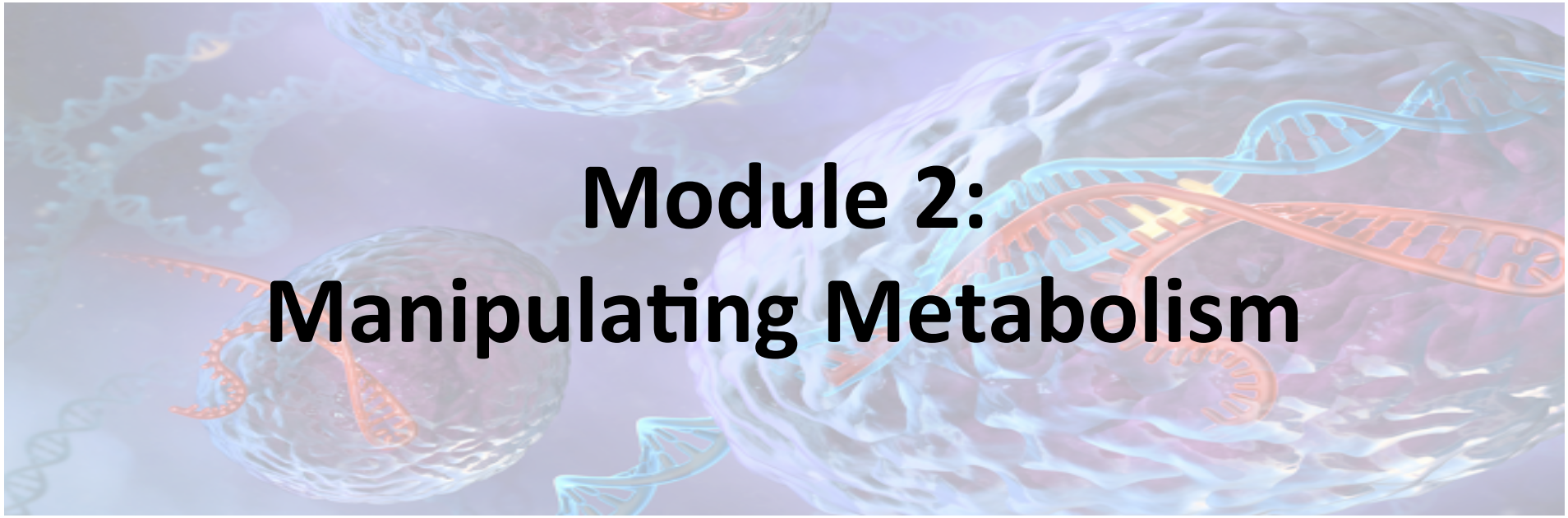


Module 2: Manipulating Metabolism

dCas9 and the CRISPRi system

10/25/15


E COLI HYPSTER: DOING CRISPR FOR MILLION YEARS
BEFORE IT BECAME MAINSTREAM
generator-meme.com

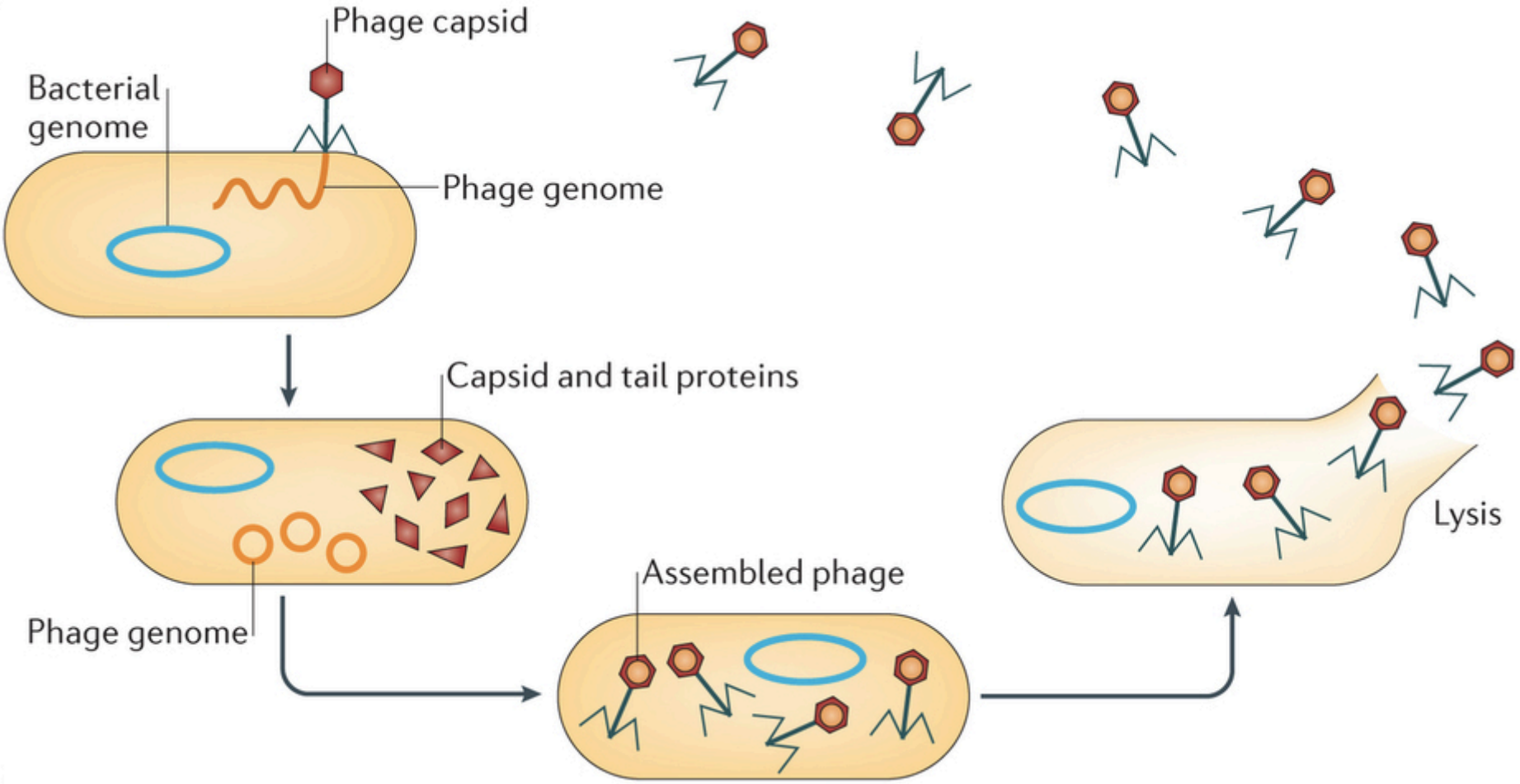
Identifying the cleavage target of Cas9

- Adaptive immune response that confers phage resistance
- Requires crRNA, tracrRNA, and Cas9

What is the target of the native system?

Incoming viral DNA or host-transcribed viral mRNA?

Lytic phage infection in bacteria



DNA vs RNA debate

Many in researchers in phage community convinced RNA interference by CRISPR too inefficient given explosive replication of phage during infection

Data support that Cas9 cleaves DNA

1. Targets to ‘sense’ DNA more efficient than those to ‘anti-sense’

2. Transformation of plasmid DNA blocked

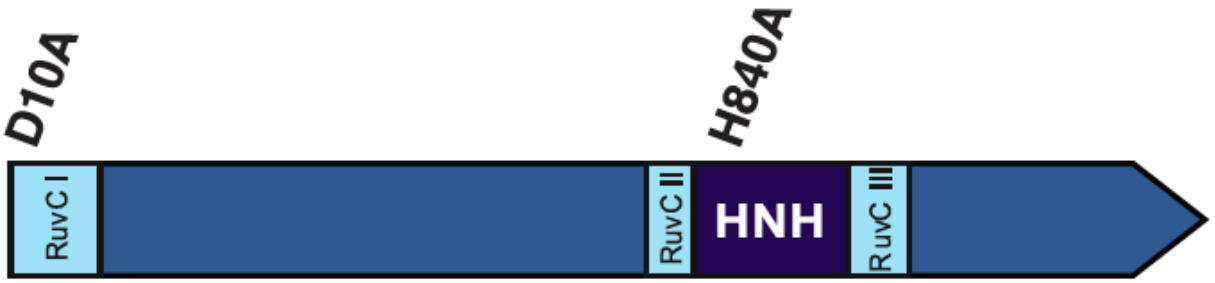
3. Presence of self-splicing RNA sequence in DNA target abolished CRISPR activity

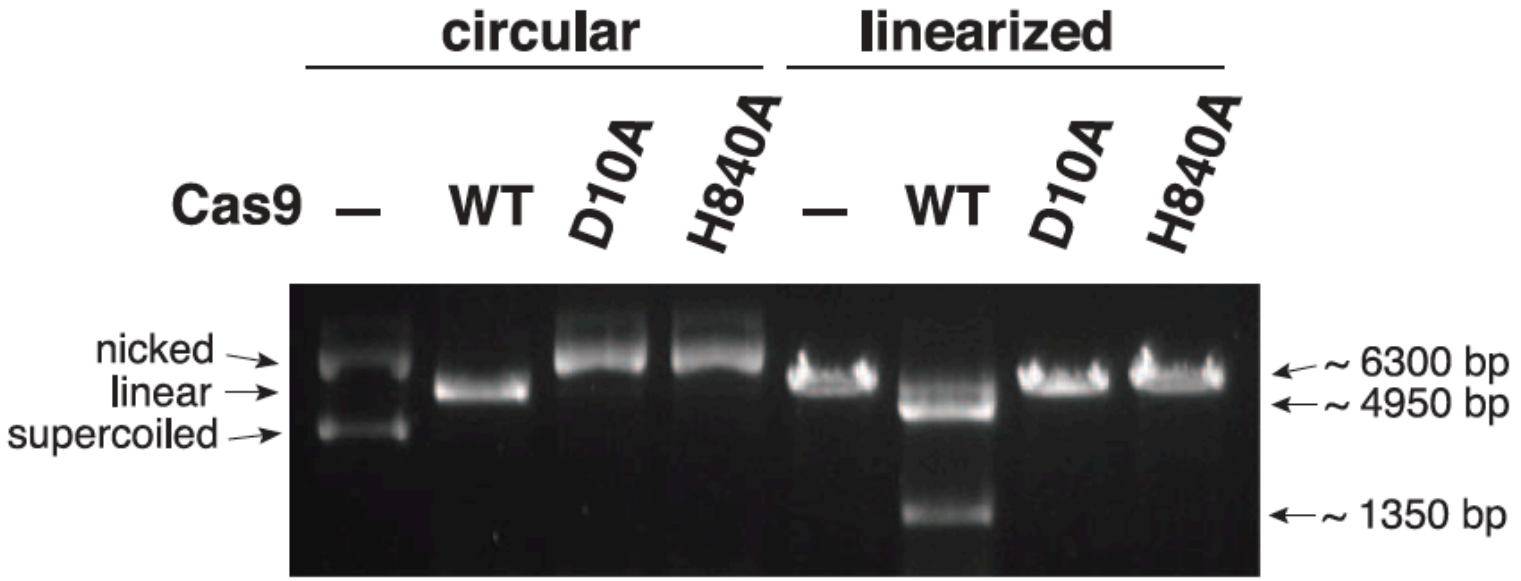
HNH and RuvC endonuclease domains

- RuvC
  - Endonuclease that resolves Holliday structure, intermediate structure in which dsDNA molecule is linked by single-stranded crossover
- HNH
  - Found in homing endonucleases, restriction endonucleases, transposases

How would you identify which domain is required for DNA cleavage?

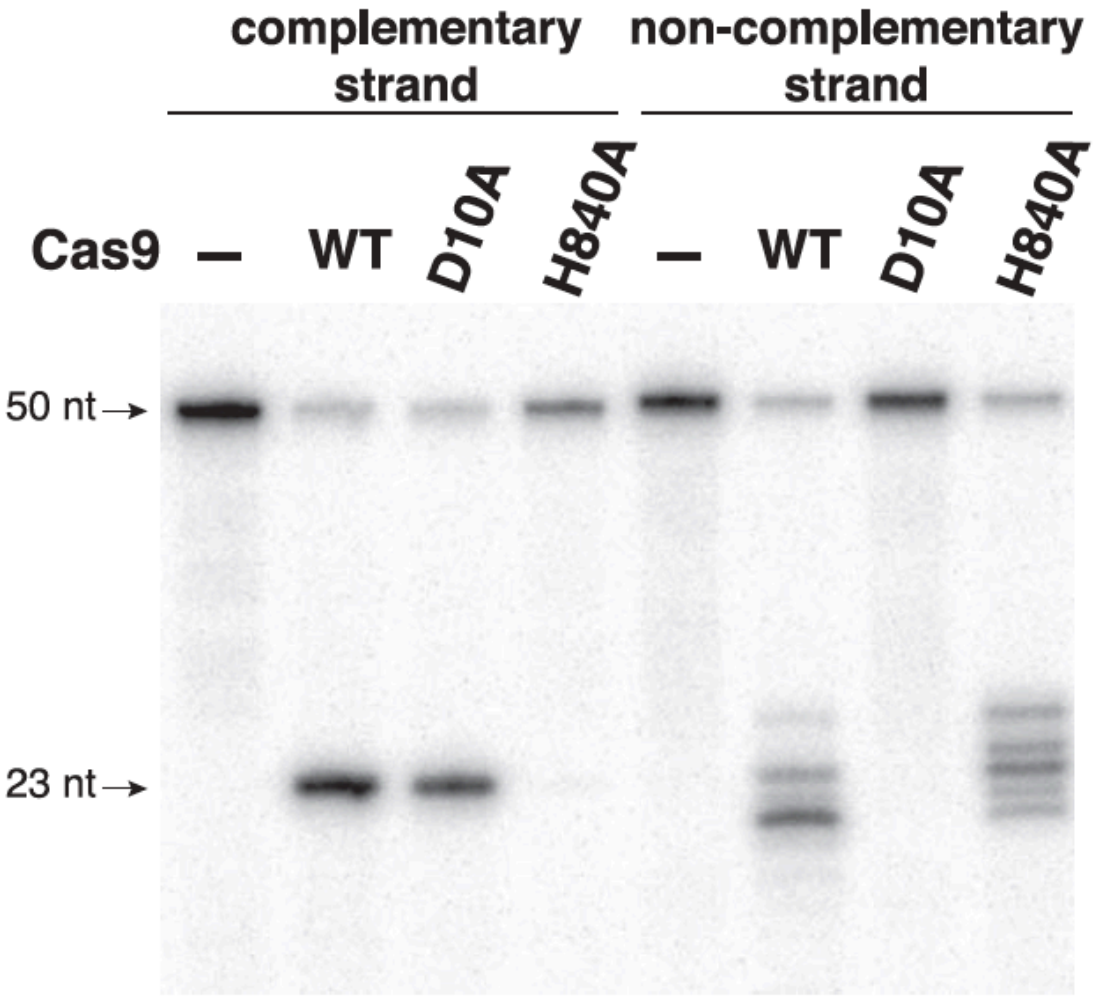
Cleavage requires HNH and RuvC domains





Jinek et al. (2012) *Science*. 337:816-820.

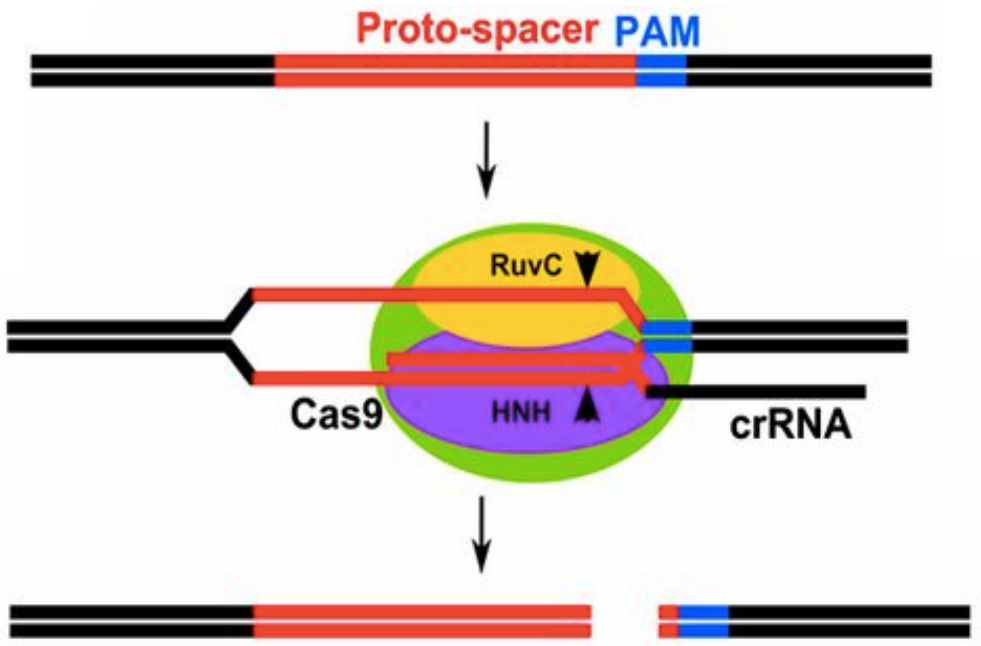
HNH and RuvC domains target specific DNA strands



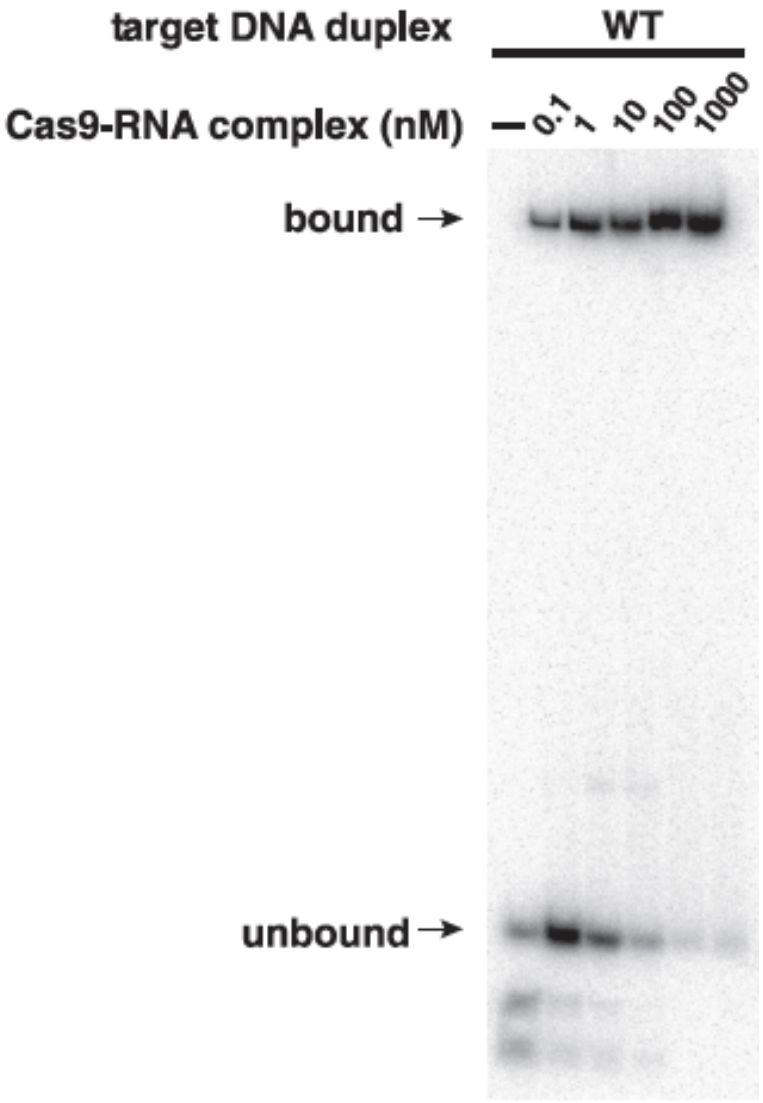
Jinek et al. (2012) *Science*. 337:816-820.

Schematic of Cas9 DNA cleavage

- RuvC domain (D10A) cleaves non-coding strand
- HNH domain (H840A) cleaves coding strand
- Result in blunt end cut 3 bp from PAM site



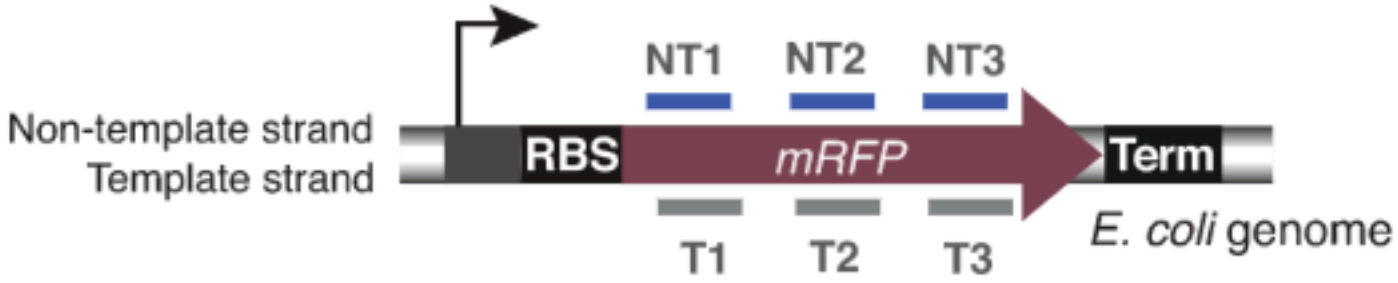
dCas9 binds target DNA sequence



How can this variant be used for gene regulation and pathway manipulation?

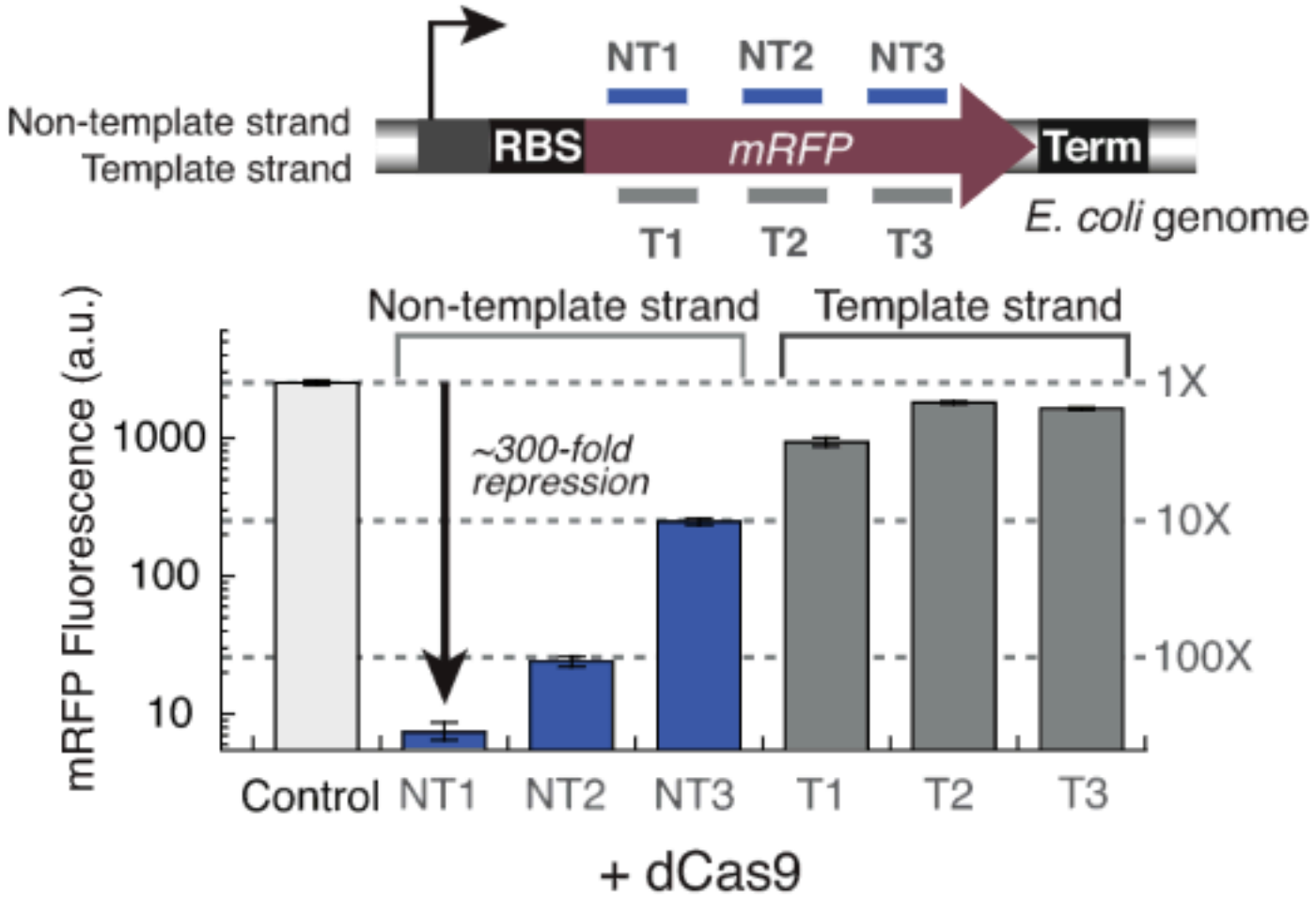
Jinek et al. (2012) *Science*. 337:816-820.

Testing the effect of dCas9 on transcription



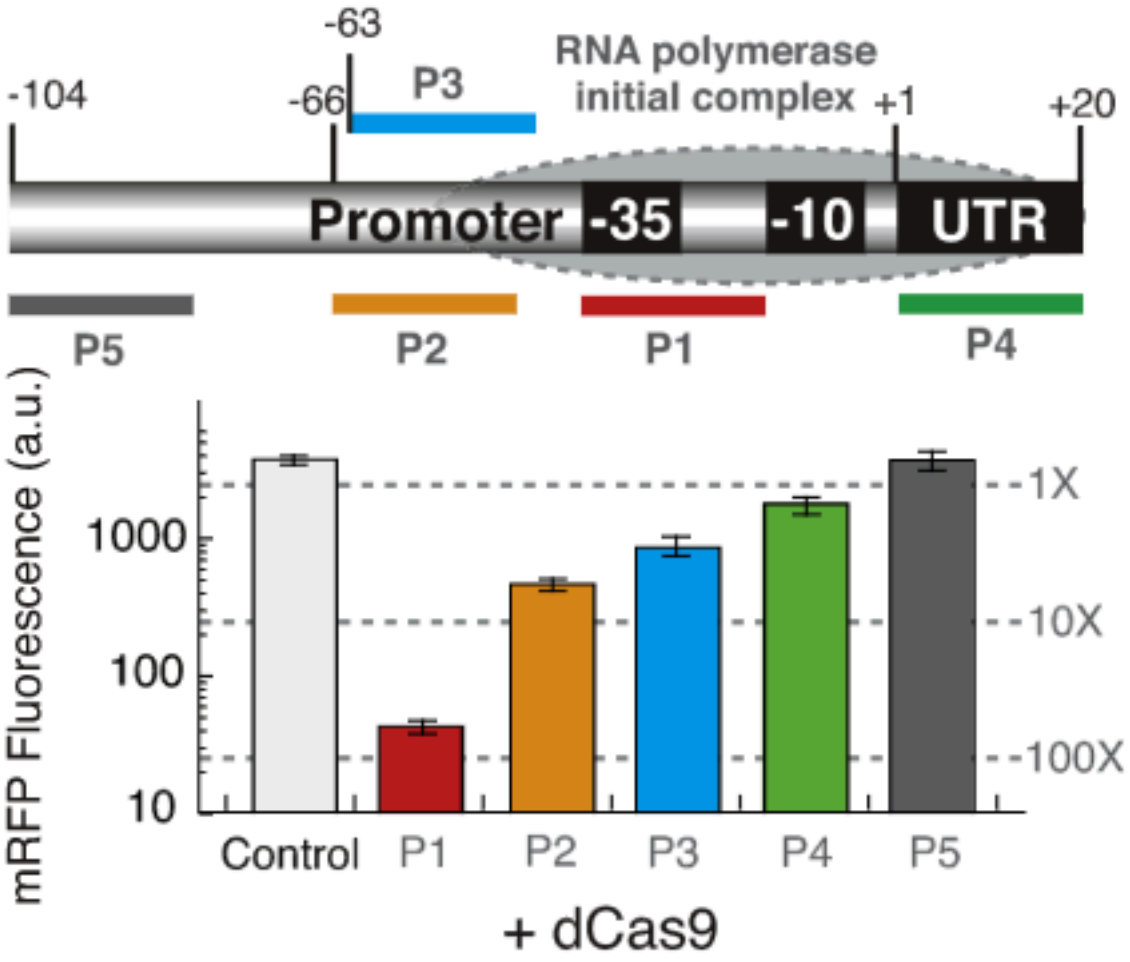
- Red fluorescent protein (RFP) cloned into *E. coli* genome
- gRNAs designed to target non-template strand and template strand within RFP sequence

dCas9 inhibits transcript elongation



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

dCas9 inhibits transcript initiation



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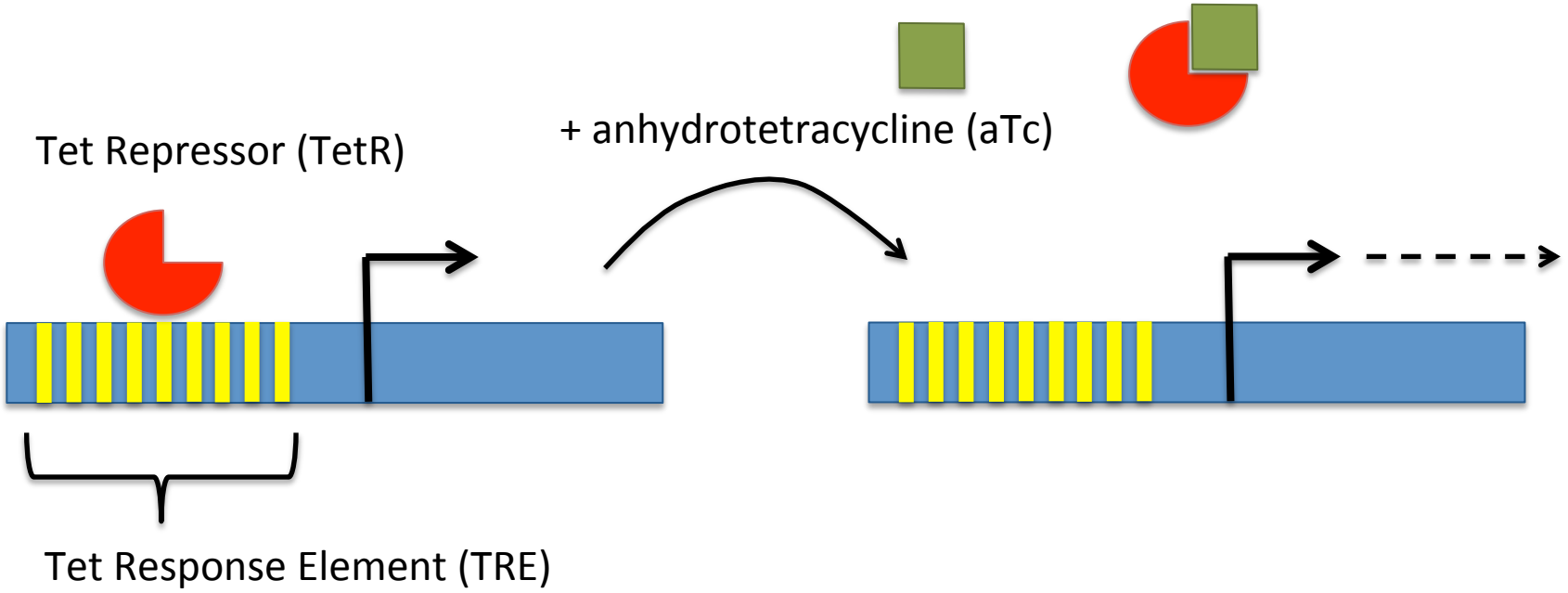
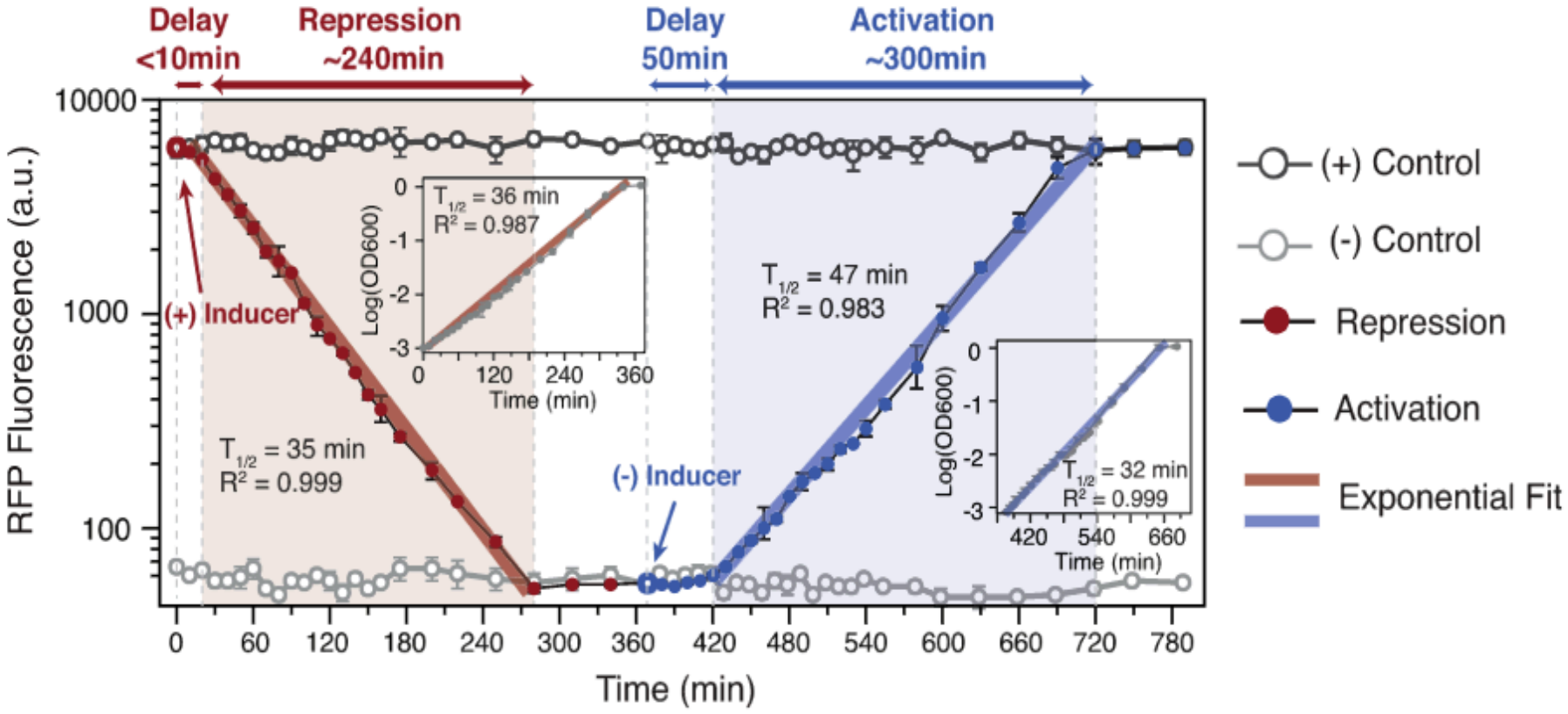
Testing dCas9 induction control switch



- aTc induction mechanism enables manipulation to be turned on and off

Inducible promoter can be used to control dCas9-mediated gene expression



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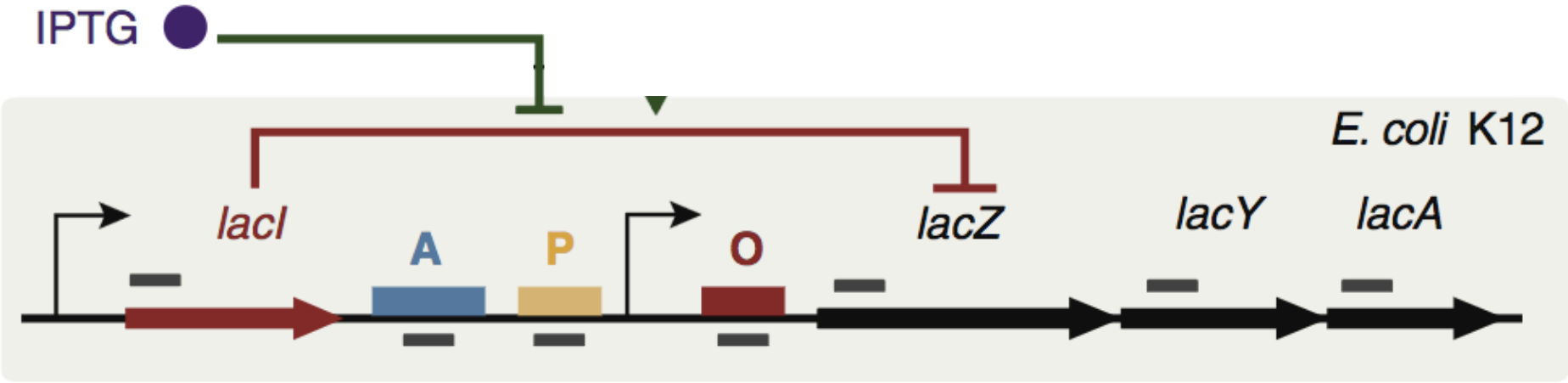
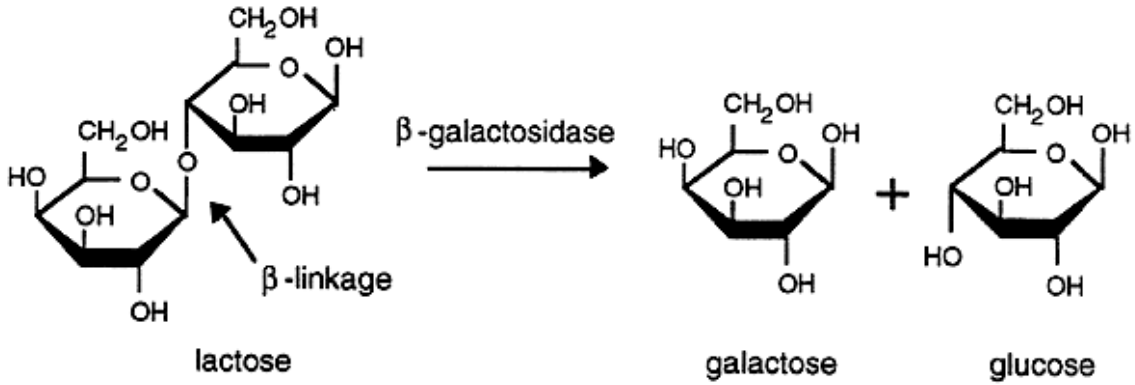
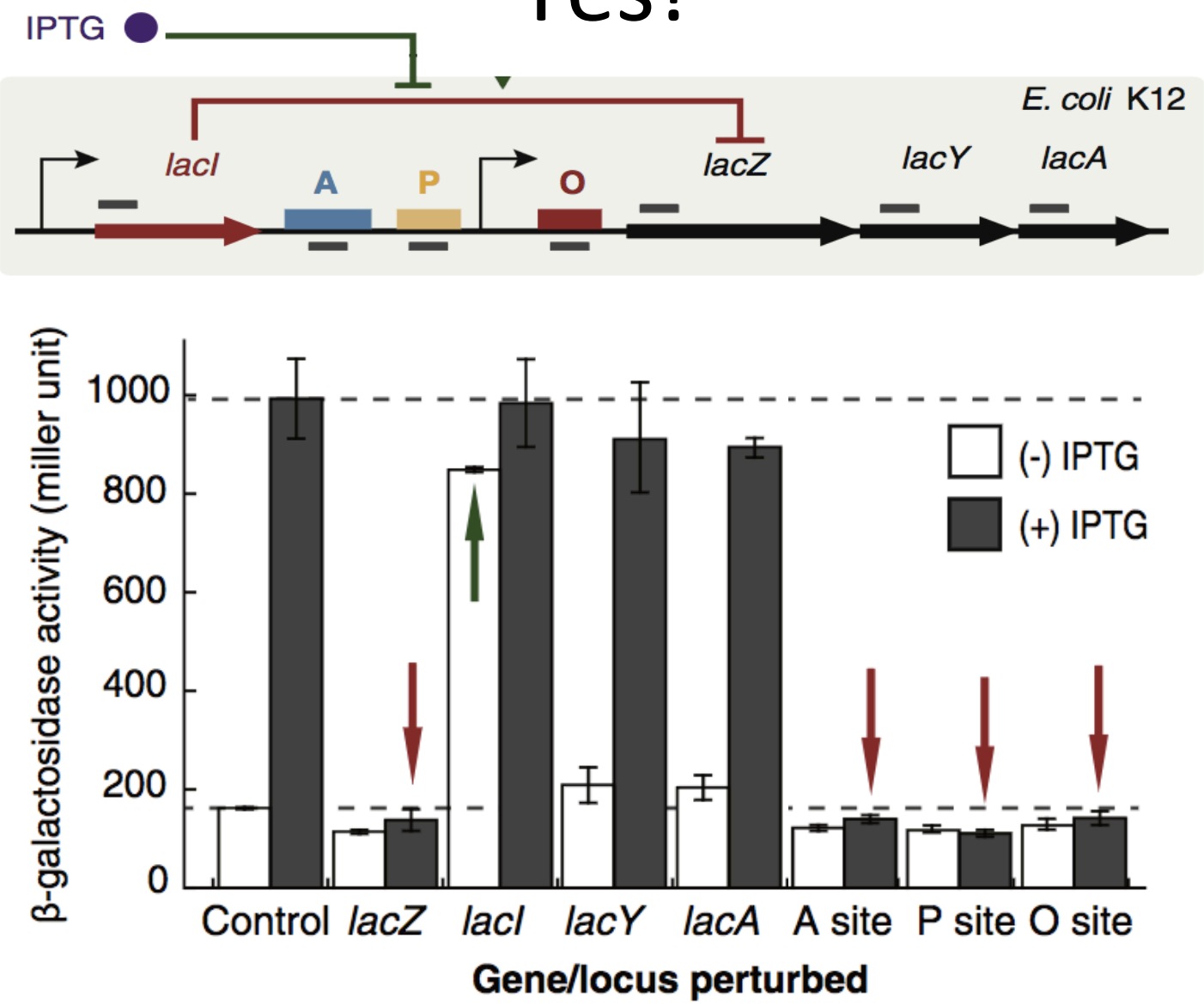
Will CRISPRi regulate native pathway?



- *lac* operon required for metabolism of lactose



Yes!



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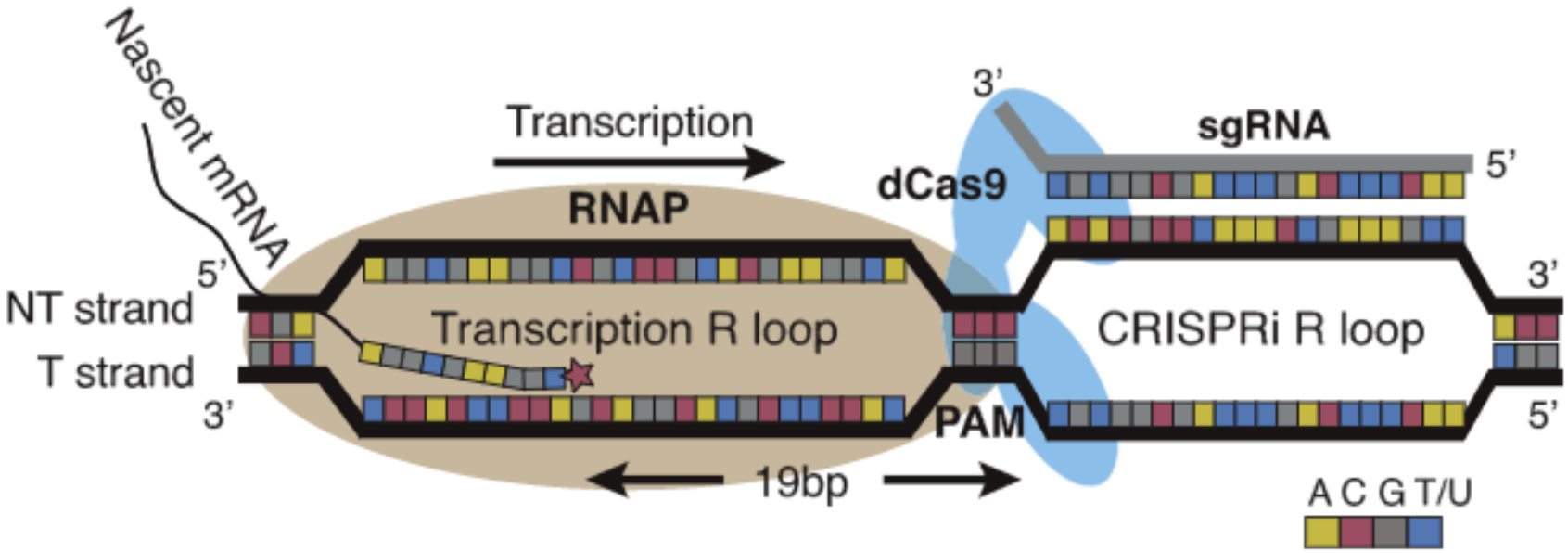
CRISPRi collision model



Future applications for Cas9

- Targeting proteins to dsDNA to mediate biology 'numbers game'
  - Recruit or prevent transcription factor binding
  - Direct chromatin-remodeling factors
- Fine-tuning the CRISPR system
  - Examine efficiency biases of spacer sequences
  - Decrease off-target Cas9 cleavage

In the *laboratory*…

- Journal club presentations
  - Meet at 1p in 16-336 for M2Q1

"Welcome to Journal Club. The first rule of Journal Club is: you practice. The second rule of Journal Club is: you practice even more."

- Former 109er