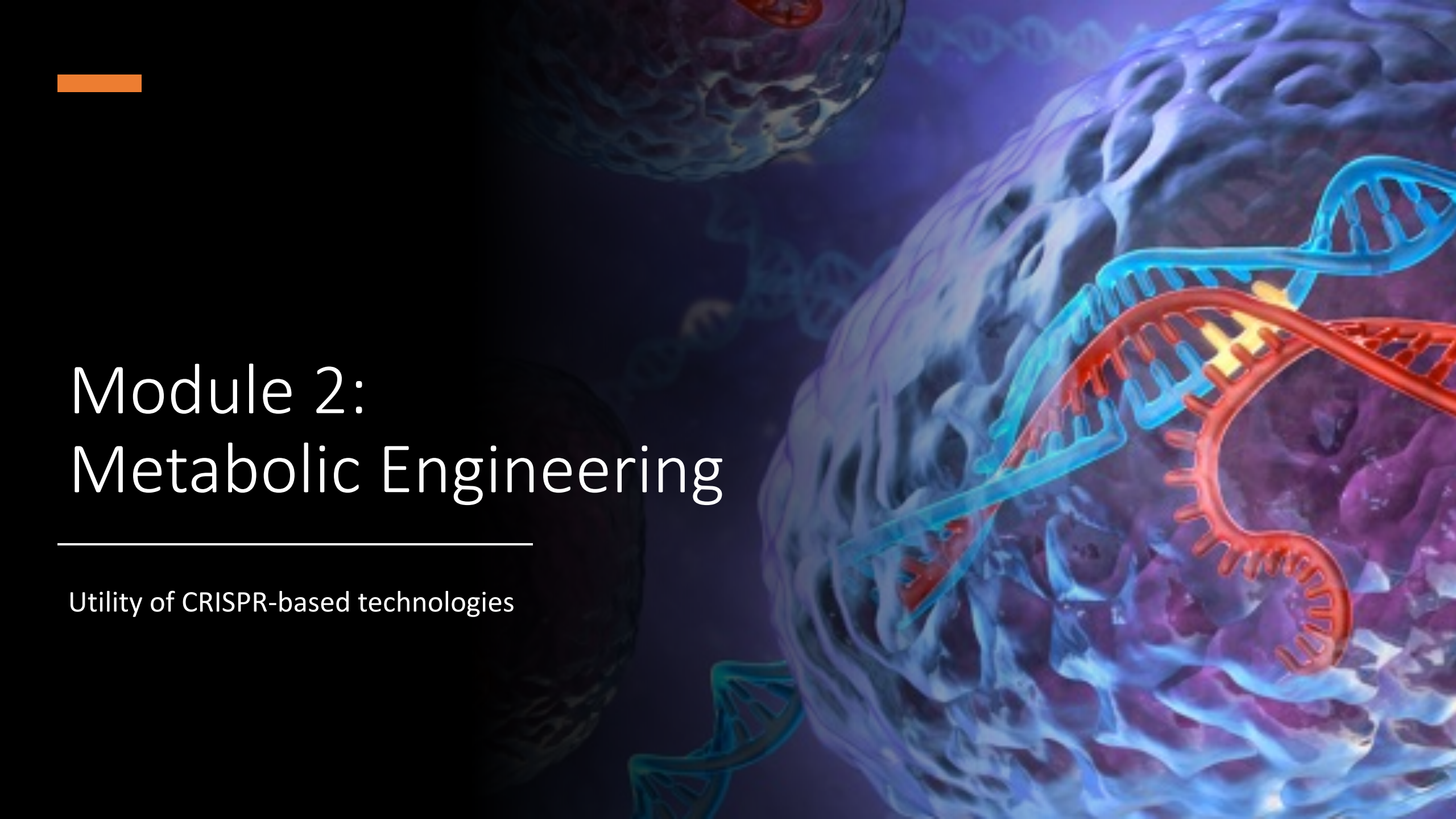
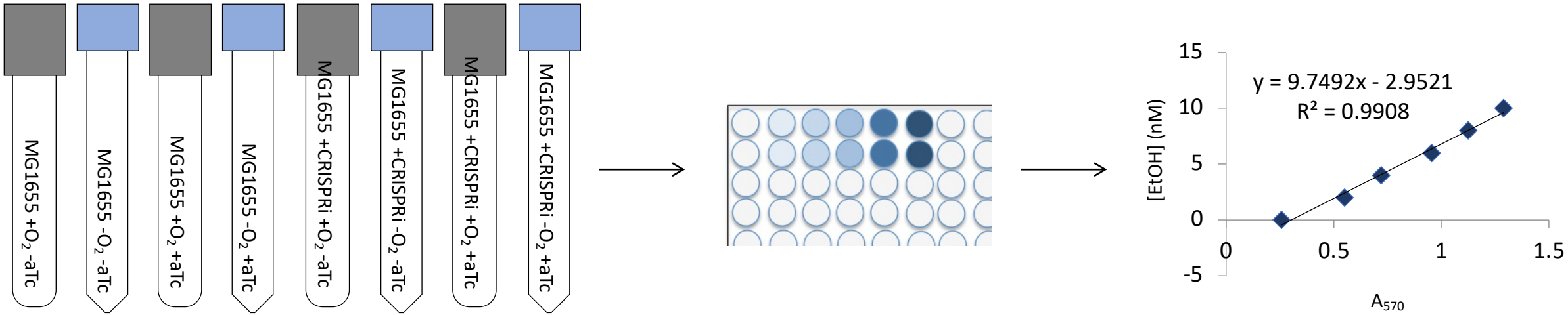


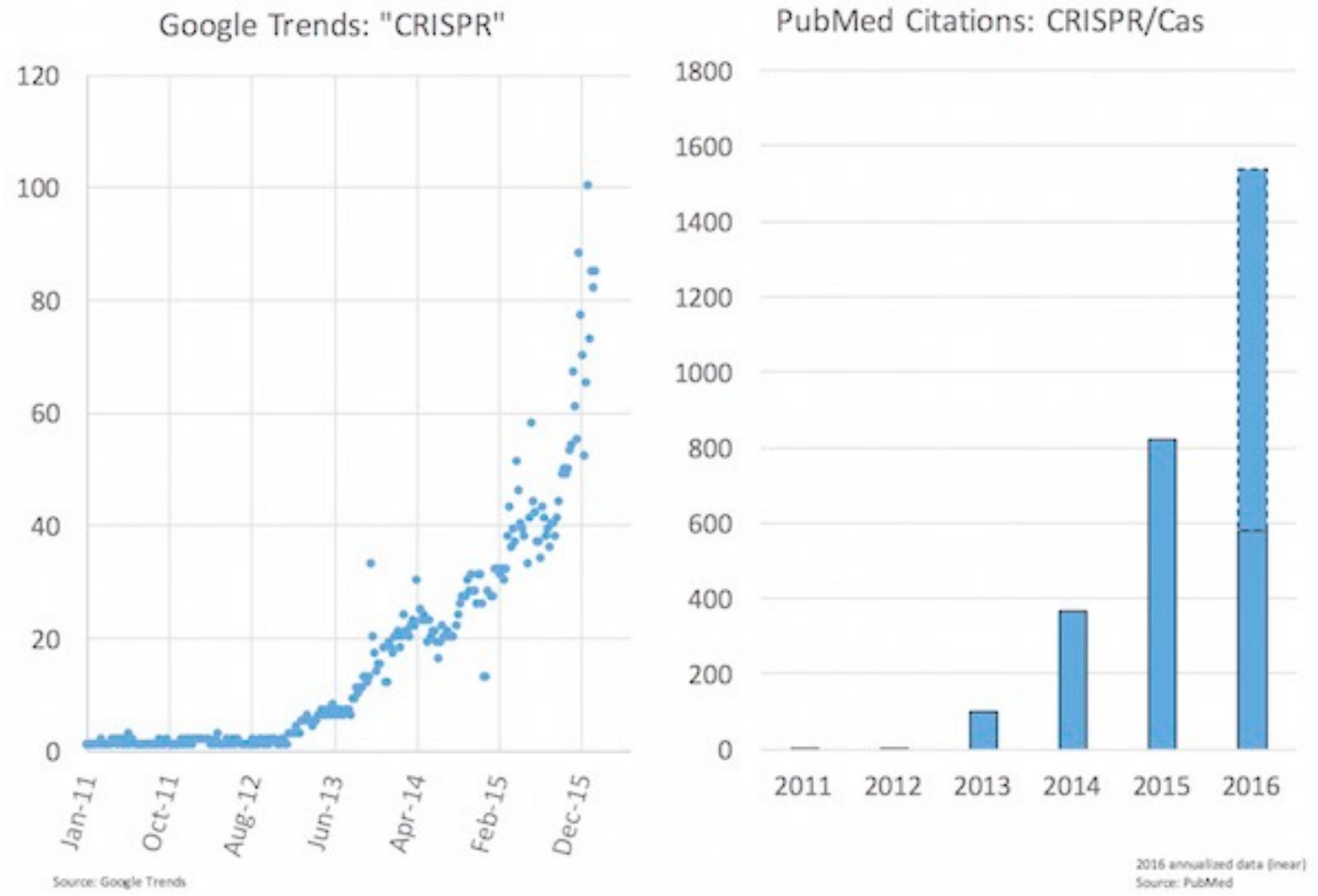
Module 2: Metabolic Engineering

Utility of CRISPR-based technologies

Review of experimental approach



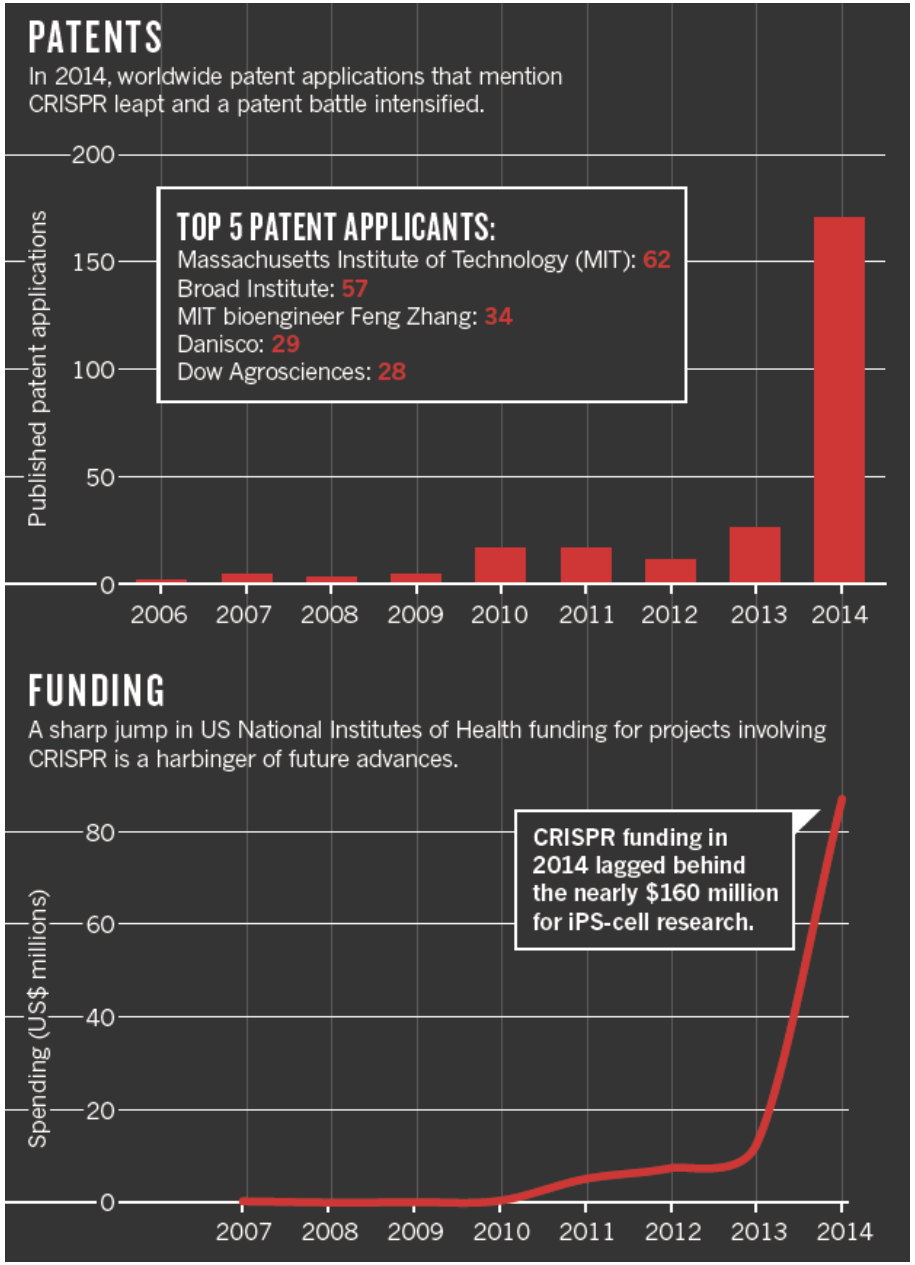
CRISPR is booming!



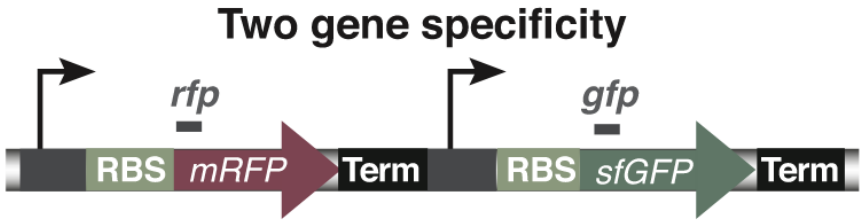
In 2018, 3000+ CRISPR-based papers were published

CRISPR technology expands research capabilities

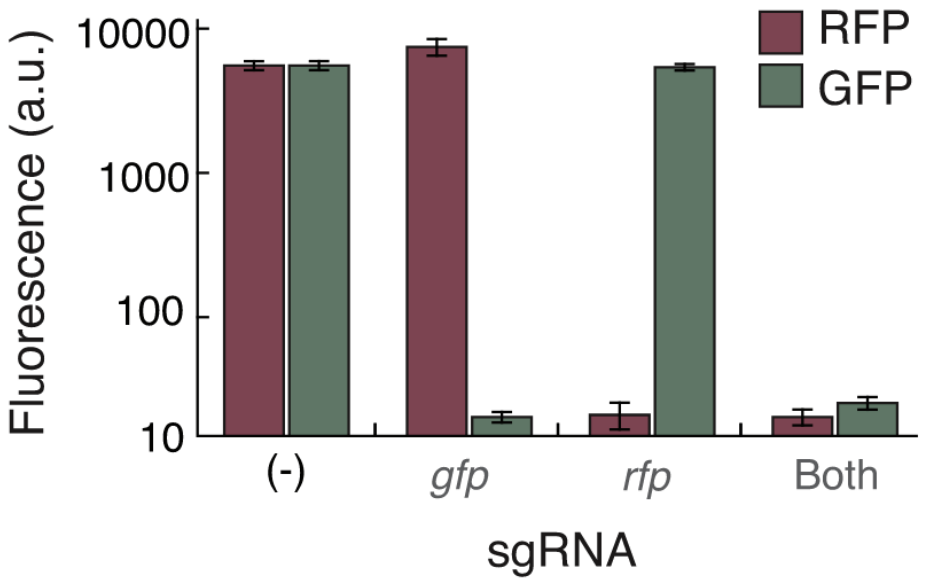
- Measuring and altering gene expression
- DNA tagging and purification
- DNA incorporation and deletion
- And so much more…



Multiplexing enables specific targeting of multiple genes



Transcription of gRNAs that target RFP and GFP controlled by distinct promoters / activators

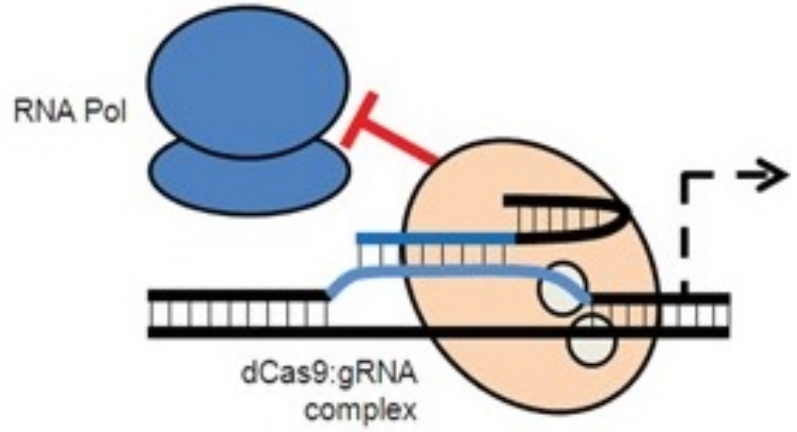


Qi et al. (2013) *Cell* 152:1173-1183

Modulating gene expression without altering DNA sequence

Catalytically inactive Cas9 is able to bind DNA and can be coupled with activators or repressors

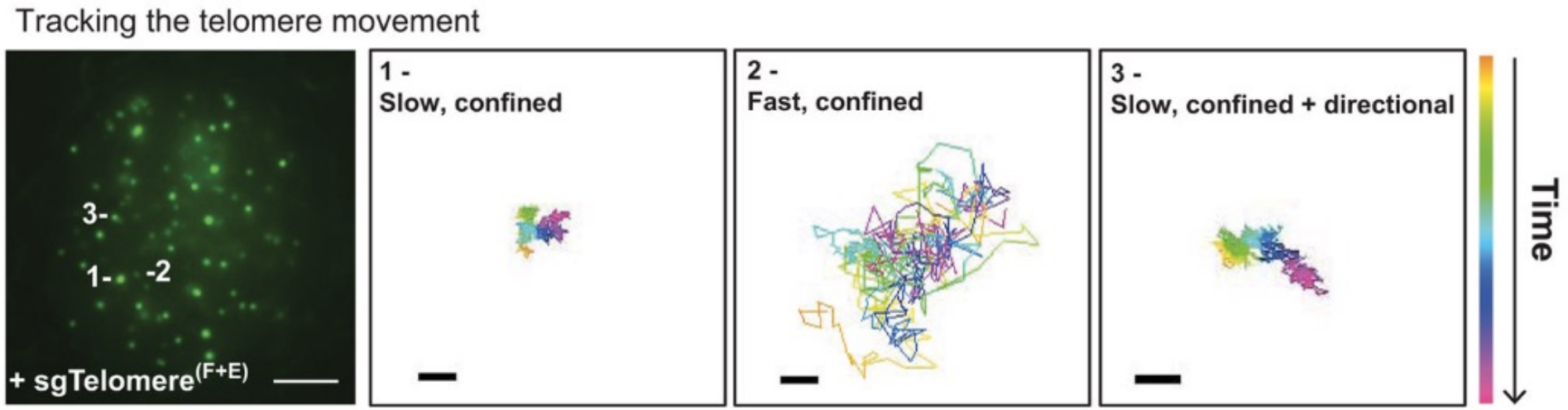
- Block transcription
- When fused with repressor or when used with multiple gRNAs, gene expression further decreased
- When fused with activator, gene expression increased



dCas9 applications: DNA tagging

dCas9 can be used to add fluorescent tags to specific genetic sequences

- Can enable visualization of spatiotemporal dynamics within live cells by tracking the movement of specific molecules

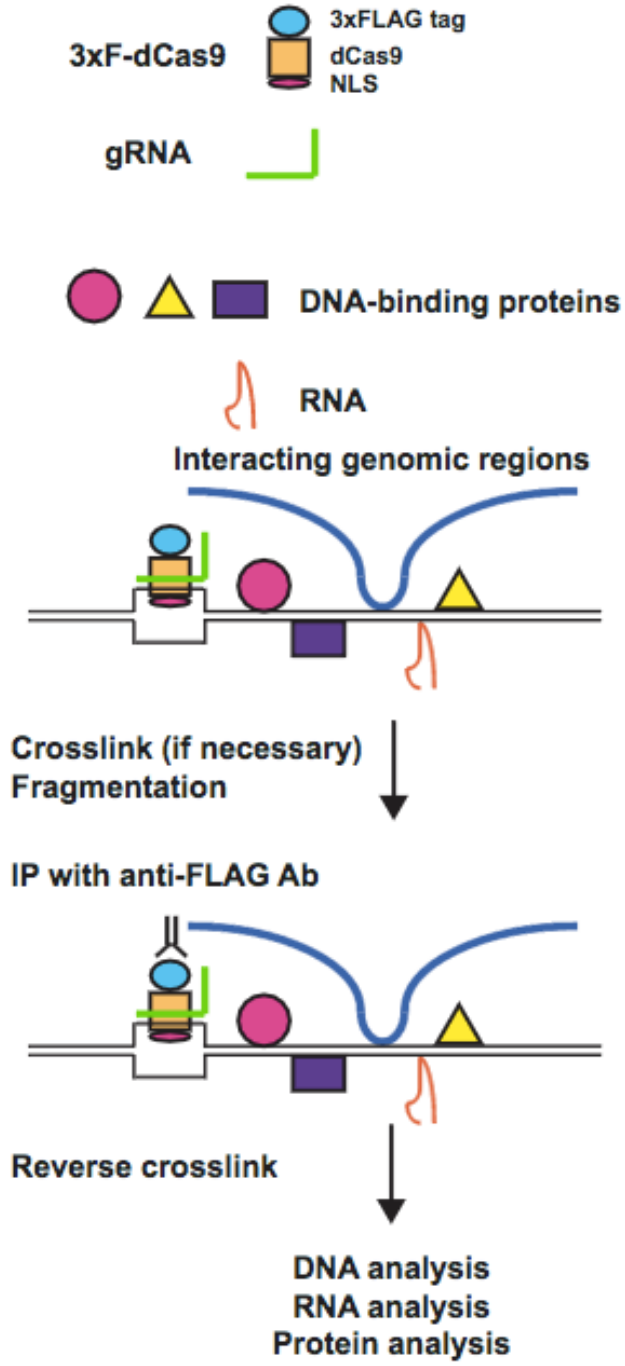


Chen et al. (2013) *Cell* 155:1479-1491

dCas9 applications: DNA purification

dCas9 can be used to purify specific DNA sequences

- dCas9 acts as a ‘handle’ by which bound DNA can be retrieved from cellular lysate
- Can also provide information on which proteins / regulatory elements bind at a specific loci

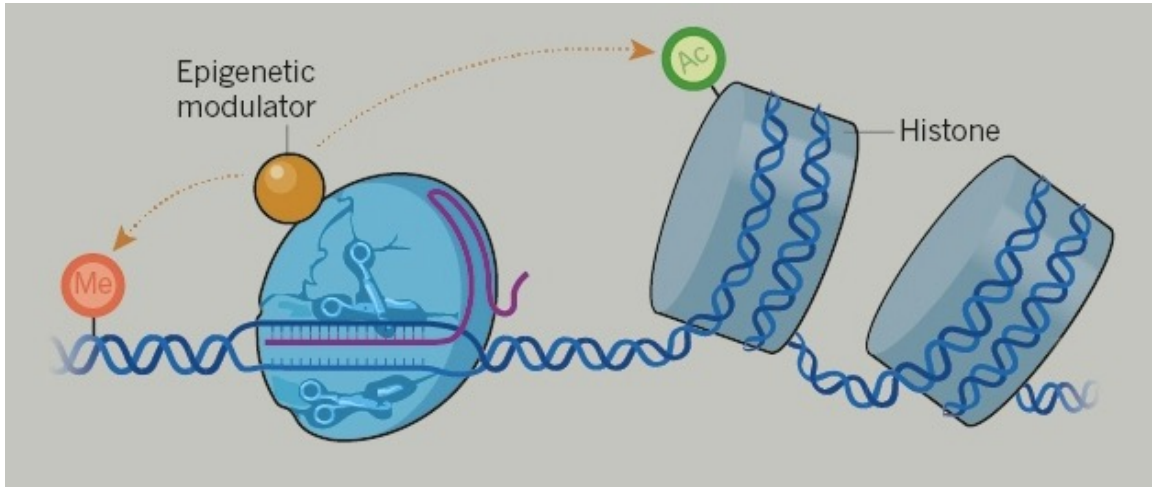


Fujita and Fujii. (2013) *Biochem Biophys Res Comm* 439:132-1336

dCas9 applications: epigenetics

dCas9 can be fused to epigenetic modulators to alter gene expression

- Acetyltransferase promotes activation by changing the charge of the histone and thereby relaxing the chromatic structure
- Methylases can either decrease or increase expression (dependent on location and extent of methylation)



Hilton et al. (2015*) Nature Biotech* 33: 510-517

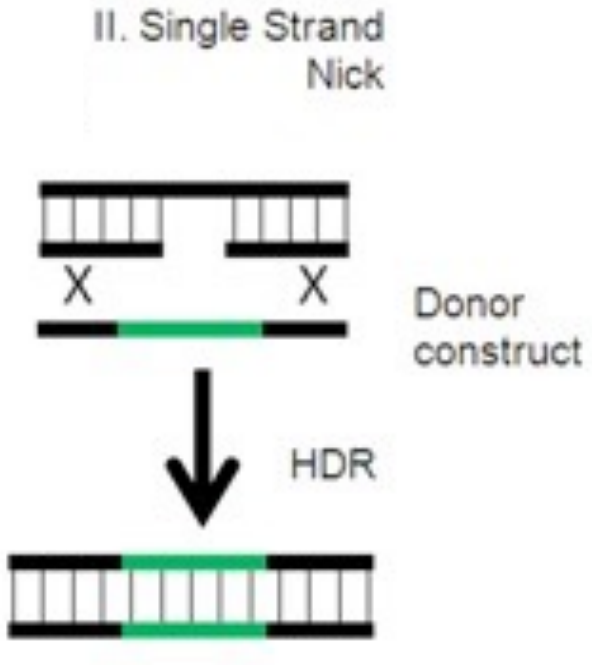
Cas9 applications in mammalian cells

- Cystic fibrosis mutation corrected in primary human intestinal cells, mouse model
- Oncogenic mutation corrected in human induced pluripotent stem cells
- Cataract-causing mutations corrected in mouse zygotes, spermatogonial stem cells
- HIV proviruses removed from infected cells
- HepB and HepC targeted in infected cells

Troubleshooting off-target effects

- Generating ssDNA nicks rather than dsDNA breaks for incorporating ‘new’ sequence(s)
  - ssDNA nicks in locations without homology to donor DNA w be repaired by host machinery
  - ssDNA nicks in locations with homology will incorporate donor DNA sequence

- Using inducers to control activity of Cas9
  - Photocaging
  - Light / Chemical activation



Science

CRISPR bombshell: Chinese researcher claims to have created gene-edited twins

By Dennis Normile | Nov. 26, 2018 , 1:10 PM

Did CRISPR help—or harm—the first-ever gene-edited babies?

By Jon Cohen | Aug. 1, 2019 , 11:30 AM

SCIENTIFIC AMERICAN®

Despite Controversy, Human Studies of CRISPR Move Forward in the U.S.

The technology that produced a global scandal in China last year has entered into clinical trials to treat sickle cell anemia and an eye disease

By Karen Weintraub on August 13, 2019

nature

NEWS | 05 March 2020

CRISPR treatment inserted directly into the body for first time

Experiment tests a gene-editing therapy for a hereditary blindness disorder.

Heidi Ledford

Concerns regarding CRISPR technology

"This power is so easily accessible by labs — you don't need a very expensive piece of equipment and people don't need to get many years of training to do this... We should think carefully about how we are going to use that power."

Stanley Qi from Ledford (2015) *Nature News Feature*

How do we evaluate the impact of new technologies?

Jennifer Doudna, Ph.D.

Nobel prize in Chemistry 2020 for her work involving CRISPR

https://bigthink.com/hard-science/jennifer-doudna/

Consider the following questions:

- Who can benefit from this technology?
- Who can be harmed by this technology?
- What are the possible ethical issues raised by the benefits / harms?

What is the take-home message?

- CRISPR-based technologies are easily developed to generate new DNA engineering tools

- Consider the benefits and consequences of making DNA engineering easier / cheaper / faster