Advancements in gRNA engineering for CRISPR in Research and Clinical Applications

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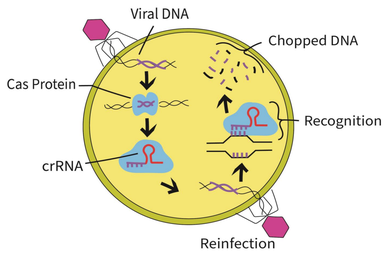
1.Background

A) CRISPR origin

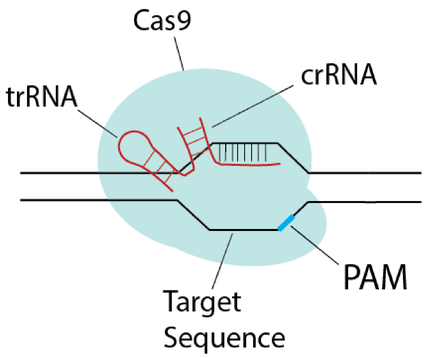
The CRISPR System has **two parts:**

1. **DNA-Cutting Protein** (CAS9)
2. **Directing Molecule(s)** (crRNA and tracrRNA),

The directing molecule f**inds specific sequences** within the DNA and destroys the DNA **– PAM sequence crucial in identifying threats**



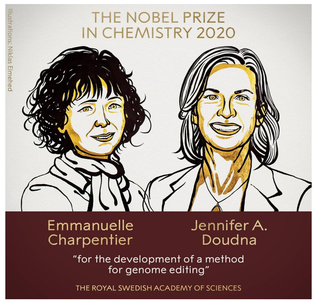
B) Repurposing CRISPR for Use in Human Cells



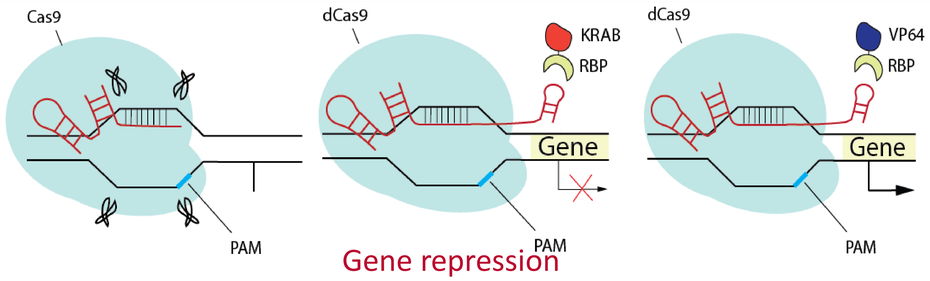
C) Awards

-FDA approved treatments for Sickle Cell Anemia

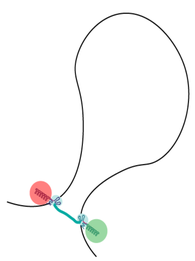
-Chemistry **Nobel Prize** in 2020



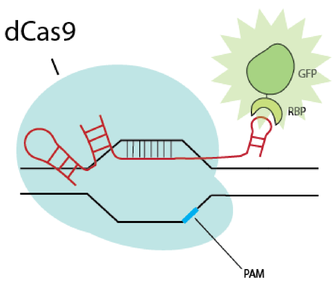
2.CRISPR Applications



CRISPR



Genome manipulation



Genome labeling

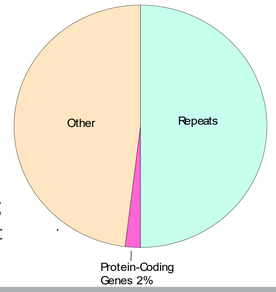
3.CRISPR-Sirius: Labeling the Genome

A) Identifying Target Sites/gRNA construction

Does the target area contain many repetitive sequences?

Yes → **One** gRNA plasmid made targeting **one** common repeat with an adjacent **PAM (NGG)** sequence

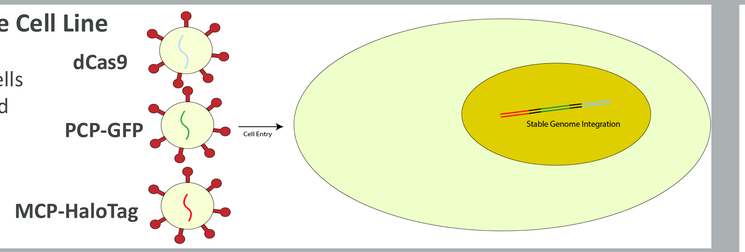
No → **Multiple** gRNA plasmids made targeting **different** sequences all with an adjacent **PAM (NGG)**



B) Establishment of Stable Cell Line

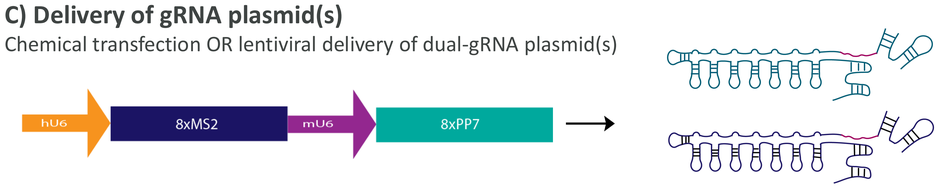
1. Lentiviral transduction
2. Flow cytometry to isolate cells containing delivered plasmid

This process is done separately for each of the following:



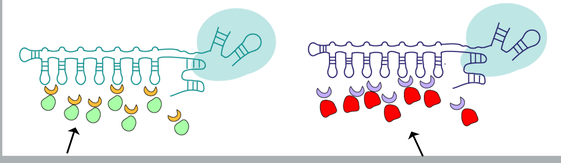
C) Delivery of gRNA plasmid(s)

Chemical transfection OR lentiviral delivery of dual-gRNA plasmid(s)

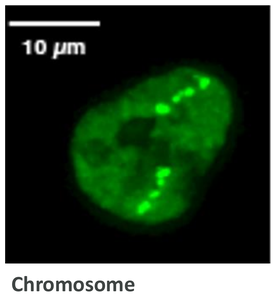


D) Assembly in the Nucleus

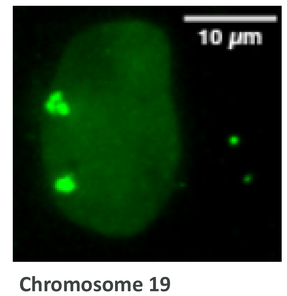
The gRNAs lead dCas9 to the target sites, and once bound, MCP-HaloTag and PCP-GFP fusions are recruited to create red and green fluorescent signals



E) Imaging and Application



Chromosome 19 conformation in standard bone cells

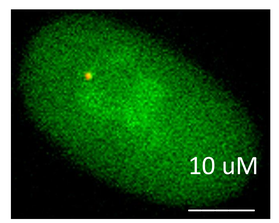


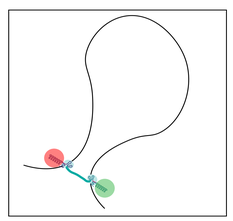
Chromosome 19 conformation in cancerous bone cells

4.CRISPR-CLIP: Manipulating the Genome

A linker takes the place of a promoter, creating one construct when the plasmid is expressed instead of two separate gRNAs

The linker fuses two Sirius gRNAs into one CLIP gRNA





Conclusion

CRISPR is a revolutionary technology that has proven to be a highly versatile tool. Using the gRNA structure as a platform for improving the CRISPR system, we have been able to achieve efficient genome labeling with CRISPR-Sirius and chromatin looping with CRISPR-CLIP. Our goal is to apply these systems to answer fundamental biological questions and pave the way for potential therapies for genetic diseases.

References

1. Ma, H., Tu, LC., Naseri, A. *et al.* CRISPR-Sirius: RNA scaffolds for signal amplification in genome imaging. *Nat Methods* **15**, 928–931 (2018). https://doi.org/10.1038/s41592-018-0174-0
2. Xiong X, Chen M, Lim WA, Zhao D, Qi LS. CRISPR/Cas9 for Human Genome Engineering and Disease Research. Annu Rev Genomics Hum Genet. 2016 Aug 31;17:131-54. doi: 10.1146/annurev-genom-083115-022258. Epub 2016 May 23. PMID: 27216776; PMCID: PMC10216851.

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