FOKUS methods lecture

CRISPR technologies

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18 January, 2022

In the last lecture…

- Discovery of CRISPR
- Types and mechanisms
- Cas9 and the sgRNA

Why did Cas9 from *Streptococcus pyogenes* become the predominant nuclease for CRISPR technologies?

(provide one reason in the chat)

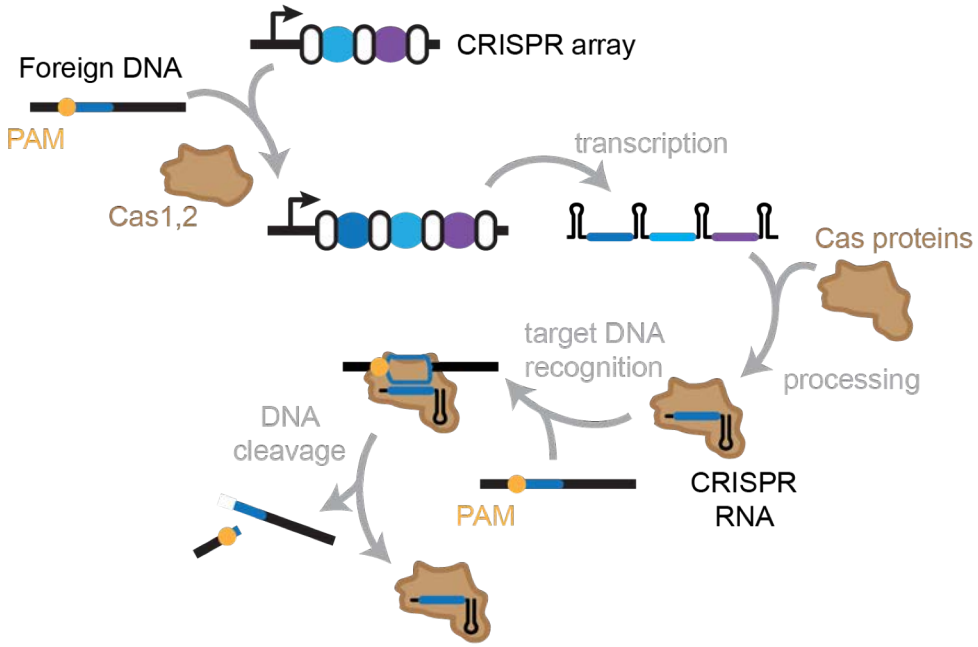
In this lecture…

- CRISPR for gene editing
- CRISPR for gene regulation
- CRISPR applications

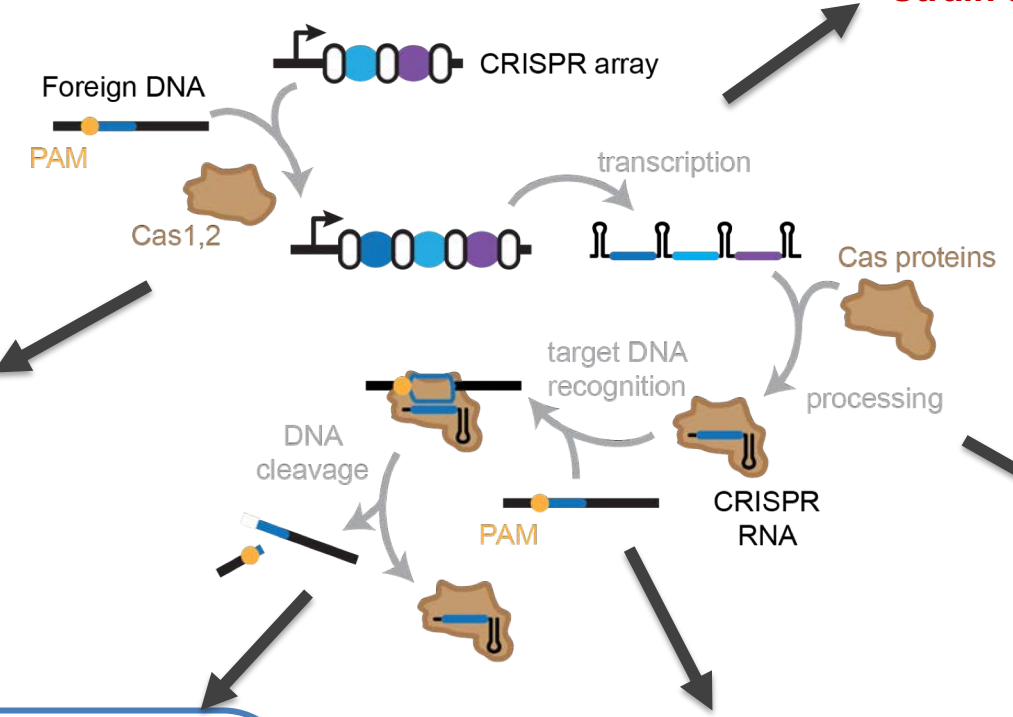
Learning objectives

- Design guide RNAs for different CRISPR nucleases
- Describe how CRISPR nucleases have been adapted for gene regulation
- Describe different applications of CRISPR and their impact on society

Steps of CRISPR-based immunity



Harnessing every step of CRISPR-based immunity



Strain typing

Biological recorders

RNA insulators

RNA purification

Genome editing

RNA editing

Gene drives

Nucleic-acid detection

DNA assembly

Antimicrobials

In vivo imaging

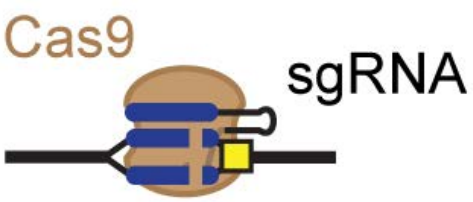
Gene regulation

Base editors

Prime editors

Protein-DNA interactions

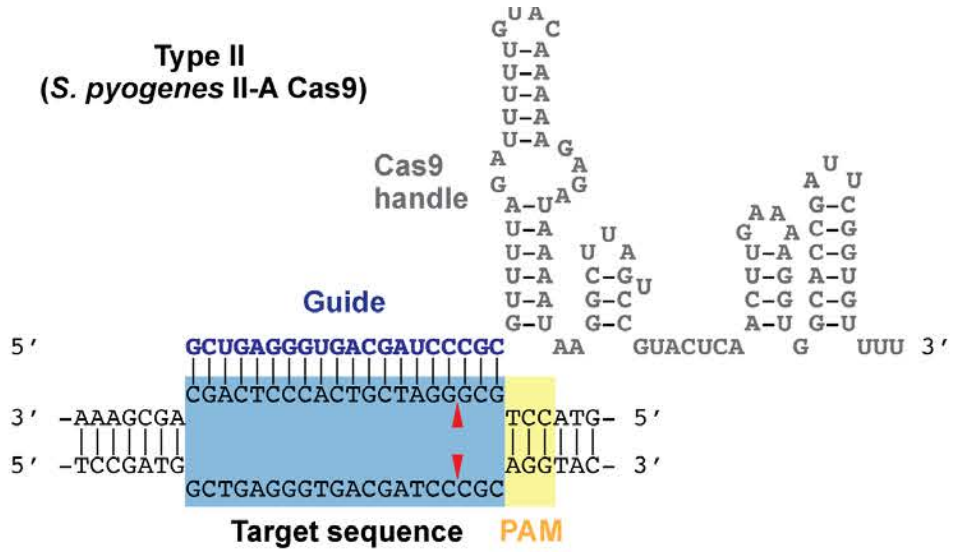
Applications of Cas9-sgRNA, a simple and programmable two-component system



- Strain typing
- Phage resistance
- Plasmid removal
- **Genome editing**
- Gene drives
- Gene regulation
- Antimicrobials
- Imaging
- RNA editing
- Genome-wide screens
- *In vitro* diagnostics
- Biological recording

Steps of guide RNA design

- Identify PAM
- Select flanking sequence
- Use that flanking sequence as guide in guide RNA



NGG PAM, 20 nts upstream as guide

Steps of guide RNA design

```
5′-AACTTGCTATATCGCCTAATATGGGAGTATATAGGCGCGTGATCATT-3′
3′-TTGAACGATATAGCGGATTATACCCTCATATATCCGCGCACTAGTAA-5′
```

Steps of guide RNA design

5′-AACTTGCTATATCGCCTAATATGGGAGTATATAGGCGCGTGATCATT-3′
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5’ CGCCUAAUAUGGGAGUAUAU + Cas9 handle 3’

Steps of guide RNA design

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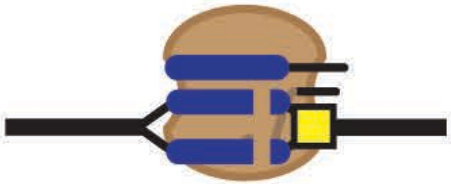
5’ CGCCUAAUAUGGGAGUAUAU + Cas9 handle 3’

Which strand (top or bottom) is bound by this guide RNA?

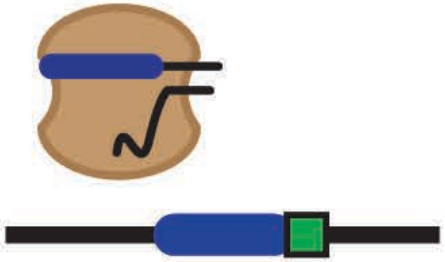
How many different guides can be designed to target this dsDNA?

Major considerations for guide selection

On-target activity



Off-target propensity



Guide design tools

- Benchling
- CHOPCHOP
- CRISPOR
- Cctop
- FORECasT
- SPROUT
- inDelphi

Unbiased off-target detection

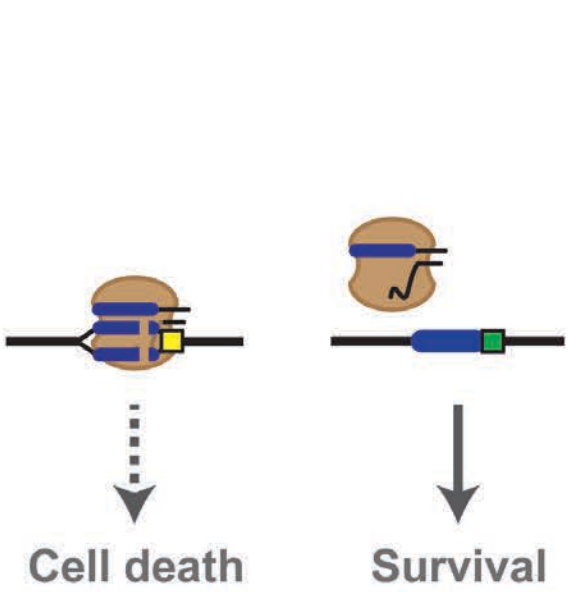
- Guide-seq
- Circle-seq
- BLISS
- Digenome-seq
- SITE-seq
- DISCOVER-seq

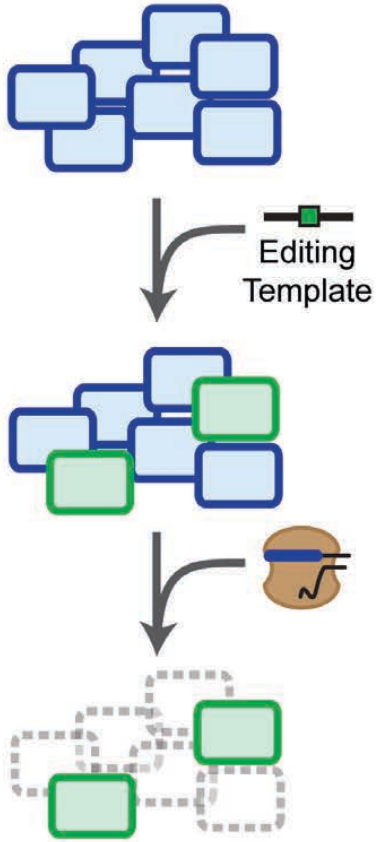
Applications extended or improved with other nucleases (natural engineered)

Other PAMs	TTTV for Cas12a NNAGAAW for Sth1Cas9 NG by xCas9, Cas9_NG; NR for SpRY
Improved specificity	Longer PAM generally means fewer off-targets Cas12a generally more sensitive to mismatches Cas9's engineered to reject mismatches
Other nucleic-acid targets	DNA and RNA for Type III systems, some Cas9's RNA for Type VI systems (Cas13)
Other targeting outcomes	Cas12a create 5-nt overhang After targeting, Cas12a degrades ssDNA After targeting, Cas13 degrades RNA

Genome editing with Cas9 (bacteria)

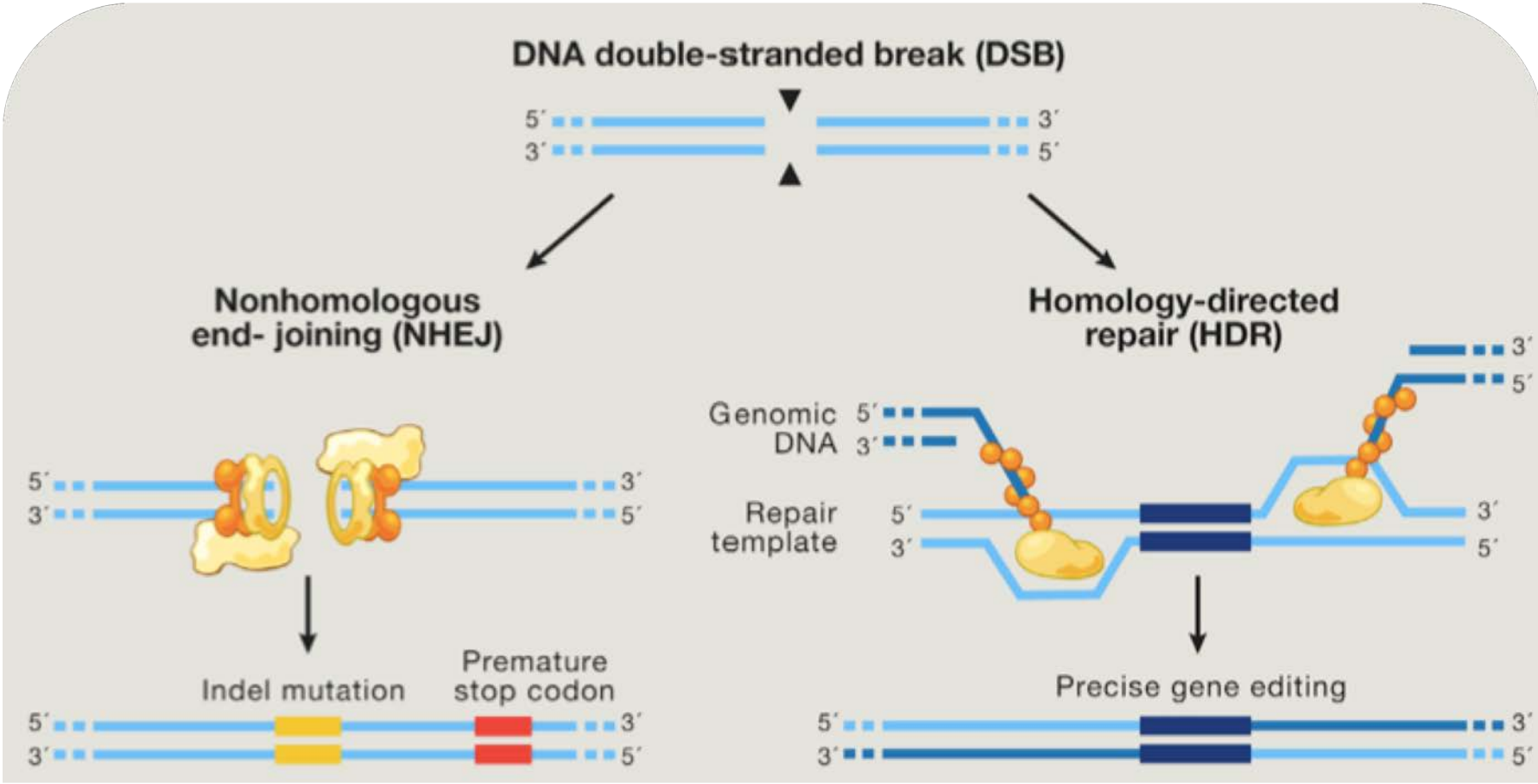
- Cleavage is often lethal in bacteria
- Use as negative selection
- Cases where homologous recombination can take place





Genome editing with Cas9 (eukaryotes)

Different outcomes of DNA cleavage in eukaryotes

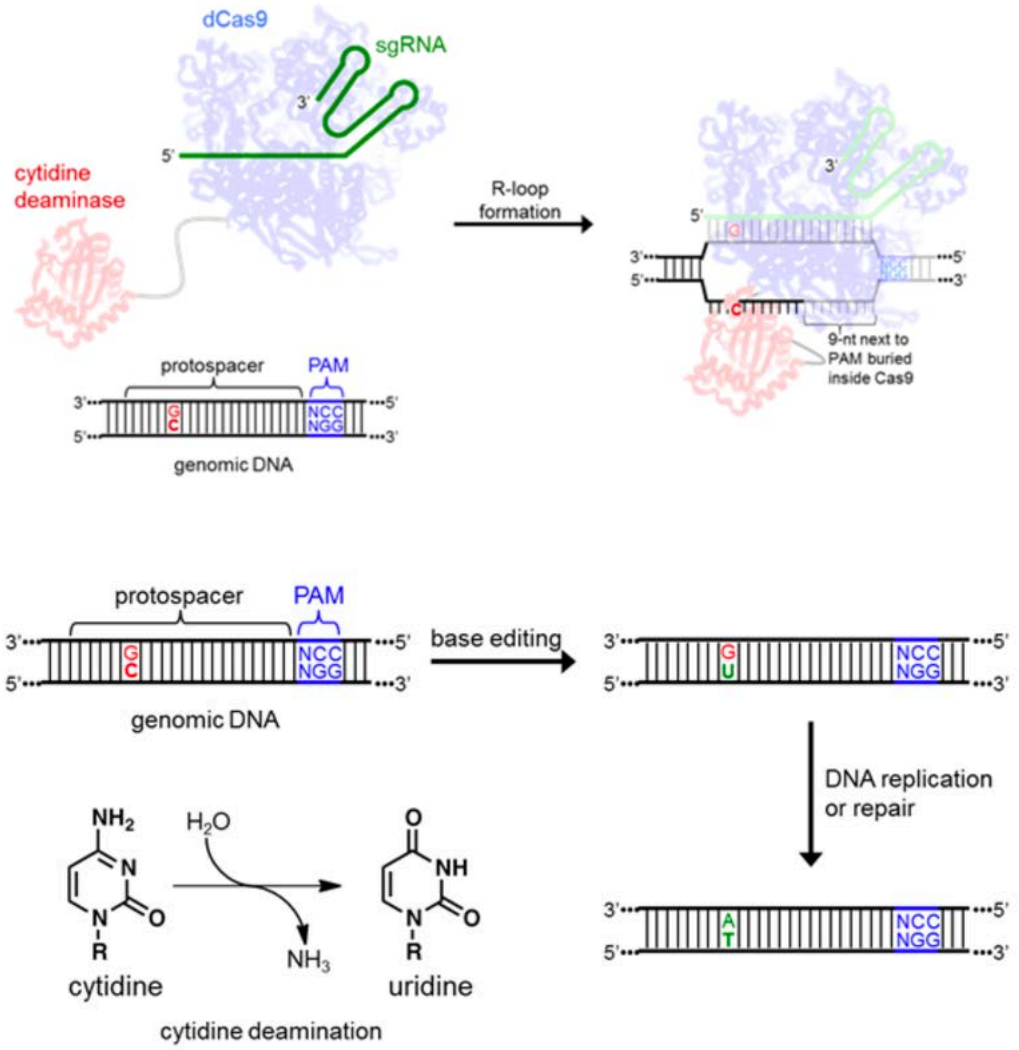


Disruption **Editing**

Base editing with Cas9

dCas9 fused to cytidine deaminase

Cytidine deaminase acts on ssDNA (i.e. displaced strand)



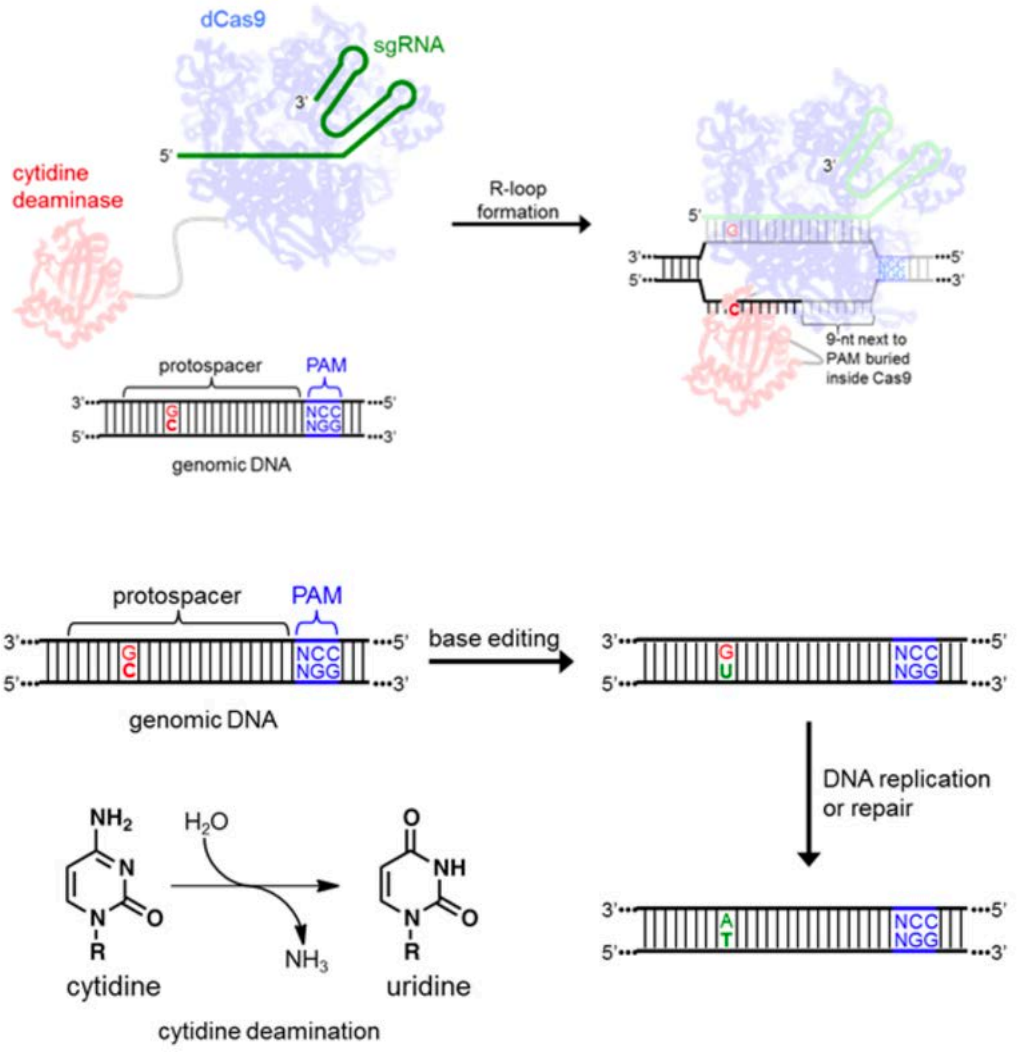
Base editing with Cas9

dCas9 fused to cytidine deaminase

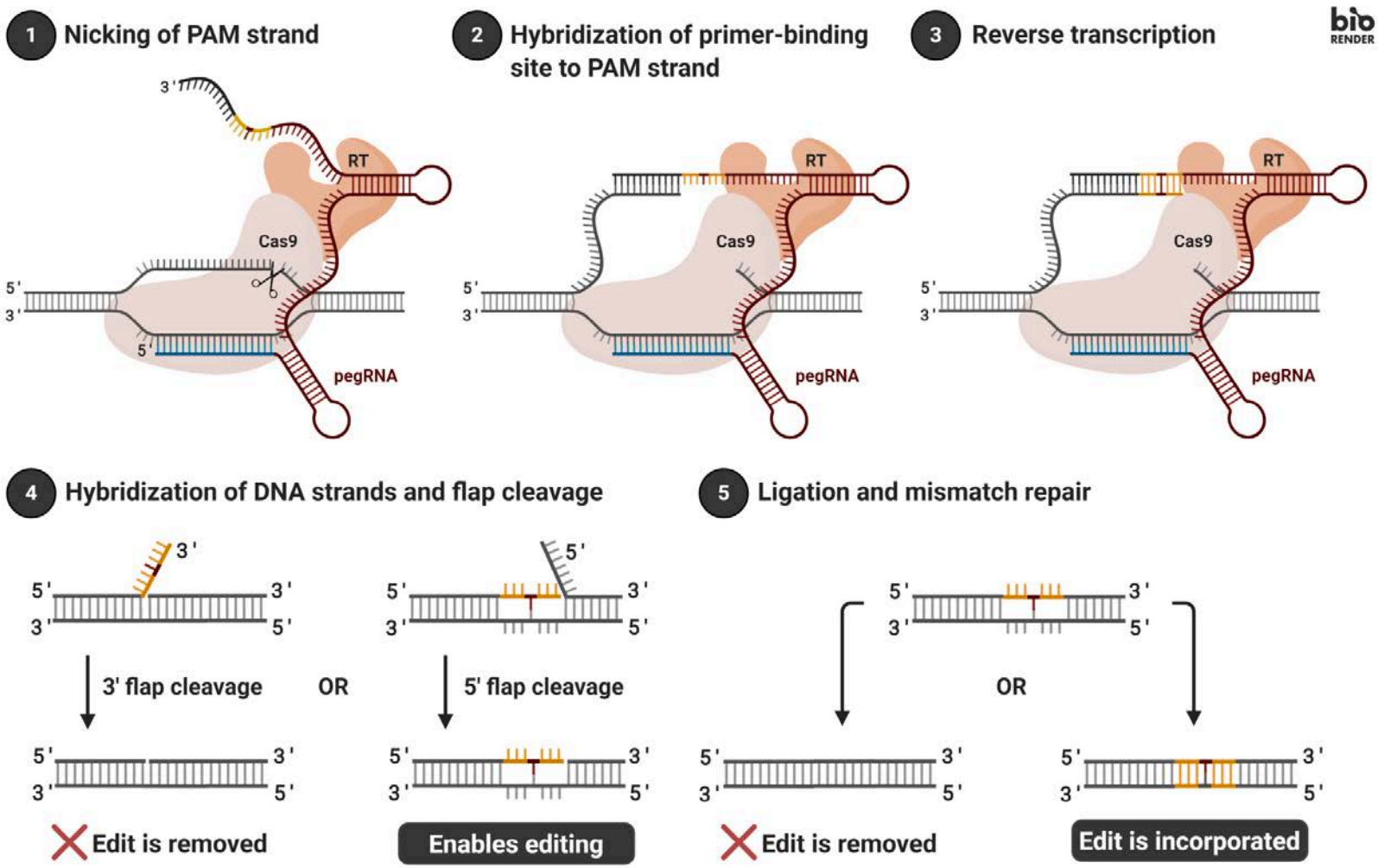
Cytidine deaminase acts on ssDNA (i.e. displaced strand)

Variants developed to improve or extend base editors

- other modifying enzymes
- Using nicking Cas9
- Using different nucleases
- Altered editing windows



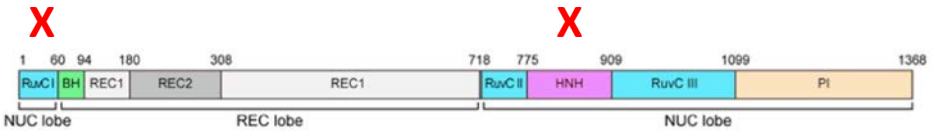
Prime editing with Cas9

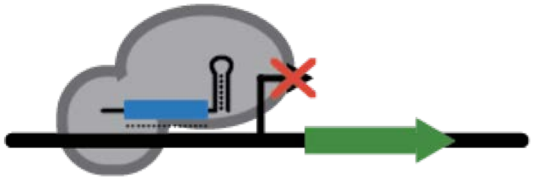


www.wikipedia.org

Gene regulation with dCas9

dCas9 (mutated RuvC, HNH)





- **Bacteria:** block transcription initiation or elongation
- **Eukaryotes:** fuse repression or activation domain

Gene regulation with dCas9

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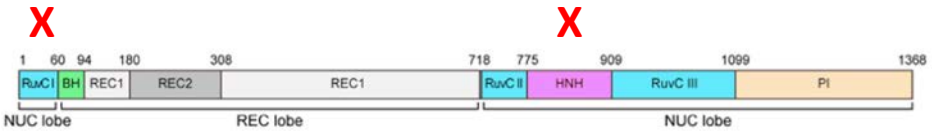

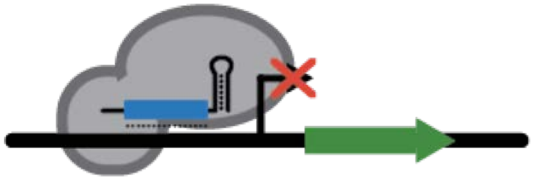

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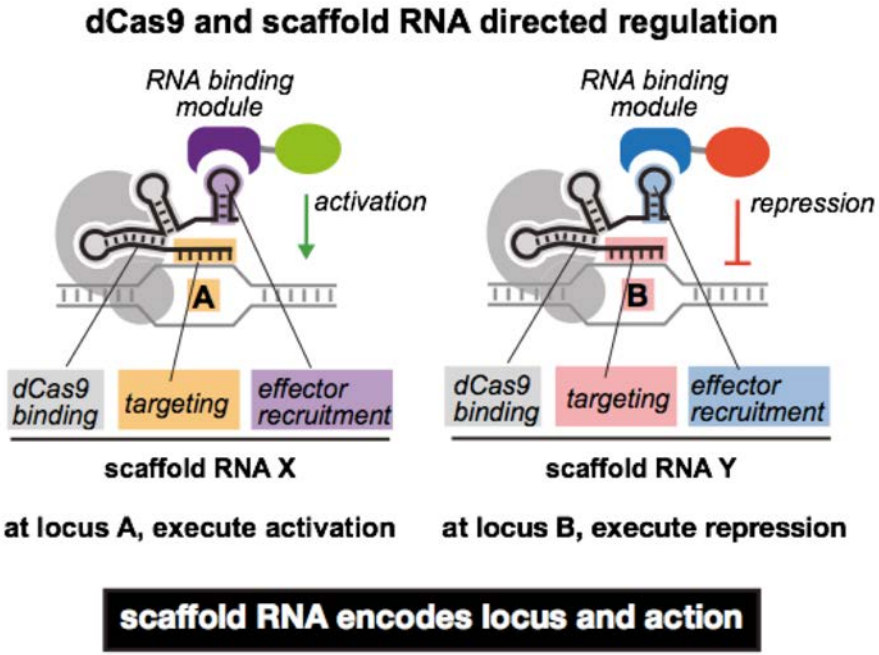
Can activate and repress in the same eukaryotic cell with scaffold sgRNAs

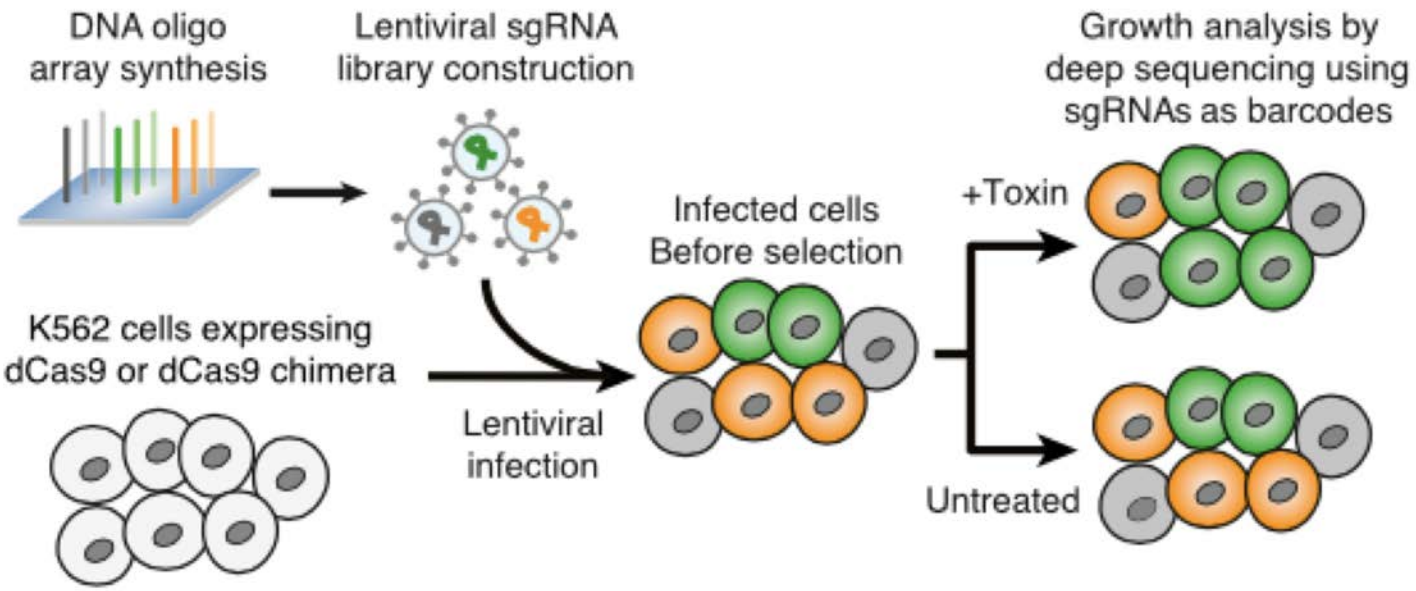


Zalatan et al. *Cell* (2015)

CRISPR-based screens

Many examples of screens using Cas9 and dCas9 in eukaryotes

Only screens using dCas9 in bacteria

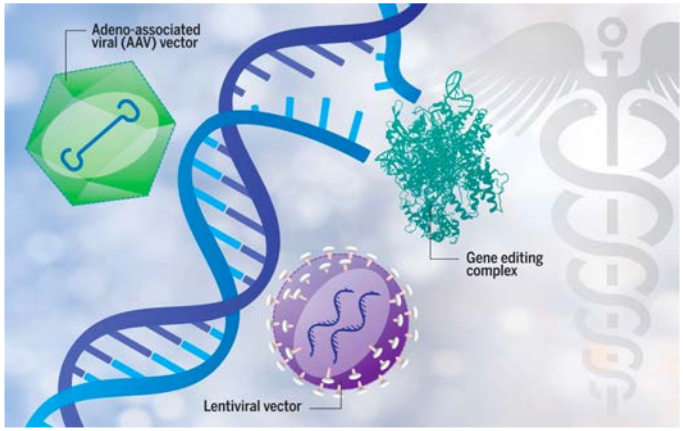


Gilbert et al. *Cell* (2014)

CRISPR applications

Many, many applications, but just highlight two today

Human gene therapy

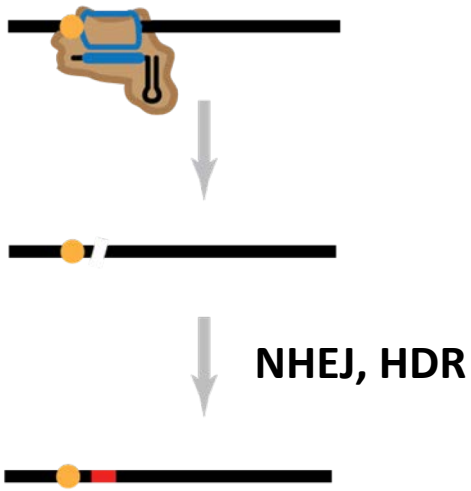


Molecular diagnostics

Human gene therapy for disease treatment, prevention

Genetic defects estimated to impact ~10% of the population

Cancer and infectious could also be treated through genetic intervention



Disease-correcting modification

Two general approaches:

- *In vivo* delivery
- *Ex vivo* manipulation

Diseases addressed in animal/cell culture

- Muscular dystrophy
- HIV-1
- Cataract
- Tyrosinemia
- Beta-thalassemia
- Blindness
- Cystic fibrosis
- Cancer
- T-cell therapy

Most recent news for CRISPR clinical trials

Jun 11, 2021

Vertex and CRISPR Therapeutics Present New Data in 22 Patients With Greater Than 3 Months Follow-Up Post-Treatment With Investigational CRISPR/Cas9 Gene-Editing Therapy, CTX001™ at European Hematology Association Annual Meeting

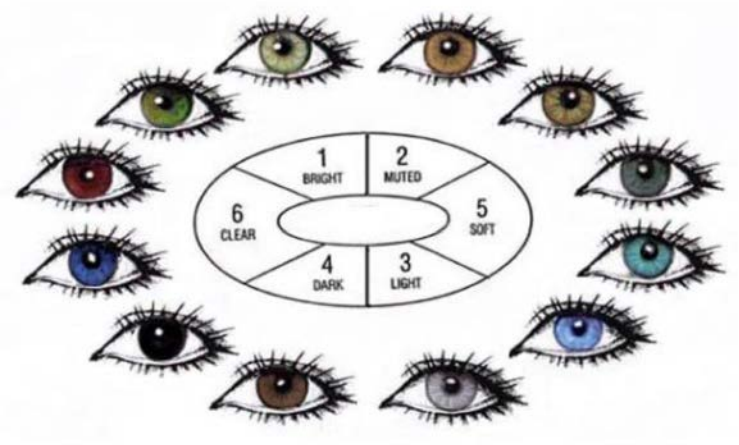
CRISPR THERAPEUTICS®

Another Milestone for CRISPR-Cas9 Technology: First Trial Data for Treatment Delivered Intravenously

Aug 18, 2021 | Clinical Laboratory Middleware, Informatics, Analytics, Digital Pathology, Laboratory Instruments & Laboratory Equipment, Laboratory News, Laboratory Pathology, Laboratory Resources, Molecular Diagnostics, Genetic Testing, Whole Gene Sequencing, Precision Medicine

Intellia THERAPEUTICS

Other potential uses of human gene therapy



The big CRISPR debate

Editing somatic cells versus germline cells

CRISPR babies

First gene-edited babies reported in China

47,934 views

129 97 SHARE SAVE ...

AP Associated Press
Published on Nov 25, 2018

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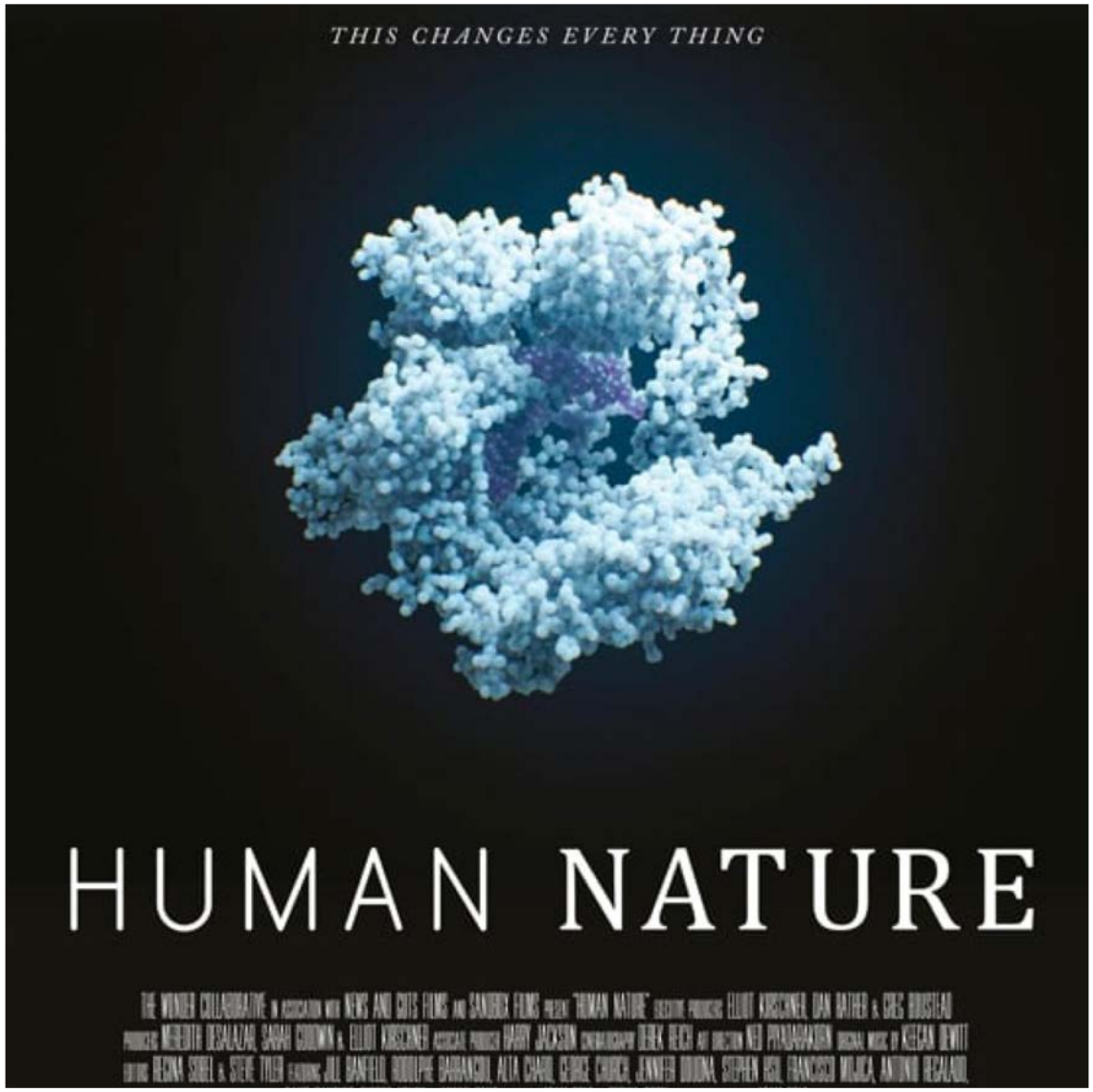
(26 Nov 2018) A Chinese researcher claims that he helped make the world's first gene-edited babies. But not everyone supports this controversial experiment. (Nov. 26)

https://www.wired.com/story/he-jiankui-crispr-babies-bucked-own-ethics-policy/

Relevant documentary



Questions for the future

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- How can we overcome the (current) major barriers to human gene therapy?
    - Editing efficiency
    - Off-targets
    - Delivery
    - Immunogenicity

Questions for the future

- What is the full diversity of CRISPR-Cas immune systems?
- How far can CRISPR be extended as technologies?
- How can we overcome the (current) major barriers to human gene therapy?
    - Editing efficiency
    - Off-targets
    - Delivery
    - Immunogenicity
- How should CRISPR technologies be used and regulated?

In this lecture…

- CRISPR for gene editing
- CRISPR for gene regulation
- CRISPR applications

Learning objectives

- Design guide RNAs for different CRISPR nucleases
- Describe how CRISPR nucleases have been adapted for gene regulation
- Describe different applications of CRISPR and their impact on society