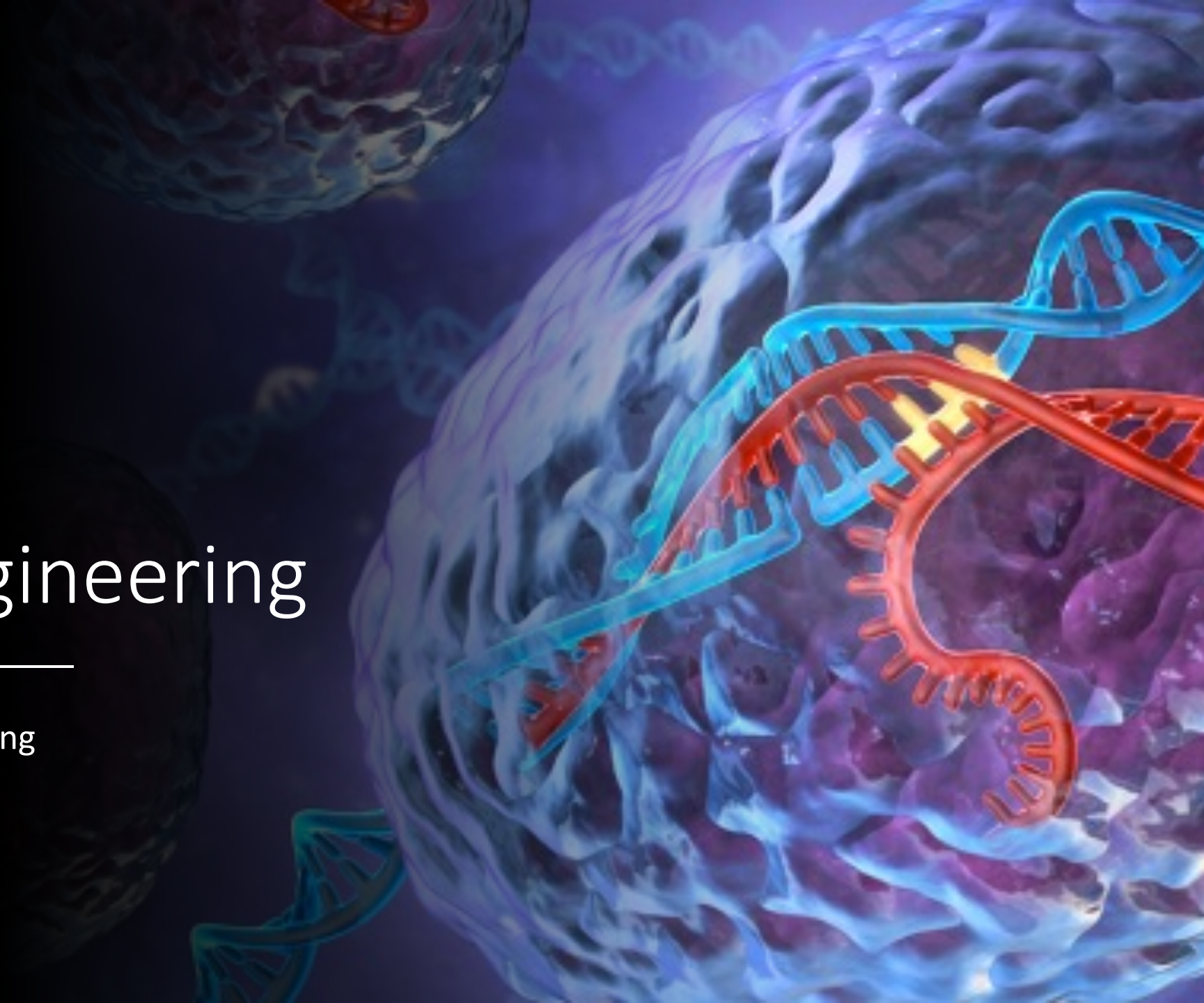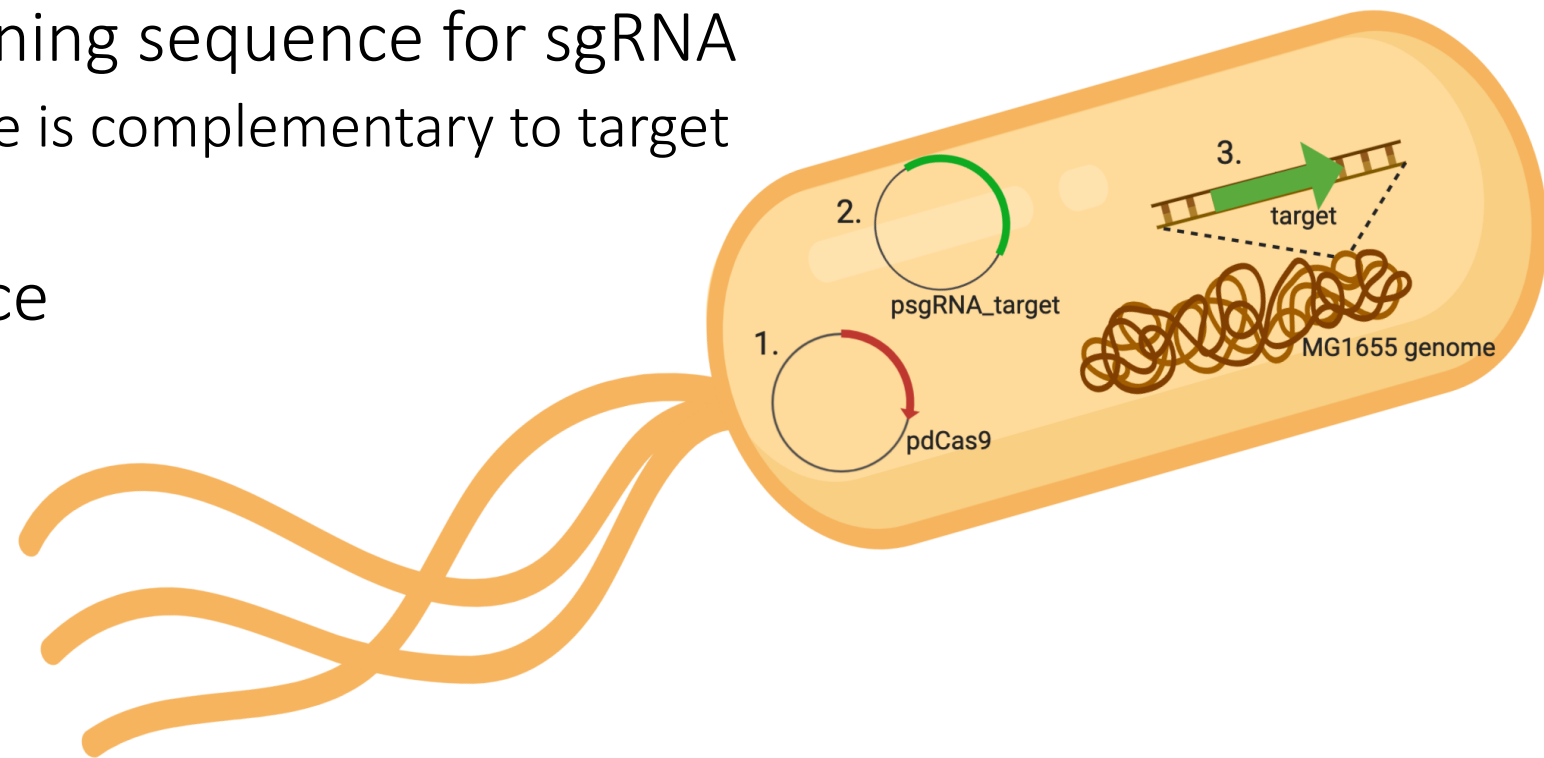# Module 2: Metabolic Engineering

CRISPRI effect on promoter binding
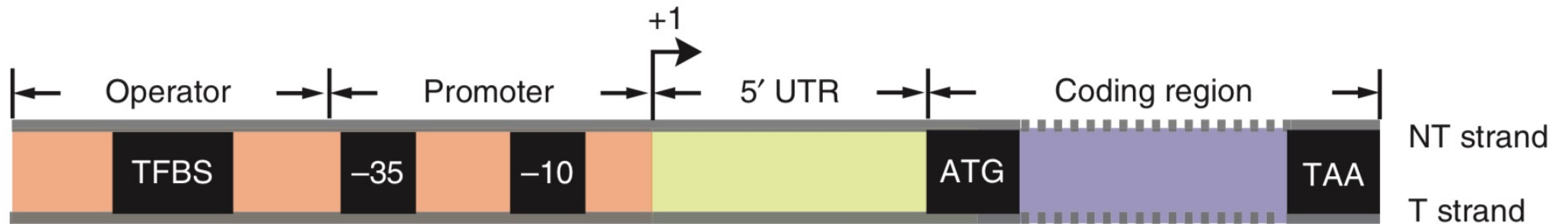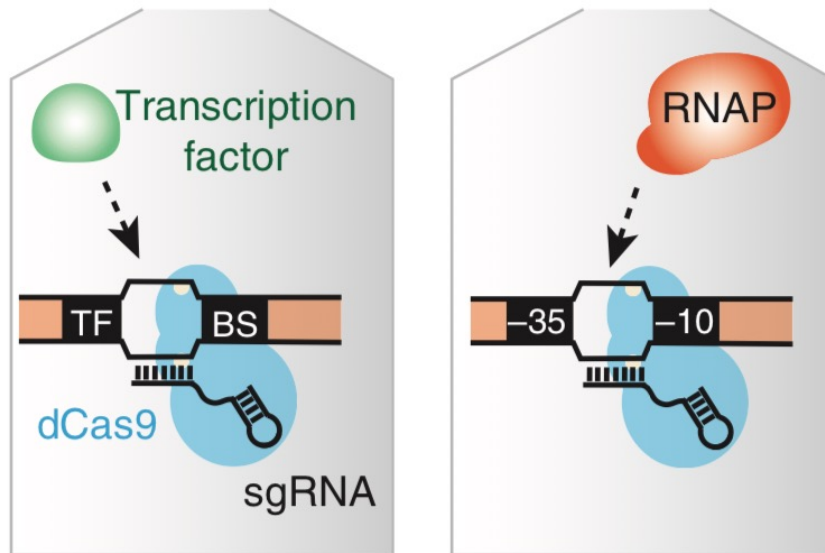
One final review…

1. Plasmid containing gene that encodes dCas9

2. Plasmid containing sequence for sgRNA
   - sgRNA sequence is complementary to target sequence

3. Target sequence

CRISPRi inhibition of gene expression

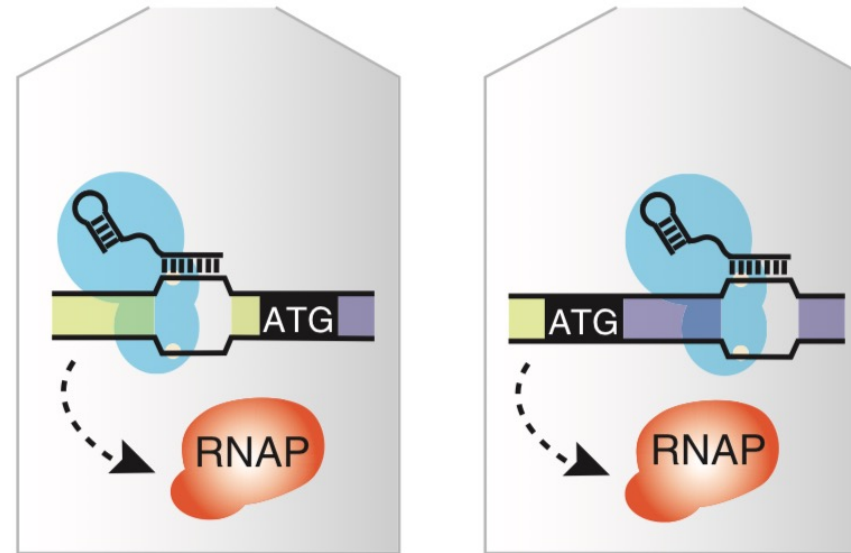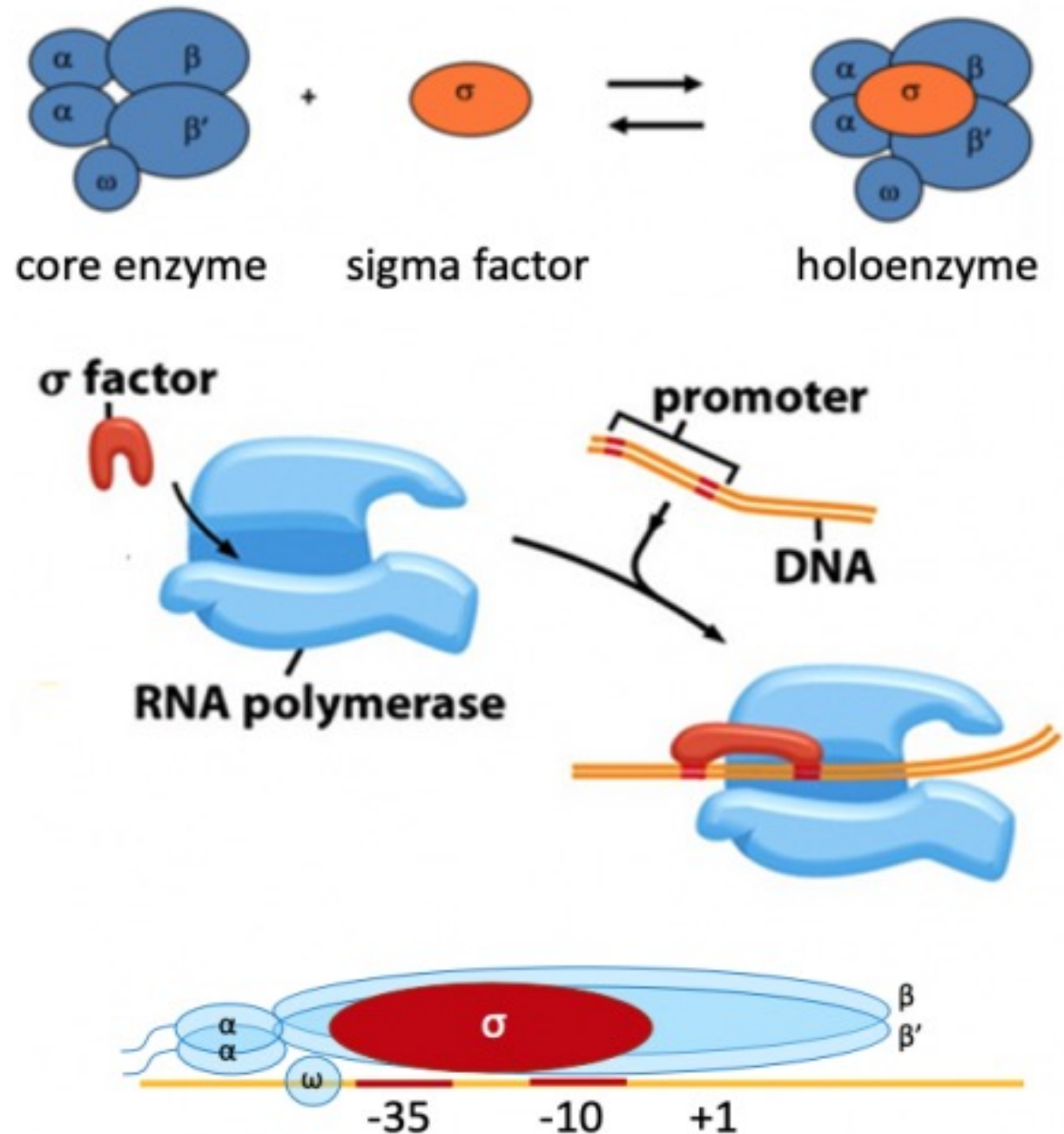Promoters enable gene expression by providing a 'landing pad' for RNAP binding

core enzyme   sigma factor   holoenzyme

σ factor

RNA polymerase

promoter

DNA

α
α
ω
σ
β
β'
-35   -10   +1

RNA binds to promoter and transcribes downstream genes
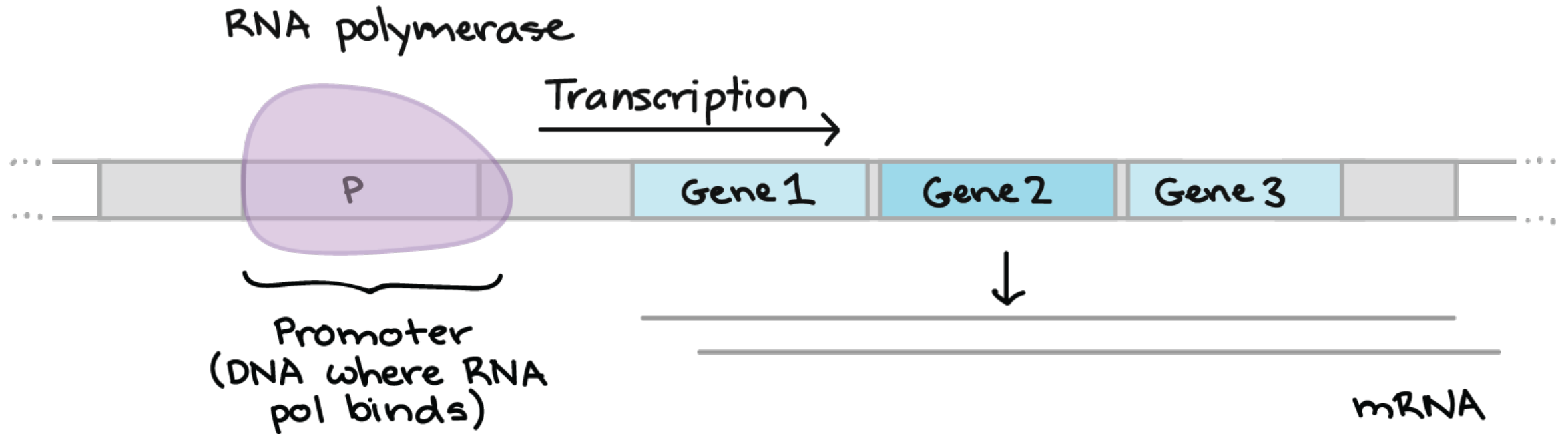
Operators block transcription

- blah

Repressor

NO transcription
✗

P | O | Gene 1 | Gene 2 | Gene 3

Operator
(DNA where
repressor binds)

↓

∅ no mRNA made
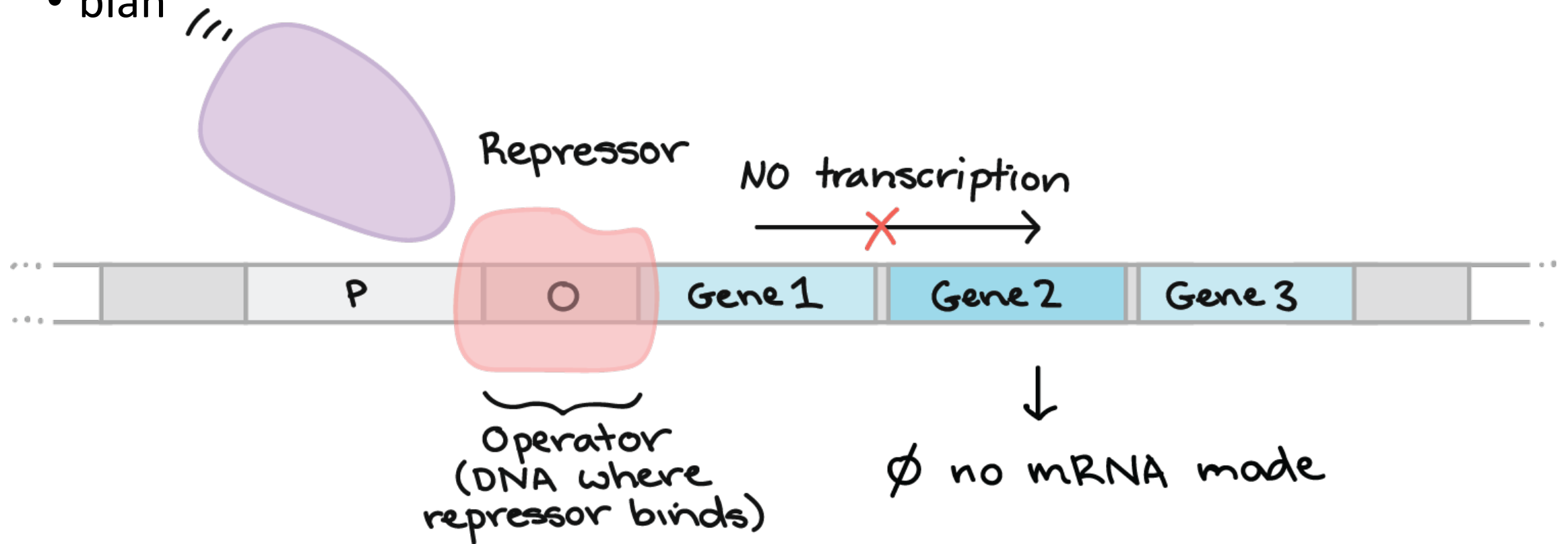
Activators promote / enhance transcription

- blah

Activator

LOTS of transcription

P   O   Gene 1   Gene 2   Gene 3

DNA where activator binds

lots of mRNA made
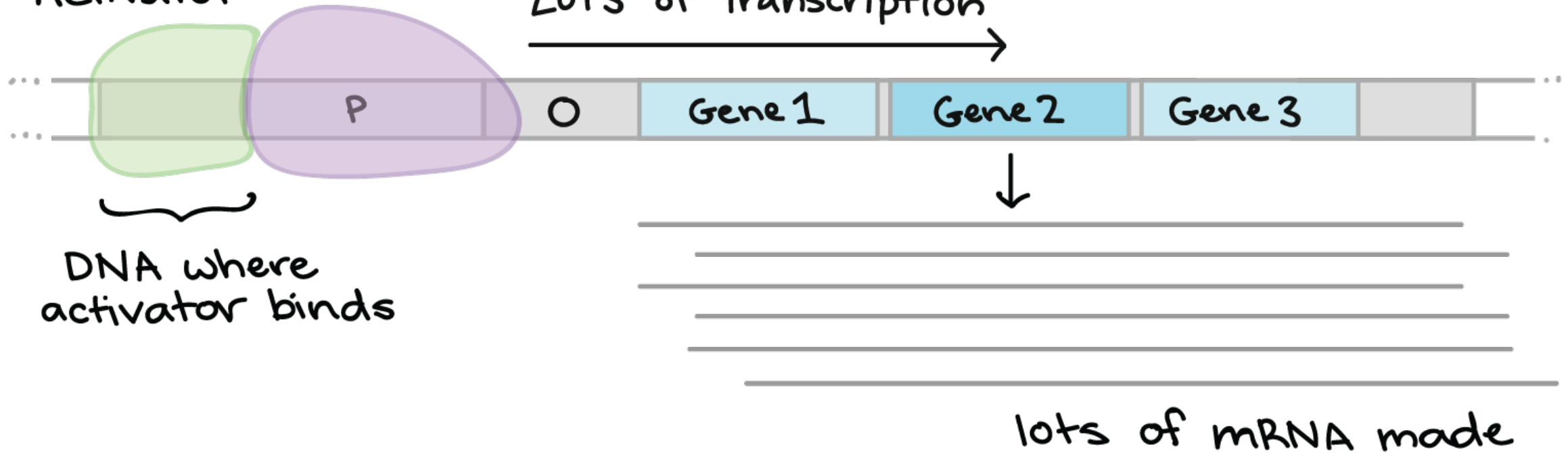
The big picture: promoters are complicated!
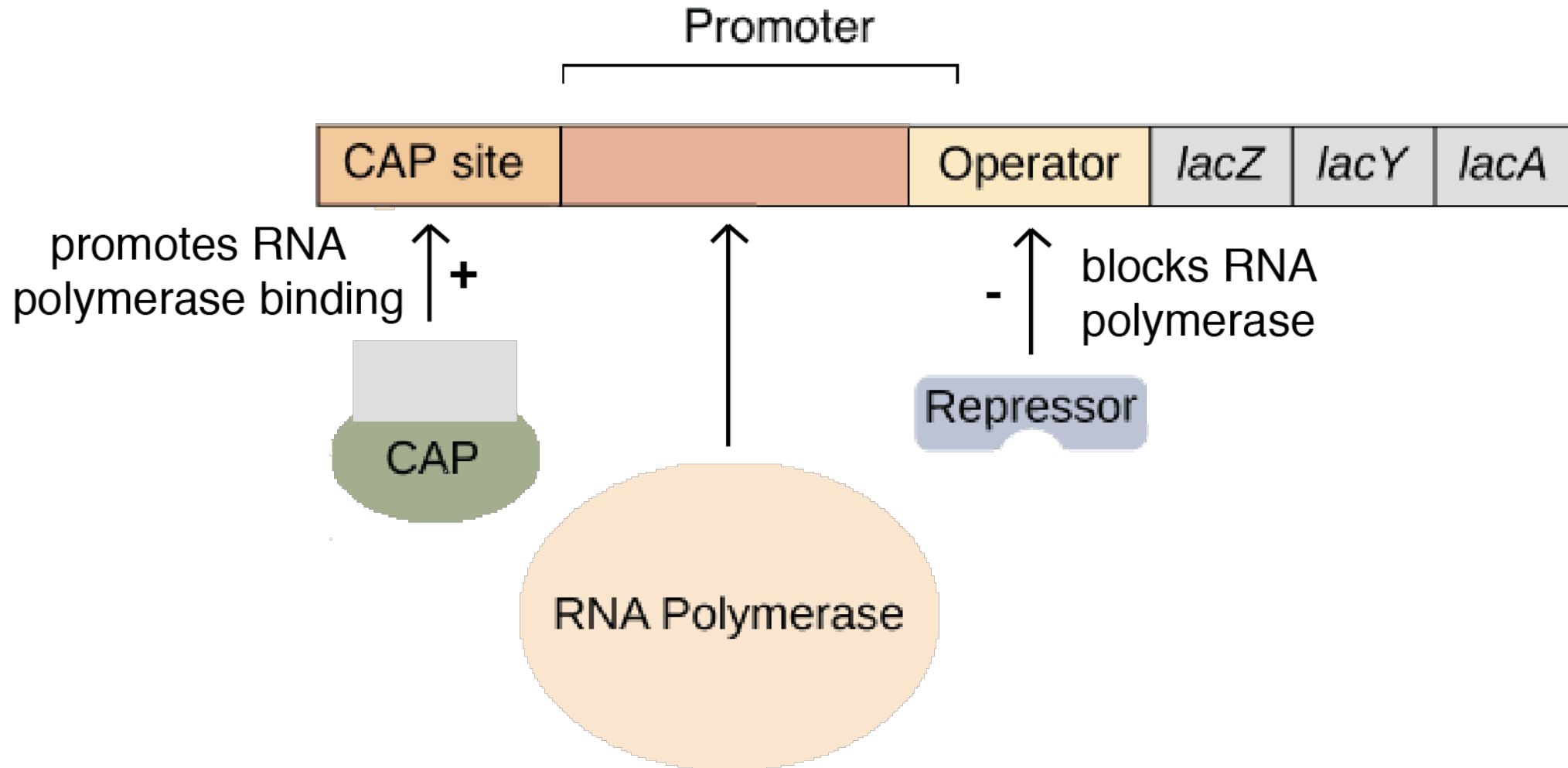
Transcription factors regulate promoter activity

- Depending on location of binding site, able to activate or repress
- Typically regulate networks of genes in response to environmental / metabolic cues
- *E. coli* encodes ~270 transcription factors

transcription factor - activator

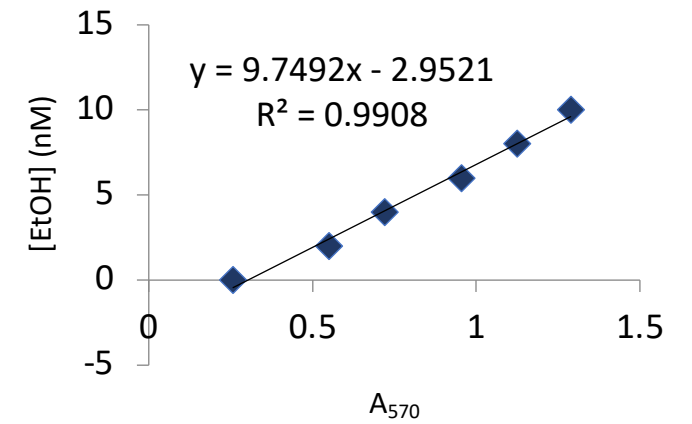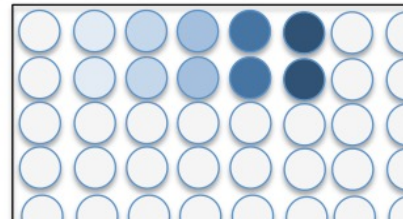transcription factor - repressor

Transcription factors also bind within genes

- ChIP used to identify sequences bound by RutR
- RutR involved in pyrimidine catabolism

Shimada *et al*. (2008) Nuc. Acids Res. 36:3950-3955.
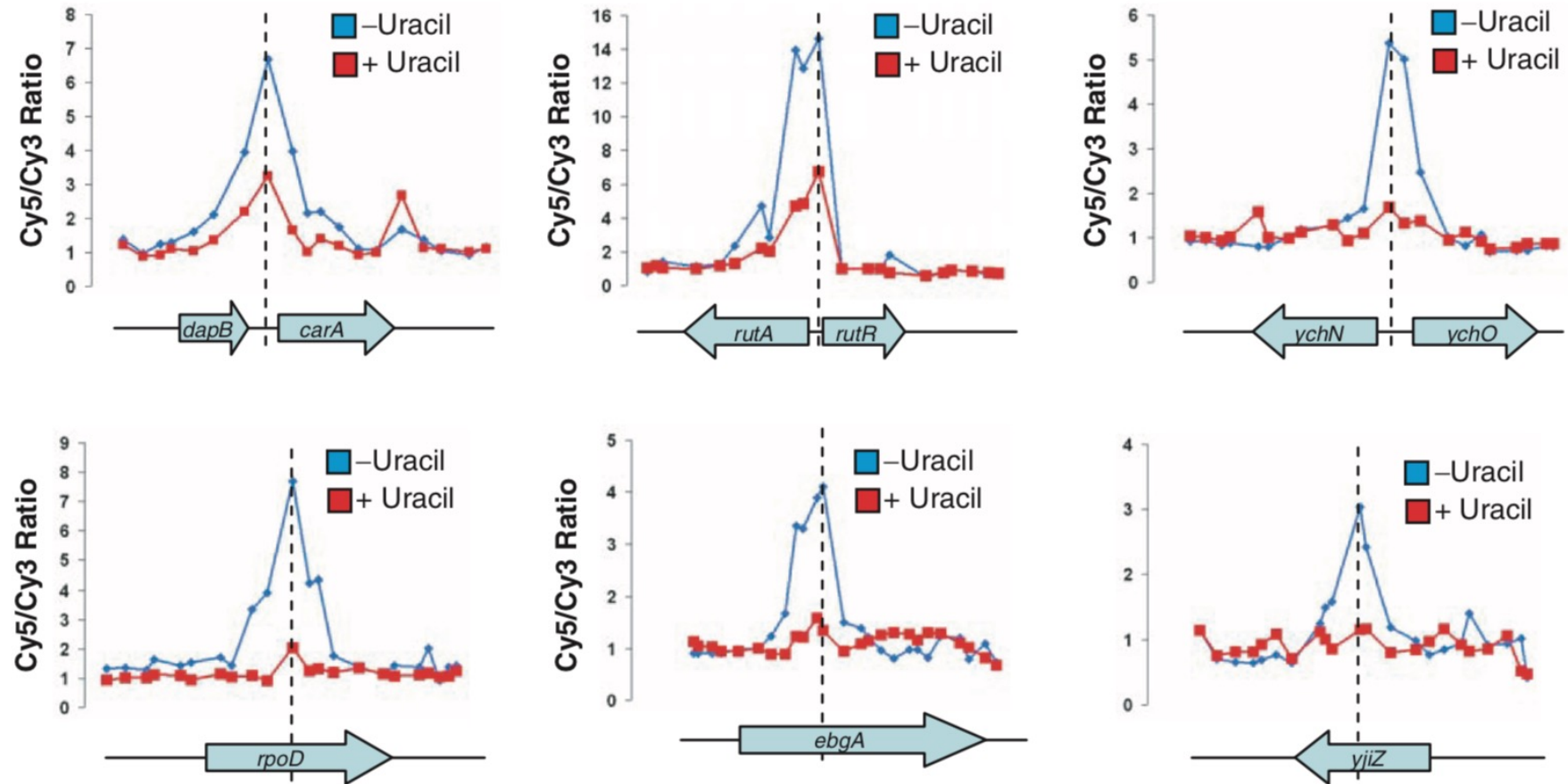
Some thoughts for the laboratory exercise…

- What transcription factor binding sites overlap with your sgRNA target sequence?

- Based on the location of the transcription factor binding site, is is activating or repressing expression of the gene?

- Consider if the transcription factors with binding sites that overlap the sgRNA targets have any effect in the context of your experiment.

How will you present your data?

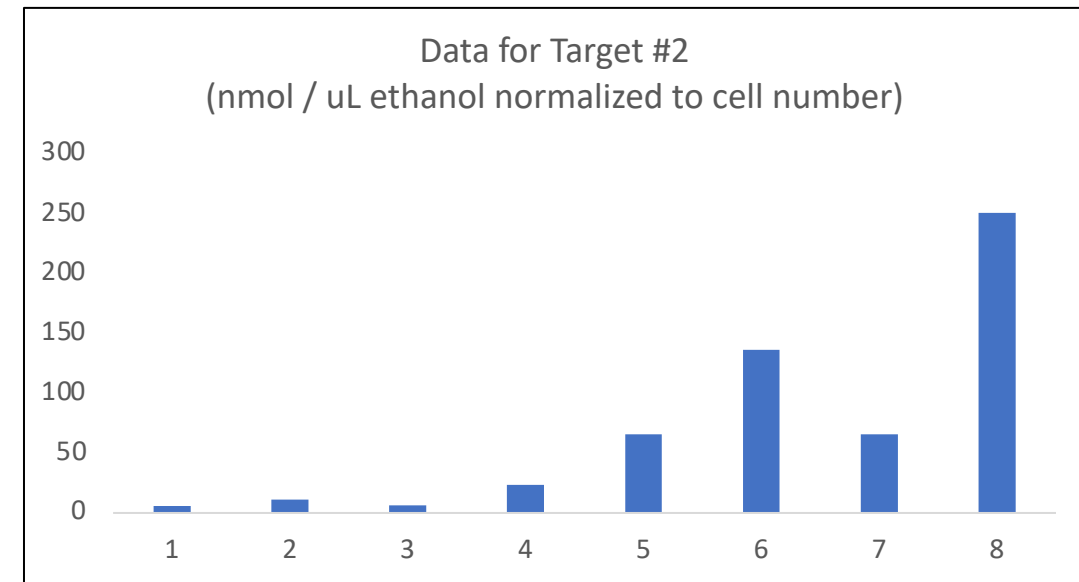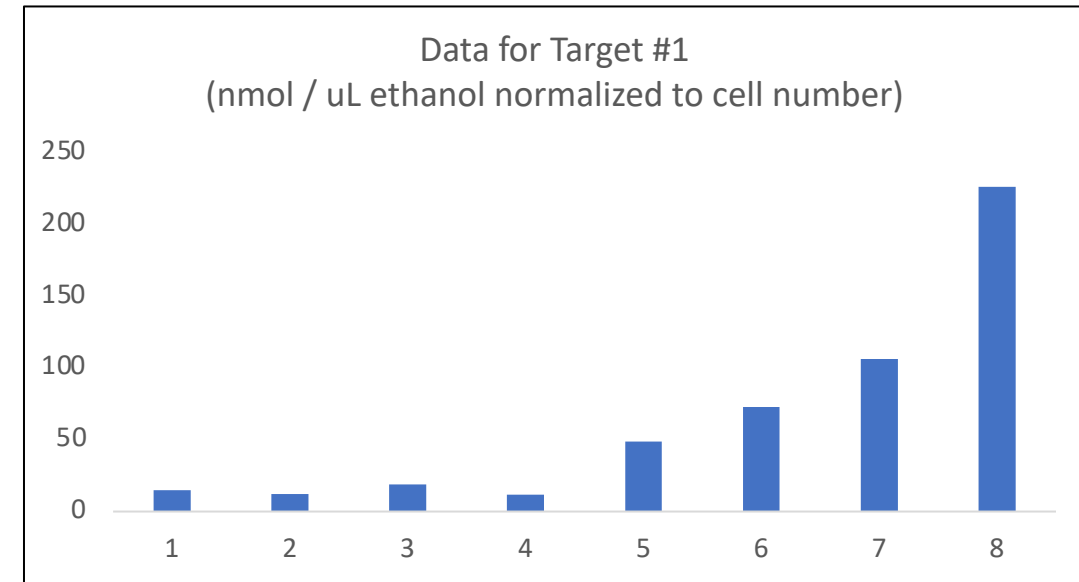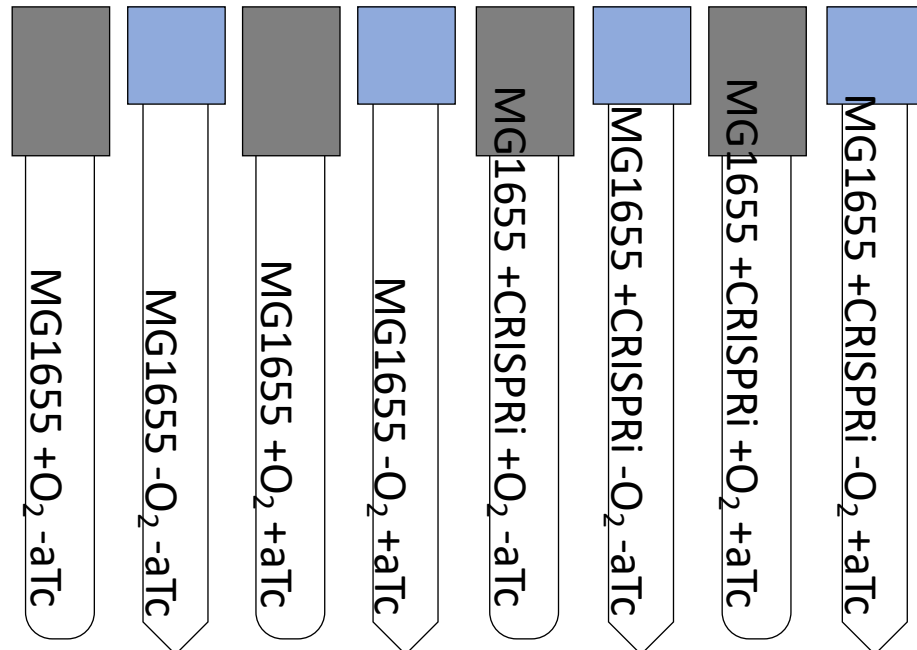# Success!

- Was ethanol yield increased?

- Is CRISPRi system working as expected?

MG1655 +O$_2$ -aTc

MG1655 -O$_2$ -aTc

MG1655 +O$_2$ +aTc

MG1655 -O$_2$ +aTc

MG1655 +CRISPRi +O$_2$ -aTc

MG1655 +CRISPRi -O$_2$ -aTc

MG1655 +CRISPRi +O$_2$ +aTc

MG1655 +CRISPRi -O$_2$ +aTc

Data for Target #1
(nmol / uL ethanol normalized to cell number)

Data for Target #2
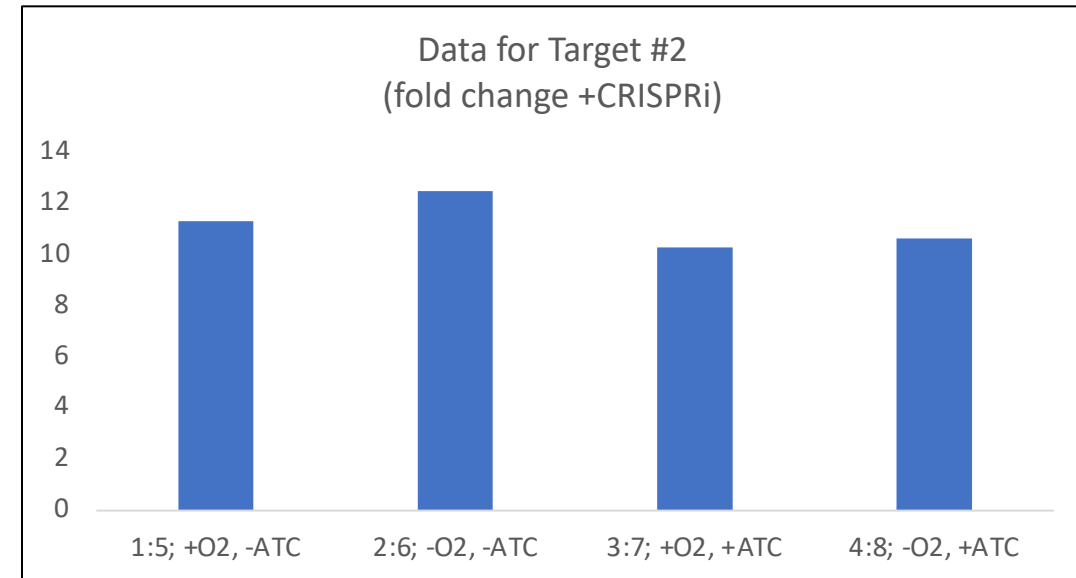(nmol / uL ethanol normalized to cell number)

Maybe a success?

- Was ethanol yield increased?

- Is CRISPRi system working as expected?

Data for Target #1
(fold change +CRISRPi)

1:5; +O2, -ATC   2:6; -O2, -ATC   3:7; +O2, +ATC   4:8; -O2, +ATC

Data for Target #2
(fold change +CRISPRi)

1:5; +O2, -ATC   2:6; -O2, -ATC   3:7; +O2, +ATC   4:8; -O2, +ATC

MG1655 +O$_2$ -aTc
MG1655 -O$_2$ -aTc
MG1655 +O$_2$ +aTc
MG1655 -O$_2$ +aTc
MG1655 +CRISPRi +O$_2$ -aTc
MG1655 +CRISPRi -O$_2$ -aTc
MG1655 +CRISPRi +O$_2$ +aTc
MG1655 +CRISPRi -O$_2$ +aTc

Be critical of the data!

- Think about your data from different points of reference
- Consider how to plot your data such that the hypothesis is answered directly

- Use class data to support your conclusion(s)
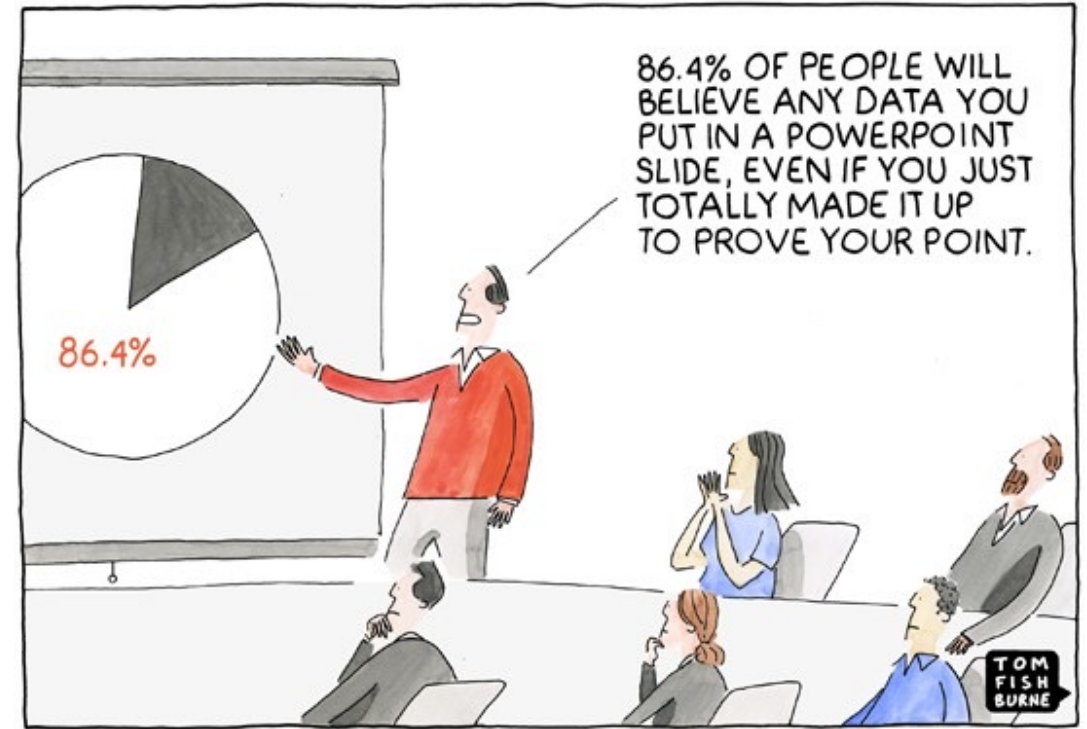
How will you use class data?

- Identify what questions the data can address

- Consider which comparisons can support your data / conclusions

- Represent the data pool honestly

- Must use at least two additional data sets (no more than three)

What is the take-home message?

- Promoters are complicated and many players are involved in regulation of gene expression

- Know the basics for how activators, operators, and transcription factors regulate transcription