Knockout animal: A knockout animal is a laboratory animal in which researchers have inactivated or knocked out, an existing gene by replacing the gene or disrupting it with an artificial piece of DNA.

Since most of the knockout animals are developed using mice, we will discuss the production and uses of knockout animals with respect to mice.

Production of knockout mice:

Current methodology for production of knockout mice is discussed below.

Embryonic stem (ES) cells are harvested from early stage mice embryo. This is done four days after fertilization. ES cells are chosen for this purpose because they are pluripotent i.e. can grow into all the cell types in mice. This property of ES cells ensures that if a gene is knocked out then its effects can be observed in any tissue in an adult mouse.

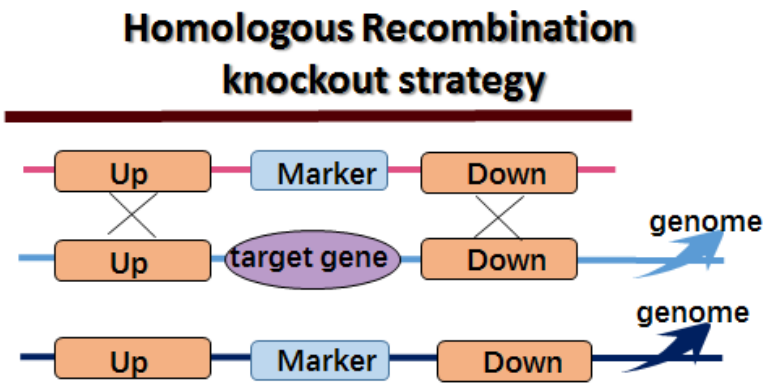
All these processes are carried out in vitro i.e. in cultured cells that have been grown in the laboratory conditions.

There are two strategies which can be used to produce knockout mice.

1. **Homologous recombination:** This is also called as gene targeting and a specific gene is manipulated in the nucleus of the ES cells. To achieve this, an artificial DNA which is homologous to the sequence of the gene is used. This is placed in a vector and on both the ends of the artificial gene, the vector contains sites that will promote recombination with the genome of the host cells.

   This recombination event replaces the correct copy of the gene with the artificial incorrect copy of the gene in the vector. Due to this now the genome has faulty copy of the gene which will not be expressed. This leads to absence of the function for the particular gene and animal is said to be knockout for the gene. Since this process involves use of recombination at precise locations, we call it site specific recombination.

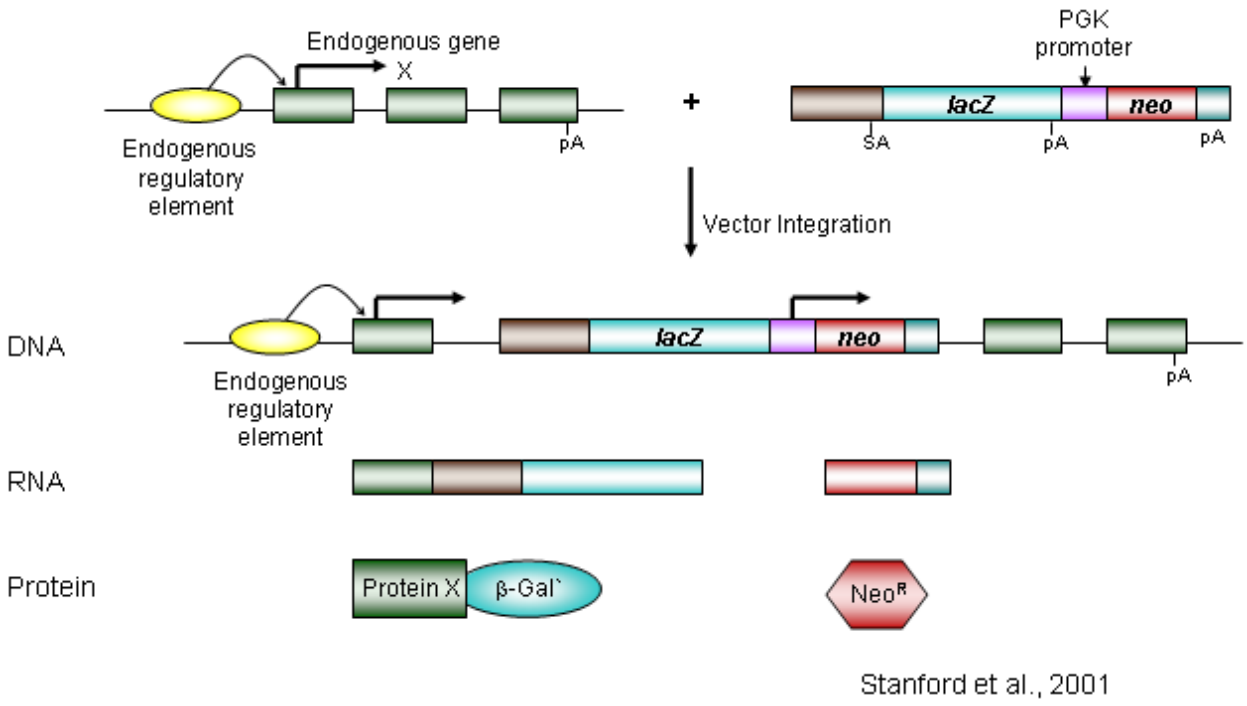
   The figure below is showing the use of homologous recombination for generation of the knockouts. In this figure, an active target gene is being replaced by an inactive marker sequence.



2. **Gene trapping:** In this method we perform manipulations in the ES cells but particular gene is not targeted. This process relies on randomness. The vector used here contains an artificial DNA which is designed to insert randomly into any gene in the host genome.

This inserted piece of artificial DNA prevents the RNA splicing of the gene after insertion. Thus, the gene is unable to produce functional protein and its function is knocked out.

The figure below is showing the use of gene trap method for inactivation of the gene.



Stanford et al., 2001

Uses of knockout mice:

Knockout mice are exclusively a research tool and are used extensively for genome related research. This is possible because we know that elimination of a gene's activity can provide understanding about its function/s.

Understanding these functions can be useful for

- gene annotation
- finding unknown functions of genes
- finding the role of gene in development of a disease
- finding the role of gene in protection from a disease
- role of gene in response against stimuli such as drugs etc.

Some examples of knockout mice:

- p53 knockout mice have inactive p53 gene and they are used to study effects of p53 mutation on the organism.
- Methuselah is a knockout mouse which is known for longevity and is used for research into it.
- Frantic is a knockout mouse which is used in the study of anxiety disorders.