Improvements to CRISPR/Cas editing technologies

Better targeting, improved functionality, and increased efficiency

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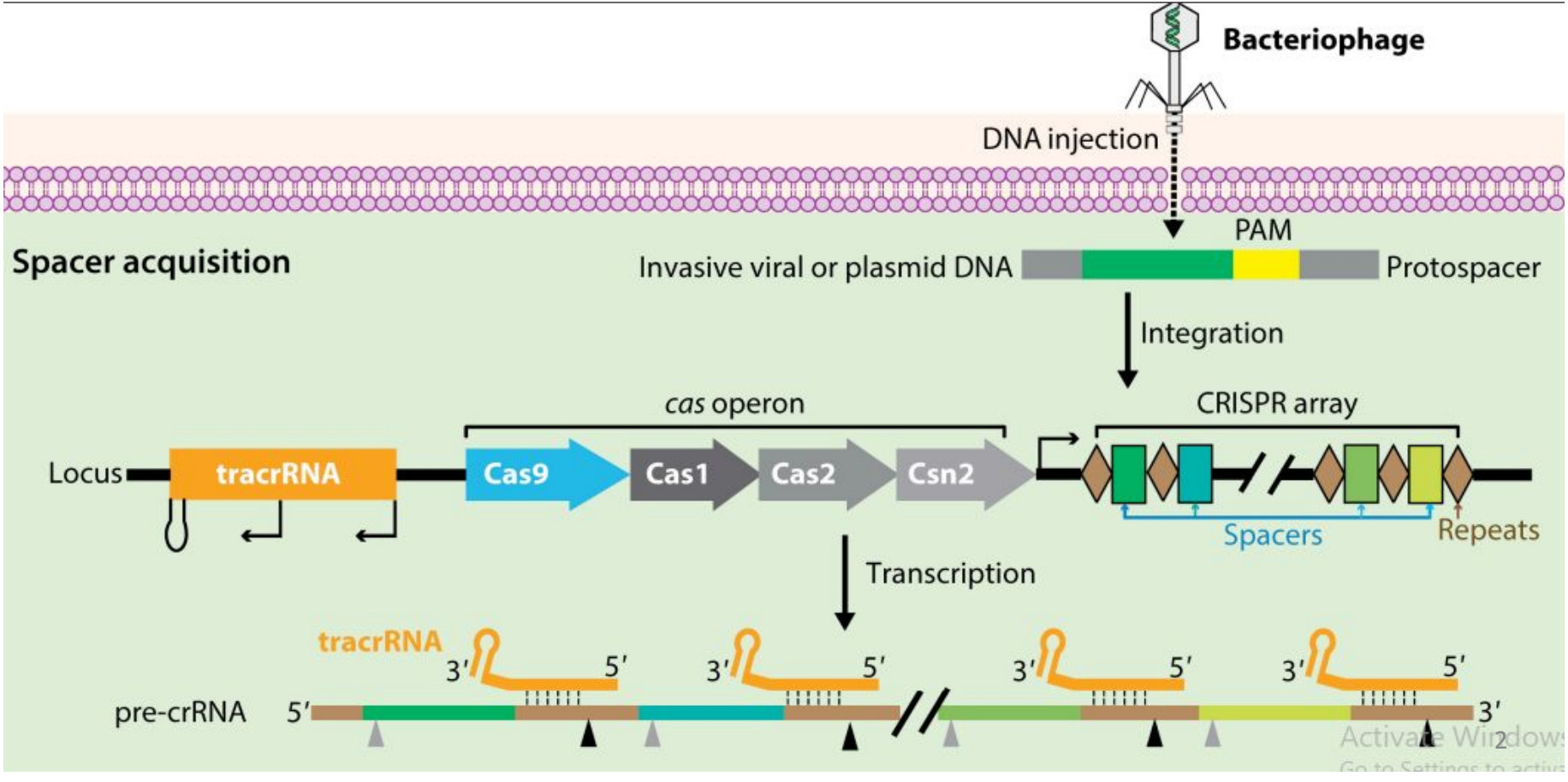
NSERC CRSNG

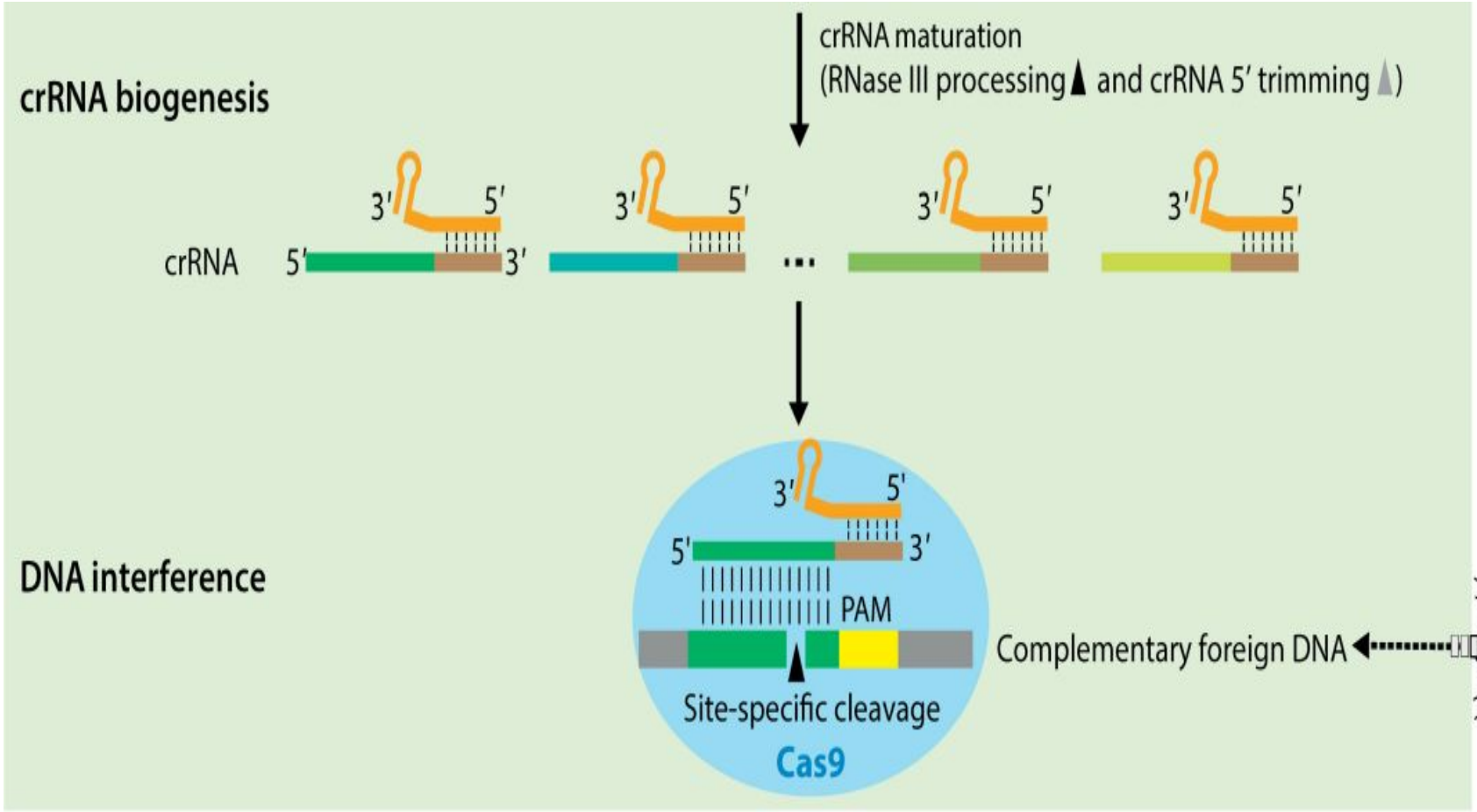
Genome Editing for Food Security and Environmental Sustainability

McGill

Queen's University

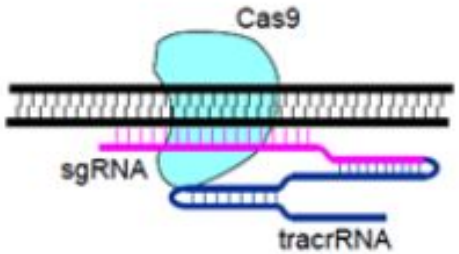
Bacterial Defence System CRISPR/Cas9

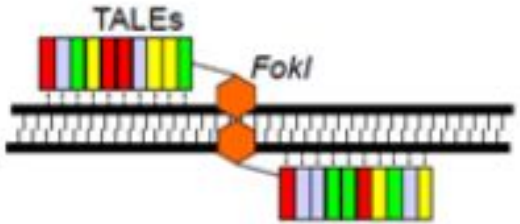


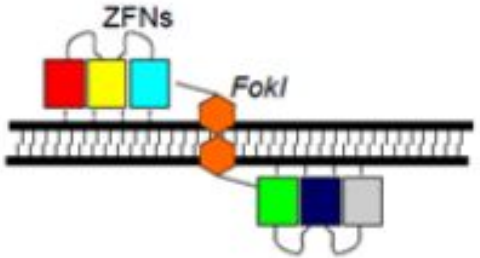


Jiang, F., & Doudna, J. A. (2017). CRISPR–Cas9 structures and mechanisms. *Annual review of biophysics*, *46*, 505-529.

CRISPR vs TALEN vs ZFN







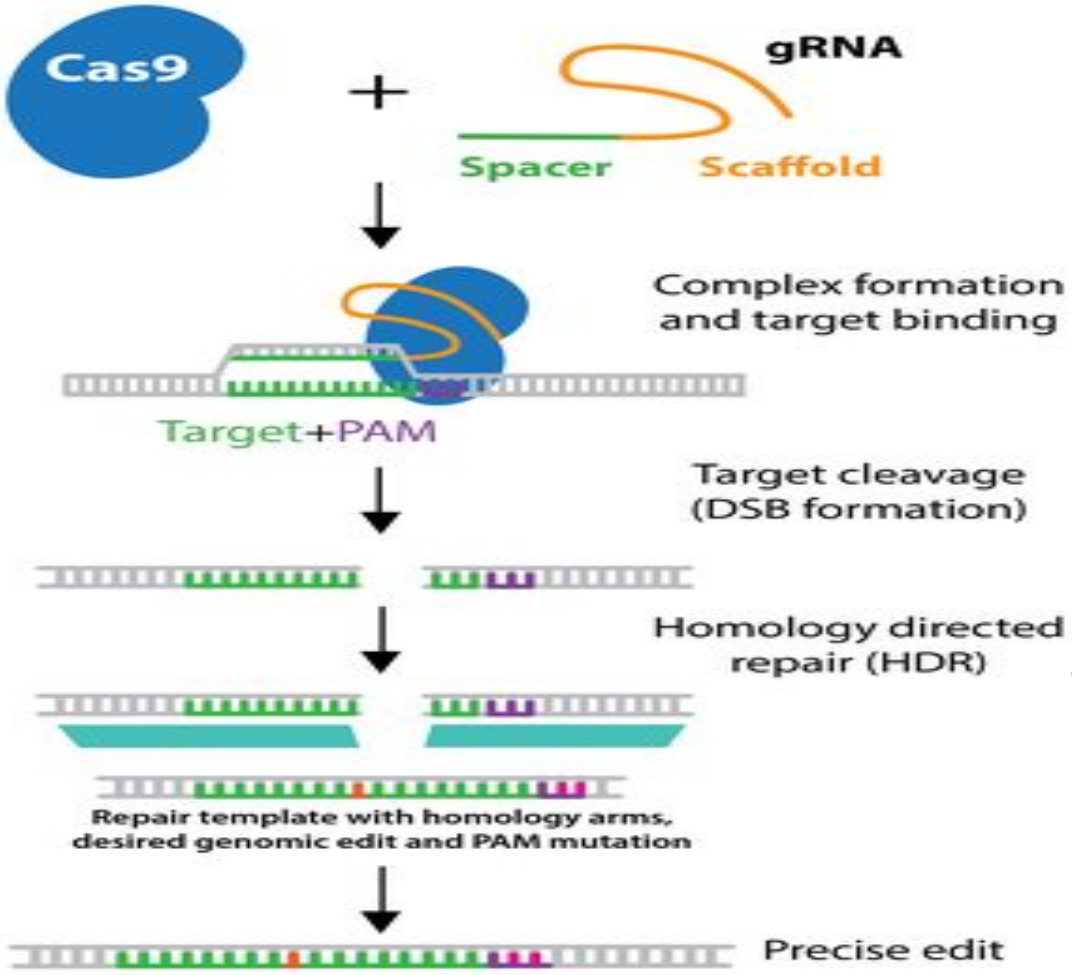
Szczesna, K. (2018, October 24). *CRISPR-Cas9, TALENs and ZFNs - the battle in gene editing*. Retrieved from https://www.ptglab.com/news/blog/crispr-cas9-talens-and-zfns-the-battle-in-gene-editing/

CRISPR vs TALEN vs ZFN

Feature	ZFNs	TALENs	CRISPR-Cas9
Length of recognized DNA target	9–18 bp	30–40 bp	22 bp + PAM sequence
Mechanism of target DNA recognition	DNA–protein interaction	DNA–protein interaction	DNA–RNA interaction via Watson-Crick base pairing
Mechanism of DNA cleavage and repair	Double-strand break induced by *FokI*	Double-strand break induced by *FokI*	Single- or double-strand break induced by Cas9
Design	Challenging. Available libraries of zinc finger motifs with pre-defined target specificity, but zinc finger motifs assembled in arrays can affect specificity of neighboring zinc finger motifs, making the design challenging.	Easy. TALE motifs with target specificities are well defined.	Easy. SgRNA design based on complementarity with the target DNA.
Cloning	Requires engineering linkages between zinc finger motifs.	TALEs do not require linkages. Cloning of separate TALE motifs can be done using Golden Gate assembly.	Expression vectors for Cas9 available. SgRNA can be delivered to cells as a DNA expression vector or directly as an RNA molecule or pre-loaded Cas9-RNA complex.

- Specificity of target
- Time to design
- Expertise required
- Complexity of troubleshooting
- Toxicity/off target effects

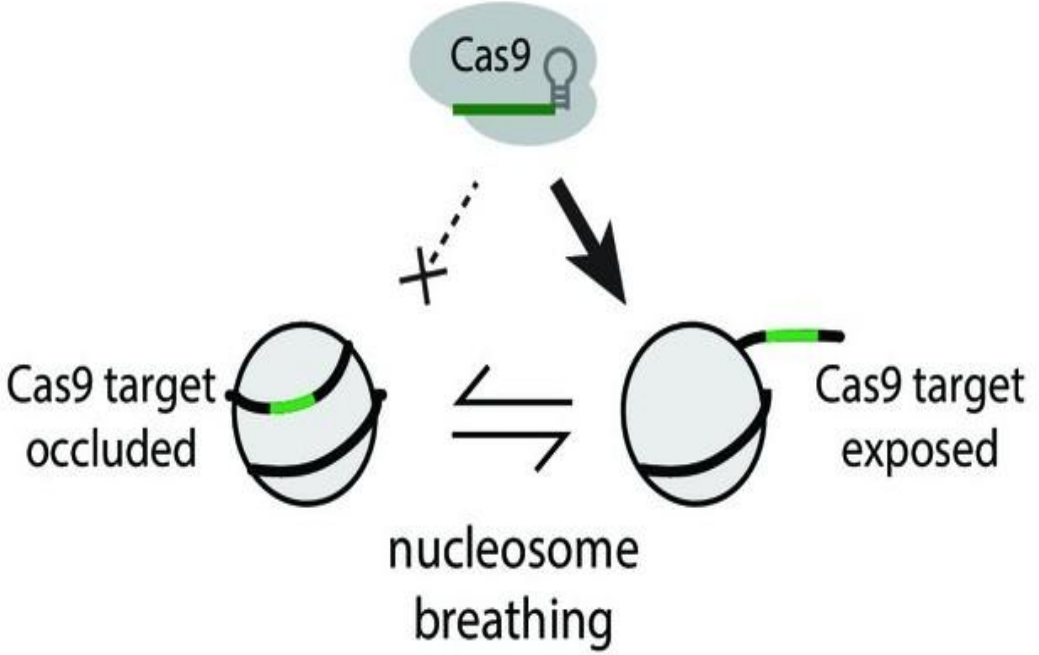
Engineered CRISPR system

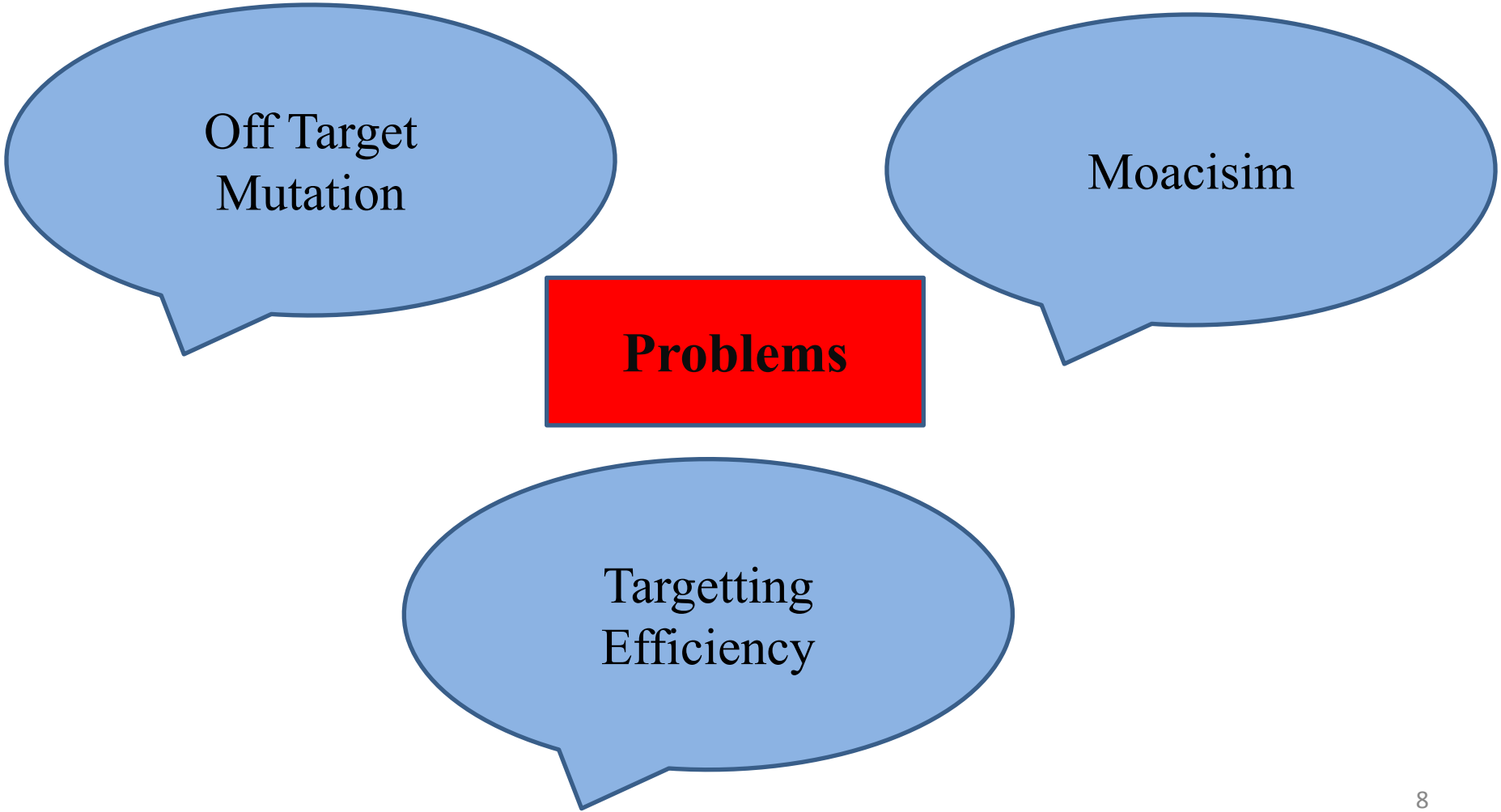


https://www.addgene.org/guides/crispr/

DNA Breathing

Local conformational transitions occurring within double-stranded DNA.



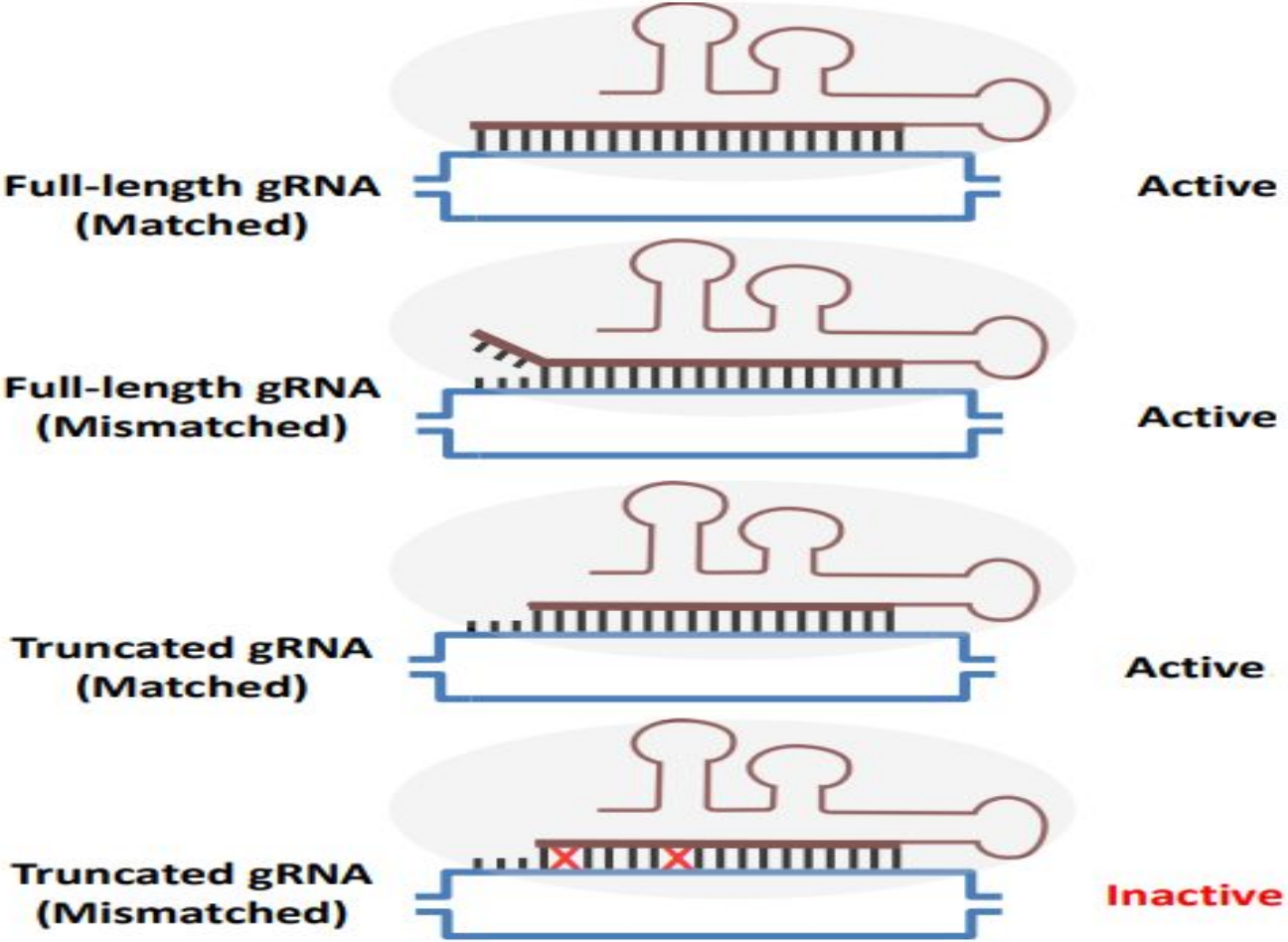

Off Target
Mutation
Moacisim
Problems
Targetting
Efficiency

Truncated Guide RNA

- The CRISPR/ Cas9-17 achieved the effect of reducing the off-target rate

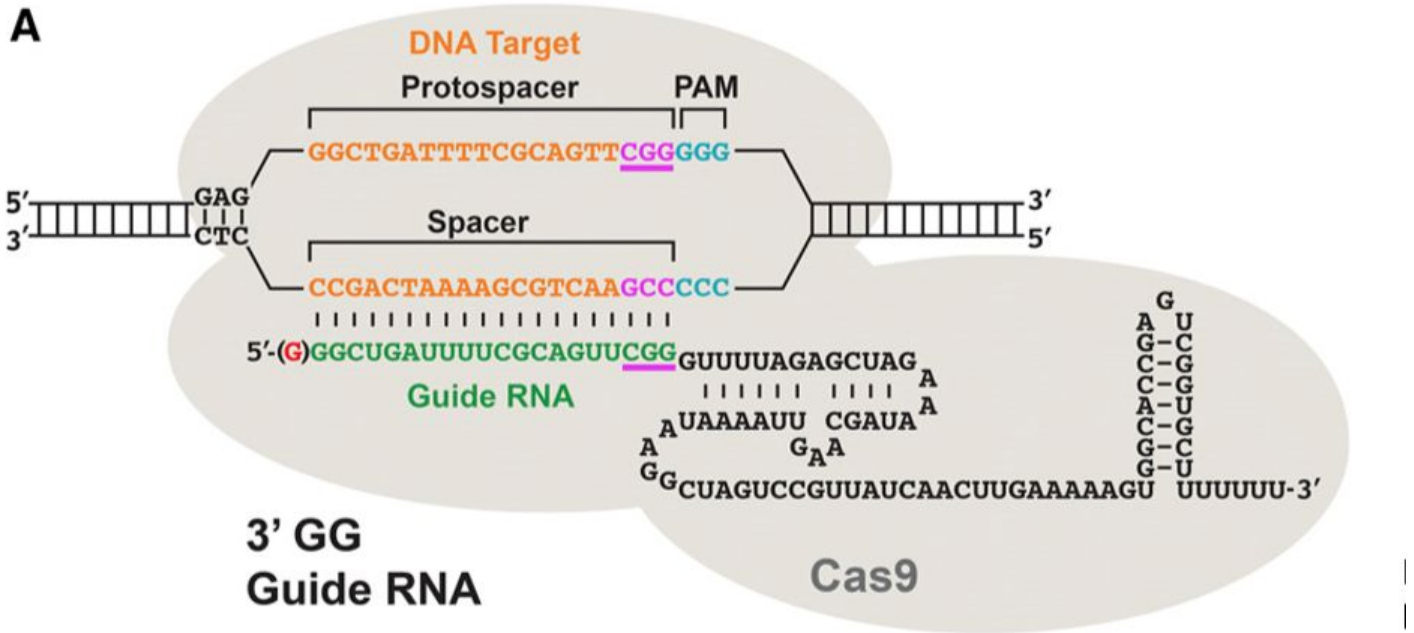
- Not affecting the gene targeting efficiency

ZHOU, Z. W., CAO, G. H., Zhe, L. I., HAN, X. J., Chen, L. I., LU, Z. Y., ... & LI, X. L. (2019). Truncated gRNA reduces CRISPR/Cas9-mediated off-target rate for MSTN gene knockout in bovines. *Journal of Integrative Agriculture*, *18*(12), 2835-2843.

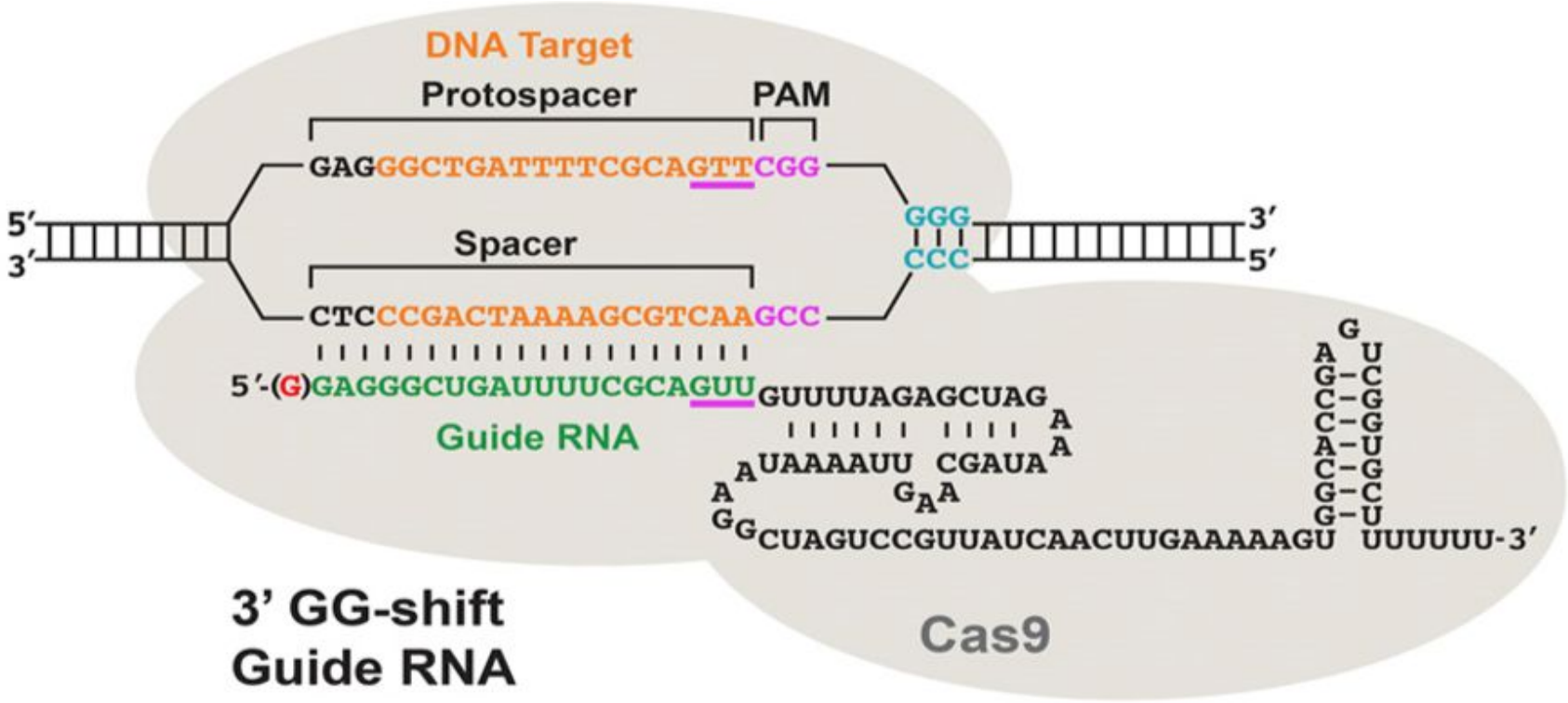


Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nature Biotechnology*, (January), 1–8. https://doi.org/10.1038/nbt.2808

Improved Guide RNA Design



Article, H. (2015). *Dramatic Enhancement of Genome Editing by CRISPR / Cas9 Through Improved Guide RNA Design*. *199*(April), 959–971. https://doi.org/10.1534/genetics.115.175166



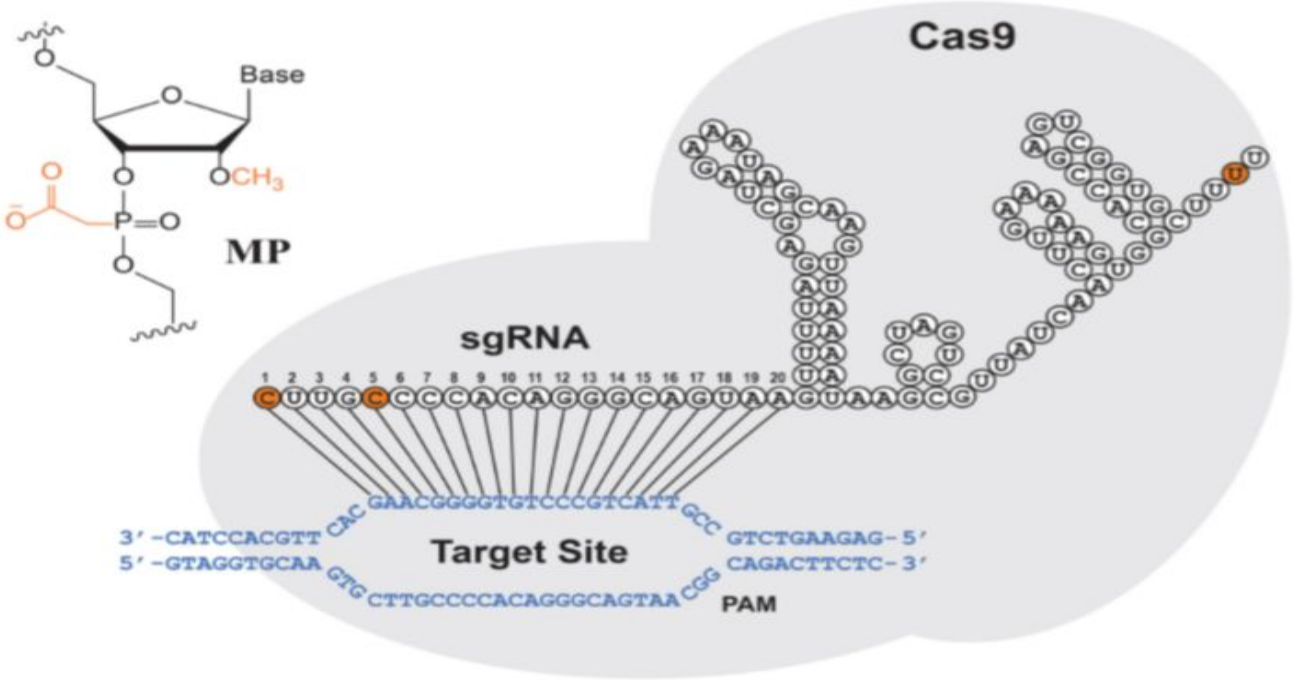
3’GG guide RNA result in high rate of mutagenesis as compare to 3’ GG shift guide RNA

Chemical Modification

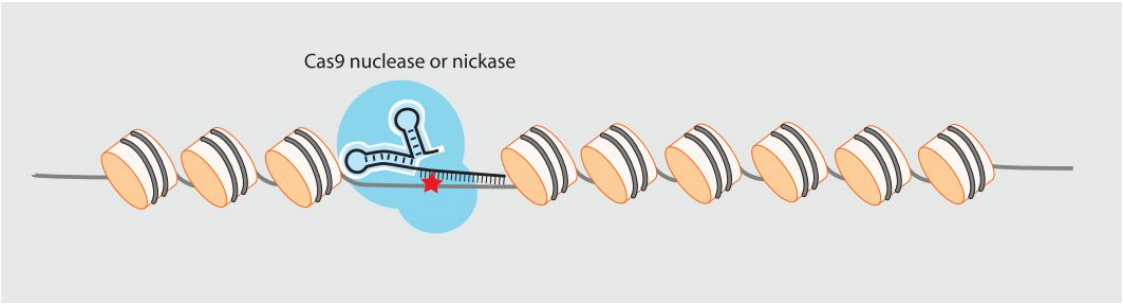
- Done the chemical modification at 5’ end of guide RNA
- Alter the melting temperature of RNA-DNA duplex
- Lower the stability of Hybrids

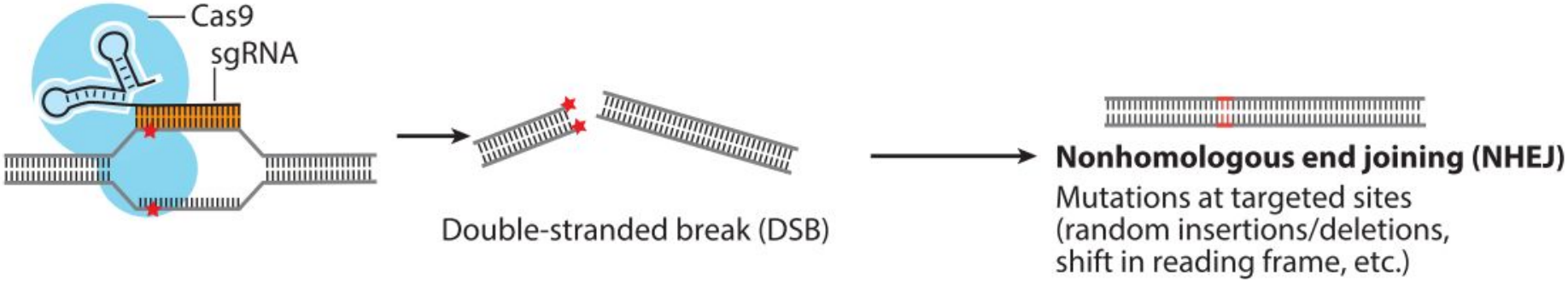
Ryan, D. E., Taussig, D., Steinfeld, I., Phadnis, S. M., Lunstad, D., Singh, M., … Dellinger, D. J. (2018). *Improving CRISPR – Cas specificity with chemical modifications in single-guide RNAs*. *46*(2), 792–803. https://doi.org/10.1093/nar/gkx1199

2’-O- Methy l-3’-phosphonoacetate (MP)



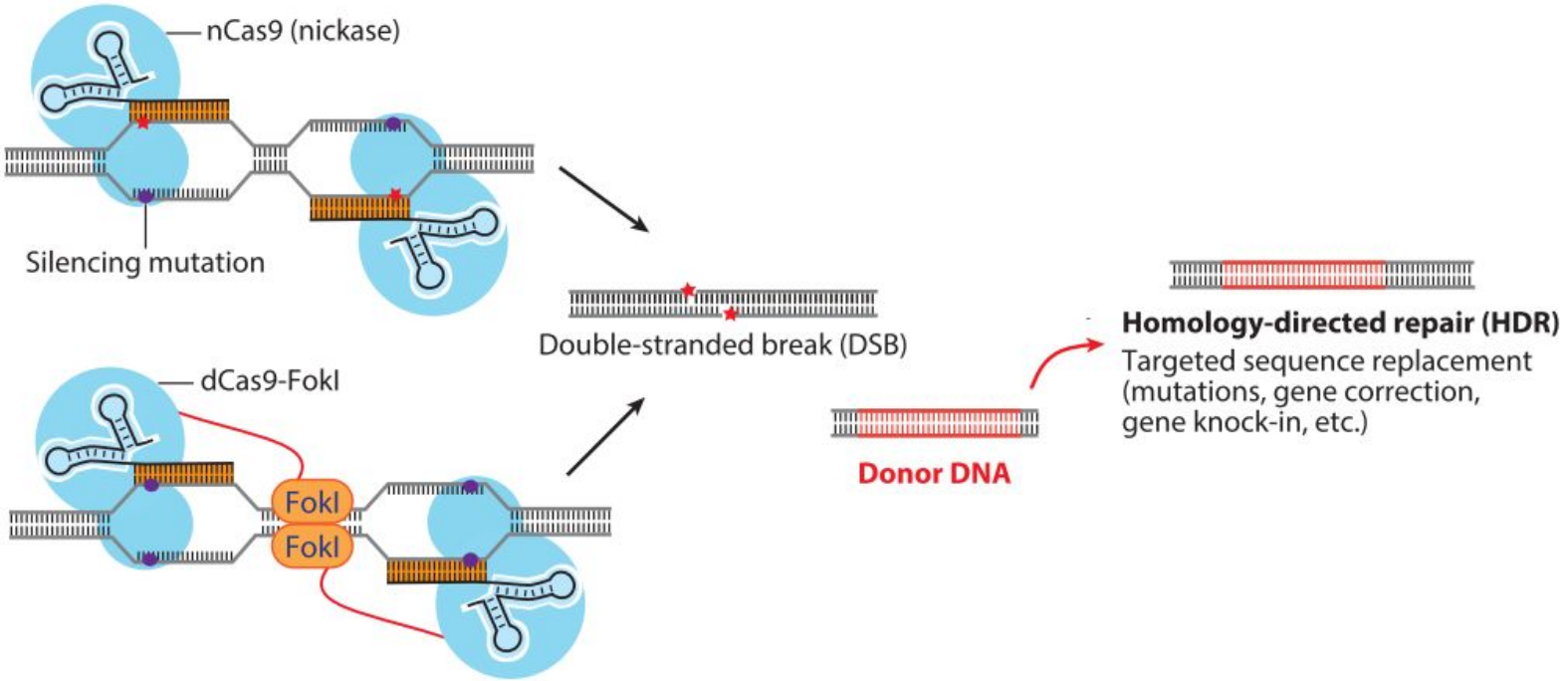
Cas9 knockouts - the early days





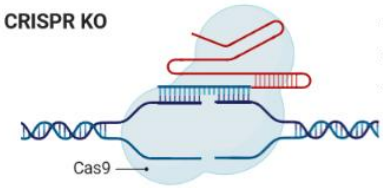
Wang, H., Russa, M. La, & Qi, L. S. (2016). CRISPR / Cas9 in Genome Editing and Beyond. *Annu Rev Biochem*, *85*, 227–264. https://doi.org/10.1146/annurev-biochem-060815-014607

Cas9 knockouts version 2.0



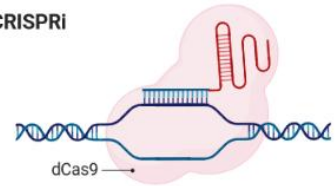
dCas9 tools

CRISPR KO



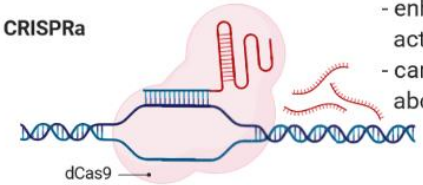
- Double-stranded break
- Random insertions and deletions (indels)
- Improved by making nCas9 (nickase) + donor DNA for single strand cut and precise control over indel

CRISPRi



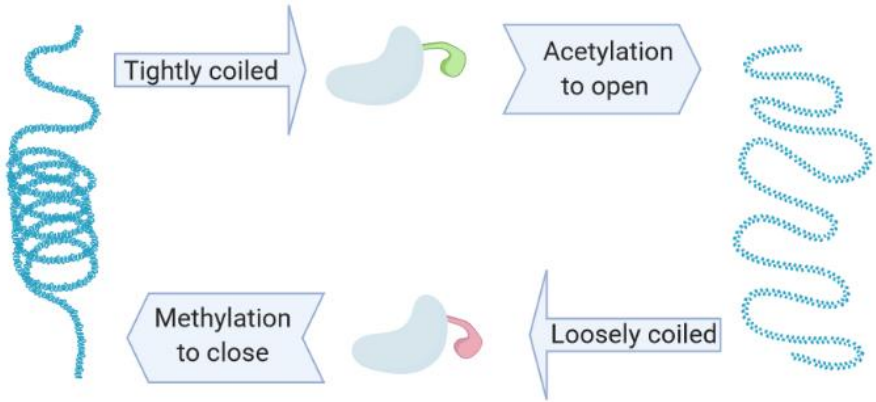
- inhibits expression by using fusion of represser domain
- can generate gradients of expression below normal

CRISPRa

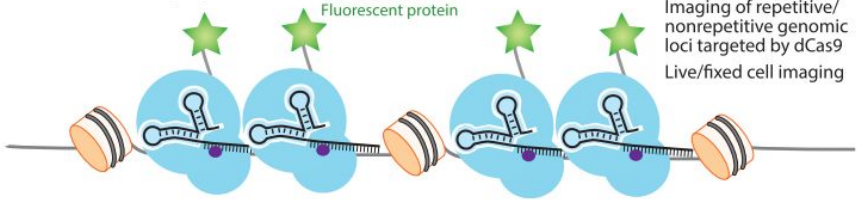


- enhances expression by using fusion of activating domain
- can generate gradients of expression above normal

Histone modification



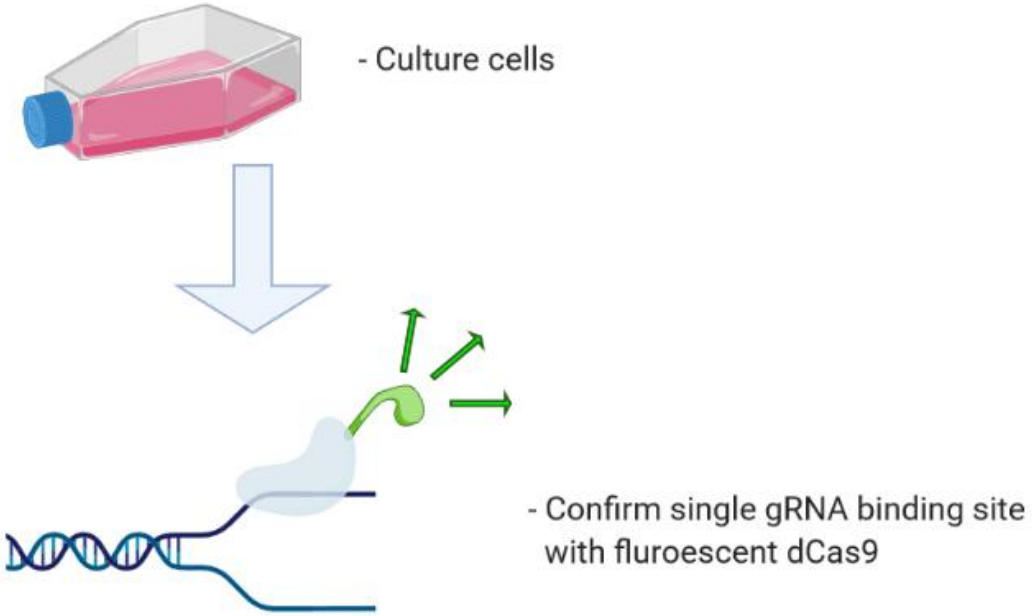
Genomic imaging



Imaging of repetitive/ nonrepetitive genomic loci targeted by dCas9

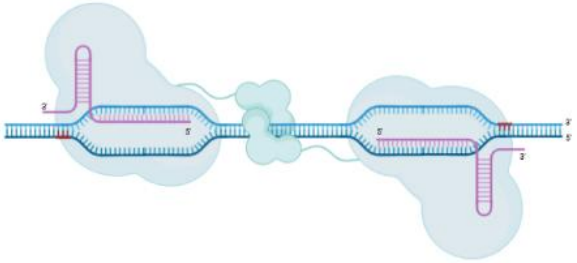
Live/fixed cell imaging

dCas9 application



dCas9 application

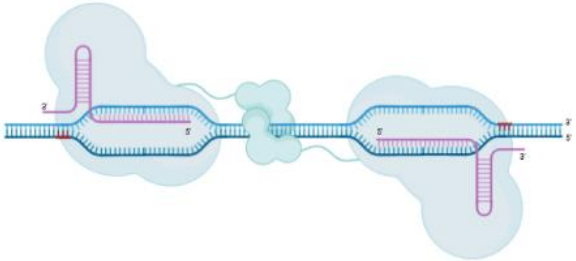
- Culture cells



- Insert/deletion

- Other application

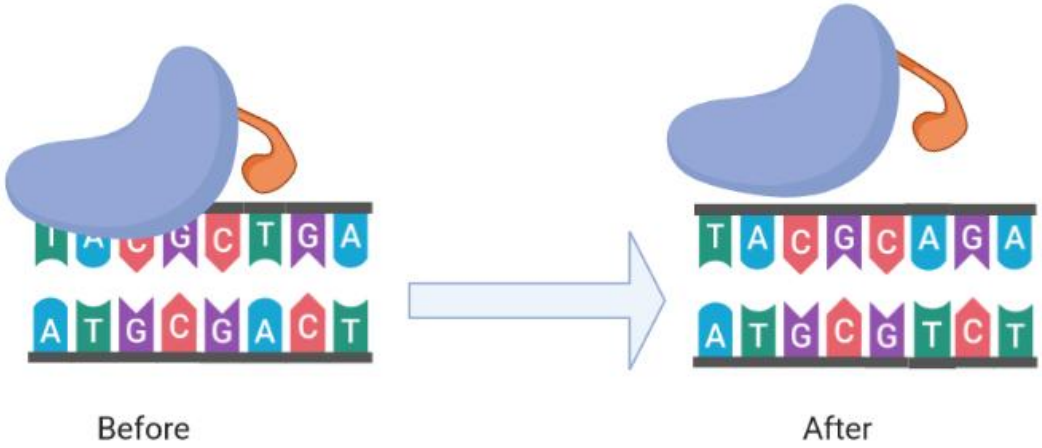
dCas9 application



- Insert/deletion

- Other application

The future of CRISPR: Base Editing



The future of CRISPR: Base Editing

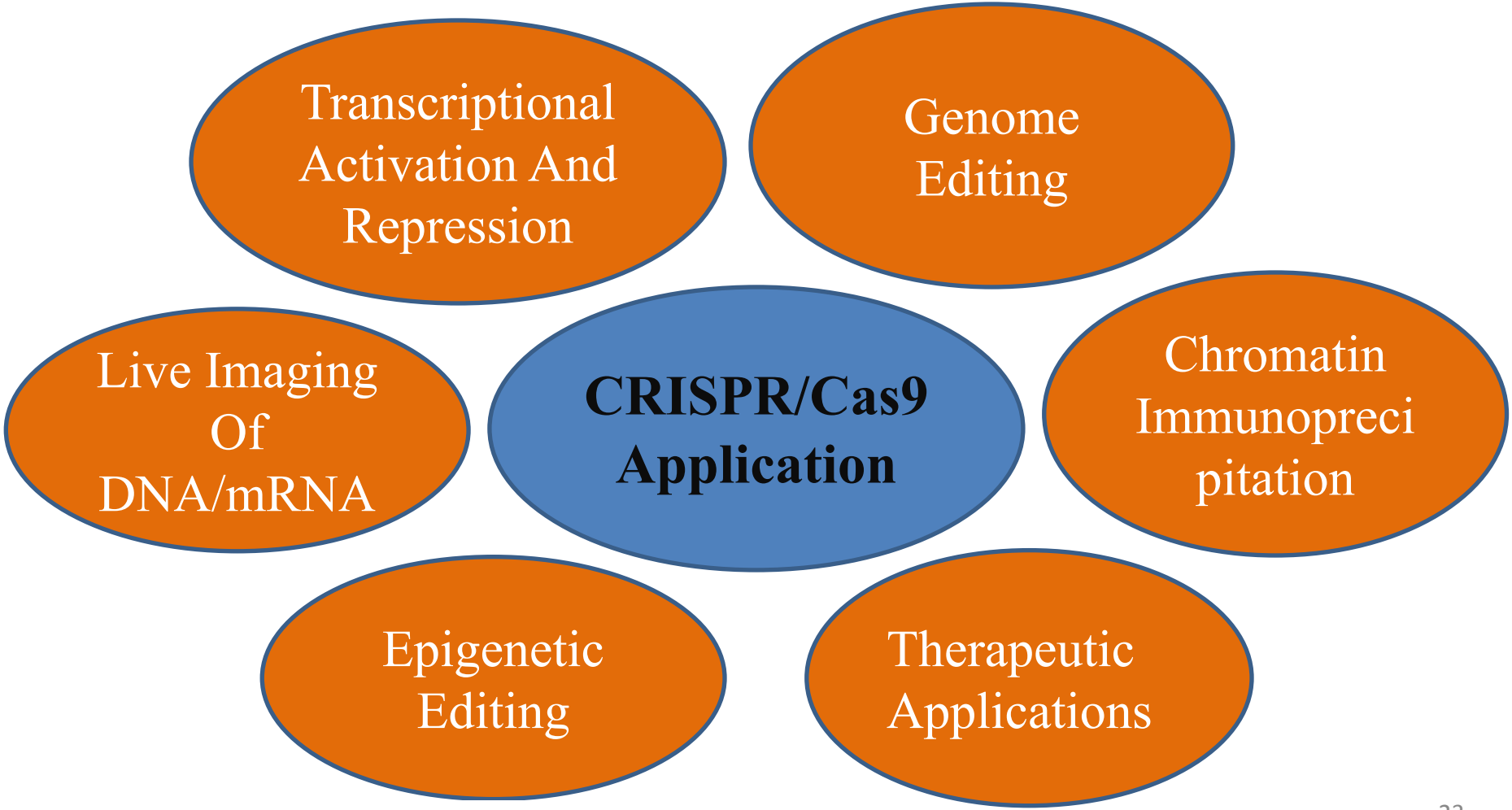
Article | Published: 21 October 2019

Search-and-replace genome editing without double-strand breaks or donor DNA

Andrew V. Anzalone, Peyton B. Randolph, Jessie R. Davis, Alexander A. Sousa, Luke W. Koblan, Jonathan M. Levy, Peter J. Chen, Christopher Wilson, Gregory A. Newby, Aditya Raguram & David R. Liu

Nature **576**, 149–157(2019) | Cite this article

- Reverse transcriptase fusion w/ dCas9
- pegRNA
- Targets: - sickle cell anemia
  - Tay-Sachs disease



https://www.genscript.com/using-crispr-libraries-for-screening

Summary

- Where CRISPR/Cas came from
- How CRISPR/Cas measures up against predecessors
- How we've improved on targeting
- CRISPR/Cas enhancements currently available
- Rough guide to therapeutic development
- CRISPR/Cas future work

Questions?