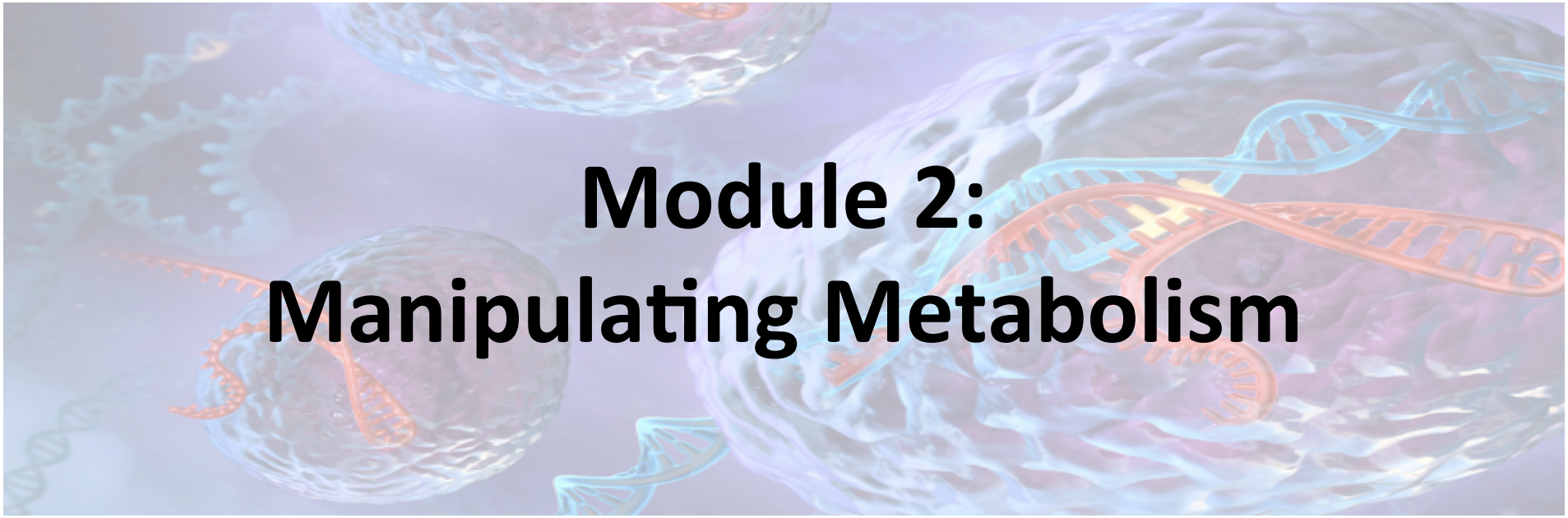


Module 2: Manipulating Metabolism

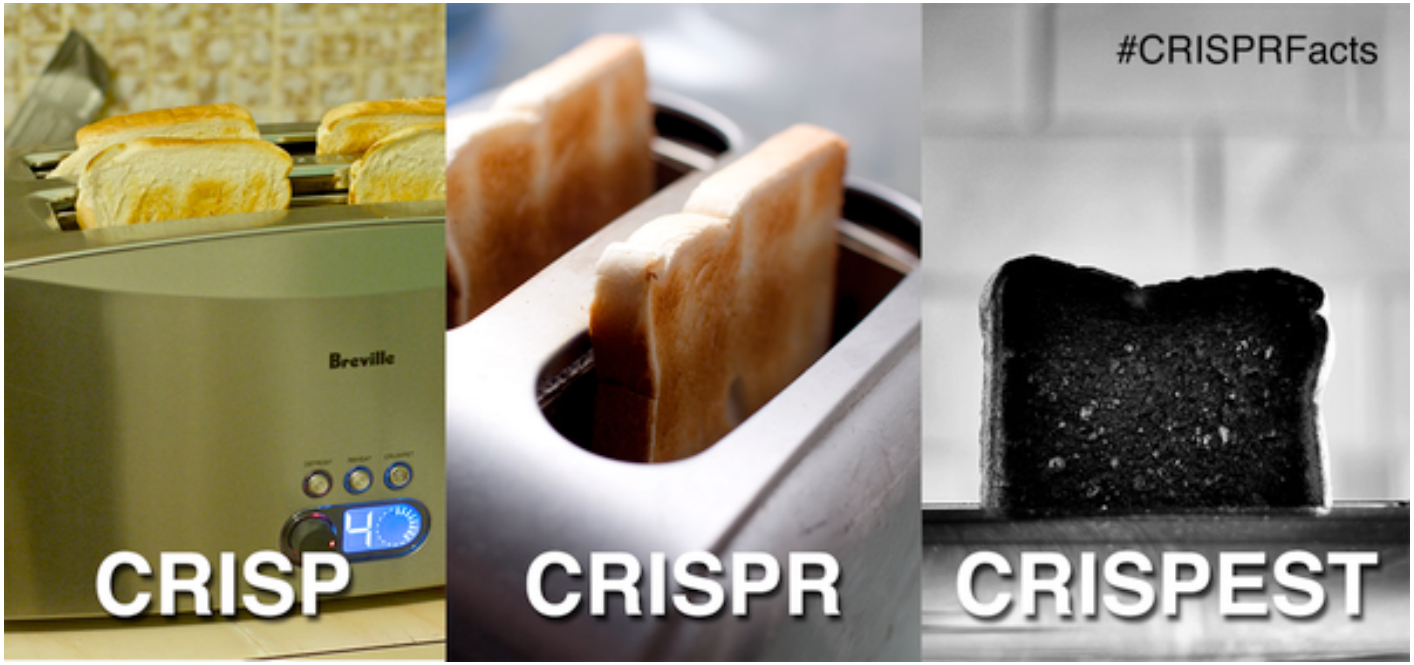
Applications of CRISPR-based systems

11/8/15

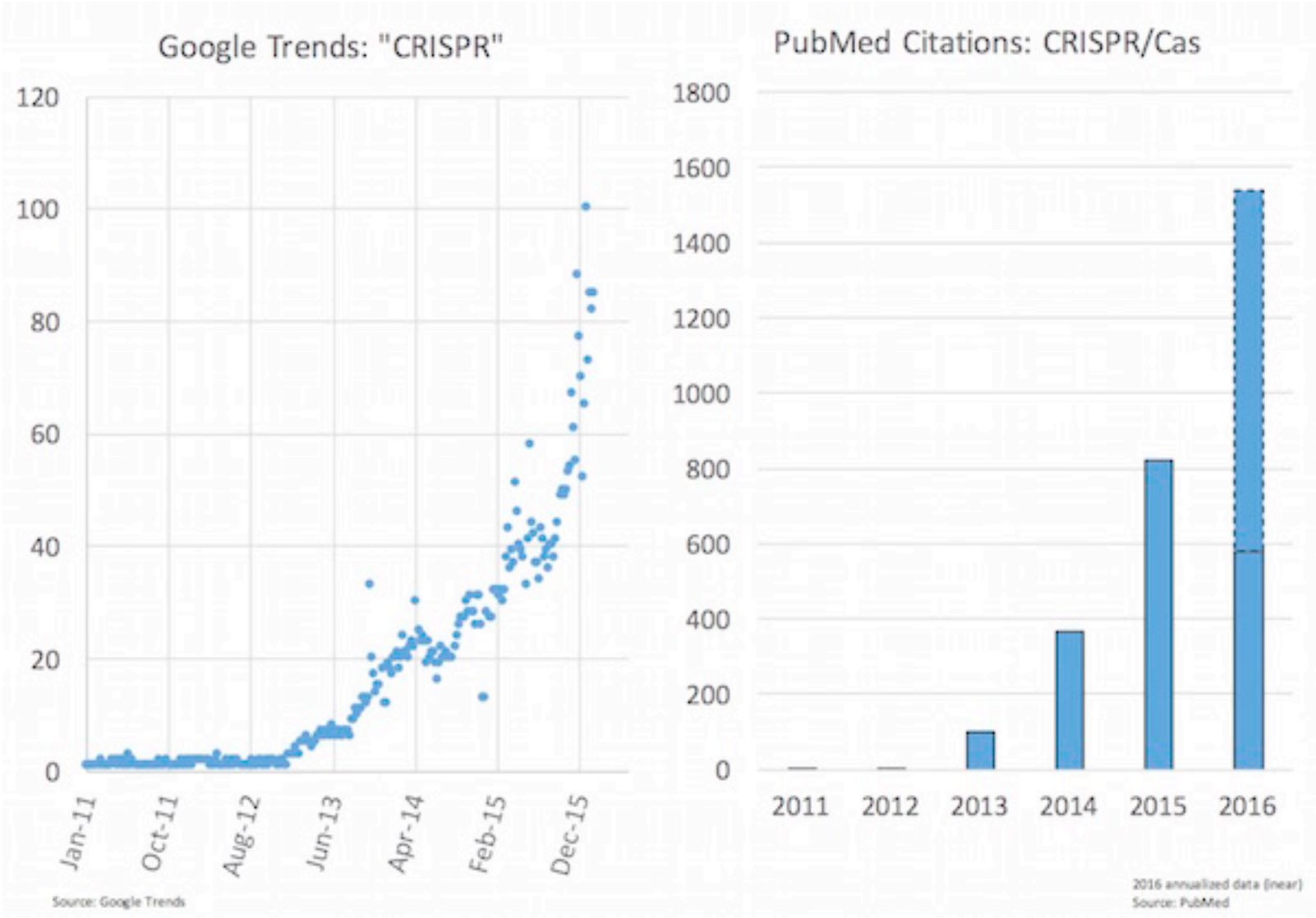
Update to Mod 2 due dates

- Research article due Sunday, Nov 20 by 5 pm
- Open office hours on Saturday, Nov 19
  - Leslie: 10 am – 12 pm
  - Maxine: 12 pm – 2 pm
  - Noreen: 2 pm – 5 pm

- Blog post due Monday, Nov 21 by 5 pm


Breville
CRISP
CRISPR
#CRISPRFacts
CRISPEST

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."

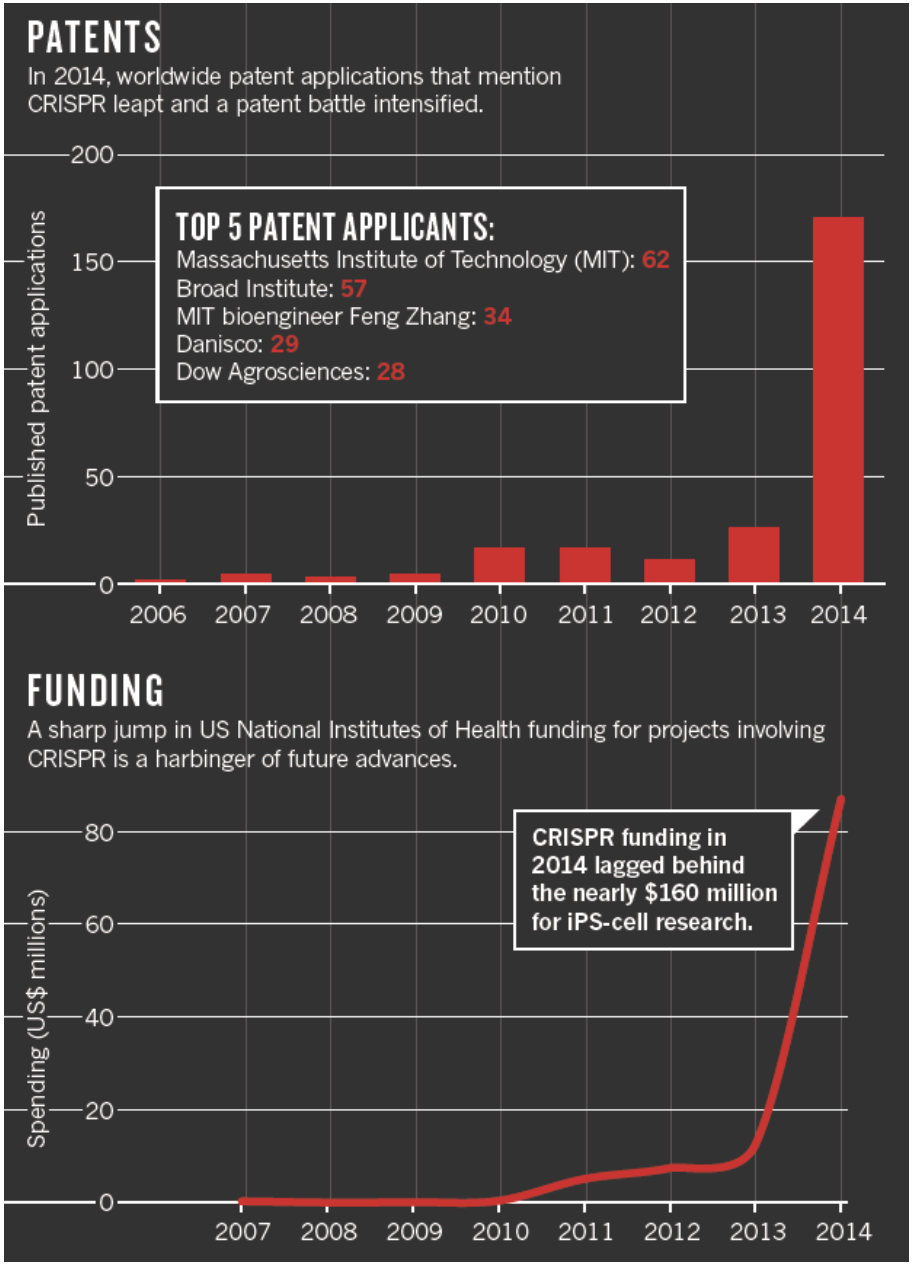
<u>Pre-CRISPR</u>
1 year
$20,000

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

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

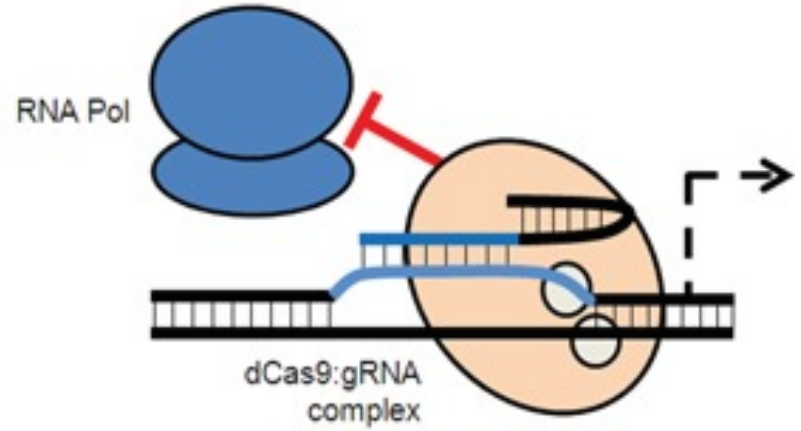
CRISPR technology is advancing research capabilities

- Gene expression
- DNA tagging / purification
- DNA incorporation



Modulating gene expression

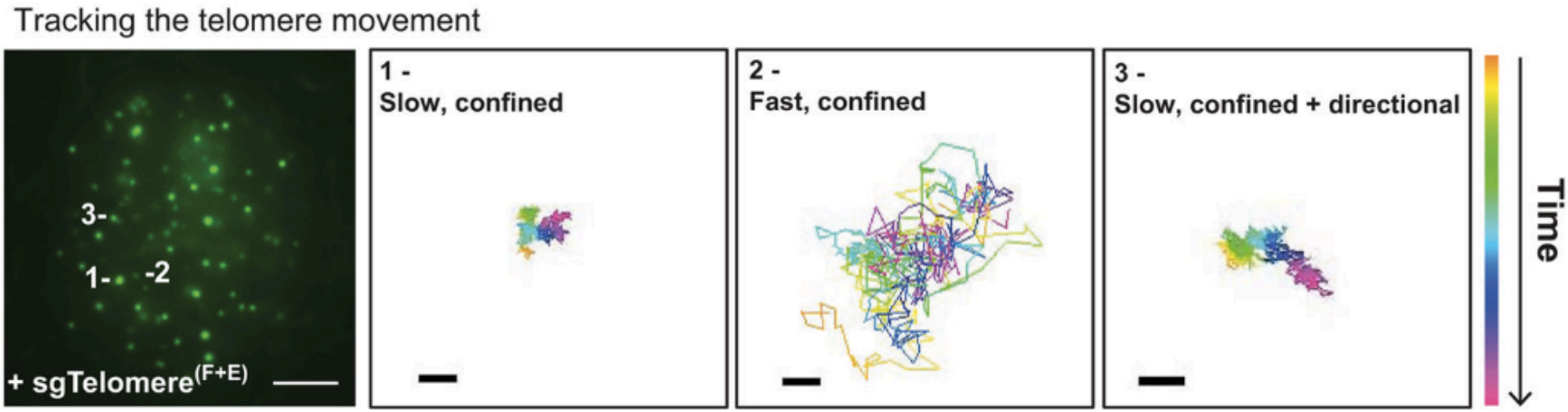
- Catalytically inactive dCas9
  - Block transcription



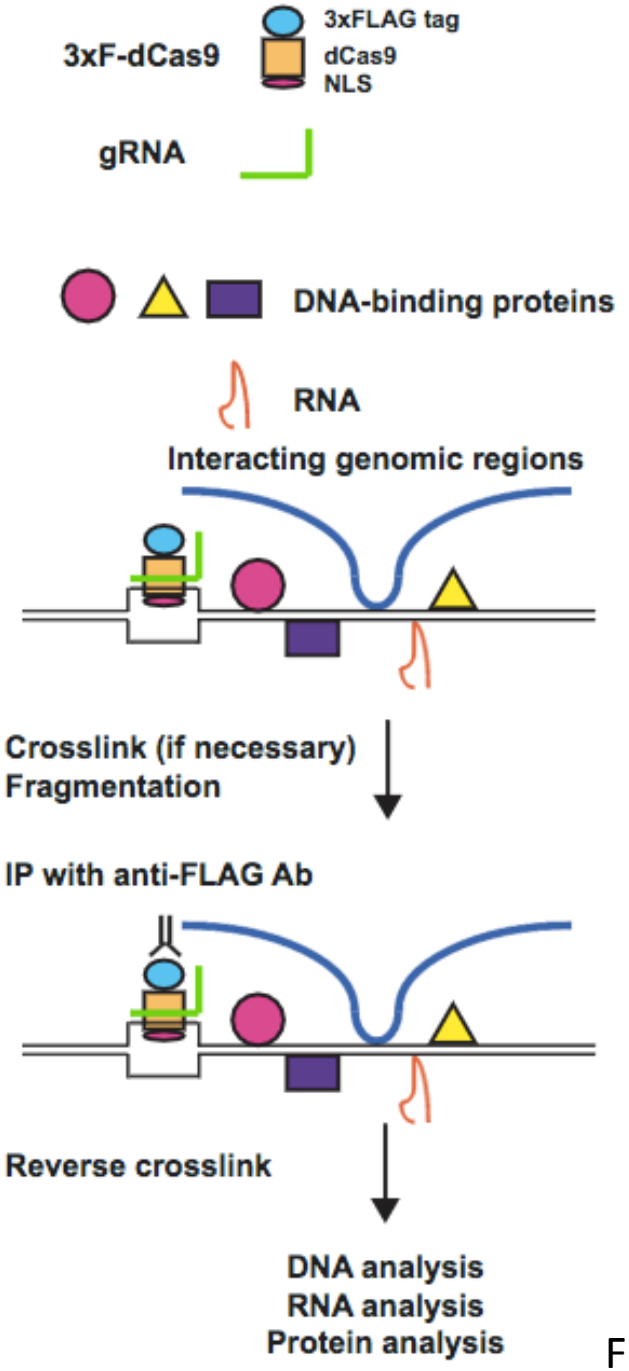
  - When fused with repressor or when used with multiple gRNAs, gene expression further decreased
  - When fused with activator, gene expression increased

dCas9 applications: DNA tagging

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



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



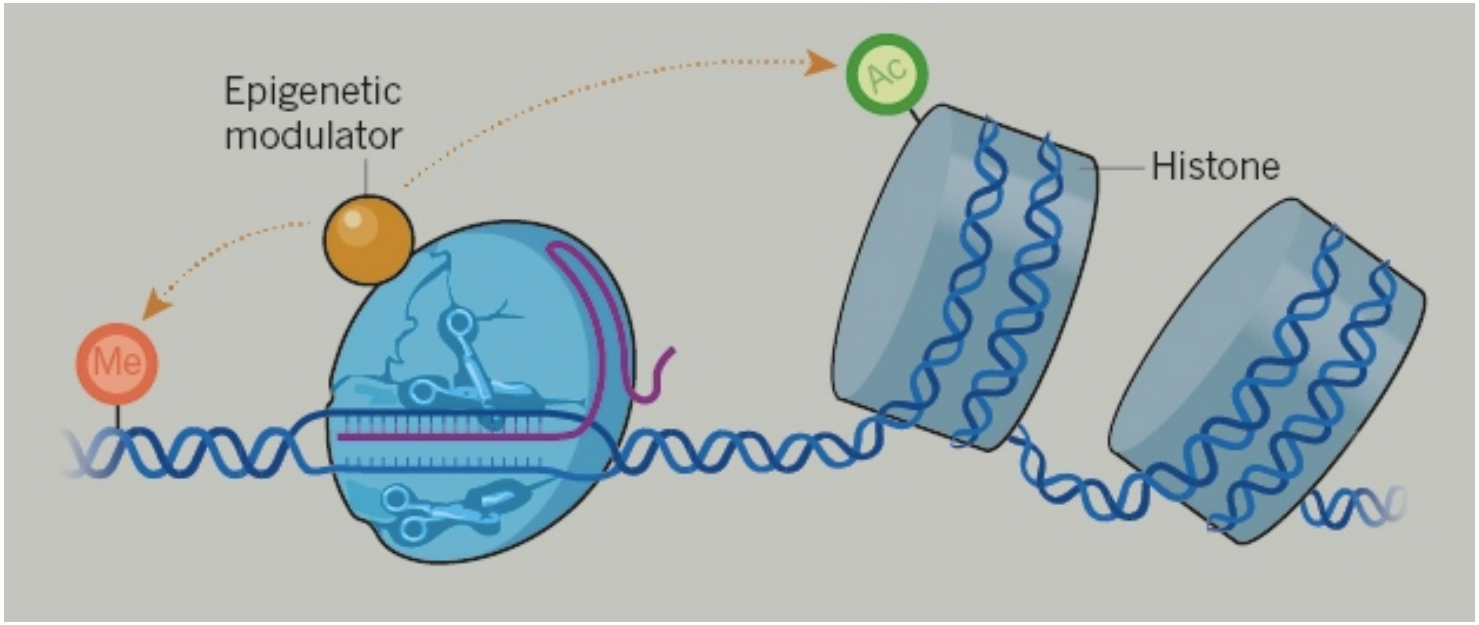
dCas9 applications: DNA purification

- 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

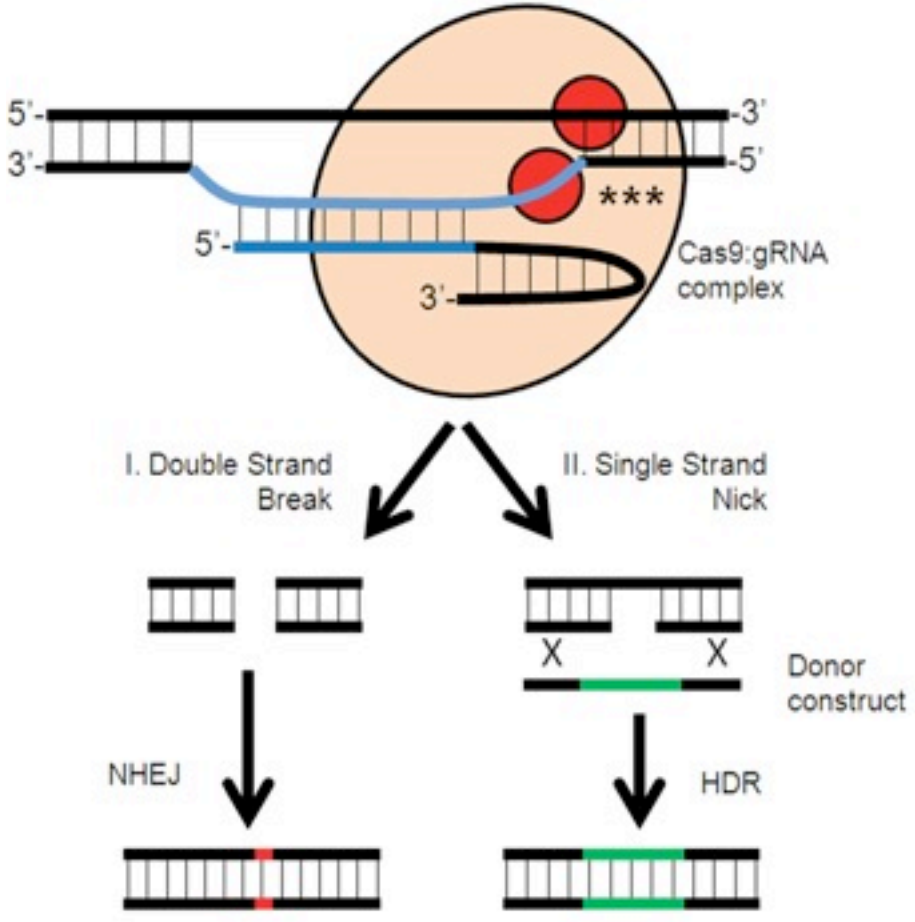
dCas9 applications: epigenetics

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



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

Engineering genetic loci



- Cas9 cleaves specific DNA sequences
  - NHEJ introduces random mutations
  - H(D)R incorporates 'new' genetic information

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

Why is CRISPR not used now in therapeutics?

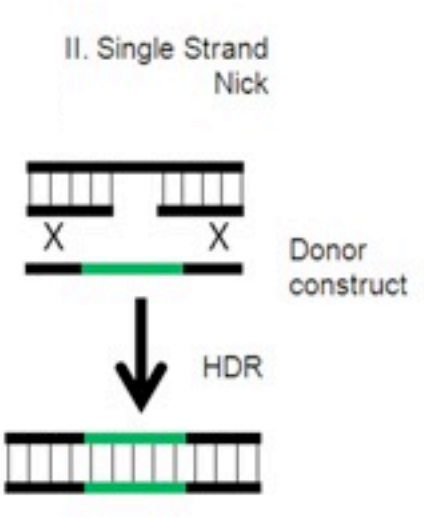
Why is CRISPR not used now in therapeutics?

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



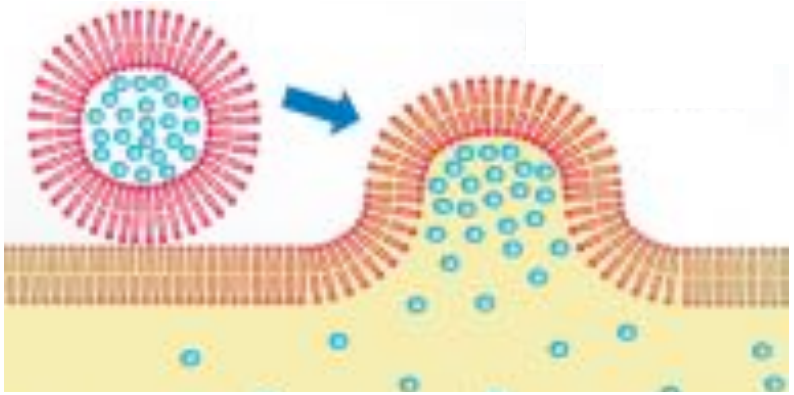
- Using photocaging to control activity of Cas9

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

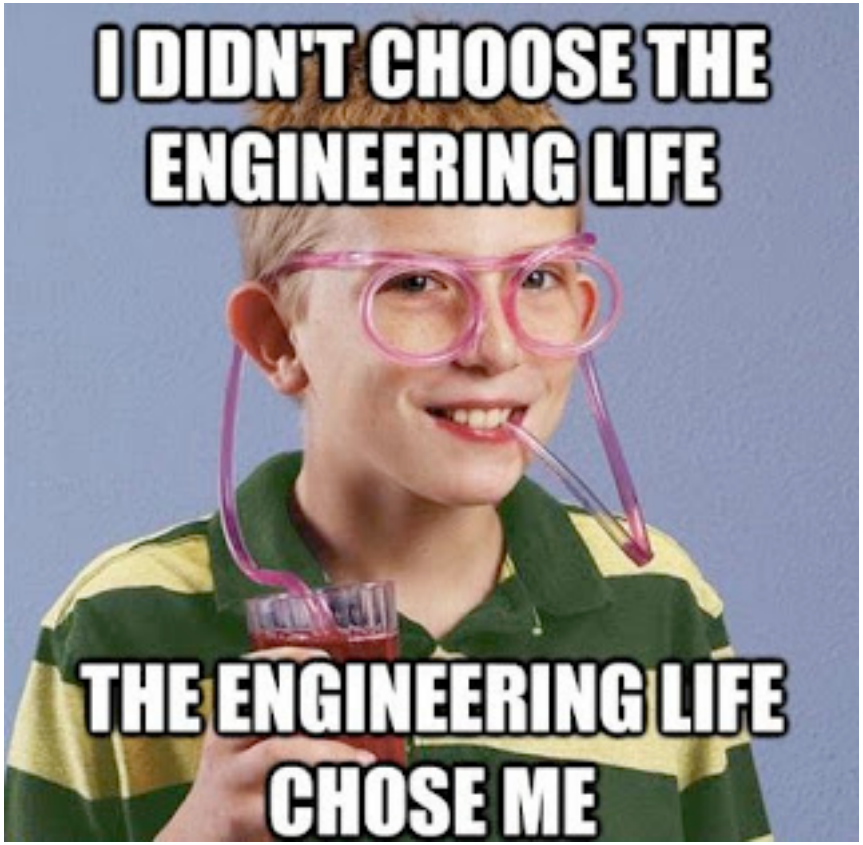
Concerns regarding CRISPR technology

"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:

- No lecture Thursday and no laboratory Thursday / Friday
- Module 3 begins next week (Tuesday, Nov 15) with Angie Belcher