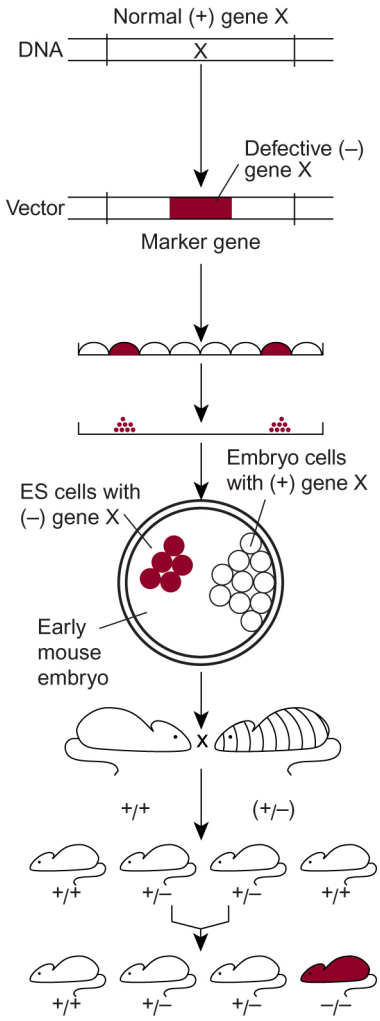
Strategy for generating conventional knockout mice



Isolate gene X and insert it into a short piece of DNA that can be easily manipulated (i.e., a vector). Inactivate the gene by inserting a marker gene that makes the cells resistant to certain antibiotics.

Transfer vector with (–) gene X into embryonic stem (ES) cells.

Grow ES cells in antibiotic-containing medium; only cells that have incorporated (–) gene X survive in this medium.

Inject ES cells with (–) gene X into early mouse embryos.

Transfer embryos into surrogate mothers for embryonic development; the resulting pups (i.e., chimeras) contain (+) gene X in some cells and (–) gene X in other cells. Mate chimeras with normal mice.

Identify pups that carry one (+) and one (–) copy of gene X and mate those animals with each other.

Analyze mouse pups; about 25 percent will have inherited the (–) gene from both parents and will completely lack the (+) gene (i.e, "knockout mice").

Source: Homanics, G.E., and Hiller-Sturmhofel, S. New genetic technologies in alcohol research. *Alcohol Health & Research World* 21(4):298–309, 1997.

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