Schematic and Time Line for the Generation of Knockout Mice

Aurora Burds Connor, Feb 2007

Making the DNA construct
(in your lab)

Time Line

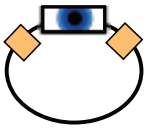
The Transgenic Facility also has a "Beginner's Guide to Gene Targeting" on the website in Methods. We are also happy to assist with advice and reagents to help you make an effective targeting construct.

It is recommended that you contact Aurora (aaburds@mit.edu) early in the process.

Acquire the genomic DNA for your gene or locus of interest
You can either screen a 129/Sv genomic library or use genomic databases, public BACs and PCR for a C57BL/6 background

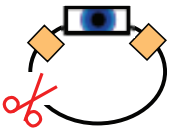
1-3 months

Make a DNA construct.
DNA from the genomic locus (orange) flanks the DNA to be inserted (blue) and it is placed in a bacterial plasmid. This construct is designed to add new pieces of DNA into the mouse genome at a desired locus. In a knockout experiment, the new DNA will replace the normal gene.



3-6 months

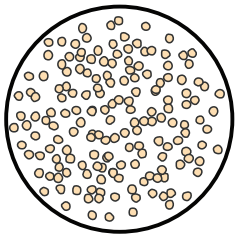
Linearize the construct and give it to the Rippel ES Cell Facility at MIT



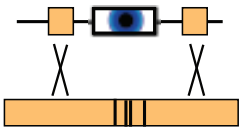
Generating Targeted ES Cells
(done by the Facility)

Time Line
Day 0

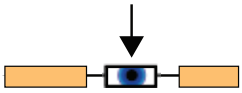
Insert the DNA into embryonic stem cells (ES cells) via electroporation.



In the cells, the homologous pieces of DNA recombine.

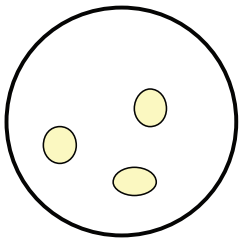


This inserts new DNA from the construct into the desired locus, usually replacing a portion of the original genome.



Place the ES cells in selective media, allowing for the growth of cells containing the DNA construct.

- The DNA construct has a drug-resistance marker
- Very few of the cells take up the construct – cells that do not will die because they are not resistant to the drug added to the media.



Day 1-10

Week 2

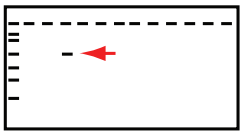
Up to 500 isolated colonies are isolated and expanded in plates.

Isolated DNA from individual colonies is shipped back to you and ES cell stocks are frozen.

Week 3

(Back in your lab…)

Use Southern blot or PCR to determine which colonies have integrated the DNA at the correct locus. Targeted recombination is generally rare, so only a few clones will usually be correct.



Week 4-8

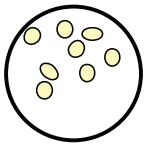
A quick email tells Aurora which colonies should be used to make mice.

Making the Mice

(done by the Facility, with DCM)

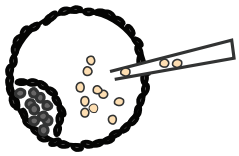
Time Line

Culture ES cells containing the correct genetic modification.



Day 0-3

Inject brown ES cells into black mouse blastocysts (3.5 day old embryos). The ES cells with the modification will contribute to a percentage of the embryo's tissues.



Day 3

Transfer injected blastocysts to the uterus of a recipient mouse which will act as a surrogate mother.

Pups containing tissues from the modified ES cells are called chimeras. The donor embryo and the ES cells are from different strains of mice with different coat colors (black and brown). This allows you to visually select chimeras – they look like striped mice.

Day 21-35 (Week 5)

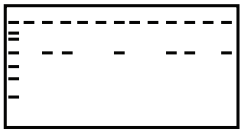
Breed 8 week old male chimeras to females with black coats.

Week 11

If any brown offspring are born, you know the modified ES cells have contributed to the germline (sperm) of the chimera.

Week 14-15

Genetic testing is performed on the offspring to determine which mice carry the genetic modification.



Week 16-17

You breed your colony and begin phenotypic analysis. The Facility has many strains of useful mice available to help with this (Cre mice, especially)

Week 22^{+}