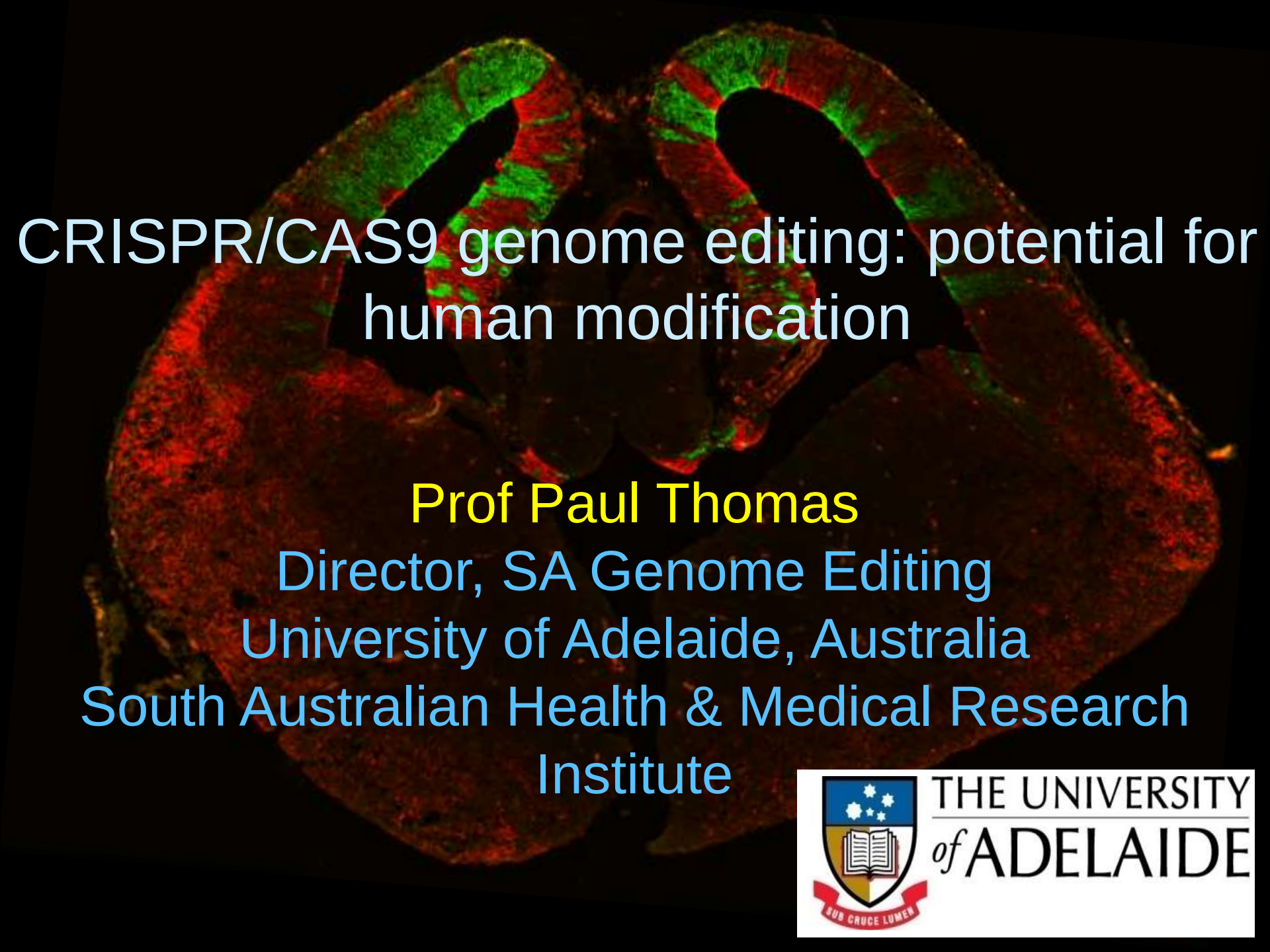

CRISPR/CAS9 genome editing: potential for human modification

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THE UNIVERSITY of ADELAIDE
SUB CRUCE LUMEN

Genome editing

Juliette Thomas, age 11

CRISPR genome editing

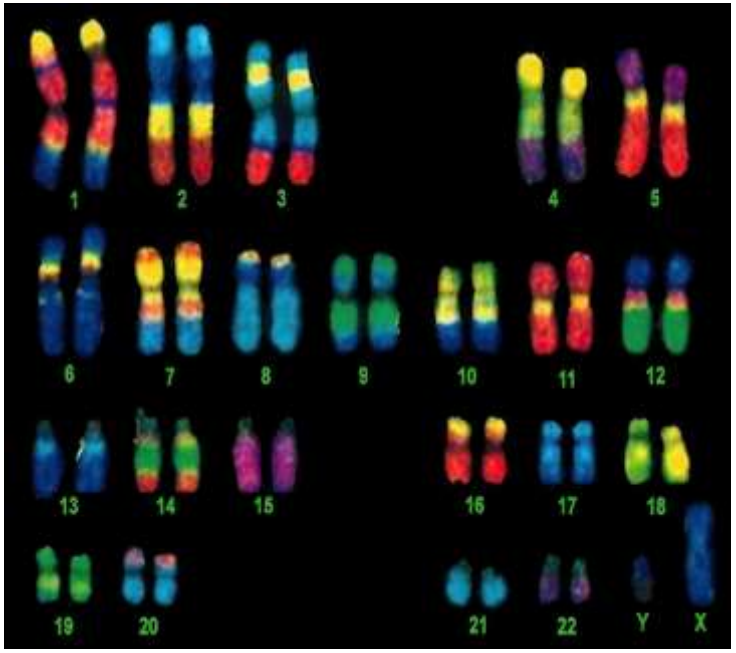
GENOME EDITING: Targeted and precise modification of any organism's genome

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

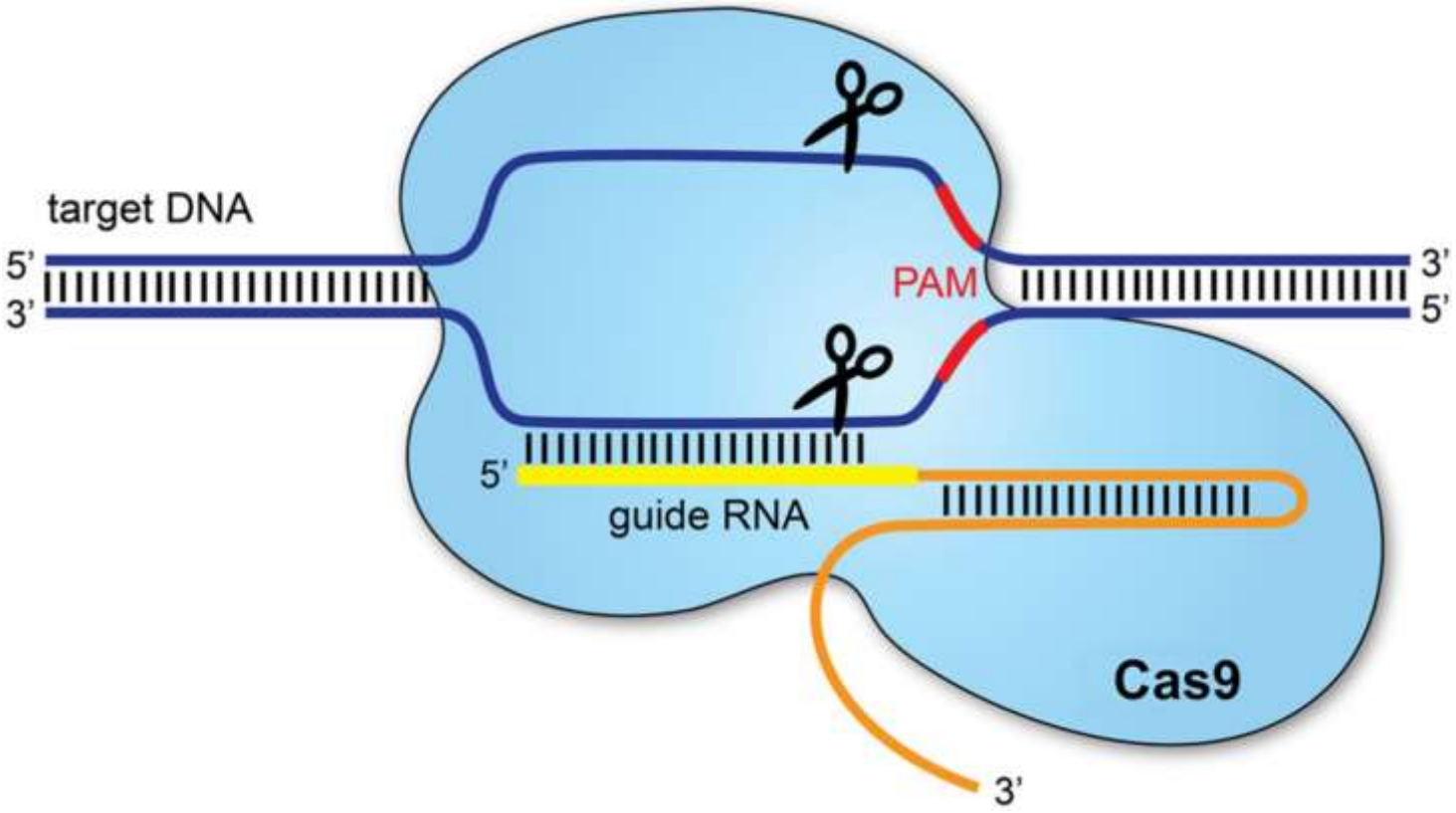
"programmable" molecular scissors

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

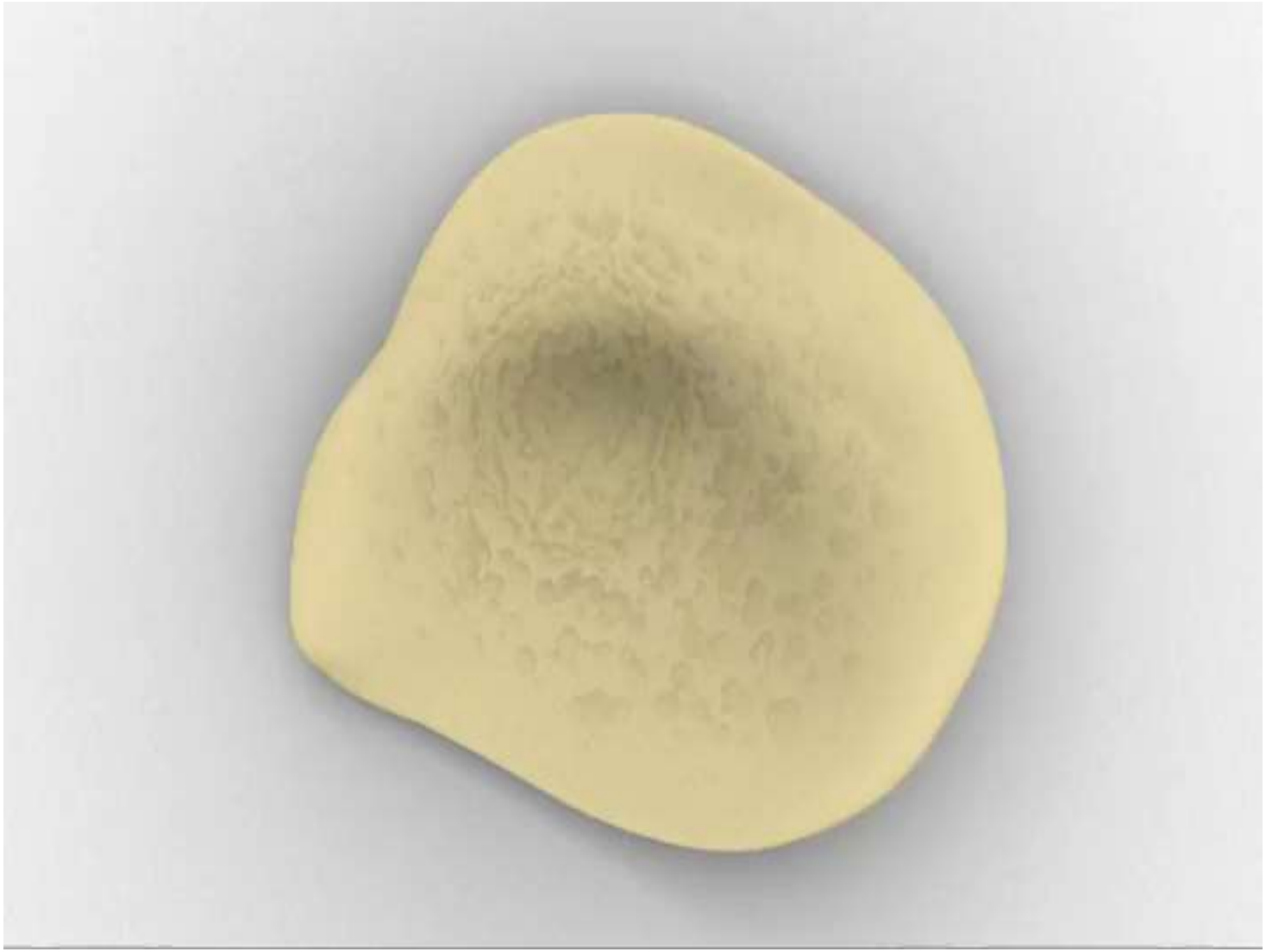
CRISPR/CAS9: Programmable genomic scissors



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

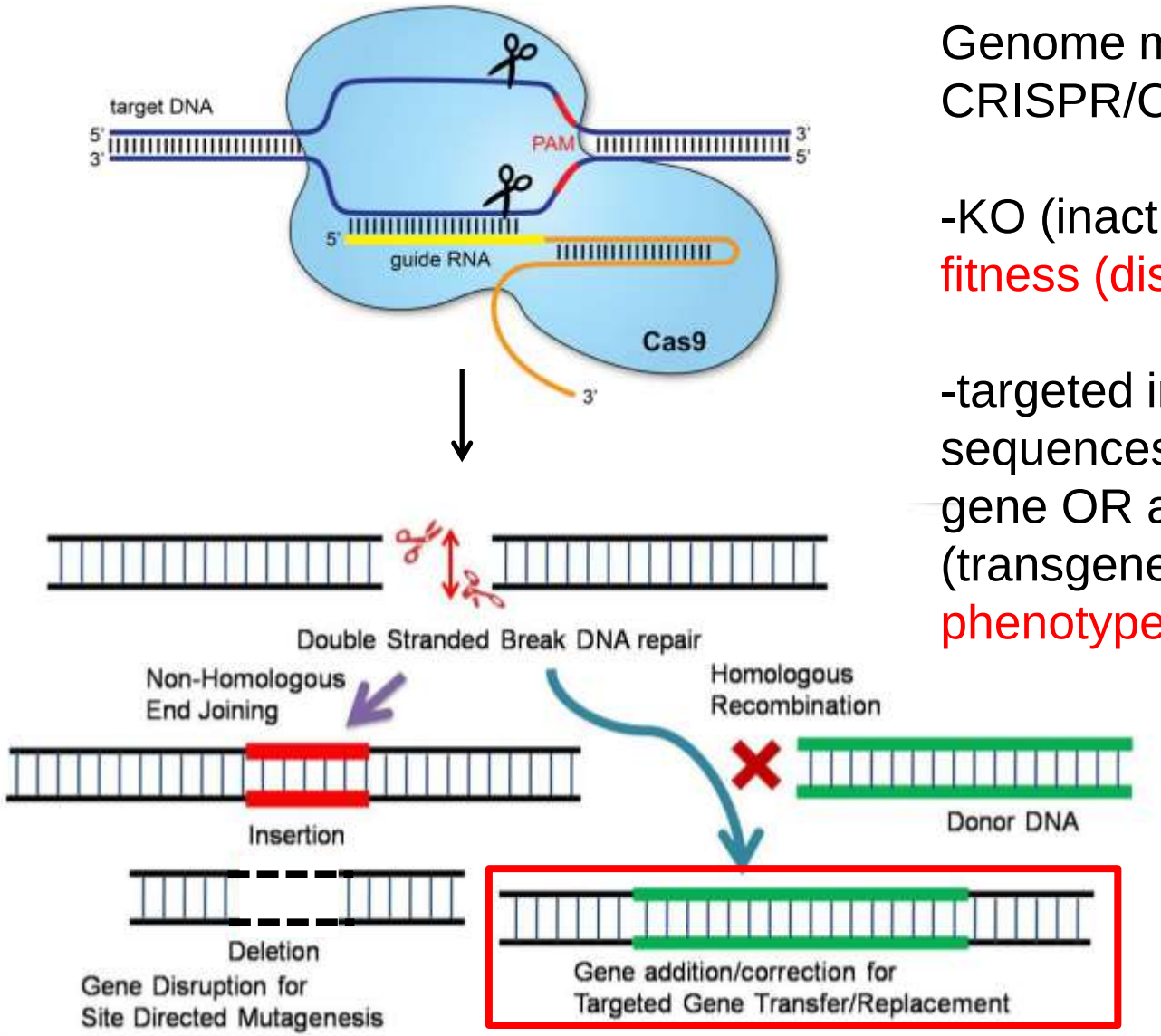


http://www.independent.co.uk/news/science/indyplus-video-crispr-technique-8925604.html

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

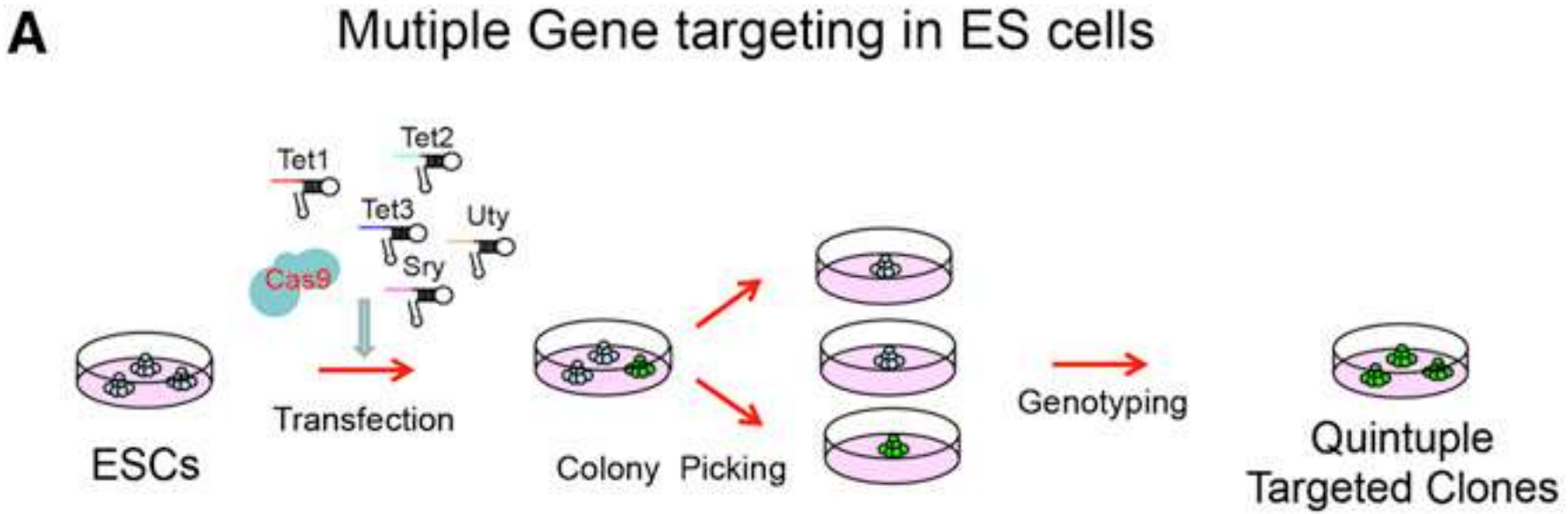


Genome modifications using CRISPR/CAS9 include:

-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)



Many other examples…

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

Haoyi Wang,[1,6] Hui Yang,[1,6] Chikdu S. Shivalila,[1,2,6] Meelad M. Dawlaty,[1] Albert W. Cheng,[1,3] Feng Zhang,[4,5] and Rudolf Jaenisch[1,3,*]

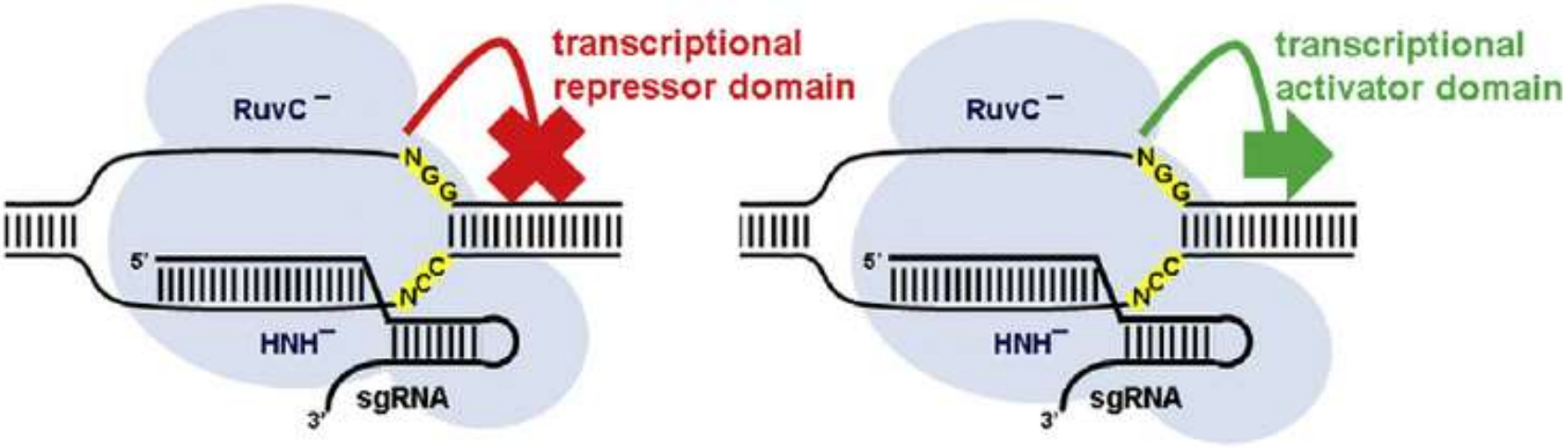
Cell (2013)

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

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

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)

Bortesi and Fischer (2014) Biotech Adv.

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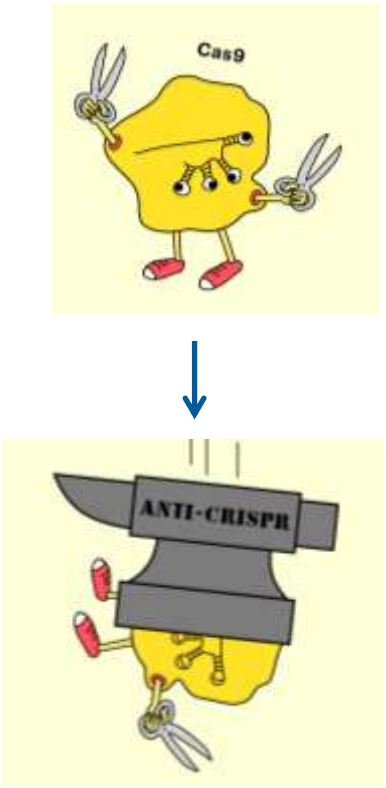
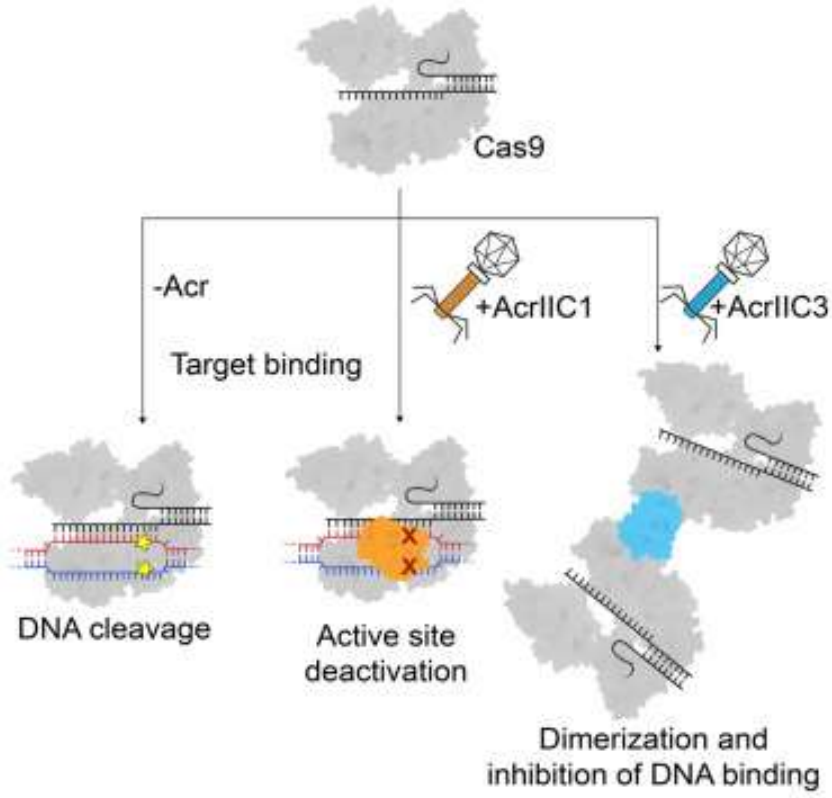
Does CRISPR technology tick the boxes?

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

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

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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

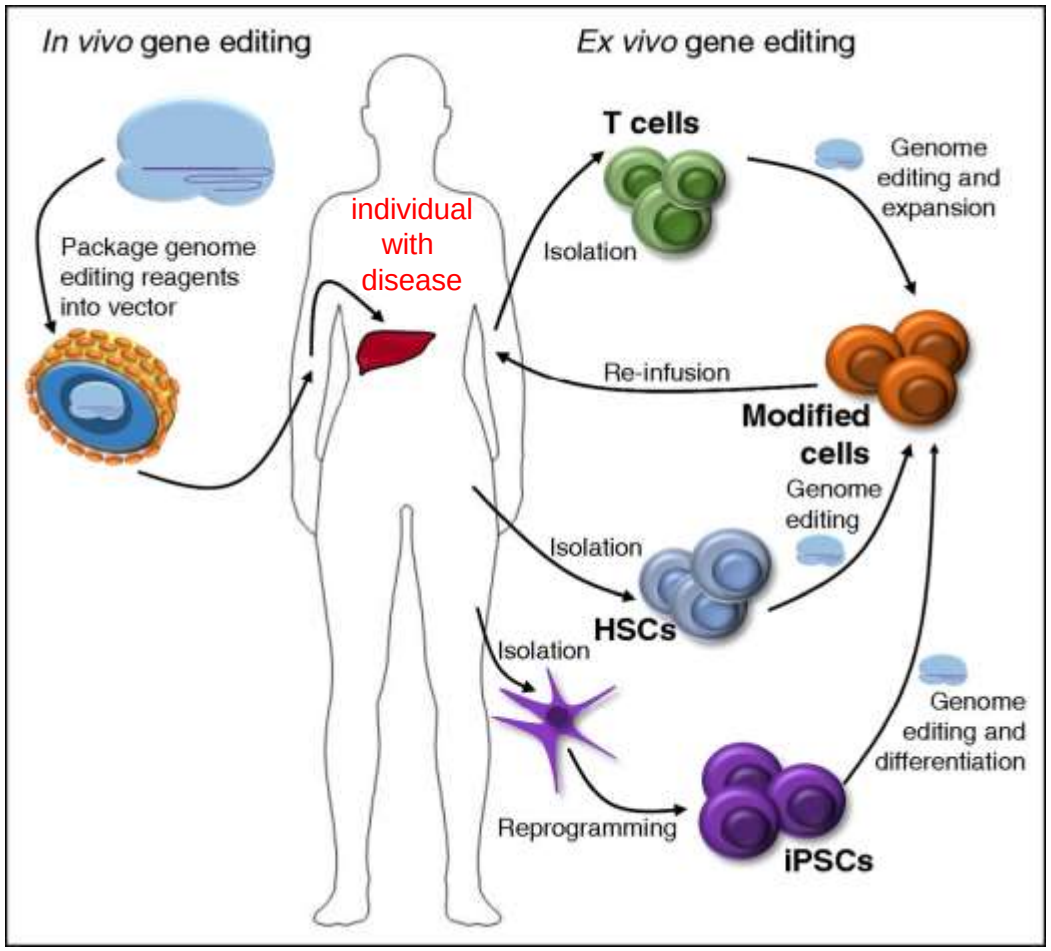
Technical
- -Delivery to target site (major issue)
- -Efficiency of generating modification (particularly inserting genes)
- -limitations of PAM requirement (disappearing)

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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SAHMRI South Australian Health & Medical Research Institute