ES cell gene targeting

A. Prerequisite: Before placing an order for gene targeting service, the following conditions should be fulfilled.
   1. Construct ready upon applying for TCF services.
   2. Method and proof to identify targeted genes; essential genotyping include testing the existence of positive selector, and the confirmation of homologous recombination of both arms.
   3. Space to store ES clones once being generated.
   4. Understanding that not every targeting construct will yield correctly

B. Gene targeting service request form, case evaluation form, agreement form, and ES gene targeting checklist should be completely filled and signed by PI. Construct map should be attached on the request form. The request form should be dated and stamped by IMB secretary upon service application.

C. For optimal process efficiency, a checklist is made based on TCF experiences. Only those requests that fit all items on the checklist can be processed with guarantee. Requests with minor discrepancy from the checklist can still be processed without guarantee. However, requests with major discrepancy from the checklist will be either subjected to committee discussion or be rejected.

D. TCF uses inbred strain C57BL/6 ES cells and Hybrid strain (129X1/SvJ´129S1/Sv-+^{P}) R1 ES cell for gene targeting.

E. Upon submitting construct, DNA material condition form should be filled and attached with a gel photo, in which the marker and the target band should be clearly indicated. Construct prep should be freshly digested in described quantity and quality .

F. Depending on the nature of gene compositions, construct design and selector(s) used, the number of clones after selection varies significantly. Incorporation of functional positive and negative selector(s) in construct will generally enrich the selection, leads to the yield of good clones around 200 to 500. Due to such wide range of the final clone number that might occur from diverse construct designs, More than 200 clones will be guaranteed.

G. TCF will redo gene targeting service when the number of clone yield falls under 200. TCF might adjust the transfection conditions for the redo experiment according to the experience from last trial. Redo service will be held once with no additional charge.

H. Additional ES clones may be generated upon request for the same construct with the full charge.

I. ES cell genomic DNA will be available about 3 to 4 weeks after electroporation. Upon receiving ES clone gDNA, a sample receiving form will be asked by TCF staff to sign. Genotyping result should be available from requester within two months from the release of ES gDNA.

J. TCF will not be responsible for the long-term storage of ES clones. All ES clones will be released after case closed; however, positive ES clones will be kept if proceeds with blastocyst injection request. Upon receiving frozen ES clones, an ES clone release form will be asked by TCF staff to sign.

K. Specifications/protocols available upon request:

1. DNA preparation for ES cell transfection.

L. Materials available upon request:

1. R1 (129X1/SvJ ×129S1/Sv-+^{p}) ES cell genomic DNA
2. C57BL/6 ES cell genomic DNA

M. Charges.

1. NT$ 50,000 for every request of ES cell transfection.
2. Charge will be bill to the order of requester at the beginning of service.
3. All service charges for orders from within IMB or other Institutes will be the same. All service charges cover some material cost and animal fee only. However, the IMB transgenic committee will subject price and service type to change according to the decision.