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Instructions on Making a Good Gene Targeting Construct

1. **The evolution of gene targeting construct:**

   A. Insertion Vector

   Nature 317:230-234, 1985.

   B. Replacement Vector

   Hprt⁻ (resistant to 6-thioguanine); G418ʳ

   Cell 51: 503-512, 1987.

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│           Transgenic Core Facility                        │
│   Institute of Molecular Biology, Academia Sinica         │
│              2789-9312, 2652-1438                         │
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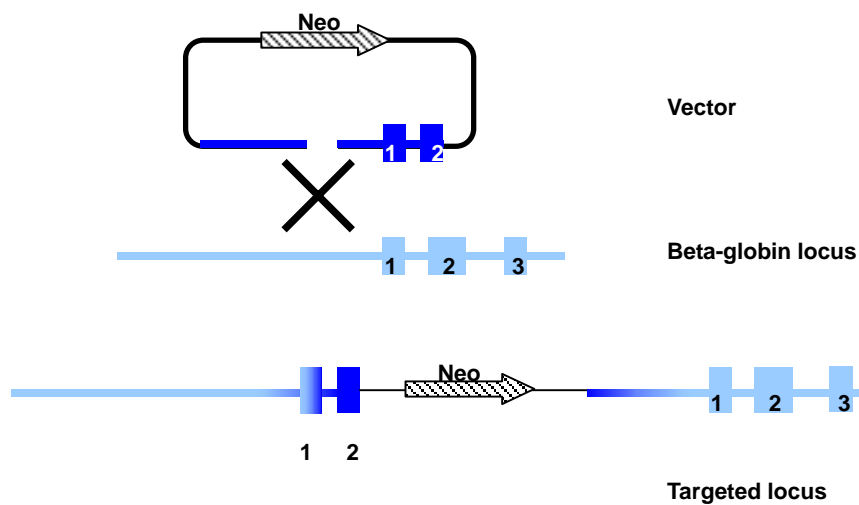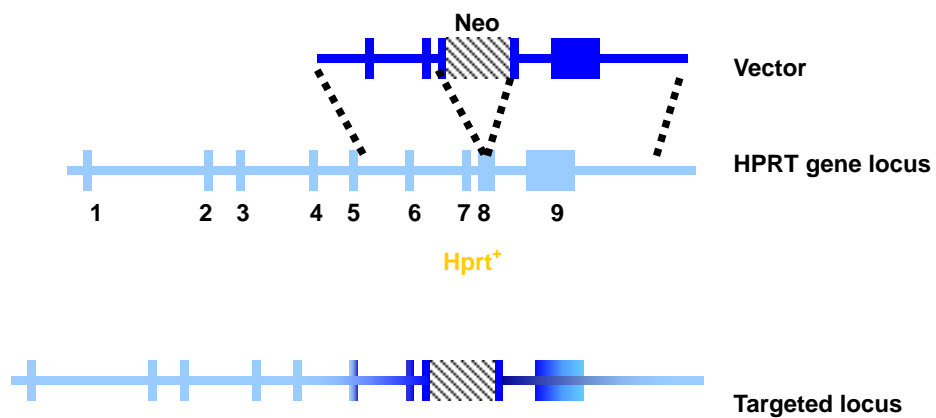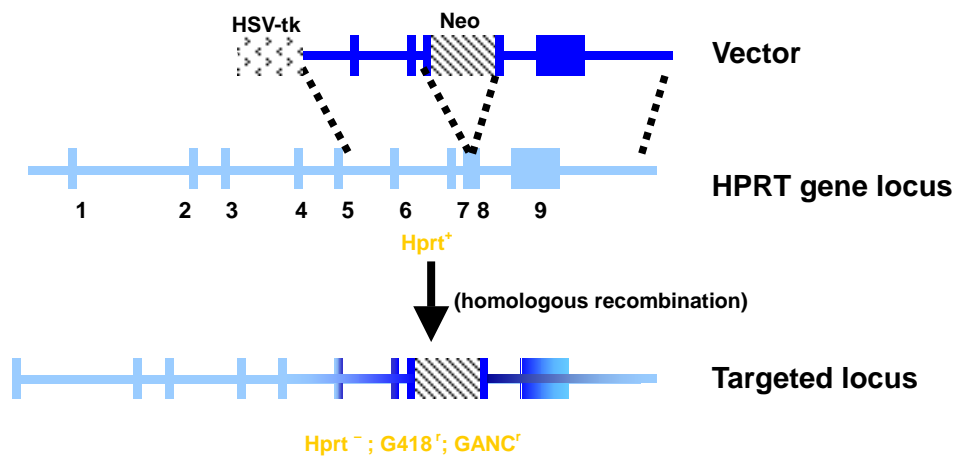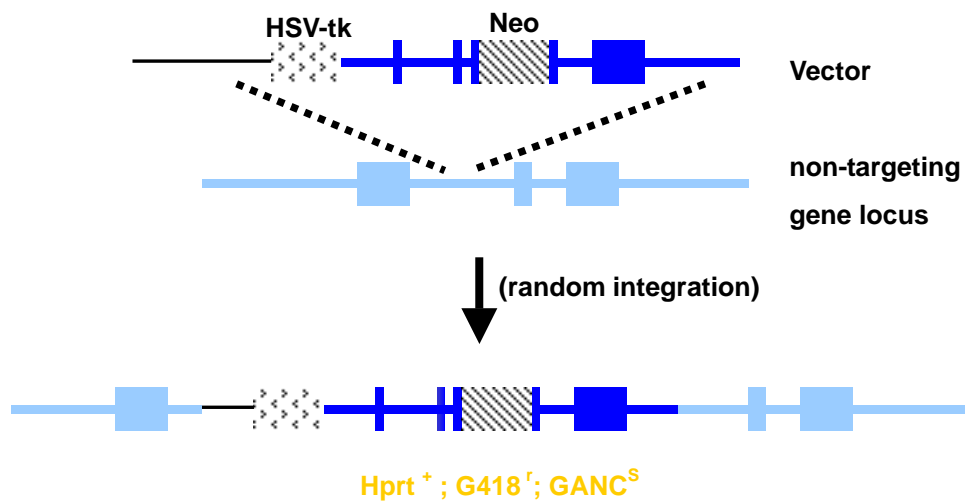
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C. Replacement Vector (enrichment strategy)

HSV-tk   Neo   **Vector**

HPRT gene locus

1   2   3   4   5   6   7   8   9

Hprt[+]

(homologous recombination)

Targeted locus

Hprt[−] ; G418[r] ; GANC[r]

Nature 336: 348-352, 1988.

HSV-tk   Neo   **Vector**

non-targeting

gene locus

(random integration)

Hprt[+] ; G418[r] ; GANC[s]

Nature 336: 348-352, 1988.

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2. Frequency of Homologous Recombination

Homology arm : $10^{-6} \sim 10^{-7}$

Homology arm + Pos. selector : $10^{-4} \sim 10^{-5}$

Homology arm + Pos. + Neg. selector : $10^{-2} \sim 10^{-3}$

That's why a gene targeting construct need **Homology arm** + **Positive selector** + **Negative selector.**

3. Critical factors on making a good gene targeting construct

A. Orientation of critical elements on gene targeting construct

B. Isogenic DNA

Different ES cell line comes from different mouse strain. Many ES cell lines were isolated from 129 mouse substrain (Mammalian Genome 8, 390-3, 1997). The DNA sequences of homology arms must be identical with the ES cell line that will be used.

C. Homology arms

Long arm + short arm < 10kb

Long arm :   4-8 kb

Short arm :   1-3 kb

Both of Long arm and short arm must be unique sequences

D. Replacement cassette versus replaced fragment

The lengths between replacement cassette and replaced fragment should be as match as possible.
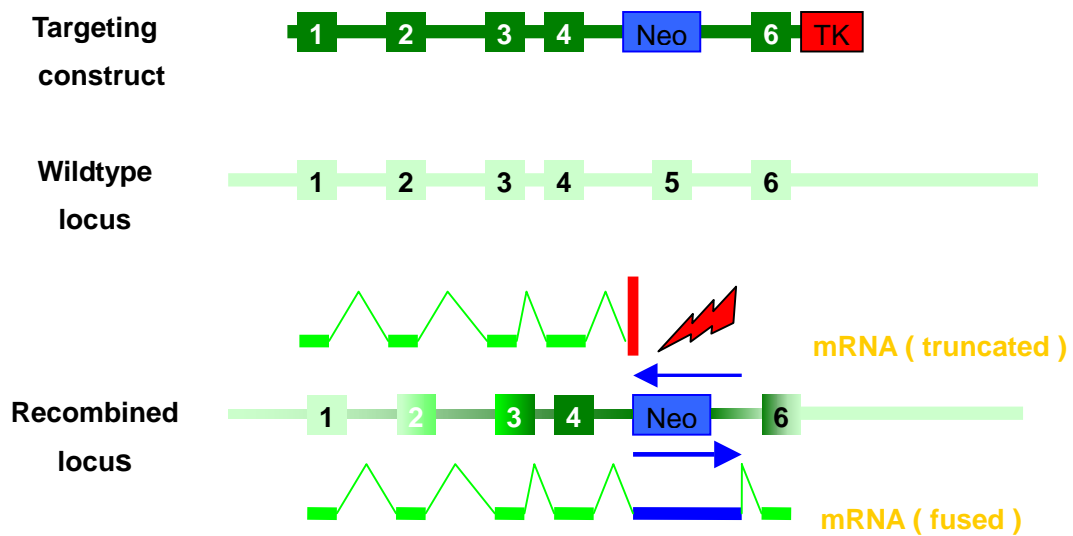
E. Linearization

Long arm exposure: increasing the rate of homologous recombination

Protect negative selector: vector sequence will protect negative selector from degradation

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F. Reciprocal interference between positive selector and targeted gene

G. Reciprocal interference between positive selector and targeted gene