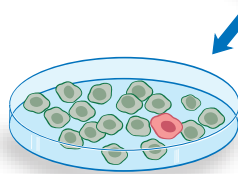
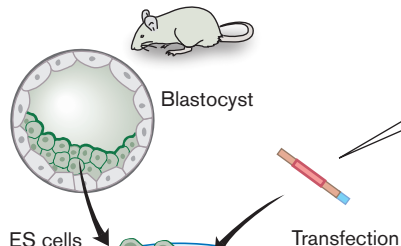


# General strategy for gene targeting in mice

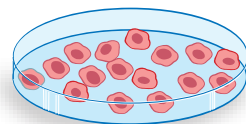
## Step 1 Gene targeting in ES cells

### 1. ES cell culture

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



Positive-negative selection

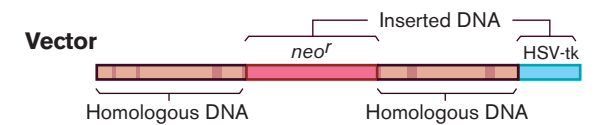


### 4. Proliferation of targeted ES cell

Selection for presence of *neo<sup>r</sup>* and absence of HSV-tk enriches targeted ES cells.

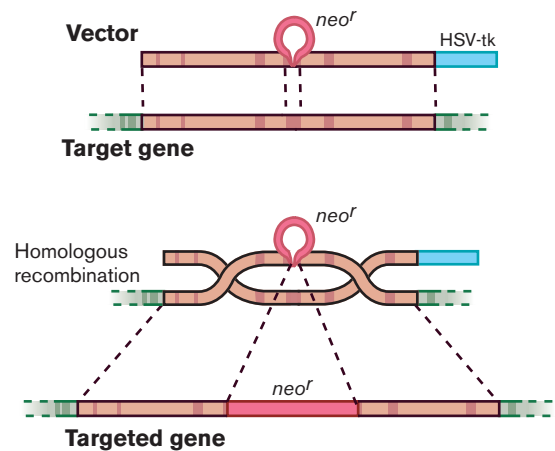
Pure population of ES cells carrying targeted gene

### 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.



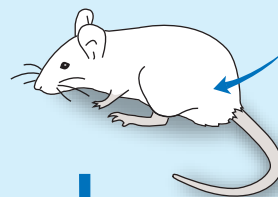
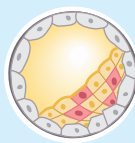
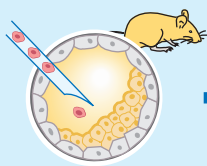
## Step 2 From gene targeted ES cells to gene targeted mice

### 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

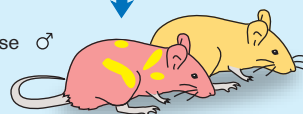
The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.

Born mosaic mice



Mosaic mouse ♂

Normal mouse ♀



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

