from 96 well plates.

2. For Southern, a clear wild type band should be evident in the majority of clones isolated from the 96well plate.

3. For PCR, a primer pair must show the correct PCR product on a artificially constructed template that represents the exact expected target in the final desired ES clones. This can be achieved by cloning a small piece genomic DNA to the right of the right arm or to the left of the left arm of homology. The PCR reaction must be done in the presence of genomic ES cell DNA from 96 well plates and the above artificial template DNA must be diluted to a concentration of one copy per mouse genome.

We will provide free practice 96 well plates with frozen ES cells that can be used to refine isolation of genomic DNA, digestion with restriction enzymes, Southern blotting and genomic PCR.

Step 1: Agreement on targeting strategy. The following points regarding the gene targeting strategy must be agreed upon before we can start growing the ES cells.

1. Mouse strain used as source of genomic DNA: ..............................................................

2. Name and passage number of ES cell line to be used: ...................................................

3. Positive selection marker and name and concentration of selection drug: ..............................................................

4. Negative selection marker and name and concentration of selection drug: ..............................................................
Step 2: Preparation of targeting vector DNA. We need 200 µg of linearized targeting vector prepared as follows: The vector DNA must be purified by Quiagen maxiprep or CsCl centrifugation. It must be linearized by the appropriate restriction enzyme. The vector DNA must then be extracted once with phenol chloroform and twice with chloroform, precipitated with 300mM Sodium Acetate pH 5 and Ethanol and finally washed with 70% Ethanol. The dried pellet must be dissolved under a tissue culture hood in 1x sterile PBS and last, the amount of DNA must be measured by UV.

Name of Vector: .................................................................

Ratio of 260/280 nm : ..............................................................

Concentration and final amount: ................................................

Photo of agarose gel:

Step 3: Electroporation and selection. We will then perform the electroporation of ES cells and select up to 192 antibiotic resistant colonies. If more resistant ES clones are generated in a single electroporation customer has the option to request freezing of up to 192 additional clones (total of 384) for an additional charge of $2000. The customer must prepare genomic DNA from the 96 well cultures according to our protocols.

Number of colonies grown for testing............................. Date .....................

Step 4: Screening of ES cell clones. Customer isolates DNA from 96 well plates according to the Penn Gene Targeting protocol and analyses DNA by PCR and/or Southern. If PCR data are ambiguous or negative, Southern analysis will be necessary to ascertain the presence or absence of positive clones. In some instances Southern with an external as well as an internal probe may be advisable. The DNA from 96 well plates will not support more than two independent Southern per clone and the utmost care must be taken not to loose any DNA. Further, high concentration restriction enzymes must be used to digest DNA for Southern, as the purity of the DNA is poor. Enzymes available as high concentration stocks are likely to work well. These are the most widely used enzymes: Nde I, BamHI, EcoRV, EcoRI, HindIII KpnI (must use high concentration preps). For other enzymes the customer must test compatibility with this DNA preparation.

Number of colonies screened ...... (by PCR); ...... (by Southern)

After we all agree that positive clones could be identified we will grow up all positive clones from the 96 well master plate and expand them sufficiently to make two liquid N2 storage vials.

Number of positive clones ............... Date....................