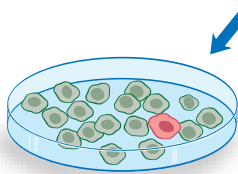
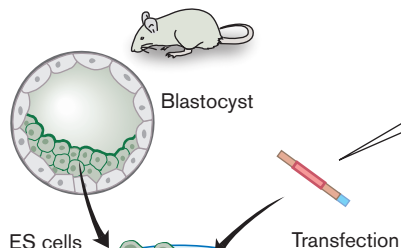


General strategy for gene targeting in mice

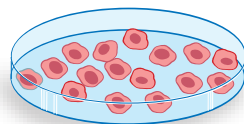
Step 1 Gene targeting in ES cells

1. ES cell culture

Embryonic stem (ES) cells are cultivated from mouse pre-implantation embryos (blastocysts).



Positive-negative selection

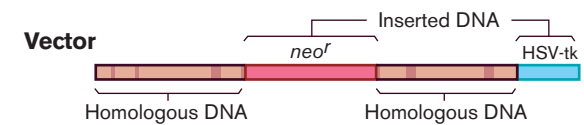


4. Proliferation of targeted ES cell

Selection for presence of *neo^r* and absence of HSV-tk enriches targeted ES cells.

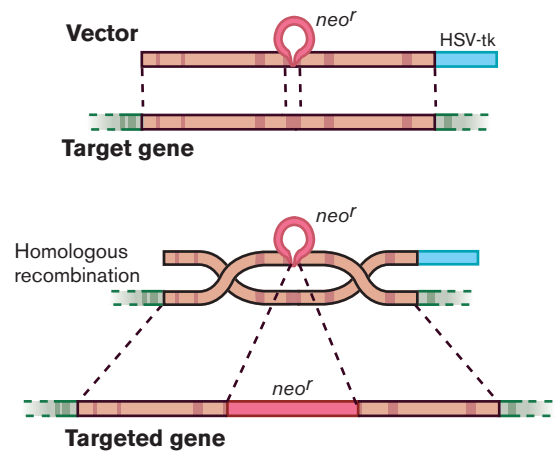
Pure population of ES cells carrying targeted gene

2. Construction of targeting vector



3. ES cell transfection

The cellular machinery for homologous recombination allows the targeting vector to find and recombine with the target gene.



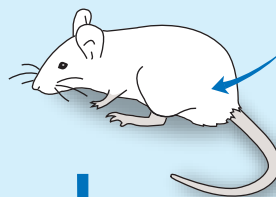
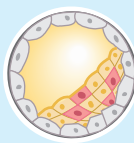
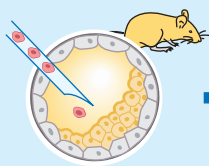
Step 2 From gene targeted ES cells to gene targeted mice

5. Injection of ES cells into blastocysts

The targeted ES cells are injected into blastocysts...

...where they mix and form a mosaic with the cells of the inner cell mass from which the embryo develops.

The injected blastocysts are implanted into a surrogate mother where they develop into mosaic embryos.



6. Birth and breeding of mosaic mice

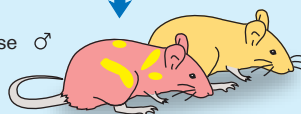
The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.

Born mosaic mice

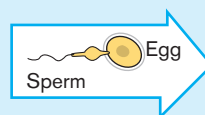
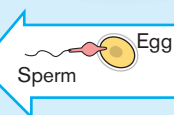


Mosaic mouse ♂

Normal mouse ♀



Gene targeted mice – called "knockout mice" when the targeted gene is inactivated



Normal mice

