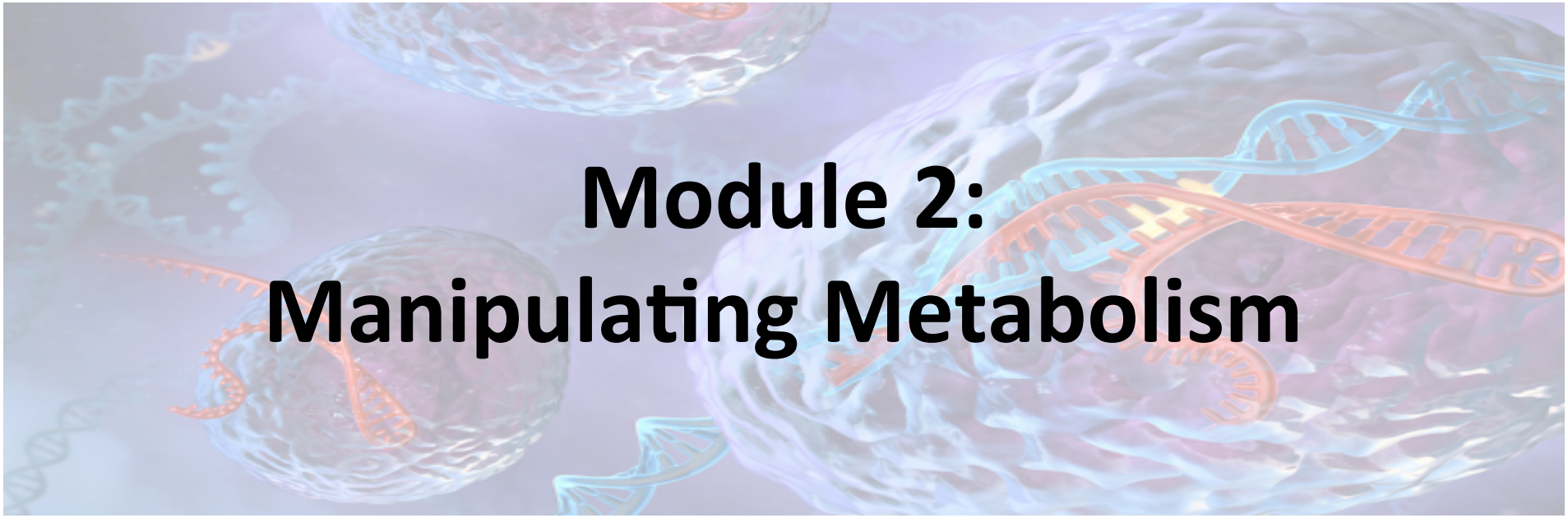


Module 2: Manipulating Metabolism

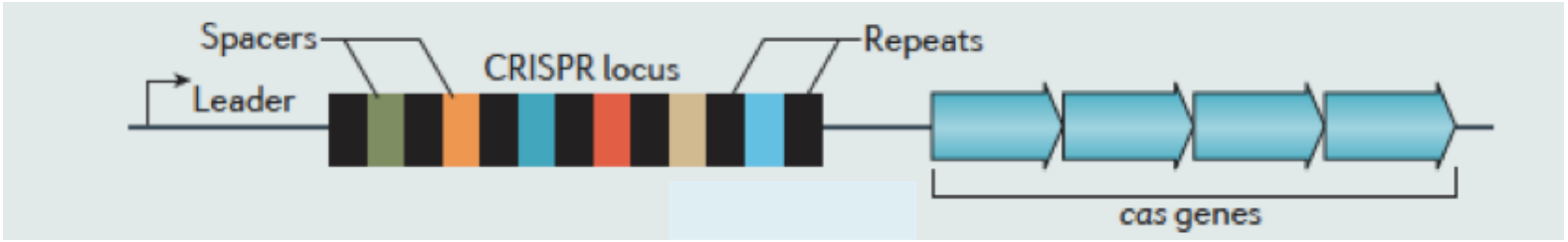
CRISPR and genetic engineering

11/1/15


TIRED.
JUST TIRED.

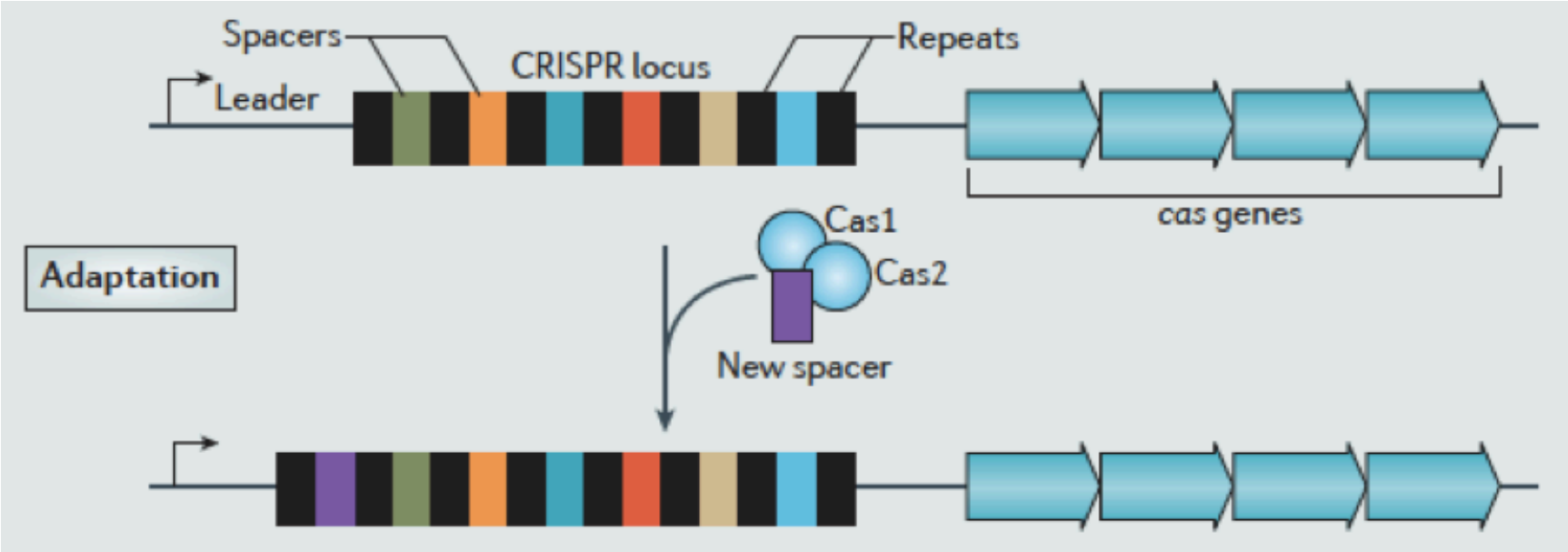
Native CRISPR system cleaves phage DNA

- Adaptive immune response encoded by CRISPR loci and Cas genes



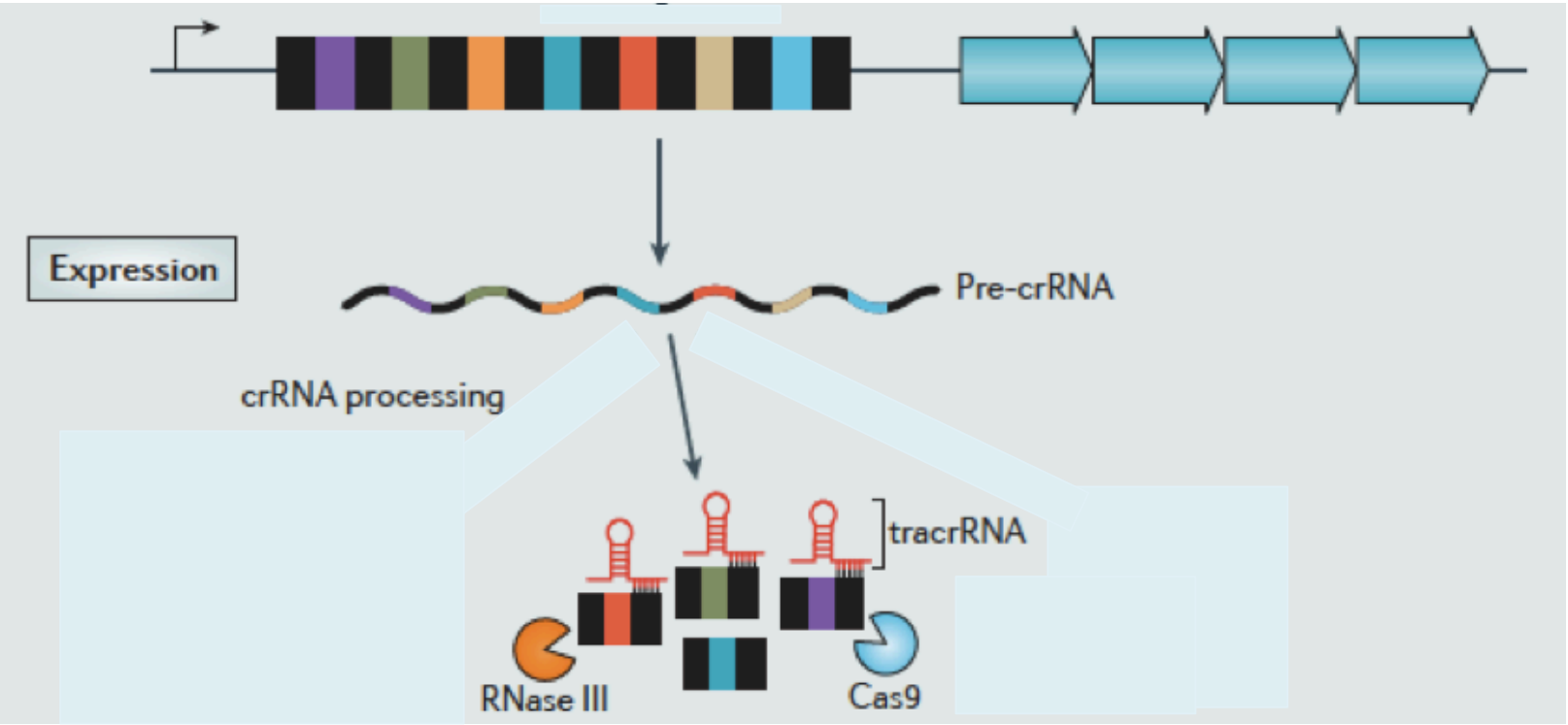
- Mechanism involves three stages:
  - Adaptation
  - Expression
  - Interference

CRISPR system: adaptation



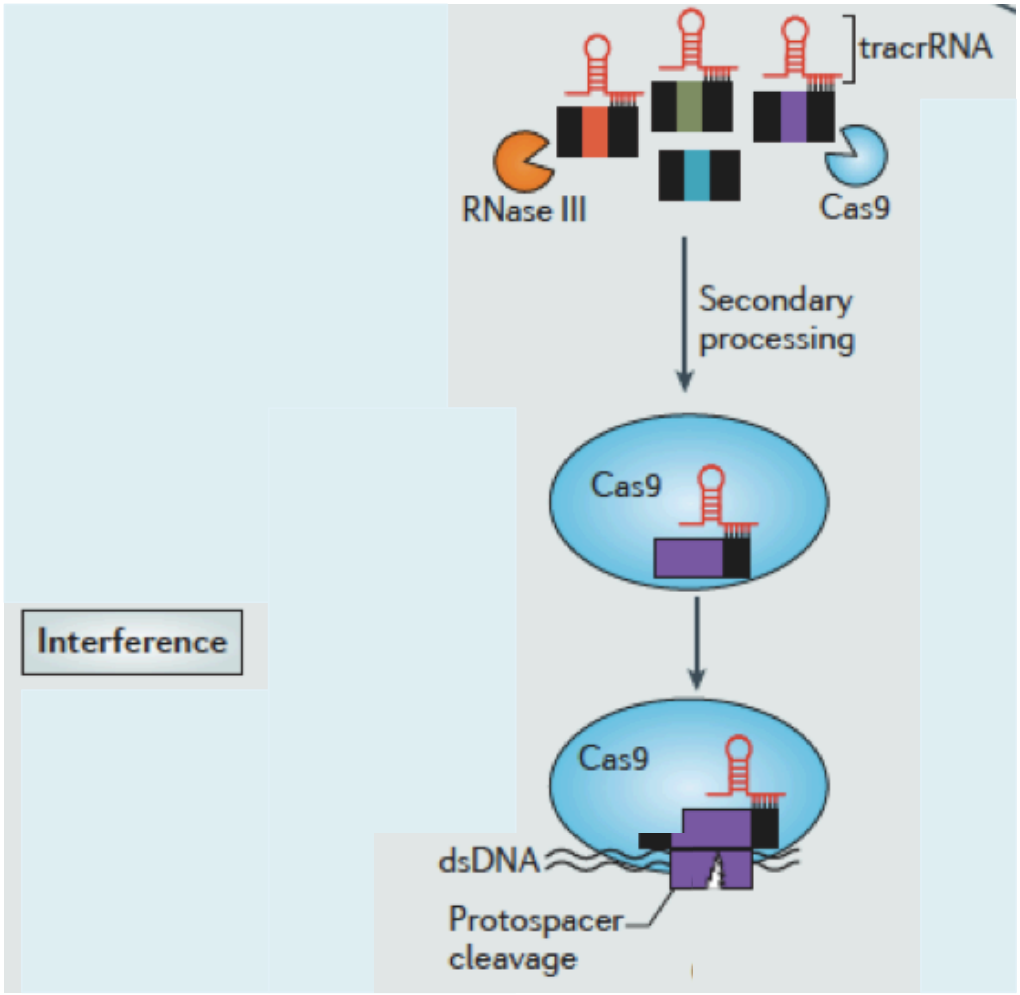
Phage DNA recognized and fragmented by restriction enzyme system, then ‘spacers’ incorporated into bacterial genome

CRISPR system: expression



Cas9 and Rnase III involved in processing pre-crRNA, then Cas9 forms complex with crRNA and tracrRNA

CRISPR system: interference



Cas9 / tracrRNA / crRNA bind invading phage DNA and cleave at target sequence that is complementary to ‘spacer’ sequence

Engineered CRISPRi system

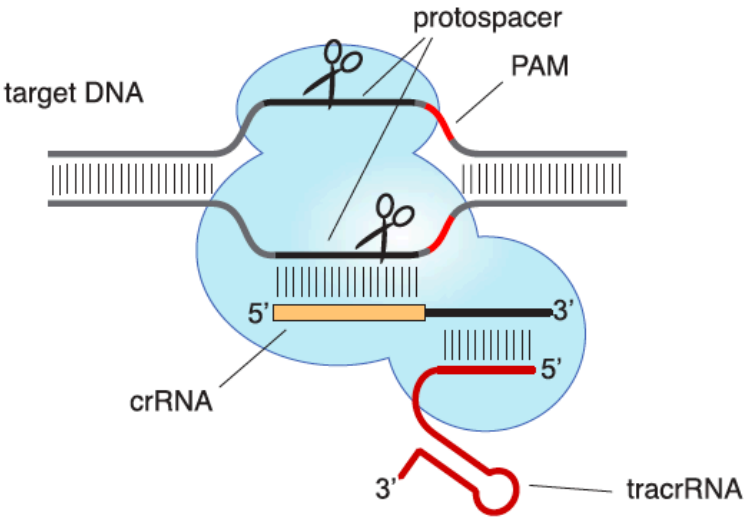
- Modifications to crRNA / tracrRNA complex?

- Modifications to Cas9?

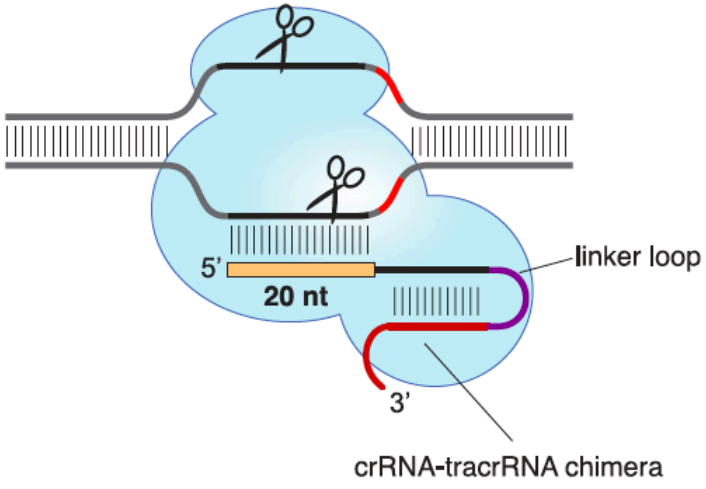
CRISPRi system: (s)gRNA

- (s)gRNA molecule is a target sequence and tracrRNA fused by a linker loop such that a single transcript used to direct Cas9 cleavage

crRNA / tracrRNA complex



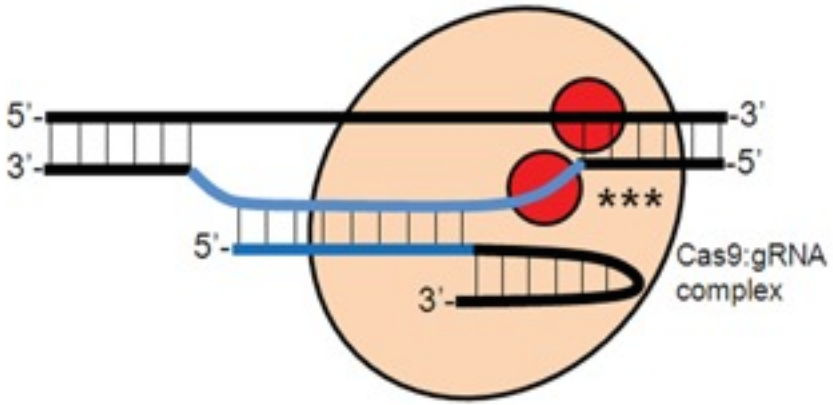
(s)gRNA



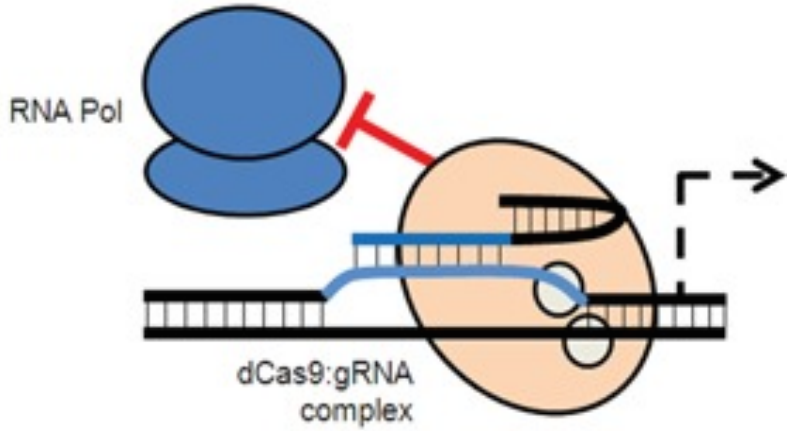
CRISPRi system: dCas9

- dCas9 protein contains mutated residues D10A and H840A that render it catalytically inactive and unable to cleave DNA, but still able to bind DNA

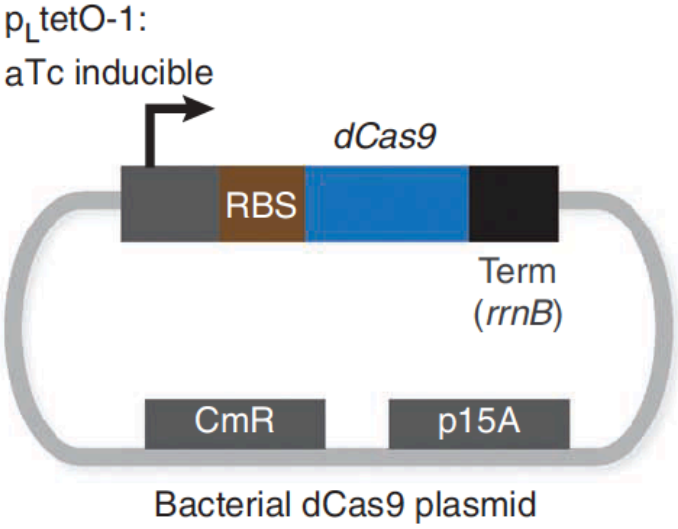
Cas9



dCas9

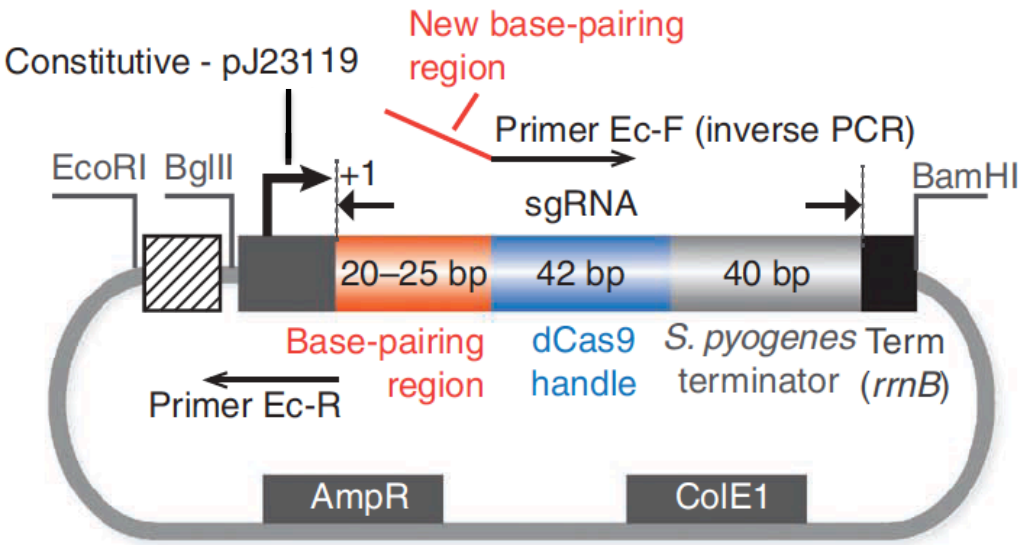


Closer look at pgRNA and pdCas9



Bacterial dCas9 plasmid

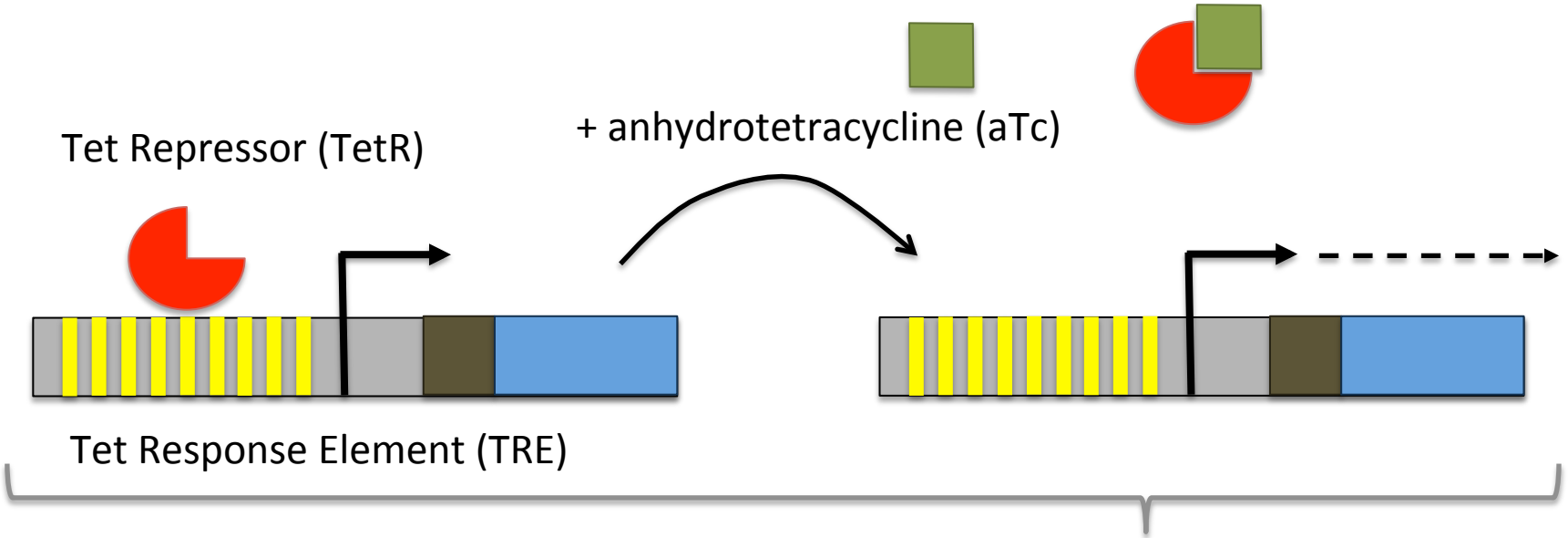
- Confirmation digest prepared on D1



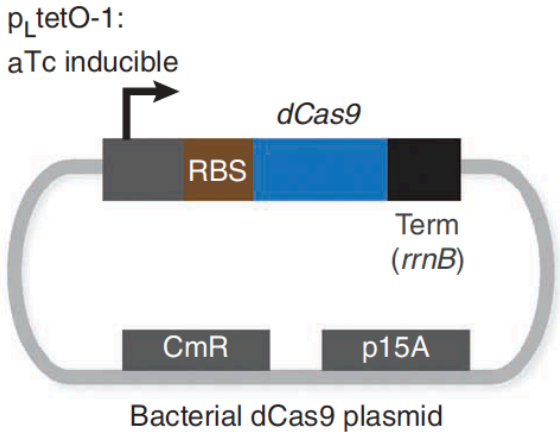
Bacterial sgRNA plasmid

- Insert (gRNA target sequence) designed on D2

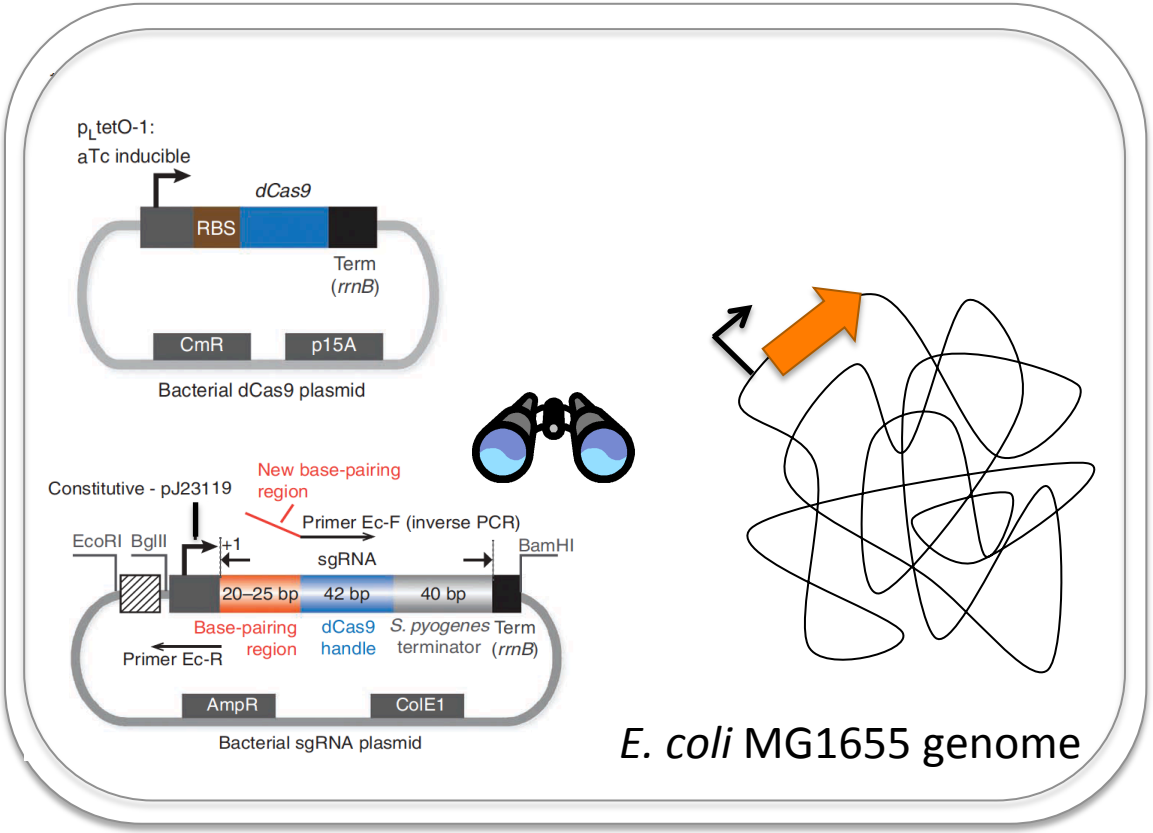
Closer look at aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene

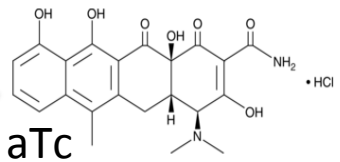


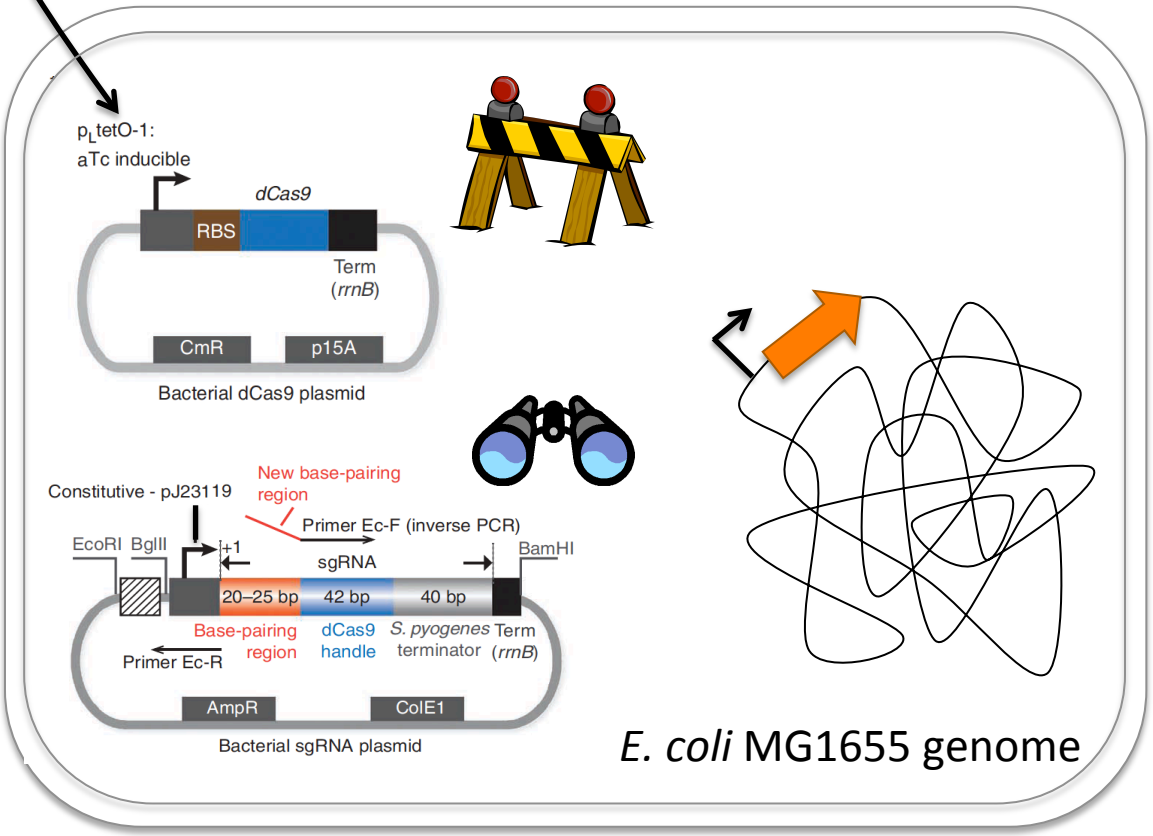
CRISPRi 'inactive' in absence of inducer



- pgRNA_target expressed constitutively
  - Always transcribed and binding to target gene

CRISPRi ‘blocks’ gene expression in presence of inducer

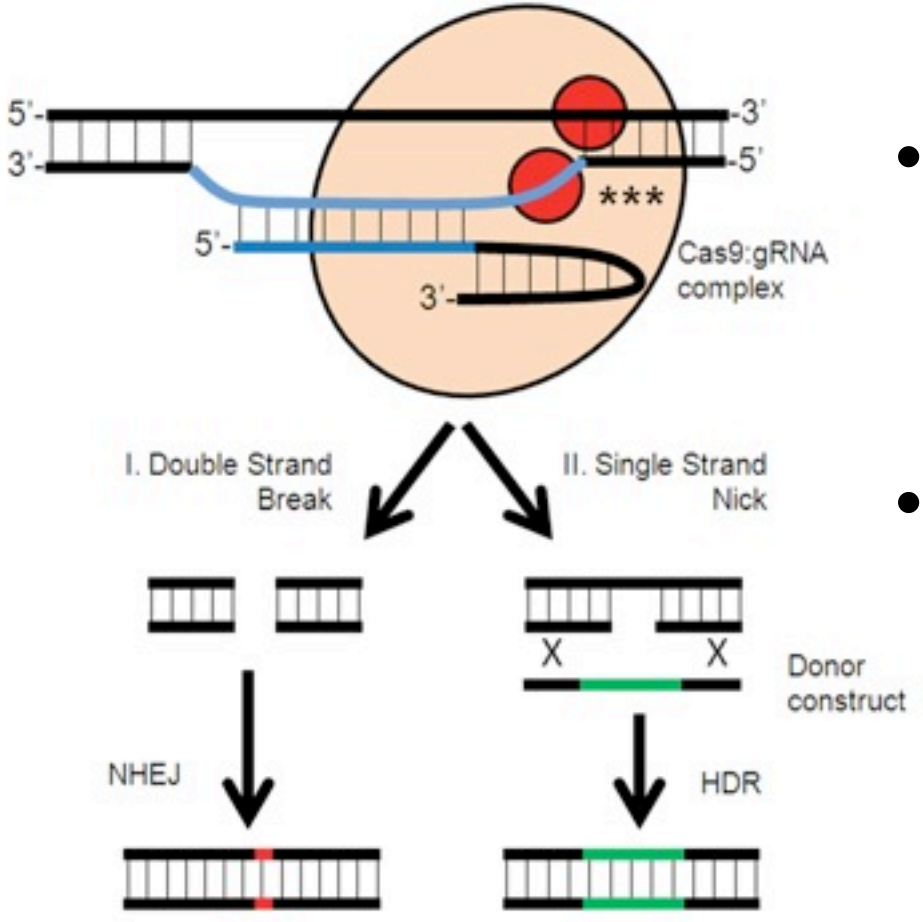




- pdCas9 expressed when aTc added
  - When transcribed associates with pgRNA_target / target gene

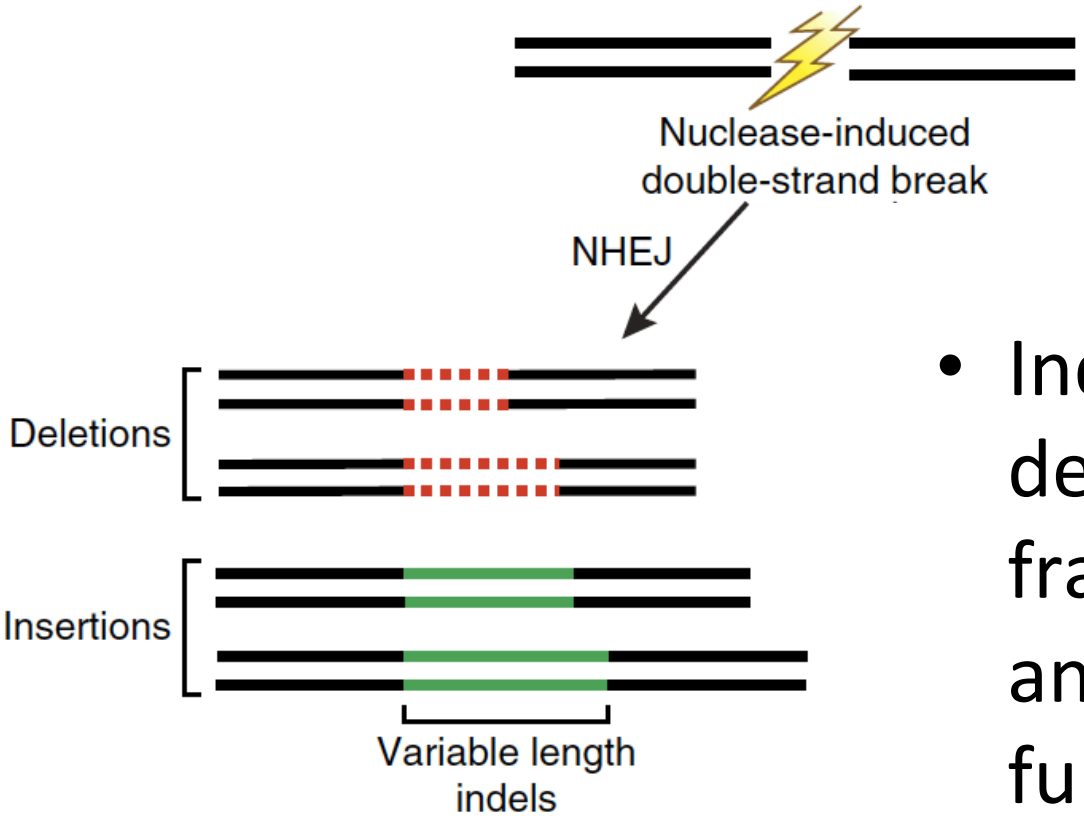
What if we want to engineer a permanent genetic mutation?

Mammalian cells able to repair dsDNA breaks



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

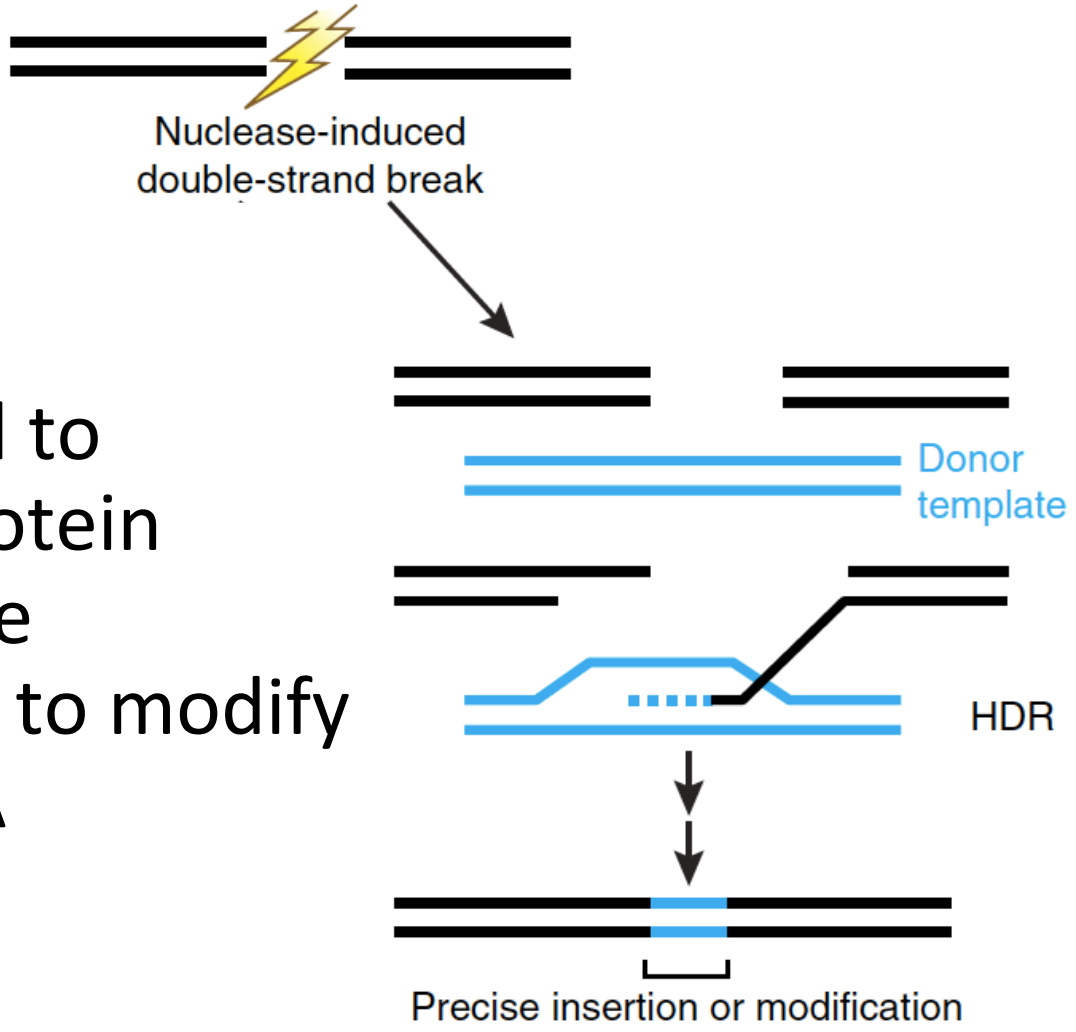
NHEJ repair generates random insertions / deletions



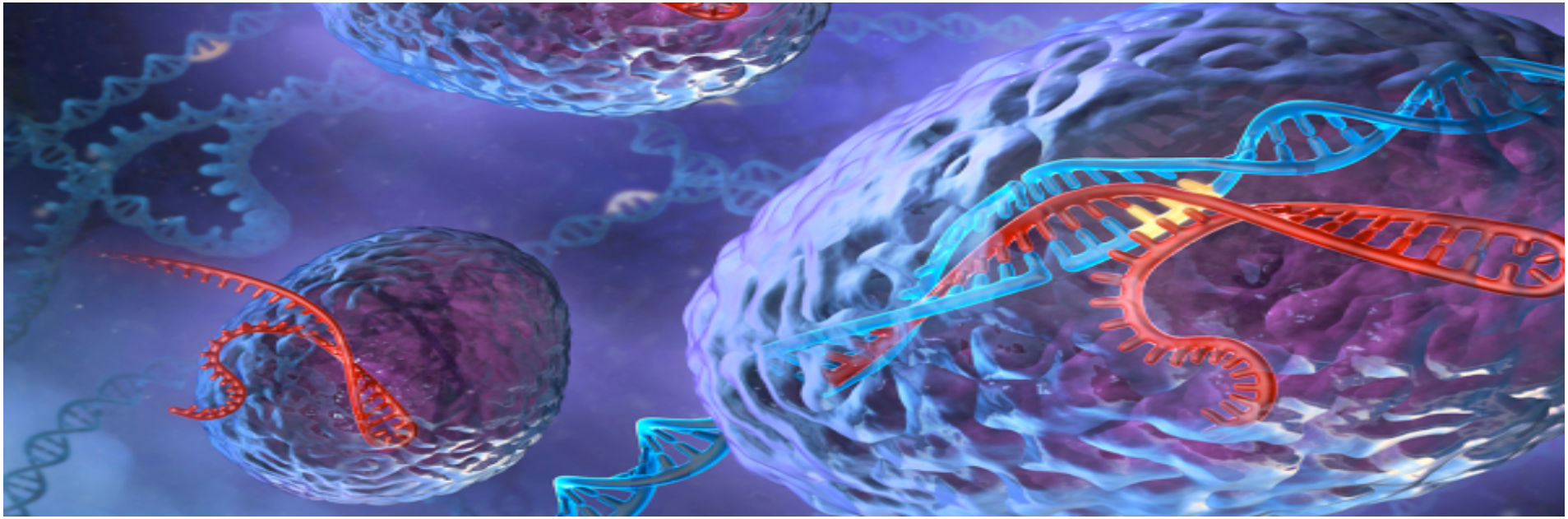
- Indels (insertions / deletions) result in frameshift mutations and loss of protein function

H(D)R repair enables specific sequence insertions

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



Final overview of CRISPR / Cas9



https://www.youtube.com/watch?v=k99bMtg4zRk

In the *laboratory*…

- Journal club presentations
  - Meet at 1p in 16-336 for M2Q2