

GENERATION OF KNOCK OUT MICE (Similar for Knockin, with slightly different construct design)

Making the DNA construct (in the researcher's lab)

Time Line

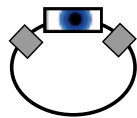
Screen a 129/Sv genomic library or use genomic databases and PCR to acquire the genomic DNA for your gene or locus of interest.



1-3 months

Make a DNA construct.

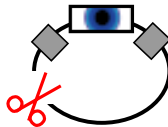
DNA from the genomic locus (grey) flanks the DNA to be inserted (blue) and it is placed in a bacterial plasmid. This construct is designed to add new pieces of DNA into the mouse genome at a desired locus. In a knockout experiment, the new DNA will replace the normal gene.



3-6 months

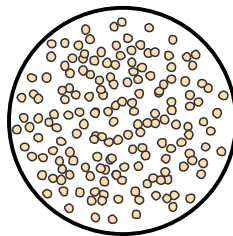
The Transgenic Facility at MIT can provide advice and reagents for making an effective targeting construct. It is recommended that you contact Aurora Burds Connor at MIT (aaburds@mit.edu) early in the process.

Linearize the construct and send it to the ES Cell Facility at MIT.



Generating Targeted ES Cells (at MIT)

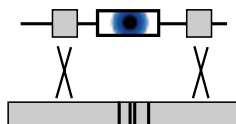
The specialists at MIT insert the DNA into embryonic stem cells (ES cells) via electroporation.



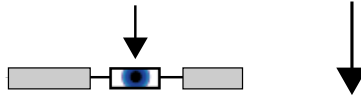
Day 0



In the cells, the homologous pieces of DNA recombine.

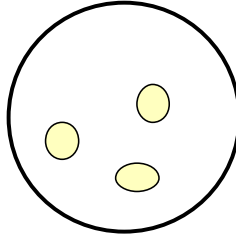


This inserts new DNA from the construct into the desired locus, usually replacing a portion of the original genome.



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

Up to 500 isolated colonies are picked by hand and expanded in plates.

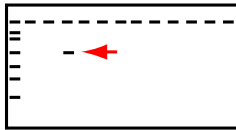
Week 2

Isolate DNA from individual colonies and send the DNA to the researchers.

Week 3

(Back in the researcher's 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.



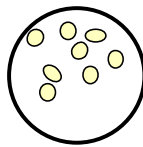
Week 4-8

A quick email to MIT tells the ES cell specialists which colonies should be used to make the mice.

Making the mice (at MIT)

Time Line

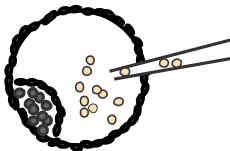
Culture ES cells containing the correct genetic modification.



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 born

Day 21

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 35
(Week 5)

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



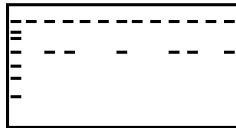
Week 11

If 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

Breed offspring.
You have now created a new line of mice!



Week 22⁺

Mice are tested for pathogens and shipped to the original researcher for analysis.