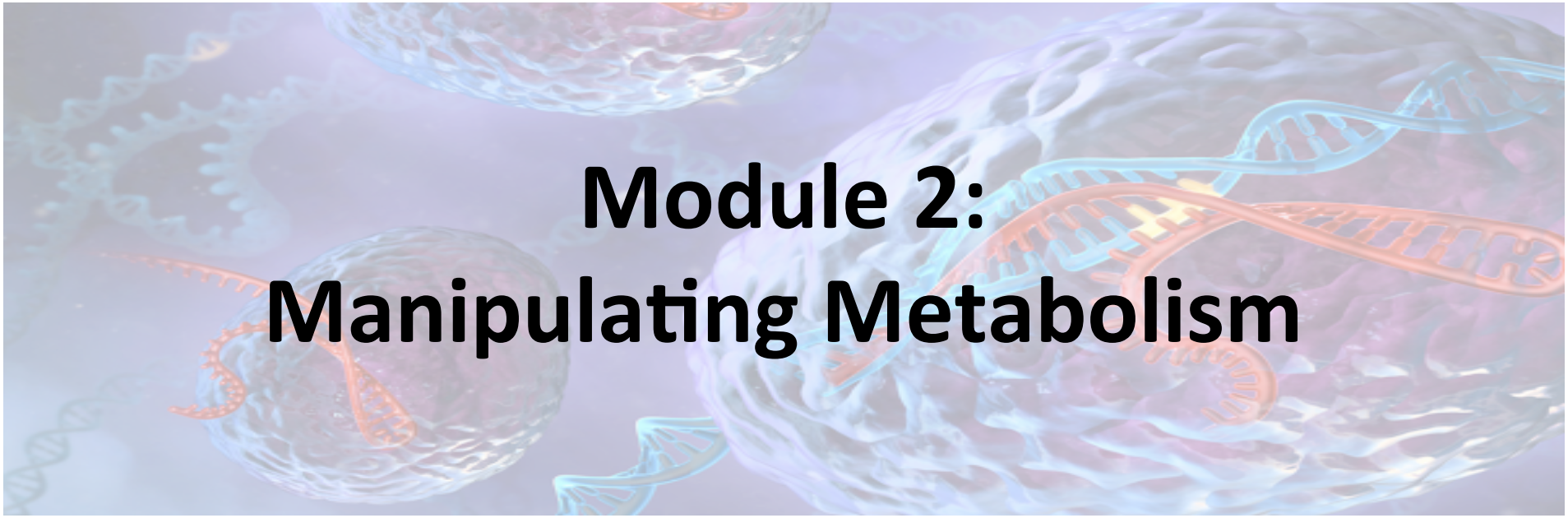
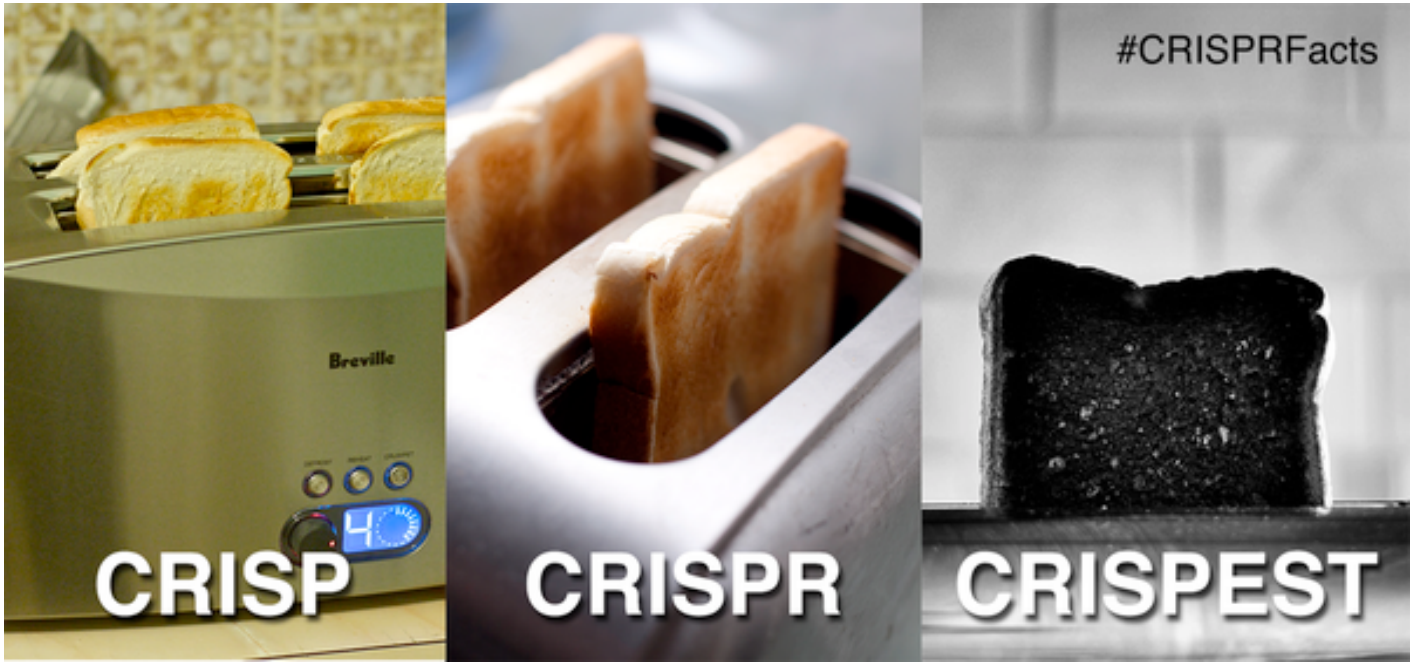


Module 2: Manipulating Metabolism

Applications of CRISPR-based systems

11/6/18


Breville
CRISP
CRISPR
#CRISPRFacts
CRISPEST

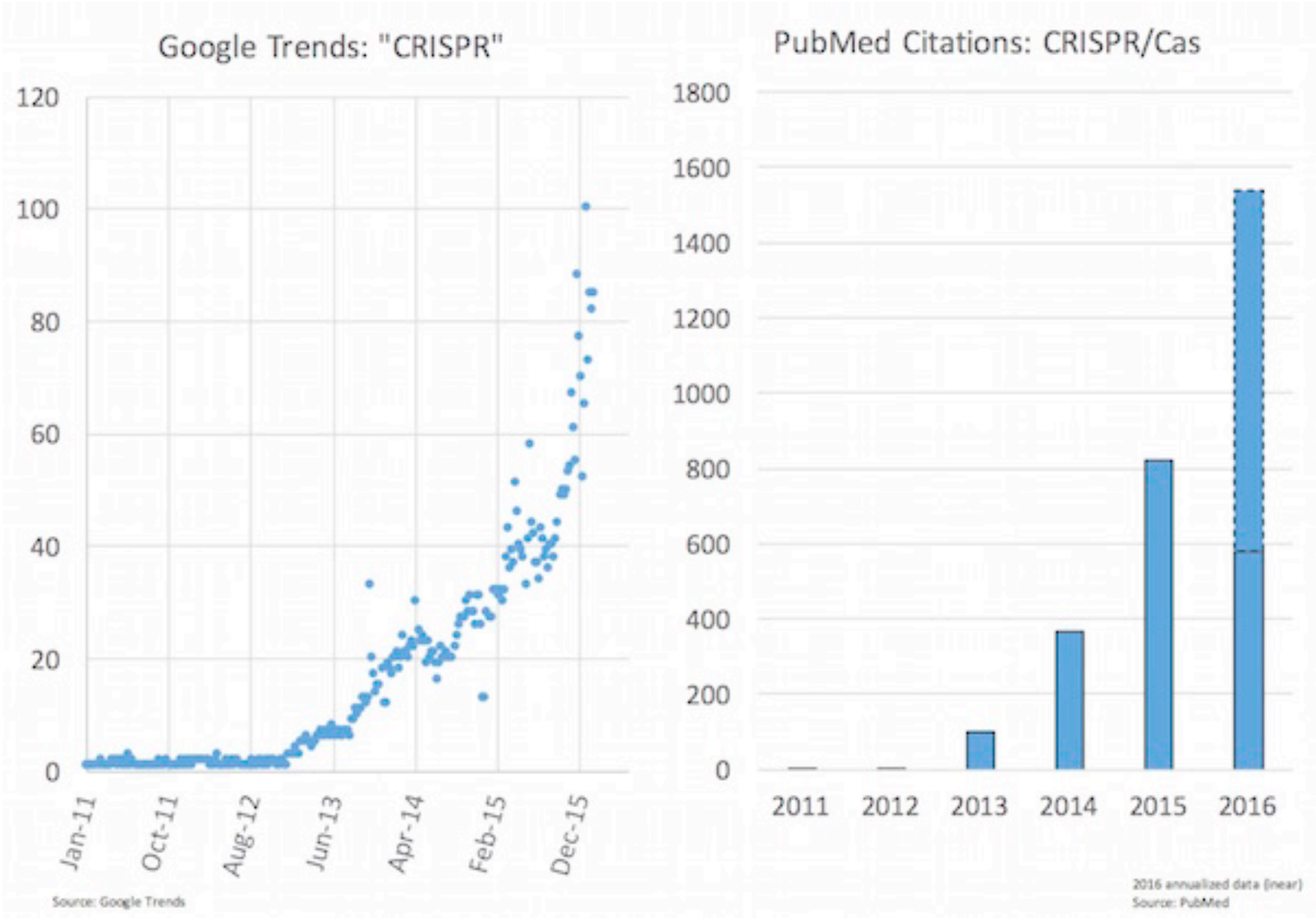
Reminder for Mod 2 due dates

- Research article due Monday, Nov 12 by 10 pm

- Open office hours:
  - Saturday, Nov 10 in 56-302 from 1-3 pm
  - Sunday, Nov 11 in 56-302 from 2-7 pm

- Blog post due Tuesday, Nov 13 by 10 pm

CRISPR is booming!



Utility of CRISPR in basic research

"I wish I had had this technology sooner. My postdoc would have been a lot shorter."

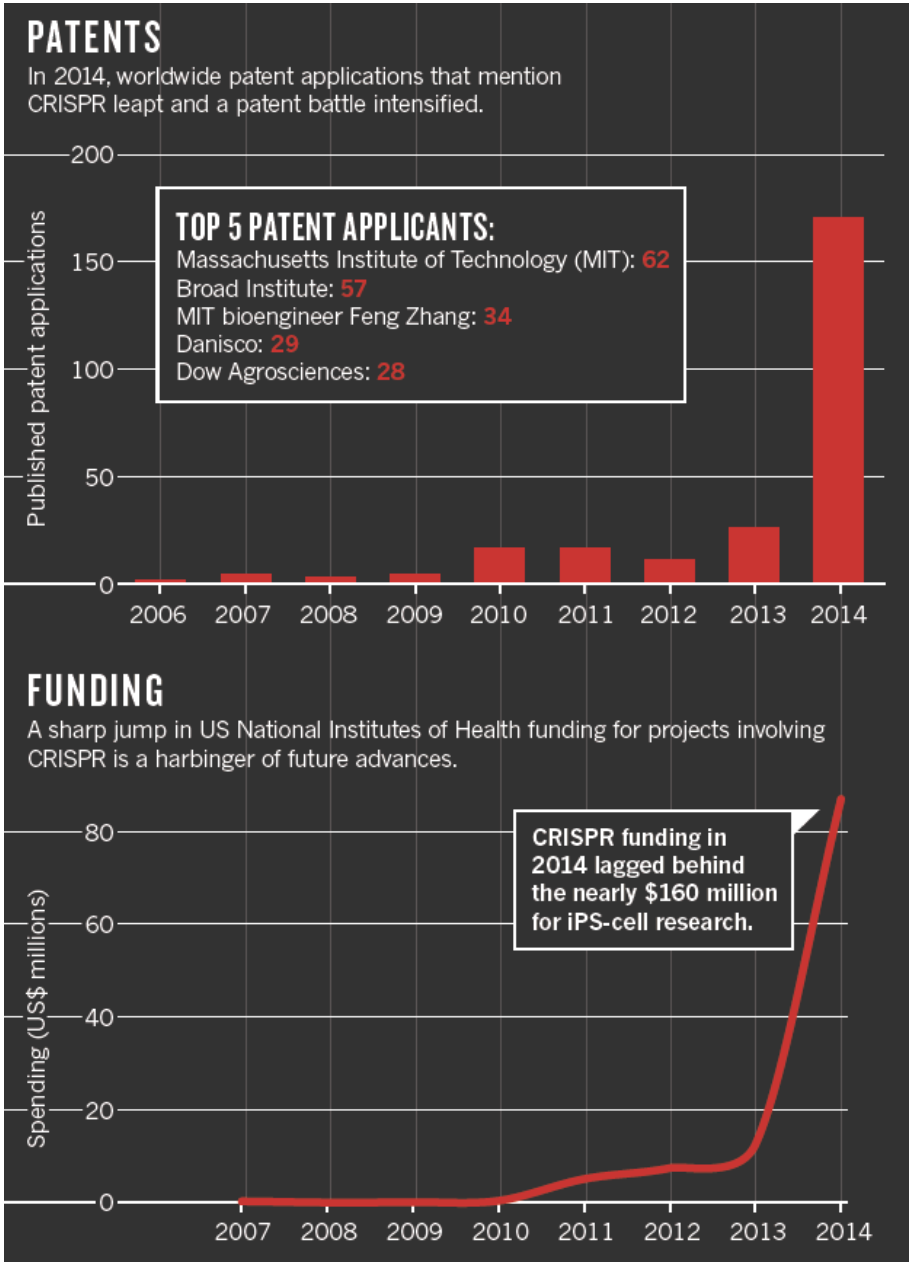
<u>Pre-CRISPR</u>
1 year
$20,000

<u>Post-CRISPR</u>
1 month

Wen Xue from Ledford (2016) *Nature News Feature*

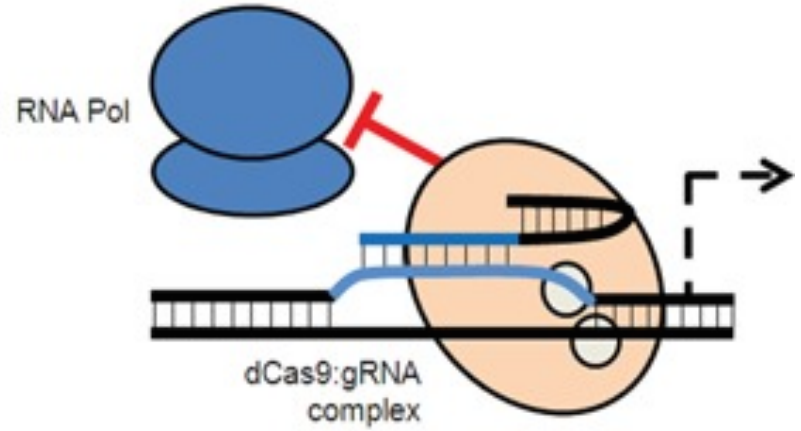
CRISPR technology is advancing research capabilities

- Gene expression
- DNA tagging / purification
- DNA incorporation



Modulating gene expression

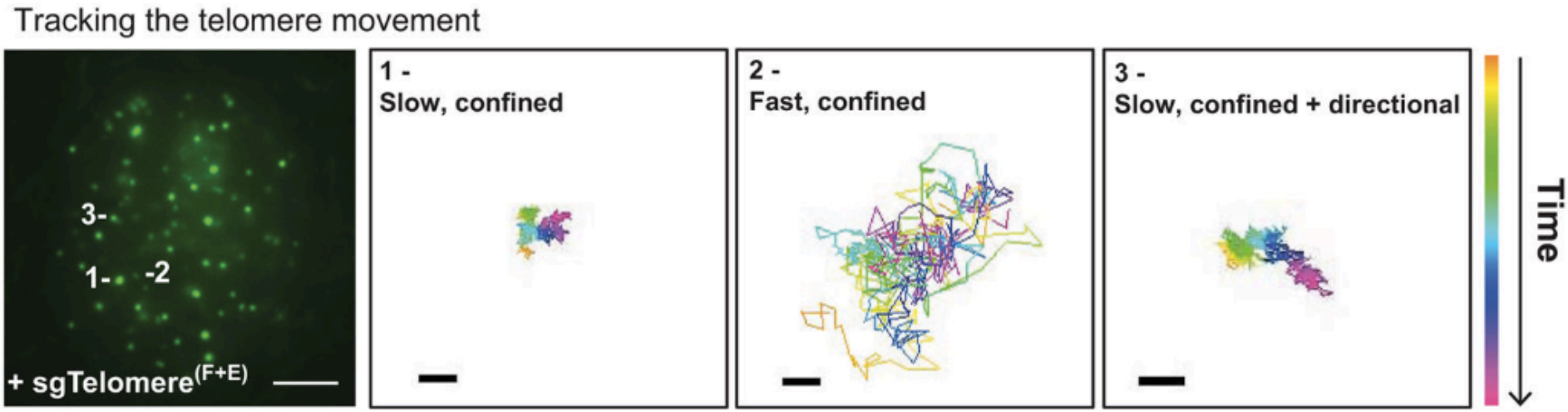
- Catalytically inactive dCas9
  - Block transcription



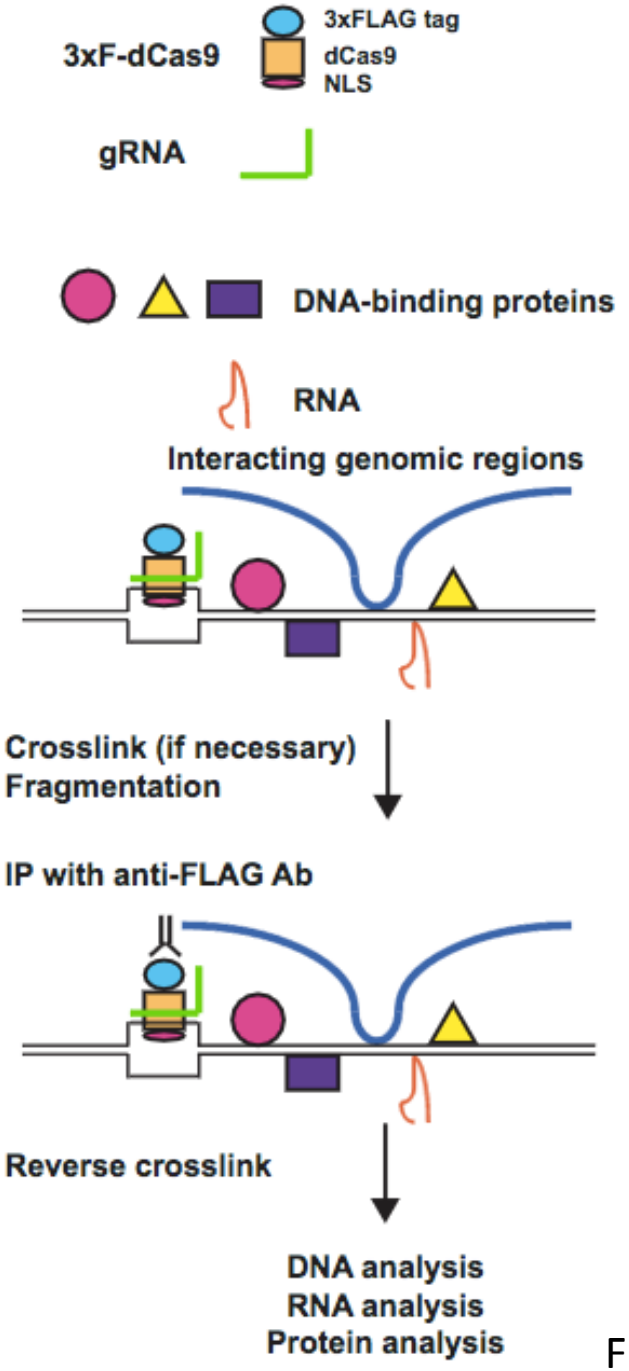
  - When fused with repressor or when used with multiple gRNAs, gene expression further decreased
  - When fused with activator, gene expression increased

dCas9 applications: DNA tagging

- Fluorescently tag genetic loci to visualize spatiotemporal dynamics within live cells



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



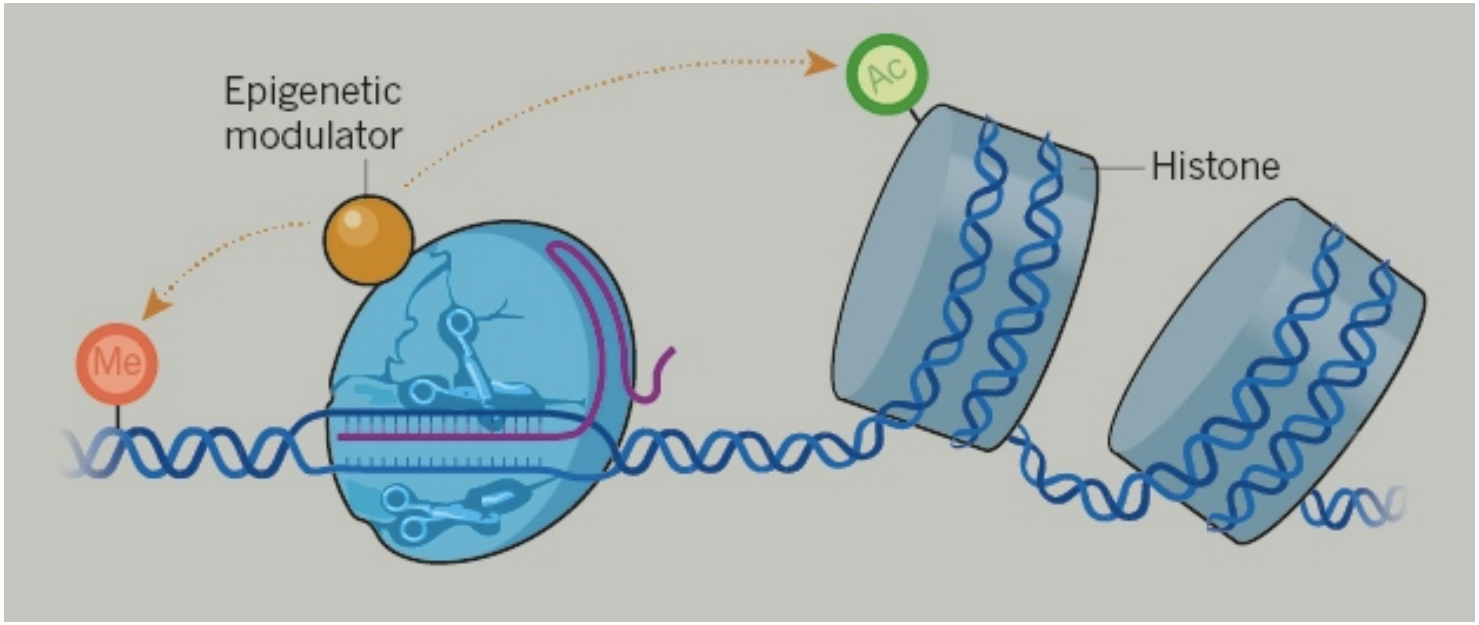
dCas9 applications: DNA purification

- Bind loci for purification to identify proteins that associate with specific sequences in DNA

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

dCas9 applications: epigenetics

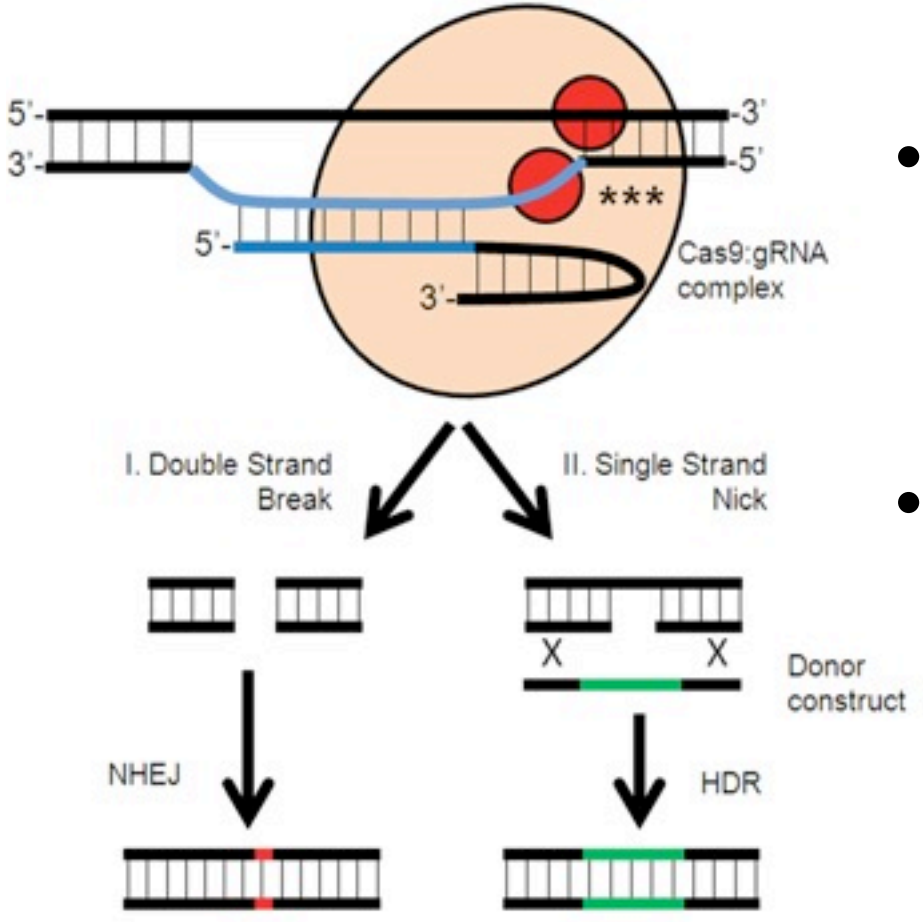
- Fused to acetyltransferase promotes activation from enhancer sites and enables heritable epigenetic changes



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

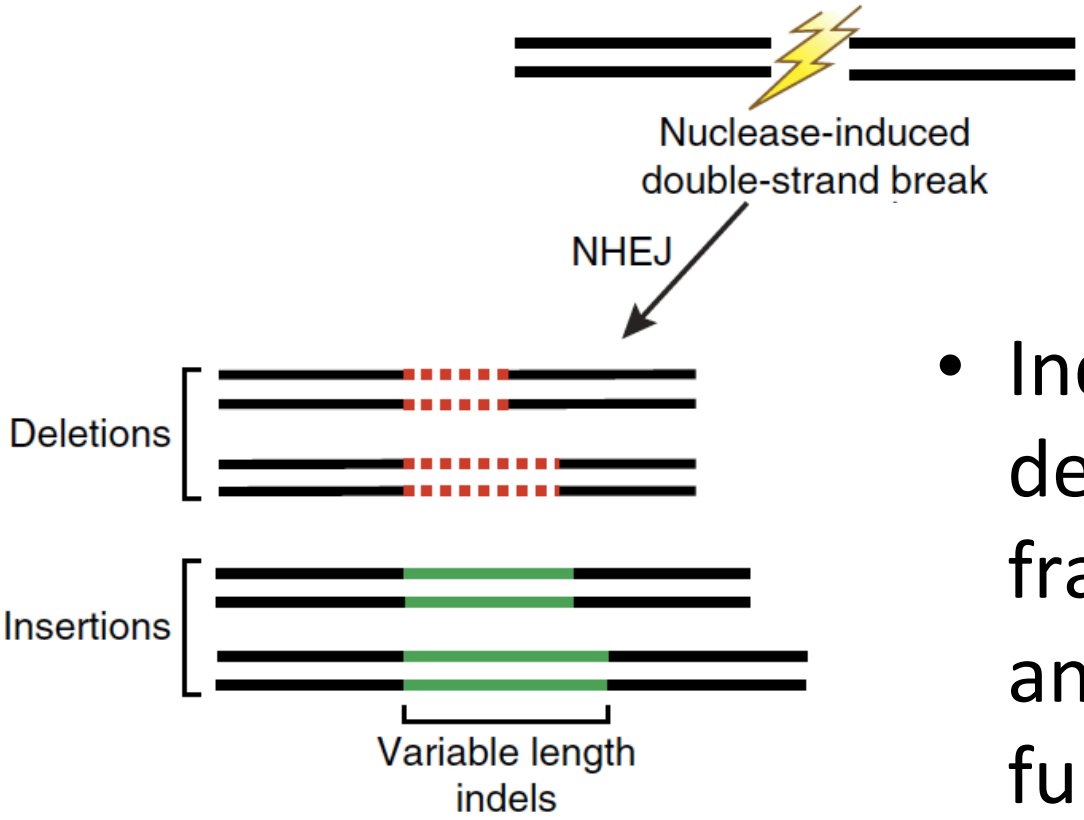
What if we want to engineer a permanent genetic mutation?

Mammalian cells able to repair dsDNA breaks



- Non-homologous end joining (NHEJ)
- Homology-directed repair (HDR) or homologous recombination (HR)

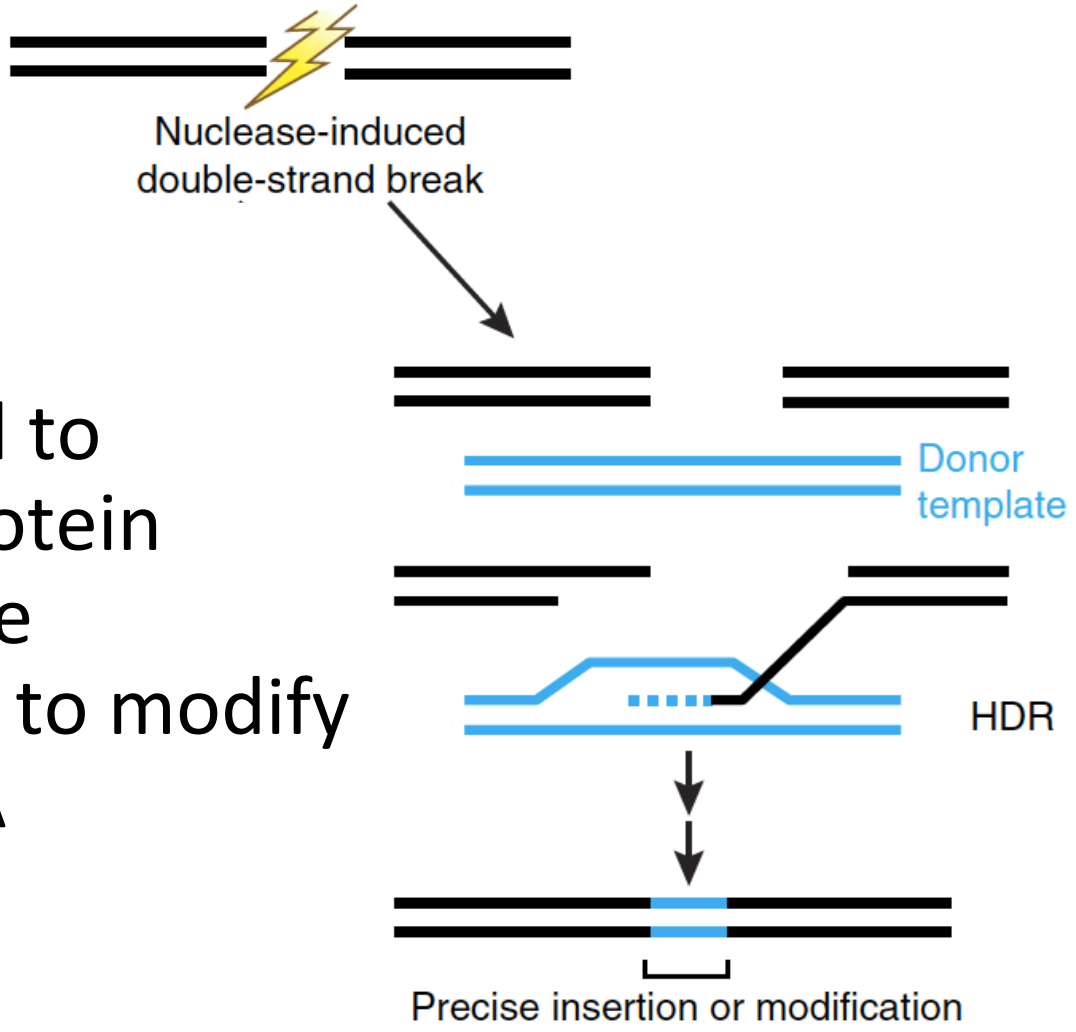
NHEJ repair generates random insertions / deletions



- Indels (insertions / deletions) result in frameshift mutations and loss of protein function

H(D)R repair enables specific sequence insertions

- Insertions used to incorporate protein tags / new gene sequences and to modify the native DNA sequence



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

MIT Technology Review

The first known attempt at creating genetically modified human embryos in the United States has been carried out by a team of researchers in Portland, Oregon, *MIT Technology Review* has learned.

NEWS · 08 AUGUST 2018

Did CRISPR really fix a genetic mutation in these human embryos?

Researchers provide more evidence for their landmark claim that gene editing rid embryos of a disease mutation — but scientists are still arguing over the results.

Why is CRISPR not used now in therapeutics?

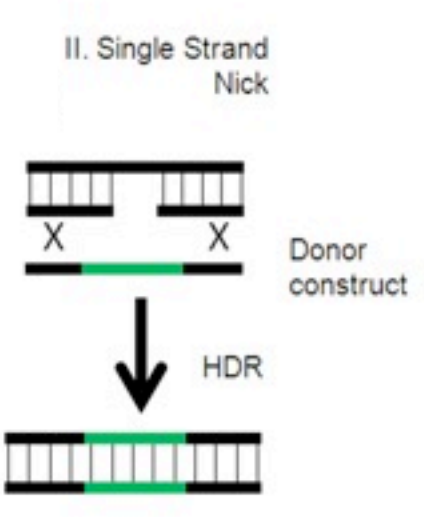
Why is CRISPR not used now in therapeutics?

What are off-target effects?

Why might off-target effects be problematic?

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 will be repaired by host machinery
  - ssDNA nicks in locations with homology will incorporate donor DNA sequence



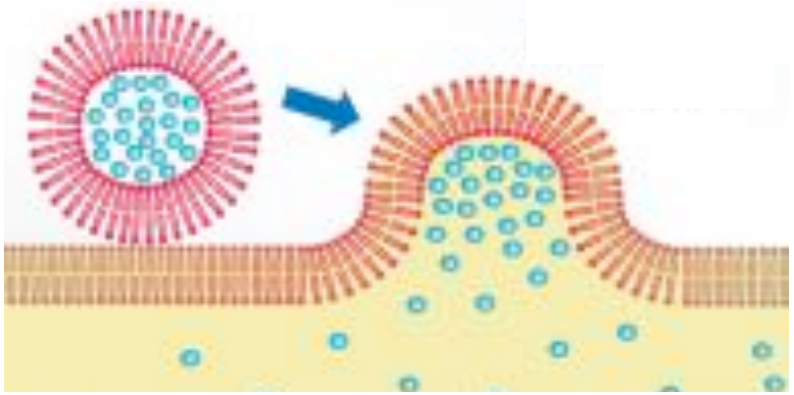
- Using photocaging to control activity of Cas9

How might CRISPR therapeutics be delivered to mammalian cells?

Developing delivery methods

- Adeno-associated virus injects system into cells

- Lipids fuse with membrane and transfect cells



- Nanoparticles or peptides penetrate cells

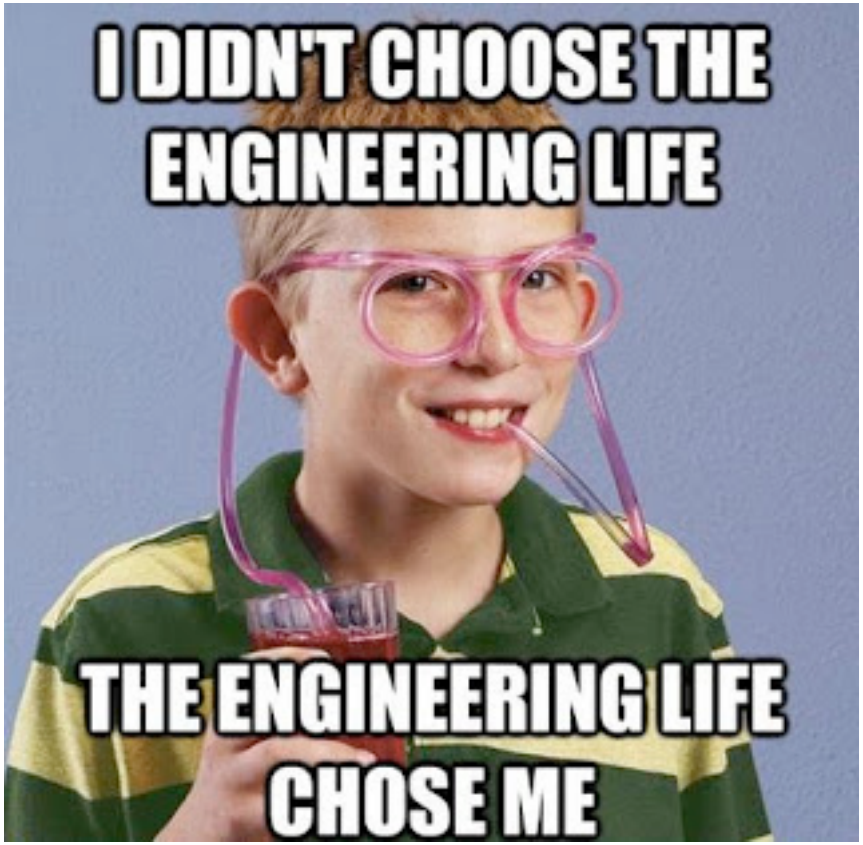
Concerns regarding CRISPR technology

"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*

What is biological engineering?

“20.109 definitely taught me more about the real world than any other class I have taken ever. Not only was it the most useful and practical class I have taken, it was one that made me finally understand what biological engineering really is.”



In the laboratory…

1. Measure fermentation products

Announcements:

- Module 3 begins Thursday, November 8 with Prof. Angie Belcher