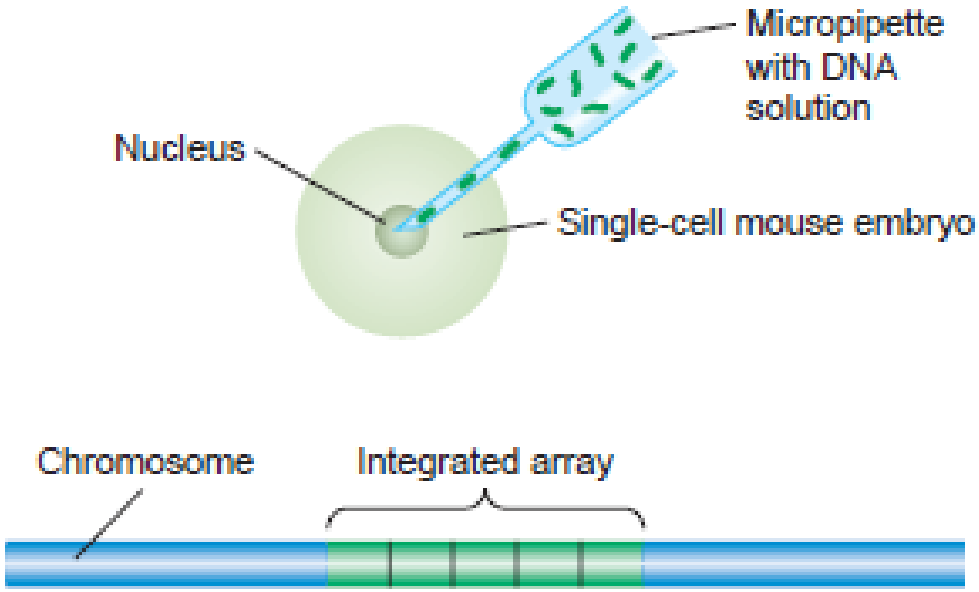


At some later stage, the transgene becomes integrated into the chromosomes of random nuclei.

The technique does give rise to some problems: (1) the expression pattern of the randomly inserted genes may be abnormal (called a **position effect)** because **the local chromosome environment** lacks the gene's normal regulatory sequences, and

(2) DNA rearrangements can occur inside the multicopy arrays (in essence, mutating the sequences). Nonetheless, this technique is much more efficient and less laborious than gene targeting.

Gene targeting in the mouse is carried out in cultured embryonic stem cells (ES cells).

In general, stem cells are undifferentiated cells in a given tissue or organ that divide asymmetrically to produce a progeny stem cell and a cell that will differentiate into a terminal cell type.

ES cells are special stem cells that can differentiate to form any cell type in the body—including, most importantly, the germ line.

To illustrate the process of gene targeting, we look at how it achieves one of its typical outcomes—namely, the substitution of an inactive gene for the normal gene.

Such a targeted inactivation is called a **gene knockout.**

First, a cloned, disrupted gene that is inactive is targeted to replace the functioning gene in a culture of ES cells, producing ES cells containing a gene knockout

To make knockout mice, design a construct which contains a stop codon or other mutation predicted to disable your gene of interest, and get it to replace the endogenous copy of the gene by homologous recombination.

First transfect mouse embryonic stem (ES) cells with your KO construct. Because homologous recombination is rare, you need a positive selection marker (usually G418 resistance conferred by a neo cassette under its own promoter) in order to select for cells that have taken up your DNA.

However, random integration will be more common than homologous recombination and will also result in a copy of the neo cassette being integrated.

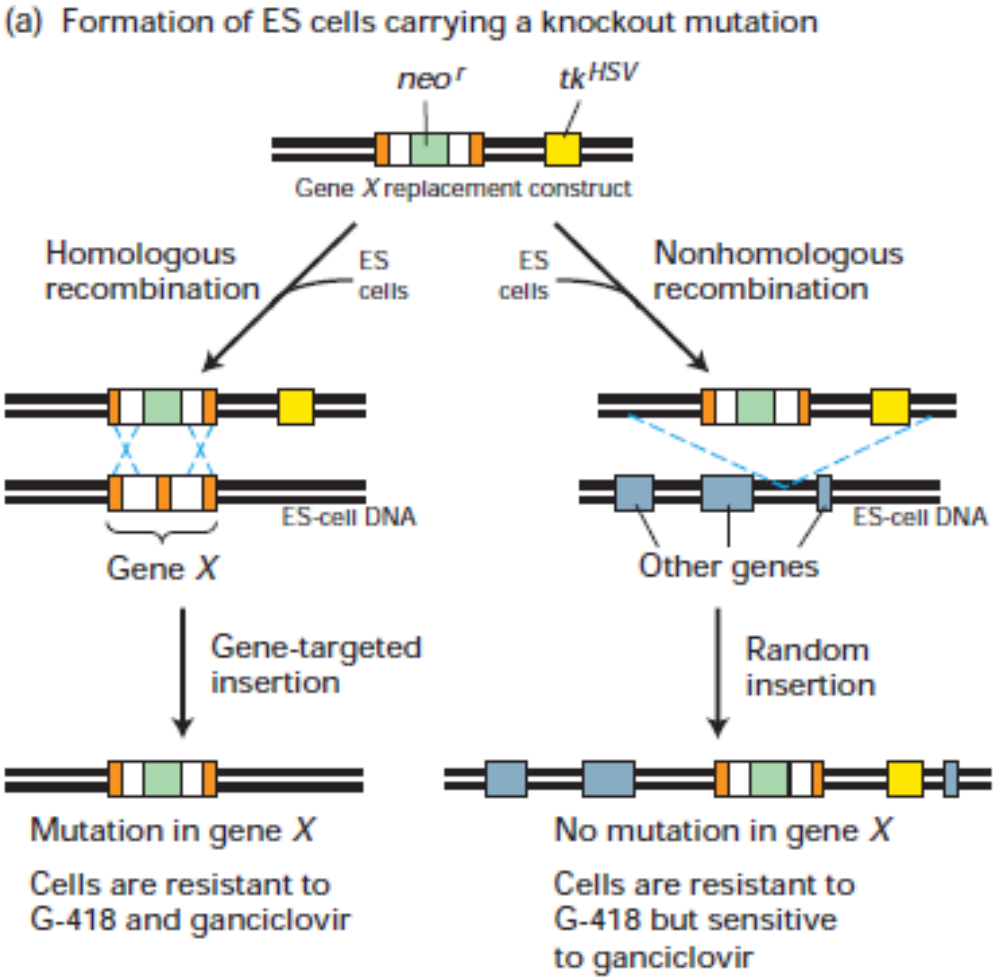
Therefore also need a negative selection marker which lies *outside* of the region of homology.

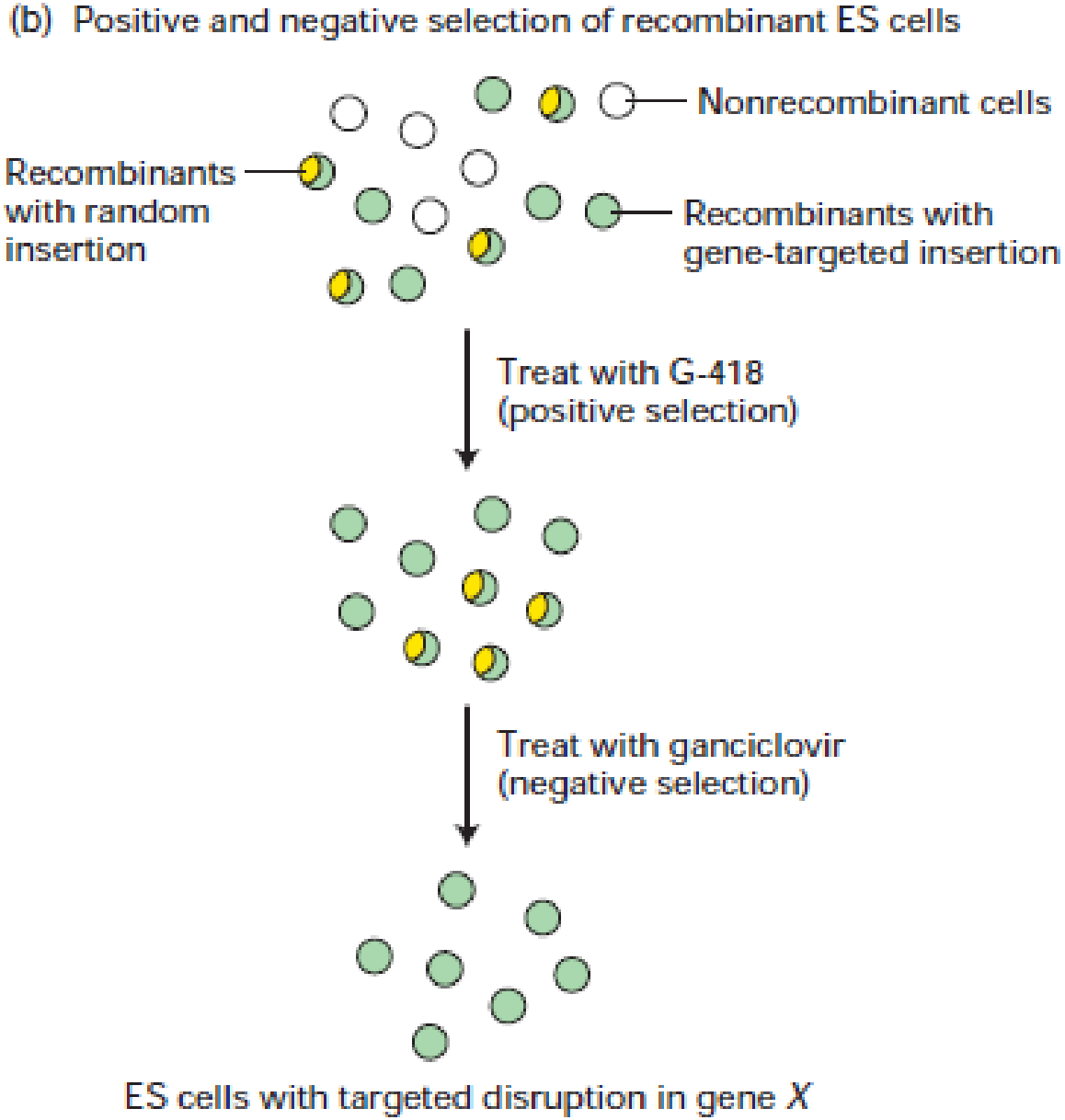
For this, usually use Herpes thymidine kinase (tk), a gene which confers sensitivity to gancyclovir.

Therefore you select ES cells first with G418 and then with gancyclovir.

After that, only cells which have the KO construct integrated into the targeted locus, replacing the endogenous gene.

Then inject these ES cells into a mouse blastocyst. Then transplant this blastocyst into a pseudopregnant mother.




(b) Positive and negative selection of recombinant ES cells
Nonrecombinant cells
Recombinants with random insertion
Recombinants with gene-targeted insertion
Treat with G-418 (positive selection)
Treat with ganciclovir (negative selection)
ES cells with targeted disruption in gene X