

Gene Knockout

A **gene knockout** (abbreviation: **KO**) is a genetic technique in which one of an organism's genes is made inoperative ("knocked out" of the organism). However, KO can also refer to the gene that is knocked out or the organism that carries the gene knockout. **Knockout organisms** or simply **knockouts** are used to study gene function, usually by investigating the effect of gene loss. Researchers draw inferences from the difference between the knockout organism and normal individuals.

The KO technique is essentially the opposite of a gene knock-in. Knocking out two genes simultaneously in an organism is known as a **double knockout (DKO)**. Similarly the terms **triple knockout (TKO)** and **quadruple knockouts (QKO)** are used to describe three or four knocked out genes, respectively. However, one needs to distinguish between heterozygous and homozygous KOs. In the former, only one of two gene copies (alleles) is knocked out, in the latter both are knocked out.

Methods:

Homologous recombination

Traditionally, homologous recombination was the main method for causing a gene knockout. This method involves creating a DNA construct containing the desired mutation. For knockout purposes, this typically involves a drug resistance marker in place of the desired knockout gene. The construct will also contain a minimum of 2kb of homology to the target sequence. The construct can be delivered to stem cells either through microinjection or electroporation.

Site-specific nucleases

There are currently three methods in use that involve precisely targeting a DNA sequence in order to introduce a double-stranded break. Once this occurs, the cell's repair mechanisms will attempt to repair this double stranded break, often through non-homologous end joining (NHEJ), which involves directly ligating the two cut ends together.

Use:

Knockouts are primarily used to understand the role of a specific gene or DNA region by comparing the knockout organism to a wild type with a similar genetic background. Knockout organisms are also used as screening tools in the development of drugs, to target specific biological processes or deficiencies by using a specific knockout, or to understand the mechanism of action of a drug by using a library of knockout organisms spanning the entire genome, such as in *Saccharomyces cerevisiae*.