What is CRISPR/Cas9?

- CRISPR, in simple terms, is part of a **bacterial anti-viral defence** system. It allows a bacterium to remember previous viral infections and to respond to these by chopping up the invading viral genome next time there is an infection. It also allows a bacterium to pass this crucial information to its daughters.
- Since 2013, scientists have exploited the **sequence specific DNA cleavage** activity to edit the genomes of multiple different species.

Why has this had such a big impact on science?

- CRISPR is much simpler and faster than previous methods for editing genomes
- Many genes can be targeted at the same time

What is CRISPR's impact on the 3Rs?

CRISPR is impacting on the 3Rs at a number of levels:

- **Replacement**:
  - Scientists can create more representative *in vitro* models of disease with much greater efficiency – opening up the use of culture systems that use primary (or early passage) human or mouse tissue
  - It is (almost) possible to work directly on patient cells without the need for introduction into animal models (*ex vivo* correction of disease)
- **Reduction**:
  - The *simplicity* of the design, *speed* of editing, and boost in *efficiency* mean disease models can be created either from…
    - direct pronuclear injection of multiple targeting constructs
    - efficient targeting of ES cells (avoiding traditional gene targeting which can take months/years for complex alleles)
- **Refinement**:
  - The flexibility to create more *representative* disease models means disease alleles are not drastically over-expressed using viral promoters, and gives confidence that discoveries made will better translate to the clinic
  - The ability to make multi-allelic animals much faster allows redesigning complex multi-allelic models, avoiding extensive breeding programmes, whole organism expression, and expression during development

In addition, this technology is therapeutically relevant and so great effort is being put into making it safe for human (and consequently animal) use.

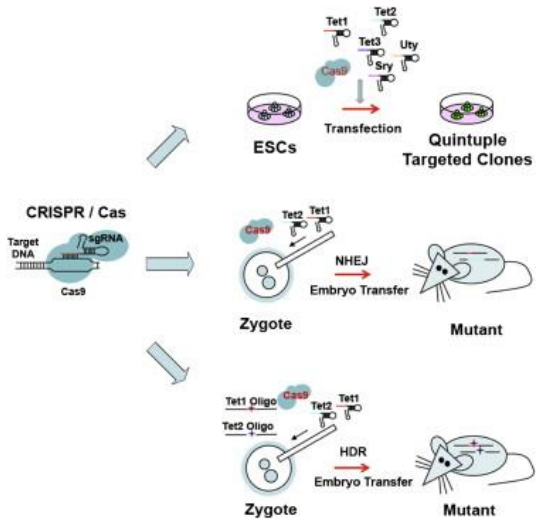

Fig 1. Multi-allelic models of disease can be efficiently created by many routes.

Wang *et al*. Cell 2013:153(04)910-918