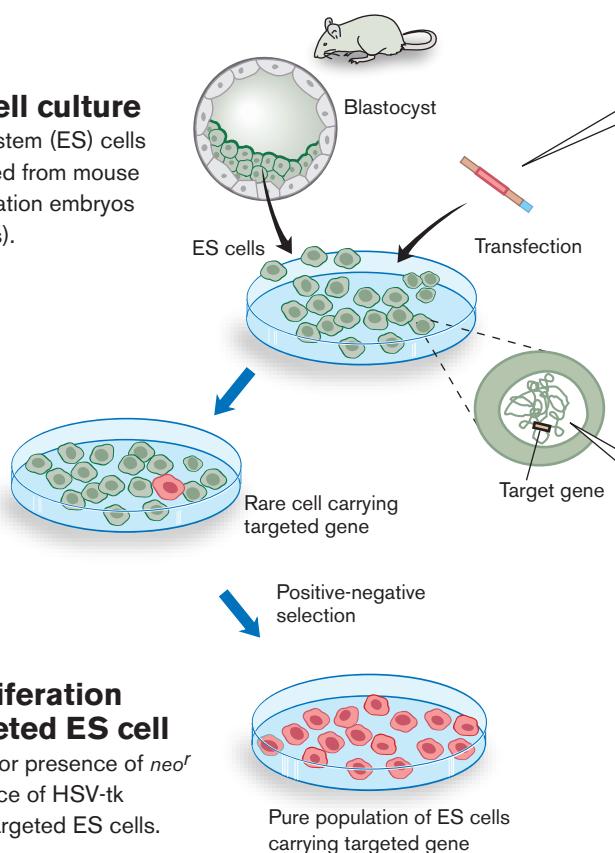


General strategy for gene targeting in mice

Step 1 Gene targeting in ES cells

1. ES cell culture

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

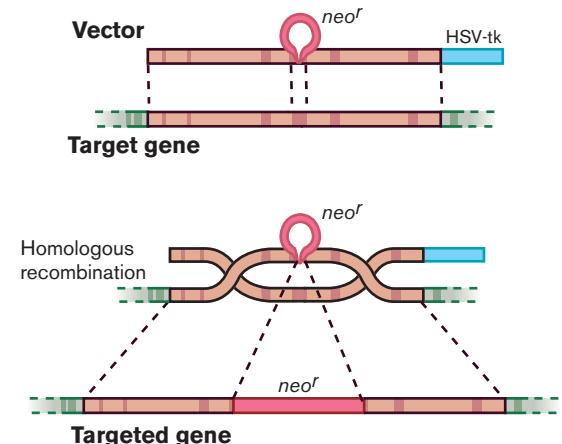


2. Construction of targeting vector

The vector contains pieces of DNA that are homologous to the target gene, as well as inserted DNA which changes the target gene and allows for positive-negative selection.

3. ES cell transfection

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



4. Proliferation of targeted ES cell

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

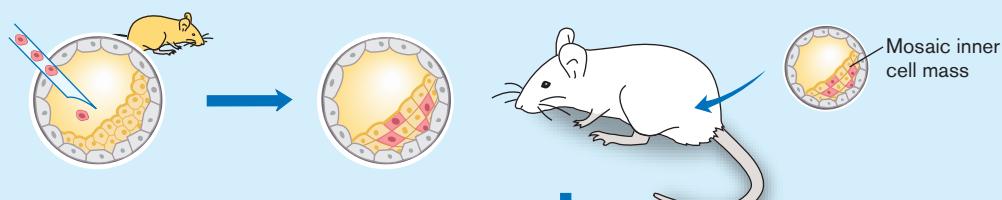
Step 2 From gene targeted ES cells to gene targeted mice

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.

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

