The ingenious Advantage

ingenious' ES Cell Lines – A Success Story

ingenious was the pioneering company to offer C57BL/6 embryonic stem cells for gene targeting. All of ingenious' robust and validated ES cell lines have a >90% euploidy rate, compared to the 70% industry standard. From ingenious' ES cell lines, over 1,400 gene targeted mouse models and targeted ES cell clones have been delivered. Hundreds of models generated by ingenious have been published, including in *Science*, *Nature*, and *Cell*.

Further optimizing our cell lines, we now introduce our new FLP ES cell lines which contain the FLP transgene for Neo deletion. Utilizing these new cell lines **saves our clients 3 months of time, and funding.**

15 Years of Published Gene Targeting Expertise:

We are one of the most highly experienced and published mouse gene targeting companies in the industry. All of the publications on our website are specifically for gene targeted mouse models that we have created in-house for our research clients over the past 15 years.
Link to our publications: http://www.genetargeting.com/Publications.htm

Intellectual Property and Confidentiality:

Your project information is handled with utmost confidentiality. We retain no intellectual property rights to any of the mouse models that we generate for our clients. We will not re-make or re-sell your model to any third party. Our clients' mouse models are produced and distributed under rights to patents and intellectual property licensed from various institutions.

Timelines:

Project timelines are a constant point of importance and satisfaction to our clients. We produce 95% of our conventional and conditional knockouts, knockins, and targeted transgenic mice within a 12-month timeframe. The full projects not fitting these timeframes represent particularly complex or novel model generation, requiring longer development times.

Project Management and Client Support:

Our clients utilize dedicated Project Managers to meet any needs and questions during all phases of their project, as well as after the mice have been delivered. Our Project Managers are all experienced molecular/cellular biologists possessing thorough mouse knockout/knockin procedural expertise. Comprehensive updates are provided monthly or more often if required. Clients can utilize our secure web-based project reports and documents where all data will be stored even after project completion.

Our Vectors

Our highly experienced construct design team can generate vectors specifically tailored to meet your scientific needs, such as:

NEW! F.A.S.T.™ KNOCKIN

A proprietary cassette is inserted into the target gene, producing a mouse model with the potential for five or more versatile functionalities, such as: inducible overexpression, knockdown/knockout, and knockout rescue. Find more information here.

KNOCKOUTS

- ***Conventional Knockout***
  The target region of interest is replaced with the Neomycin selection cassette.
- ***Conditional Knockout***
  The target region is flanked by loxP sites to facilitate Cre recombinase mediated tissue specific excision.
- ***Large Scale Deletions***
  Deletion of very large genomic sequences, such as multiple genes or whole gene clusters.

KNOCKINS

- ***Point Mutation(s)***
  The mutation(s) can be engineered base specific a) conventionally or b) conditionally, enabling the researcher to turn the mutation on/off in a specific tissue or at a specific time point.
- ***Human or Murine cDNAs and/or Reporter Genes***
  Reporters may be inserted either to replace an endogenous gene or to be fused to the endogenous gene for co-expression. In addition, to facilitate co-expression of a cDNA/reporter with an endogenous mouse gene, a 2A peptide strategy can be applied.
- ***TruHumanization***
  Insertion of entire human genes to replace a murine gene.

TRANSGENICS

- ***Targeted Transgenics***
  A cDNA or reporter gene can be inserted into the ROSA26 locus for a targeted transgenic overexpression mouse model. Tissue specificity can be achieved by utilizing a floxed stop cassette.
- ***Transgenic or BAC Transgenic Vectors***
  Constructs suitable for pronuclear injection can be generated to express or overexpress murine or human genes of interest under a specific promoter.

Project Milestones

0. Project Evaluation and Initiation

Together we carefully evaluate your needs and research goals and translate them into personalized project parameters for your particular mouse model.

Before a project is initiated, the gene of interest is thoroughly evaluated at no extra cost, and all potential strategies are discussed with you to determine which strategy would best fit your experimental objectives.

I. Vector Creation

- Complete bioinformatics analysis of gene structure and sequence content.
- Technical review of any previous targeting history of the gene of interest.
- Presentation of all relevant targeting strategies that meet your experimental objectives.
- Screening strategy design for PCR and Southern blot analysis.
- Confirmation of genomic BAC clone containing the target gene.
- Cloning and confirmation of homology arms.
- Insertion and confirmation of the Neomycin selection cassette flanked by FRT and/or loxP sites.
- Engineering/confirmation of specific mutations/deletions/insertions according to the project specifics.
- Sequencing of targeting vector.
- Linearization of DNA for electroporation.

II. Electroporation and Tissue Culture:

- Culture and expansion of ES cell s
- Electroporation of ES cells with targeting vector
- Positive/negative selection of ES cells
- Isolation of 200-300 ES cells per electroporation
- Duplication and freezing of ES cells
- Isolation of DNA for screening of ES cells

Our ES Cell Strains:

- C57BL/6
- C57BL/6 FLP
- 129SvEv
- HYBRID (129 x C57)
- HYBRID (129 x C57) FLP
- BALB/c

III. Screening:

- PCR and Southern blotting for identification and confirmation of positive clones.
- Genomic stability of ES cell clones is assessed by determining percentage of euploid cells to ensure that ES cells of high genomic stability are used for injection and mouse production.
- Expansion of positive ES cell clones and preparation for microinjection.

IV. Injection:

- Preparation of embryos and foster mice
- Microinjection of targeted ES clones into blastocysts or laser assisted injection of 8-cell stage embryos
- Implantation of blastocysts/8-cell embryos.

Breeding is performed in a Specific Pathogen Free (SPF) facility at ingenious' main production facility or in an AAALAC-approved Maximum Isolation and SPF facility with our long standing partner in animal husbandry, Stony Brook University.