

# CRISPR/CAS9 genome editing: potential for human modification

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THE UNIVERSITY  
*of* ADELAIDE

# ~~Genome~~ editing



Juliette Thomas, age 11

# CRISPR genome editing

GENOME EDITING: Targeted and precise modification of any organism's genome

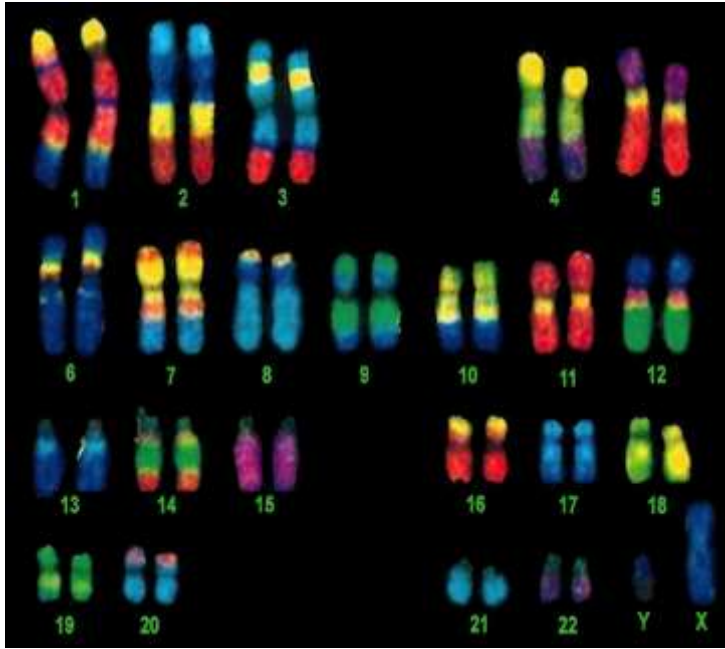
A revolution in biology, medicine and agriculture! (>9,000 CRISPR papers since 2012)

How could CRISPR technology be used for Human Modification?



<http://en.hdbuzz.net/038>

# The Genome



Every cell contains the blueprint for life.....3,000,000,000  
building blocks → 20,000 genes!

Each gene has a role → alter gene sequence or activity →  
change phenotype (properties/characteristics) of an individual

Add new genes → acquire new phenotypes

# What are desirable properties of (genomic) human modification technology?

1. Flexible (able to target any gene/genomic region in any cell type and insert new genes into the genome)
2. Multiplex (able to target multiple genes simultaneously)
3. Permanent or temporary modification
4. Readily/rapidly inhibited

Does CRISPR technology tick the boxes?

# CRISPR genome editing

“programmable” molecular scissors

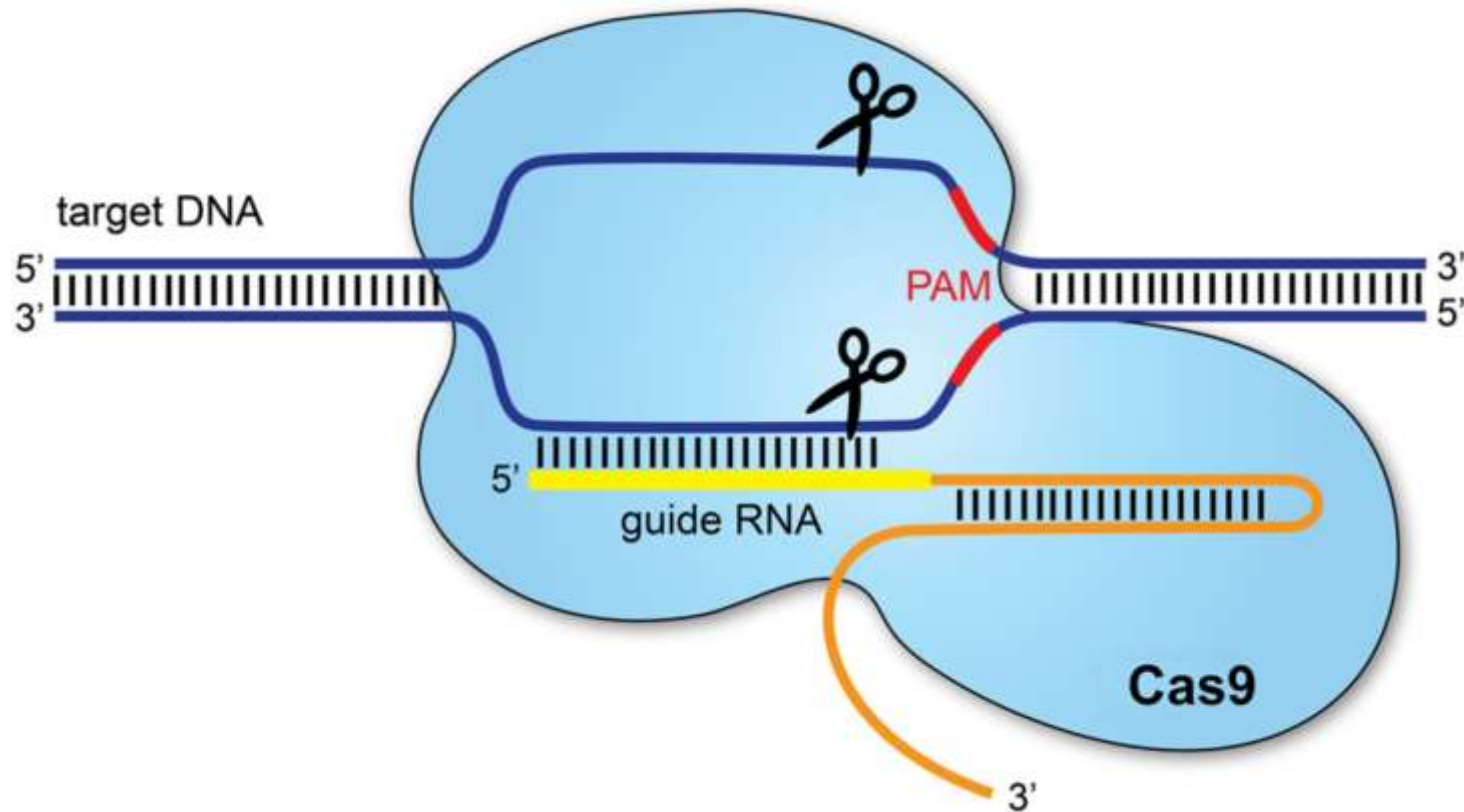
Make a cut/modify virtually any sequence in the genome →  
inactivate/alter/activate any gene

**CRISPR** = Clustered Regularly Interspaced  
Short Palindromic Repeats  
(from bacteria)



<http://en.hdbuzz.net/038>

# CRISPR/CAS9: Programmable genomic scissors



**CAS9** = endonuclease (DNA cutting enzyme)

**Guide RNA** = provides the “address code” for the cut

Repair of the DNA cut → change DNA sequence → altered gene function (or new gene added)

# CRISPR in action

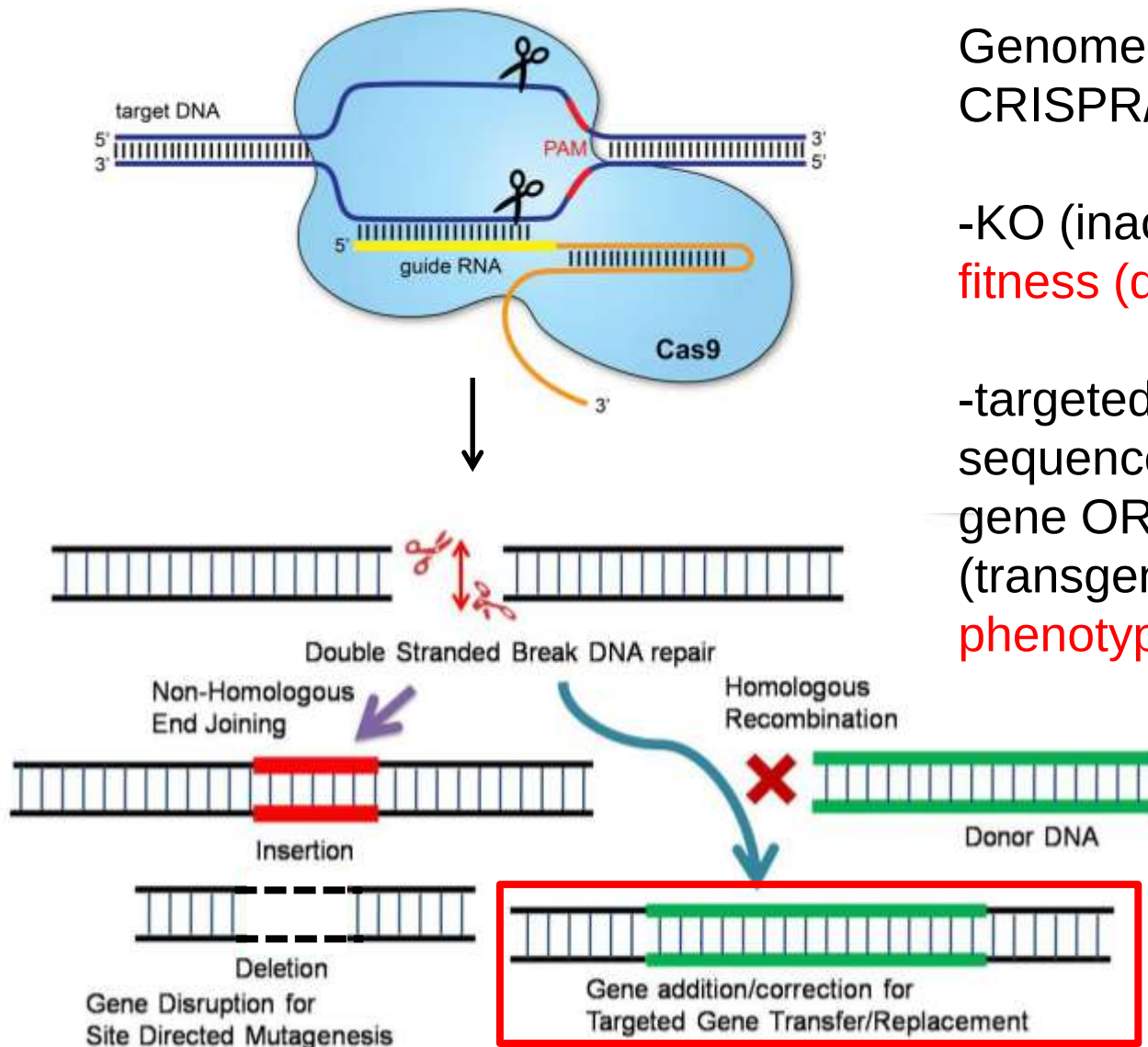


# Vast array of genomes have been modified by CRISPR/CAS9



Hundreds of genes targeted in >50 species (including humans) → CRISPR/Cas activity not limited by species or cell type.

# CRISPR-mediated KO and transgenesis



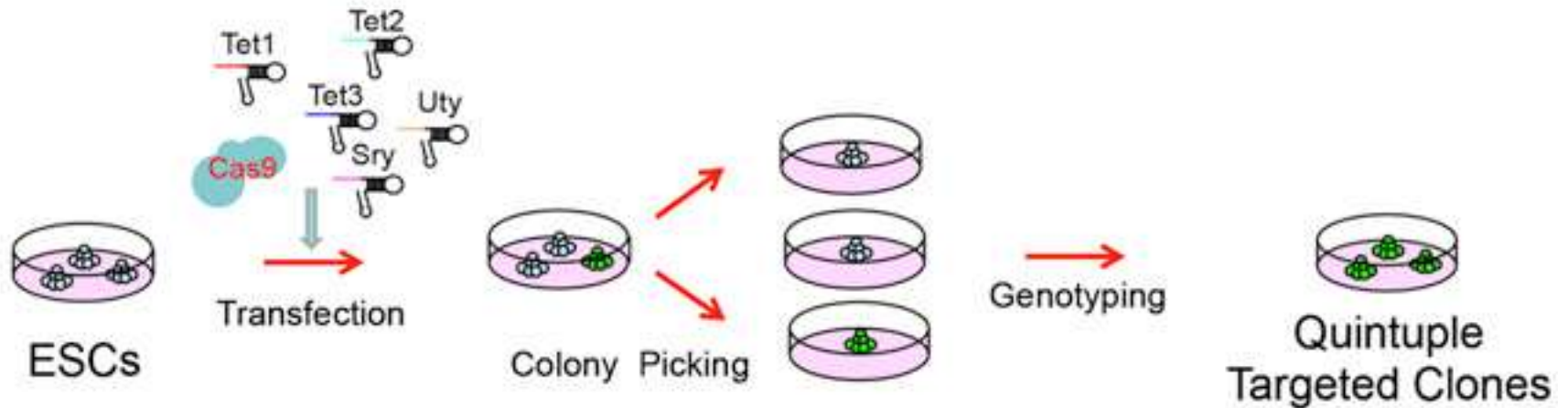
Genome modifications using CRISPR/CAS9 include:

-KO (inactivation) → **loss of fitness (disease state)**

-targeted insertion of new sequences (modify endogenous gene OR add new gene (transgenesis)) → **new phenotype**

# Simultaneous inactivation of multiple genes (multiplexing)

## A Multiple Gene targeting in ES cells



Many other examples...

## One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering

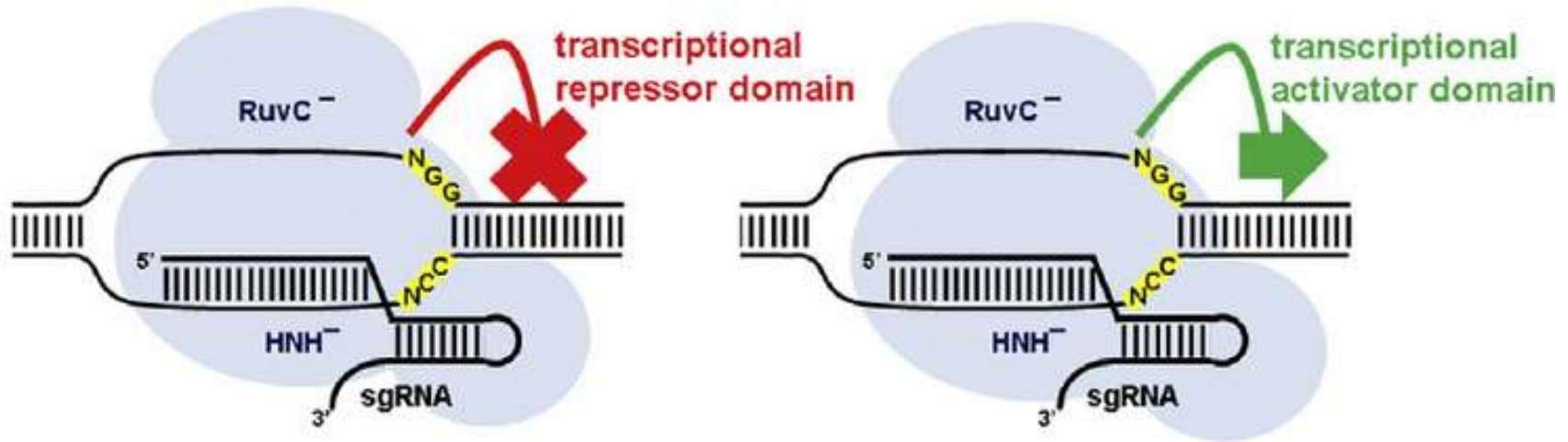
Haoyi Wang,<sup>1,6</sup> Hui Yang,<sup>1,6</sup> Chikdu S. Shivalila,<sup>1,2,6</sup> Meelad M. Dawlaty,<sup>1</sup> Albert W. Cheng,<sup>1,3</sup> Feng Zhang,<sup>4,5</sup> and Rudolf Jaenisch<sup>1,3,4</sup> Cell (2013)

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Does CRISPR technology tick the boxes?

# CRISPR/CAS9 technology can be used to *transiently* activate/repress gene activity



Uses a “dead” CAS9 protein that does not cut DNA but retains gRNA binding activity

CAS9 modified to contain “**Repressor**” or “**Transactivator**” activity  
→ transient alteration of gene activity (up or down)

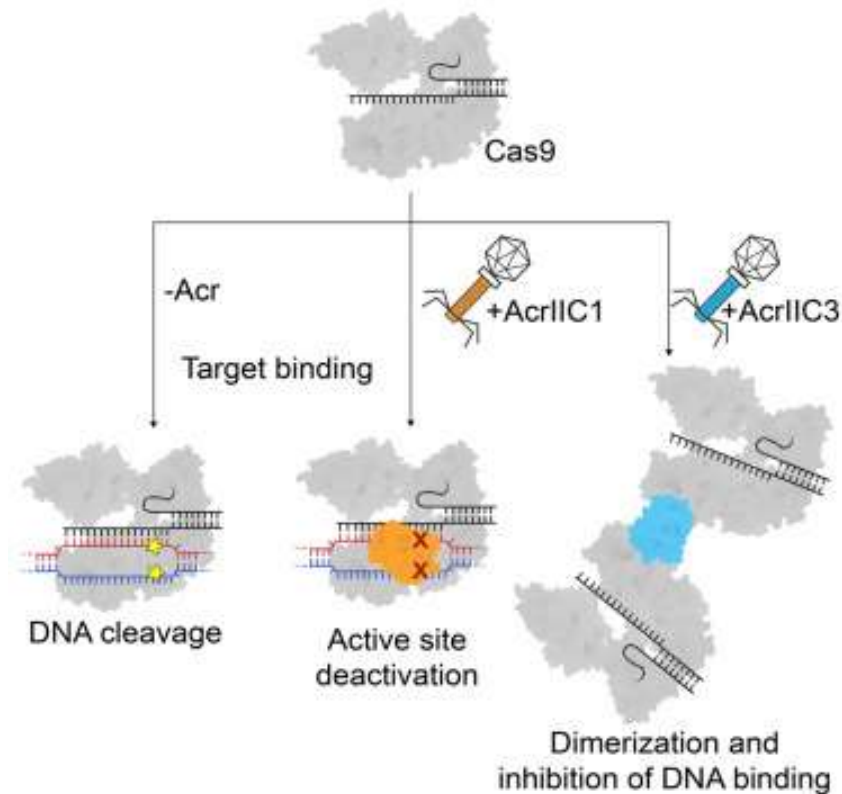
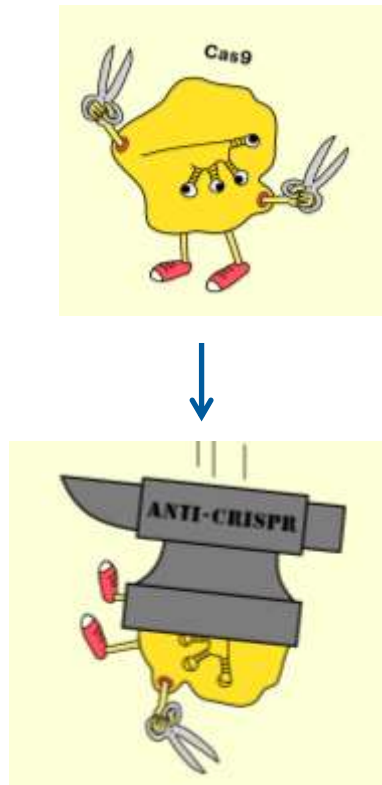
**Does not alter DNA sequence (ie. not a permanent modification)**

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Does CRISPR technology tick the boxes?

# Inhibition of Cas9 activity using small proteins



Small protein (from phage) interferes with CAS9 activity to prevent binding at target site

May be useful for rapid inhibition of CRISPR/Cas9-induced phenotypic change

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Does CRISPR technology tick the boxes?

Yes, promising technology, but there are issues...

## Barriers to use of CRISPR technology for human modification (in 2040)

## Societal – Ethics of CRISPR modification (designer babies, defense)

## Safety – Unintended consequences, off-target modifications/binding

Technical

- Delivery to target site (major issue)
- Efficiency of generating modification (particularly inserting genes)
- limitations of PAM requirement (disappearing)

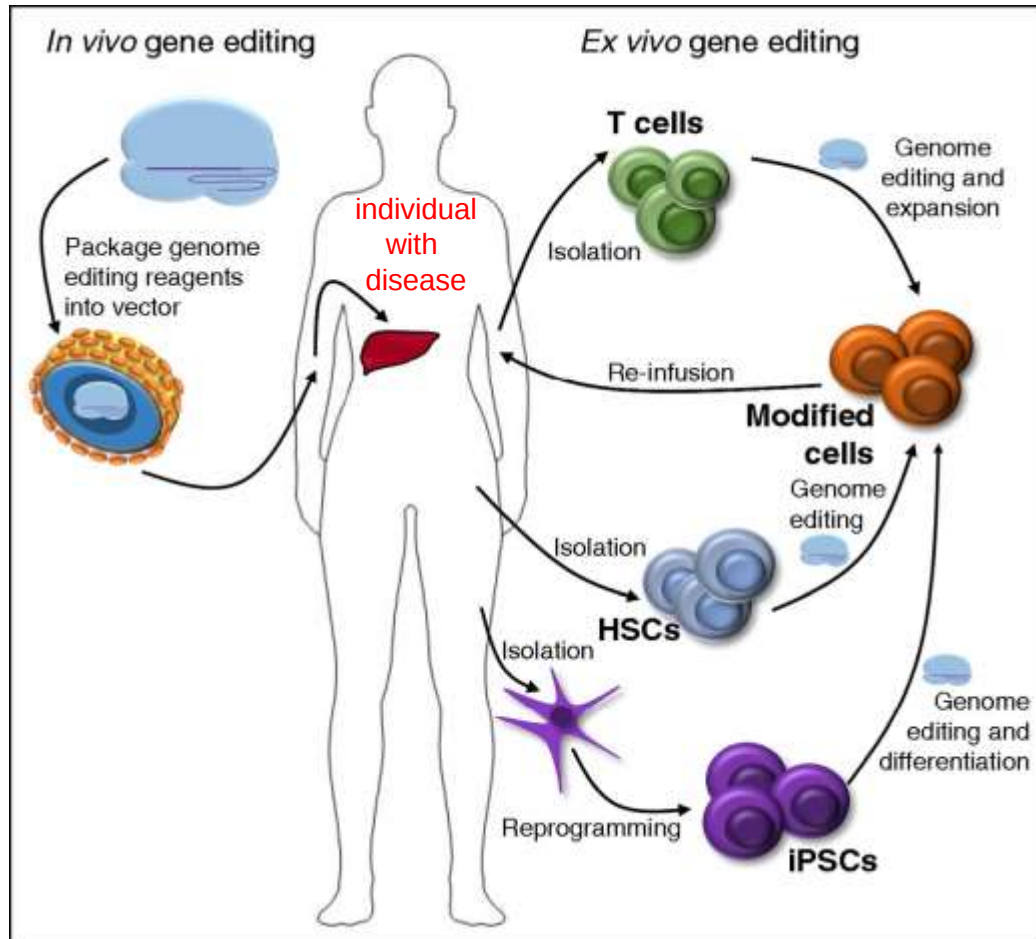
Biological

- what genes should be modified or inserted to generate the intended phenotype (vision/muscle function/metabolism)
- crossing the blood brain barrier

We can expect progress in all of the above...

# Using CRISPR/Cas9 for gene therapy

Use CRISPR gene editing to *correct* a disease-causing mutation (**human modification**)



Actively developed for a host of genetic diseases of the:

- liver
- eye
- muscle
- blood
- and others...

We have CSIRO/UA/SAHMRI Synthetic Biology Fellowship for development of DMD CRISPR therapy

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