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Editing Stem Cells with CRISPR Technology

Footprint-Free Gene Editing Using CRISPR/Cas9 and Single-Cell Cloning of Edited Human iPS Cells

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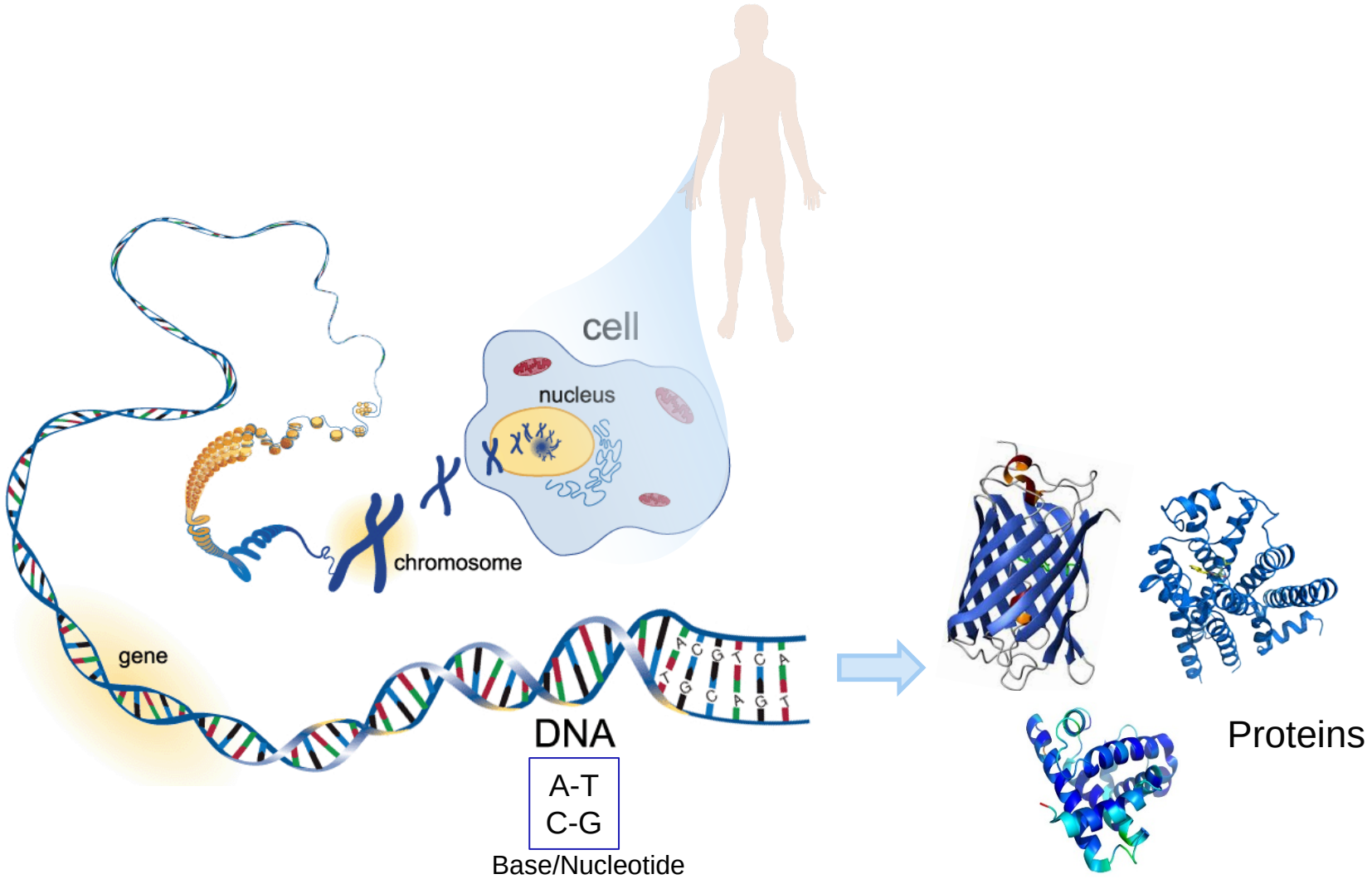
Outline

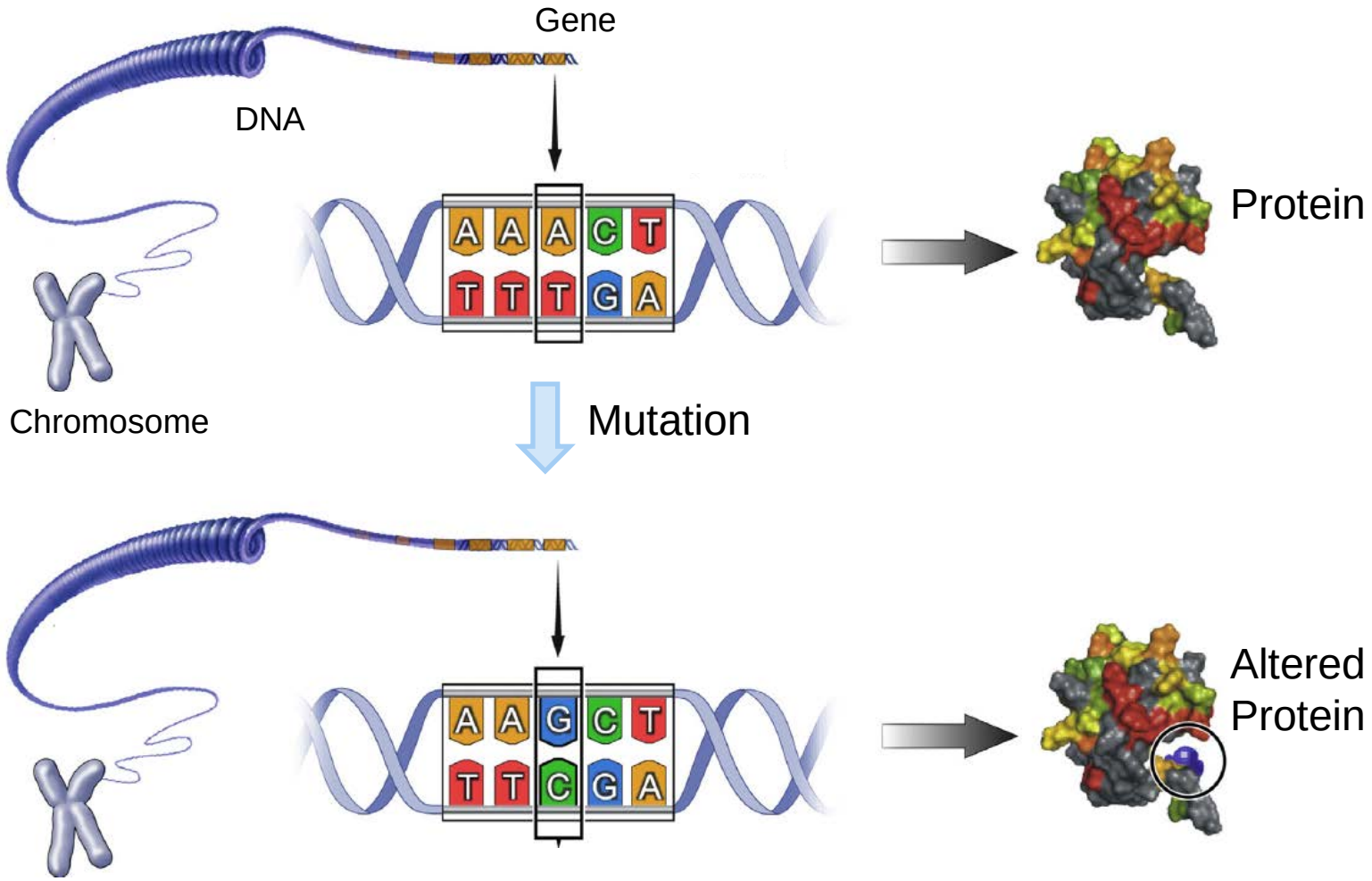
- Genome Editing
  - Importance of genome editing
  - Evolution of genome editing tools
- CRISPR/Cas9 Technology
  - Characteristics
  - Applications
- Human induced Pluripotent Stem Cells (hiPS cells)
  - Utility in disease modelling
  - Challenges of editing hiPS cells
- Workflow to Generate Edited hiPS Cell Clones
- Conclusions

Importance of genome editing
The genome encodes proteins



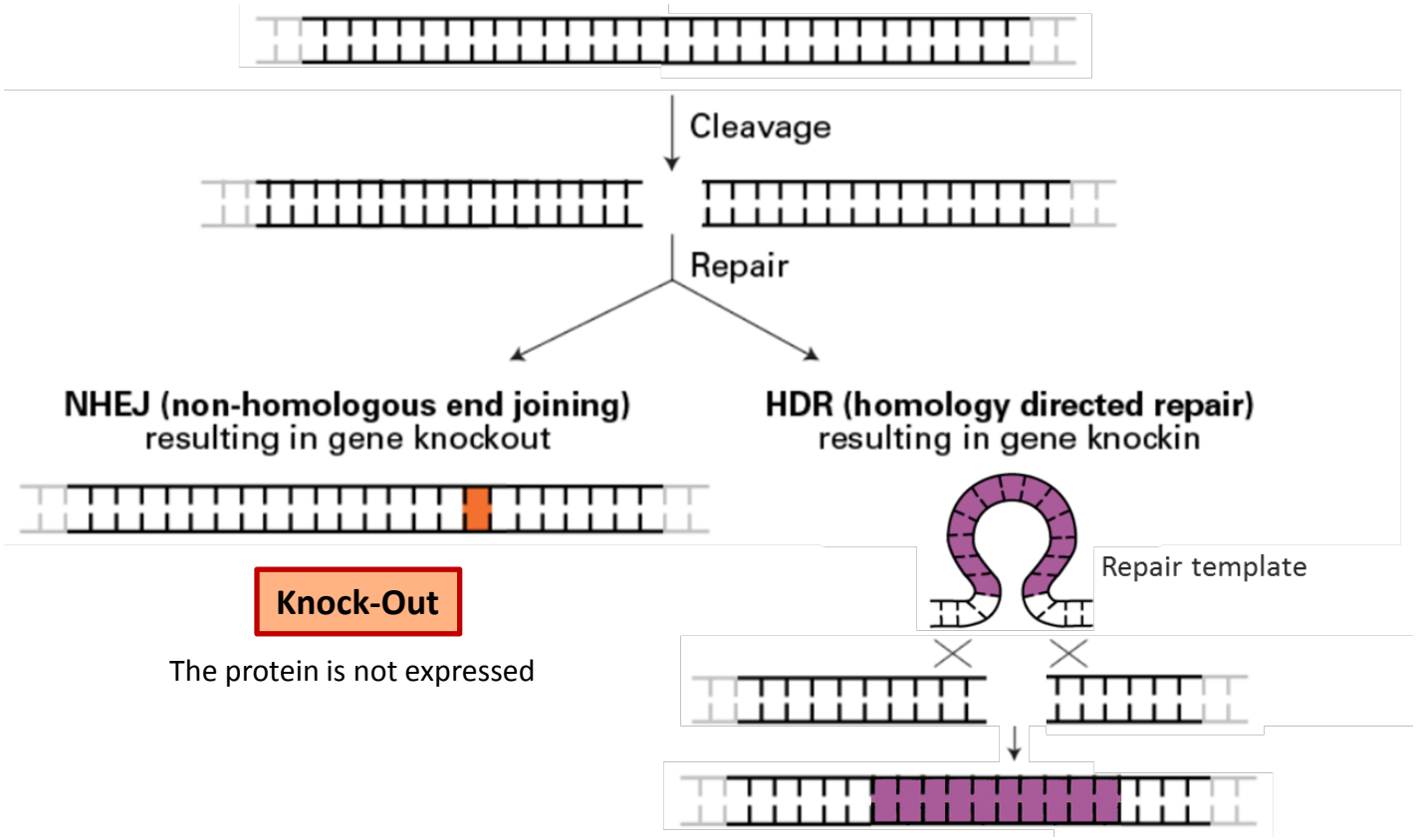
Adapted from https://ratedrtech.wordpress.com/

Importance of genome editing
Consequences of DNA mutations



Camp et al, *J Acad Nutr Diet*. **2**, 299–312 (2014)

How does genome editing work?



Knock-Out

The protein is not expressed

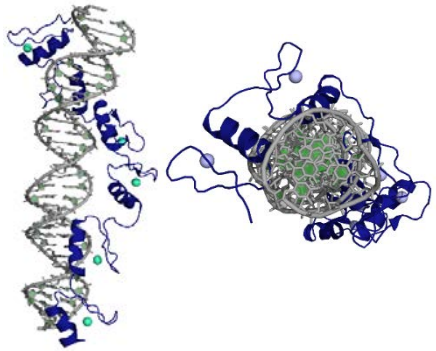
Knock-In

- Tag a protein
- Correcting a mutation

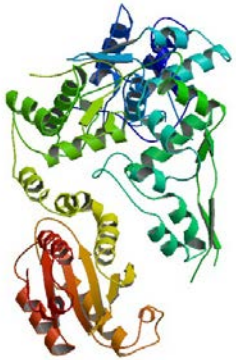
Genome editing technologies
Zinc Finger Nucleases and TALENs

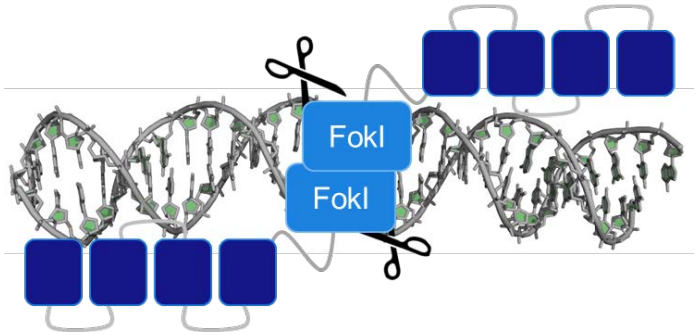
DNA target site recognition (DNA binding domains) + DNA cleavage → Genome editing tools

Zinc Fingers

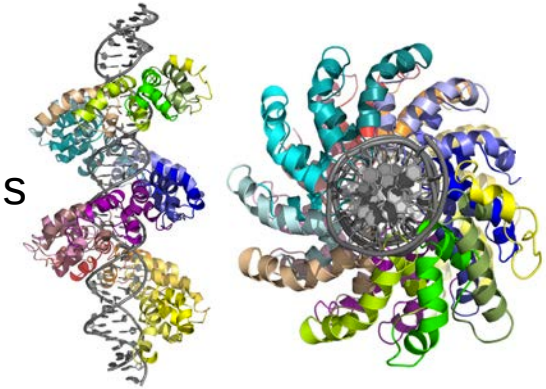


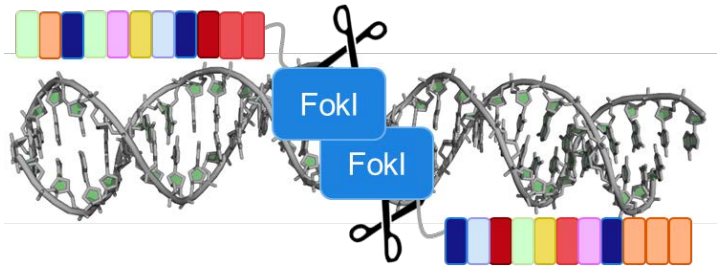
FokI nuclease





TALENs



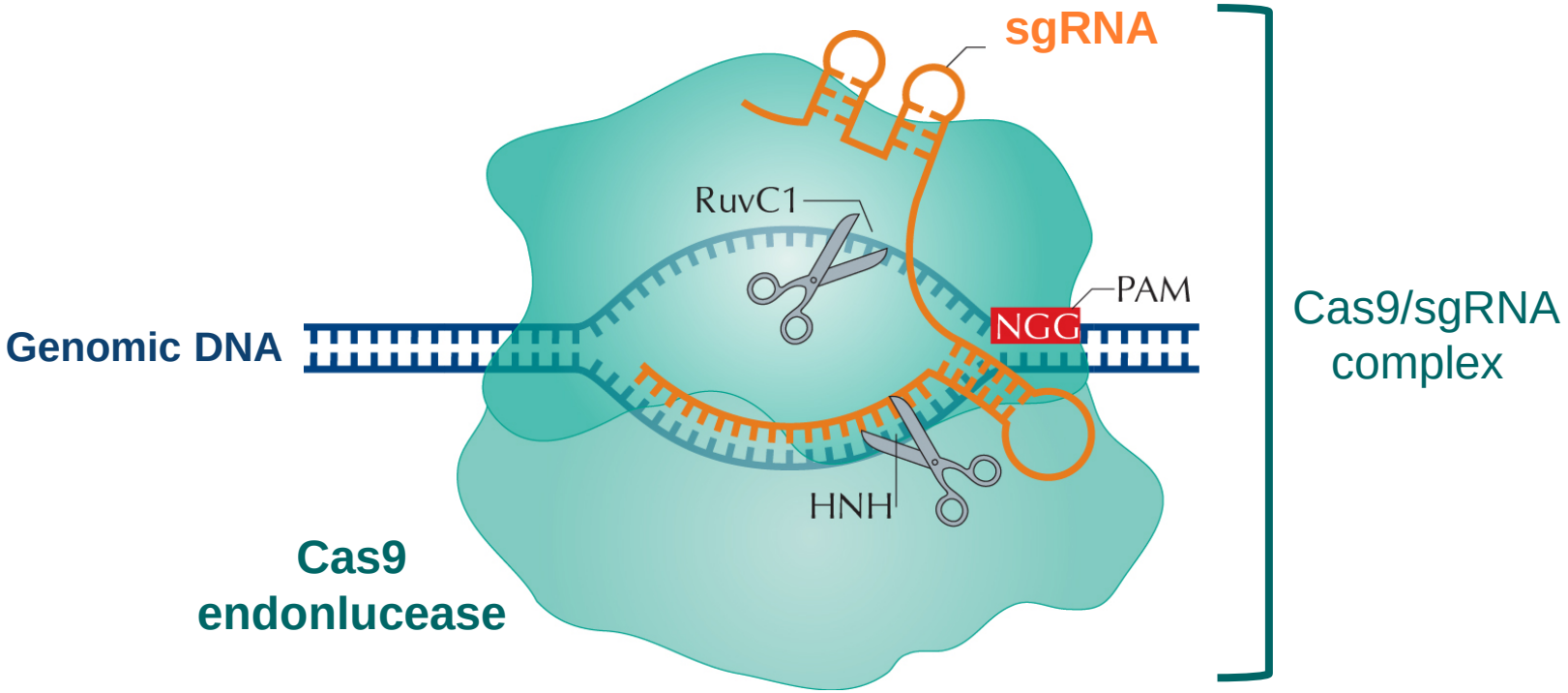


Mak et al. *Science* **335**, 716–19 (2012)

Genome editing technologies

CRISPR-Cas9: bacterial mechanism of self-defense repurposed as a genome editing tool

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

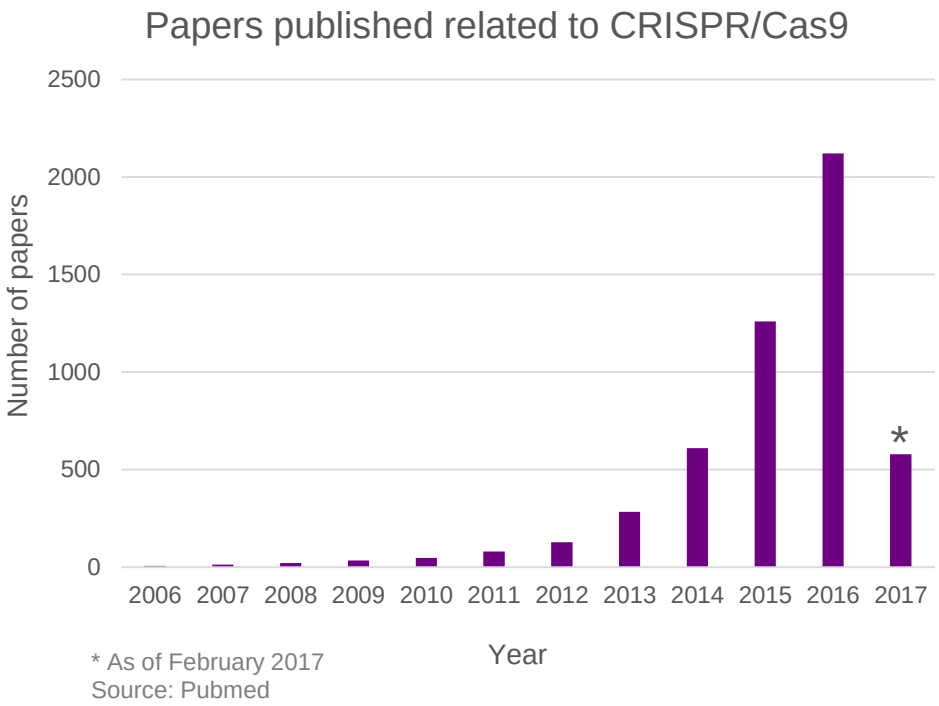


DNA target site recognition + DNA cleavage → Genome editing tool

sgRNA **Cas9 protein**

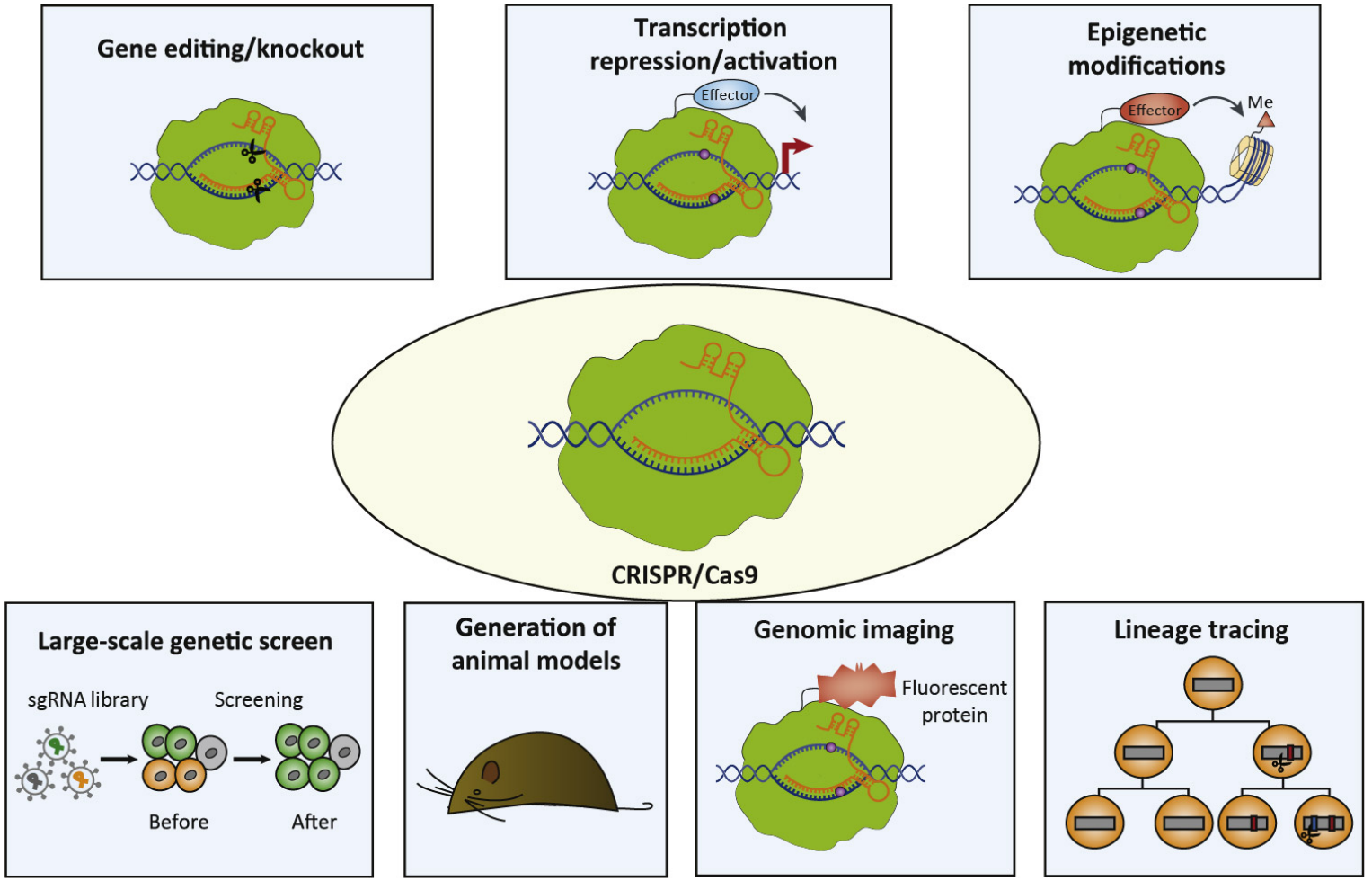
Mali et al. *Nat Methods* **10**, 957–963 (2013)
Dominguez et al. *Nat Rev Mol Cell Biol*. **17**, 5–15 (2016)

CRISPR/Cas9: a revolution in genome editing



* As of February 2017
Source: Pubmed

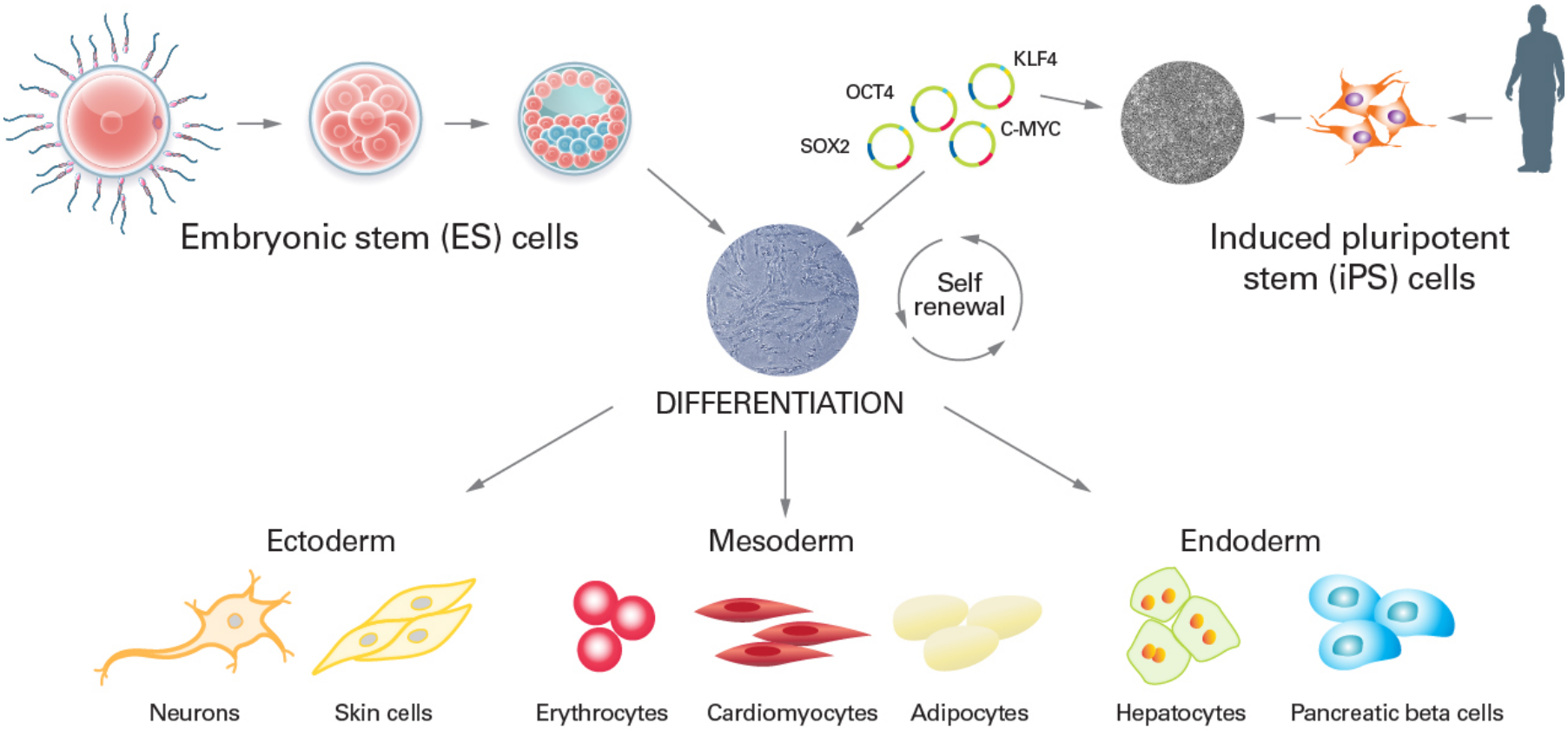
Expanding use of CRISPR/Cas9



Wang et al. *Trends Cell Biol* **26**, 5–15 (2016)

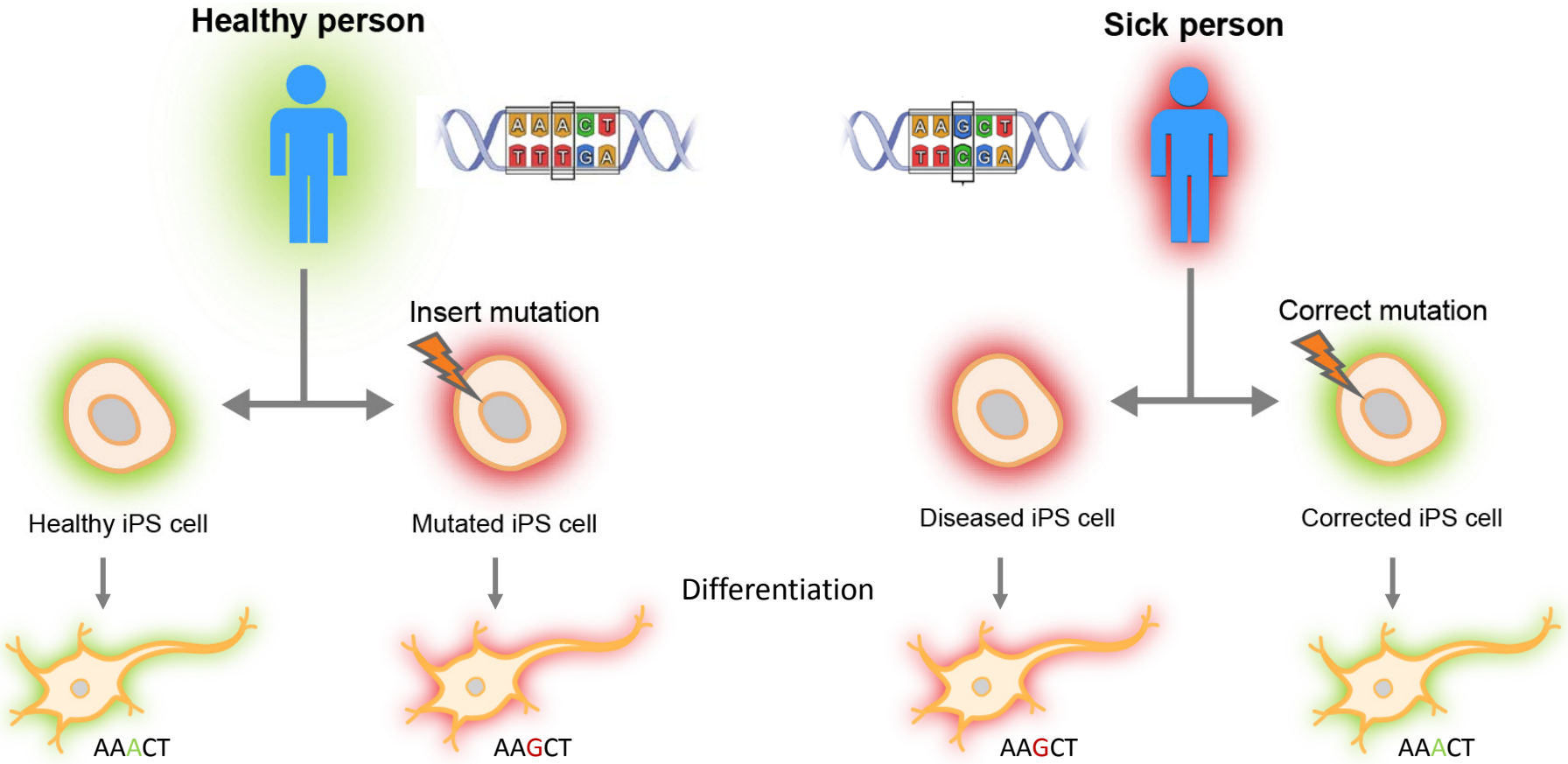
hiPS cells

Human induced pluripotent stem cells



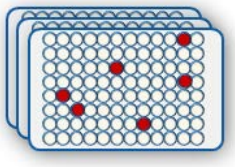
Utility of stem cells and genome editing

- hiPS cells have a robust expansion capacity and high differentiation potential



Challenges in editing hiPS cells

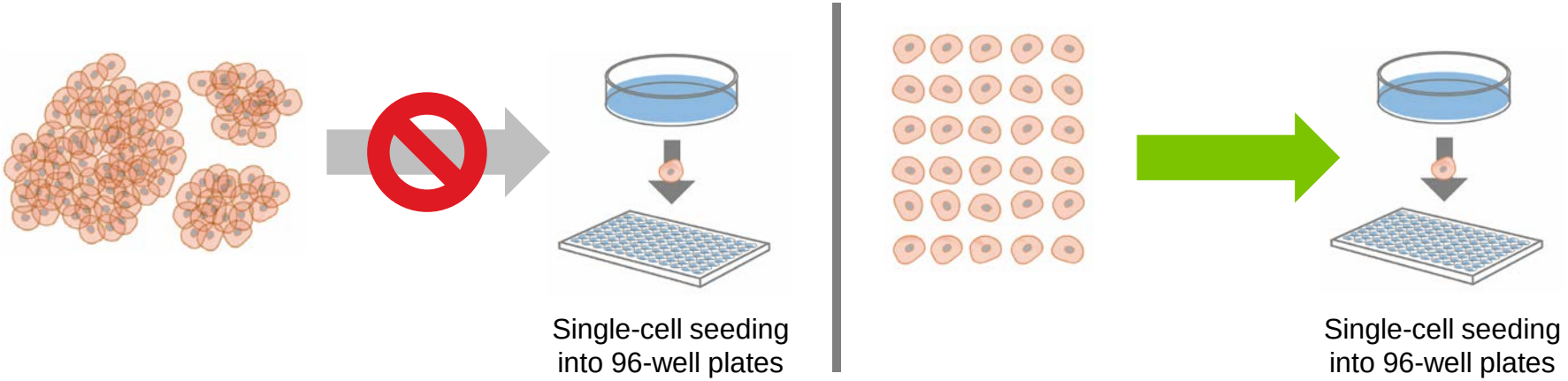
sgRNA design	Cas9/sgRNA delivery into cells (genome editing)	Single-cell cloning	Screening
• Accuracy • Specificity	• Minimize toxicity • Maximize pluripotency • Efficient editing • No off-target effects	• Maximize survival • Maintain pluripotency	• Identification • Specificity



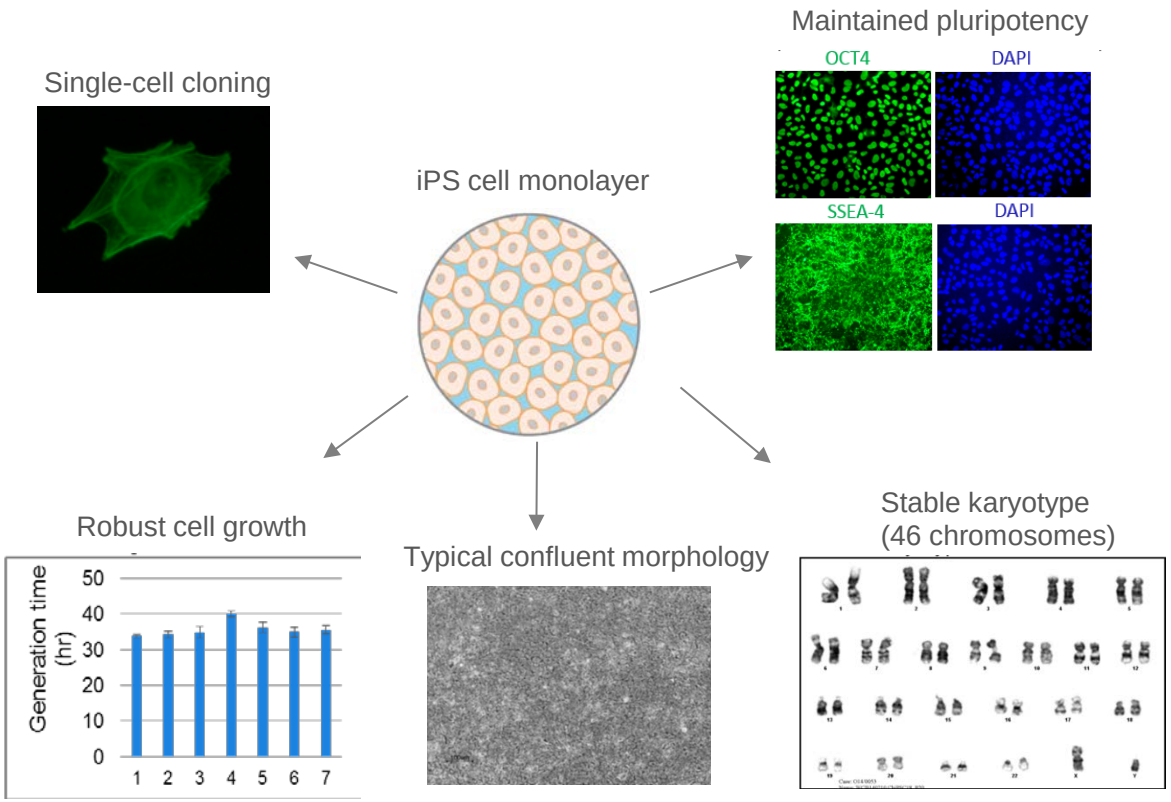
Isolate & expand an edited single cell

The bottleneck: clonal expansion of edited hiPS cells

- 100% of editing efficiency is rarely achieved
- Colony-based culture is not ideal for this application
  - Pluripotent stem cells are traditionally grown in colonies on feeders
  - Screening a colony is time-consuming and challenging
  - Single pluripotent cells typically die or differentiate
- Monolayer-based culture eliminates these challenges



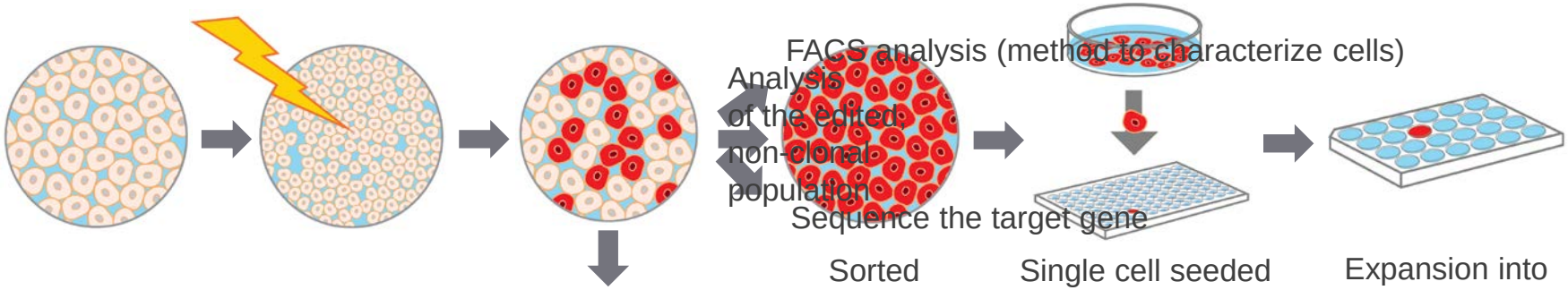
The DEF-CS™ Culture System: ideal for single-cell cloning



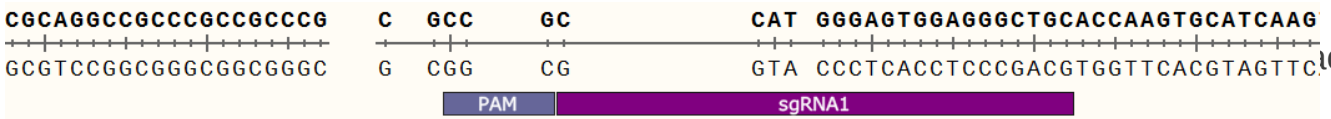
- Highly pluripotent state (capacity to differentiate into any specific cell type)
- Rapid expansion of clonal population
- Results in karyotypically stable cells
- Enables survival and expansion of single cells

Gene editing workflow

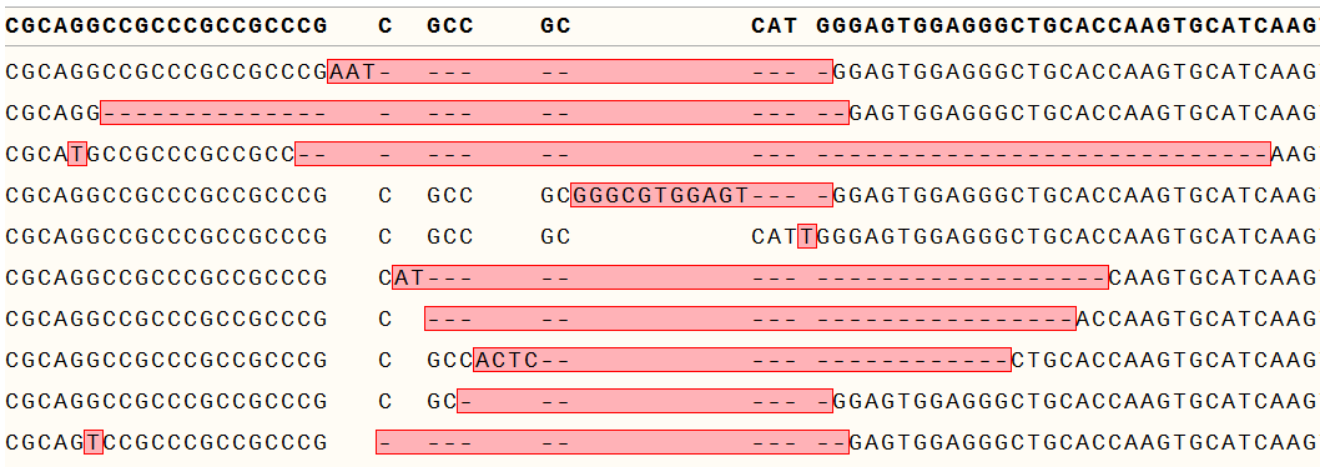
Generating clonal cell lines deficient in CD81 (a protein embedded in the cell membrane)



Wild-type sequence

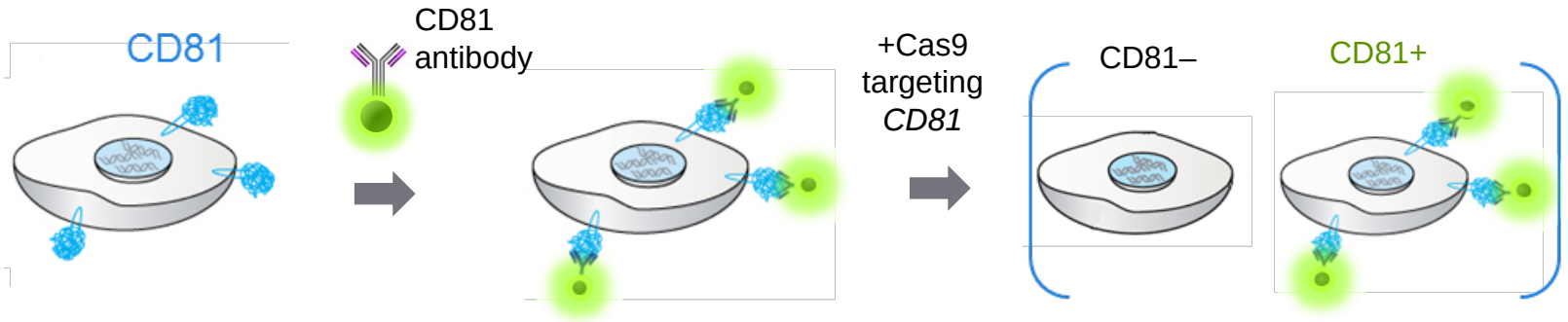


Mutations (indels) created by editing

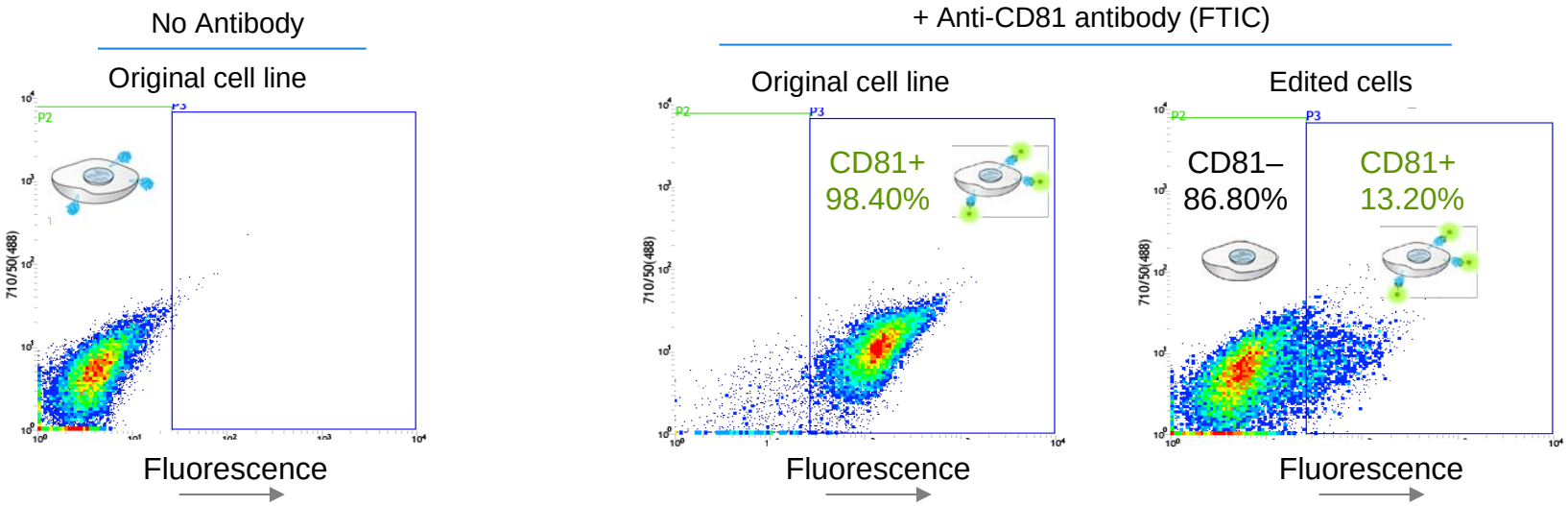


Characterization of the edited cells

Percentage of cells deficient in CD81



FACS analysis (method to characterize cells)

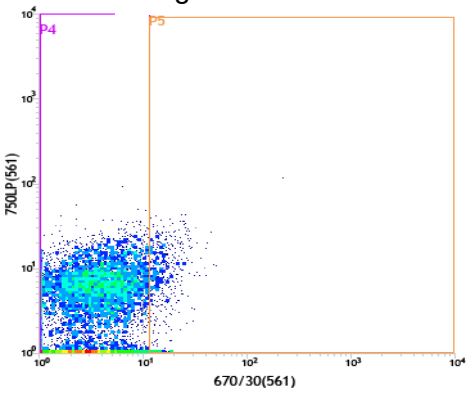


Pluripotency maintained after *CD81* KO

OCT-4 and SSEA-4 are defined as pluripotency markers

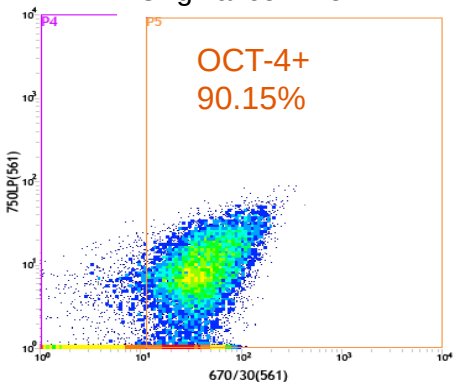
No Antibody

Original cell line

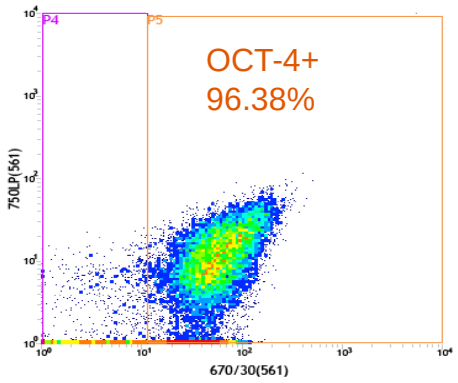


+ Anti-OCT-4 antibody (PE)

Original cell line

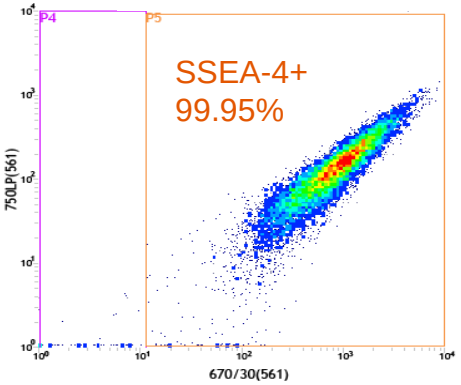


Edited cells

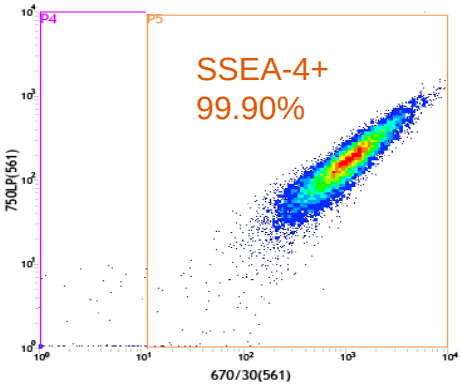


+ Anti-SSEA-4 antibody (PE)

Original cell line

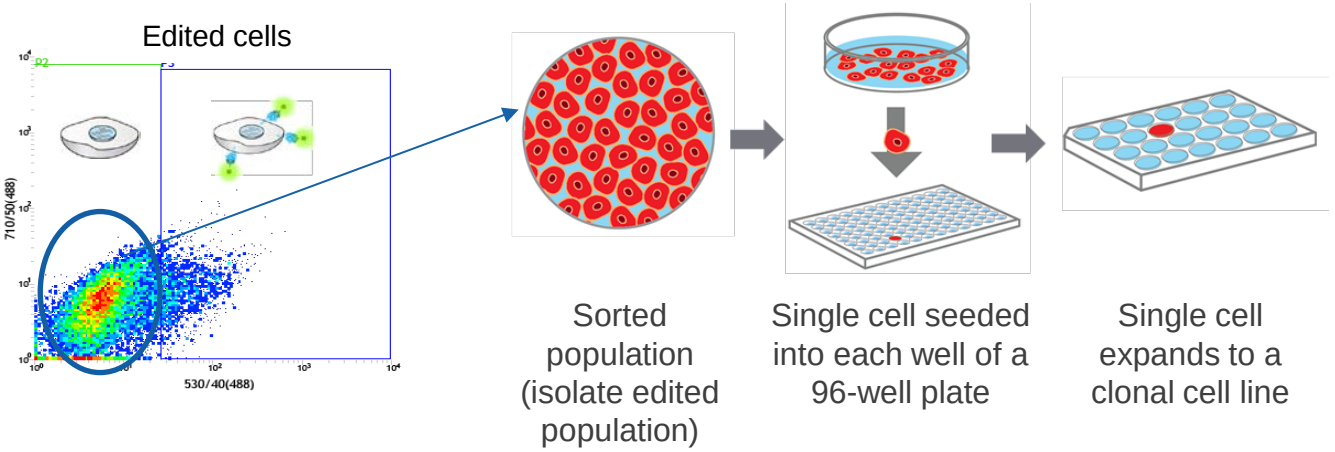


Edited cells

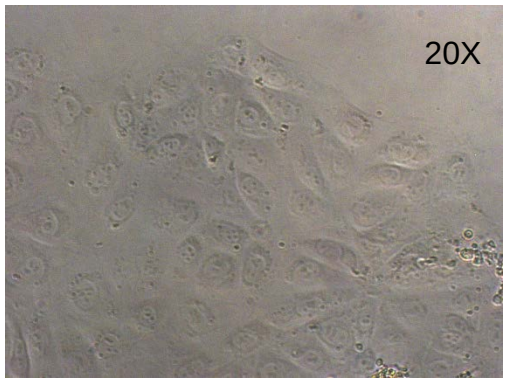


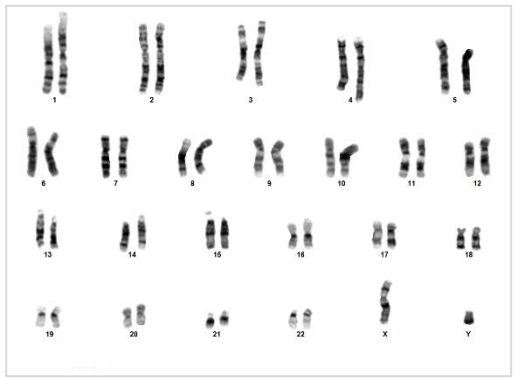
Robust expansion of edited clones

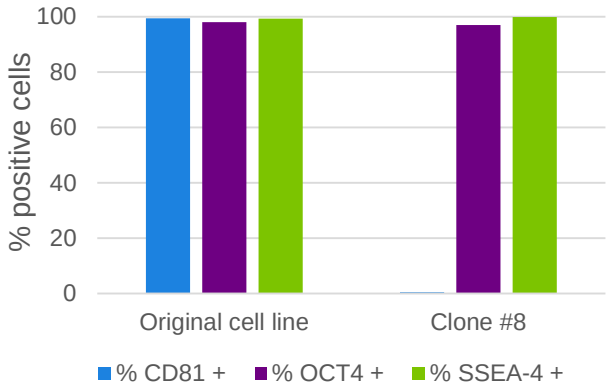
Characterization of colonies that originated from a single cell



Clonal cell line #8







Conclusions

- CRISPR/Cas9 is a powerful genome editing technique that can be applied to different fields, from agriculture to study of human genetic diseases
- The combination of two powerful technologies, human induced pluripotent stem (hiPS) cells and precise, footprint-free editing using CRISPR/Cas9, allows for a new level of sophistication in development of disease models
- The DEF-CS system is a defined, feeder-free system for culturing edited human pluripotent stem cells
  - Supports survival of edited single cells
  - Supports pluripotency in clones expanded from edited single cells

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