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).

   ![Diagram of ES cell culture](https://example.com/ES_cell_culture_diagram)

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.

   ![Diagram of vector construction](https://example.com/vector_construction_diagram)

3. **ES cell transfection**
   The cellular machinery for homologous recombination allows the targeting vector to find and recombine with the target gene.

   ![Diagram of ES cell transfection](https://example.com/ES_cell_transfection_diagram)

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

4. **Proliferation of targeted ES cell**
   Selection for presence of neoR and absence of HSV-tk enriches targeted ES cells.

   ![Diagram of proliferation](https://example.com/proliferation_diagram)

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.

   ![Diagram of injection](https://example.com/injection_diagram)

6. **Birth and breeding of mosaic mice**
   The mosaic mice mate with normal mice to produce both gene targeted and normal offspring.

   ![Diagram of birth and breeding](https://example.com/birth_and_breeding_diagram)

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