

# M2D2: Design gRNA for CRISPRi

10/16/18

- Pre-lab discussion
- Part 1: Load digests from M2D1 in agarose gels
- Mid-lab discussion: gRNA design considerations
- Part 2: Select target gene in fermentation pathway
- Part 3: Design gRNA for CRISPRi system

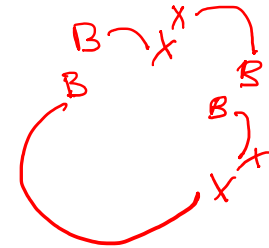
## Announcements

- Journal club starts next week! (See evaluation rubric on wiki. Also, if coming to office hours related to it, email us ahead of time so we can be sure to read your paper)
- Bring presentation slide homework for Comm Lab workshop at 1:30pm on Thursday

# From Last Time: Ligation Calculation

## Optimal backbone-to-insert ratio

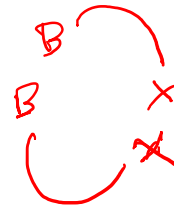
- Ideally, want 4:1 insert:backbone
  - molar ratio, **not** mass or volume



- What if too much insert?

*multiple inserts daisy-chained, inserted into backbone*

- What if too much backbone?



# Today Part 1:

## Separate DNA by gel electrophoresis

### Agarose gel electrophoresis

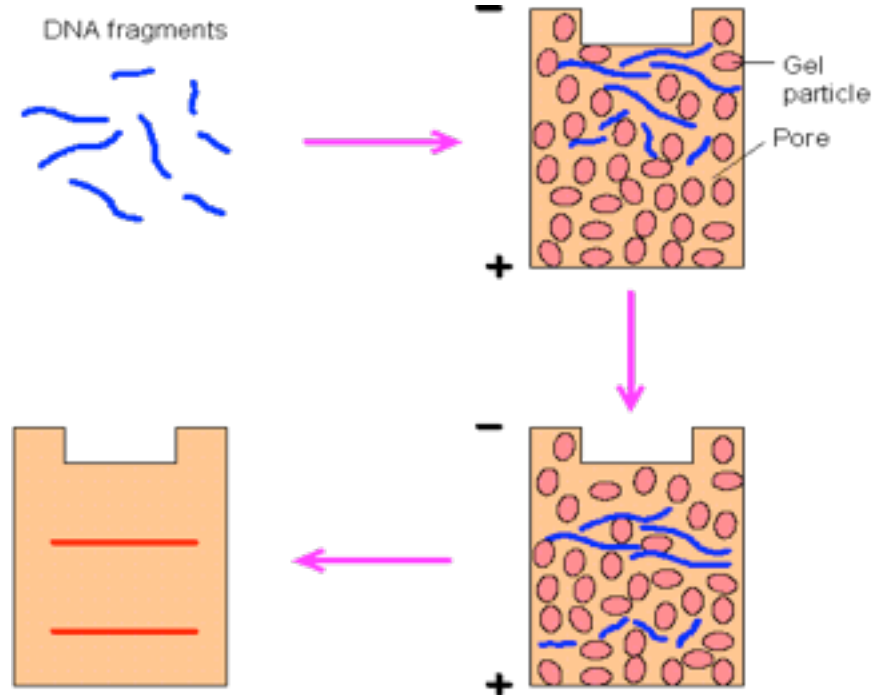
*DNA - negatively charged*

– Driving force:

*charge, electric field*

– Separates DNA by:

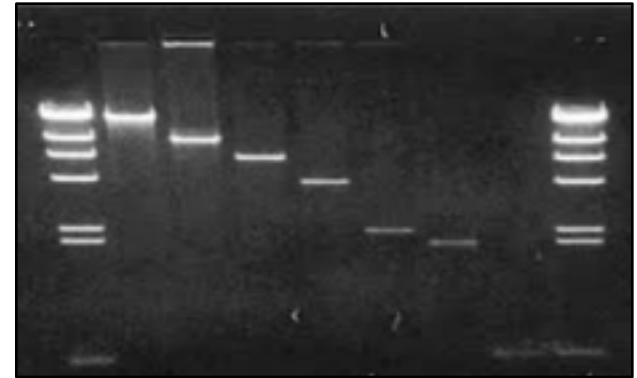
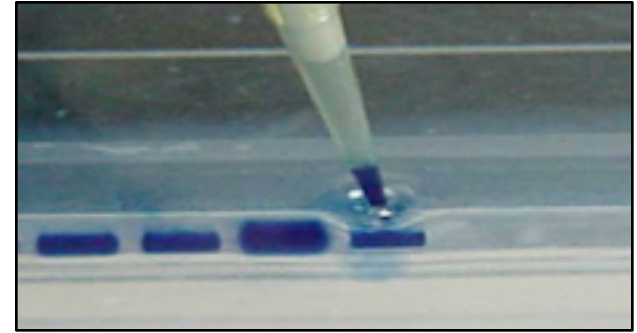
*size*



# Today Part 1:

## Visualize DNA + save a picture!

- DNA Loading dye (6X):
    - Bromophenol blue
      - visible marker of progress
      - small molecule, runs at ~500bp
    - Viscous & dense
  - Sybr-Safe DNA stain:
    - DNA intercalator
    - fluorescent
    - visualize via UV/blue light
- Safety : wear nitrile gloves



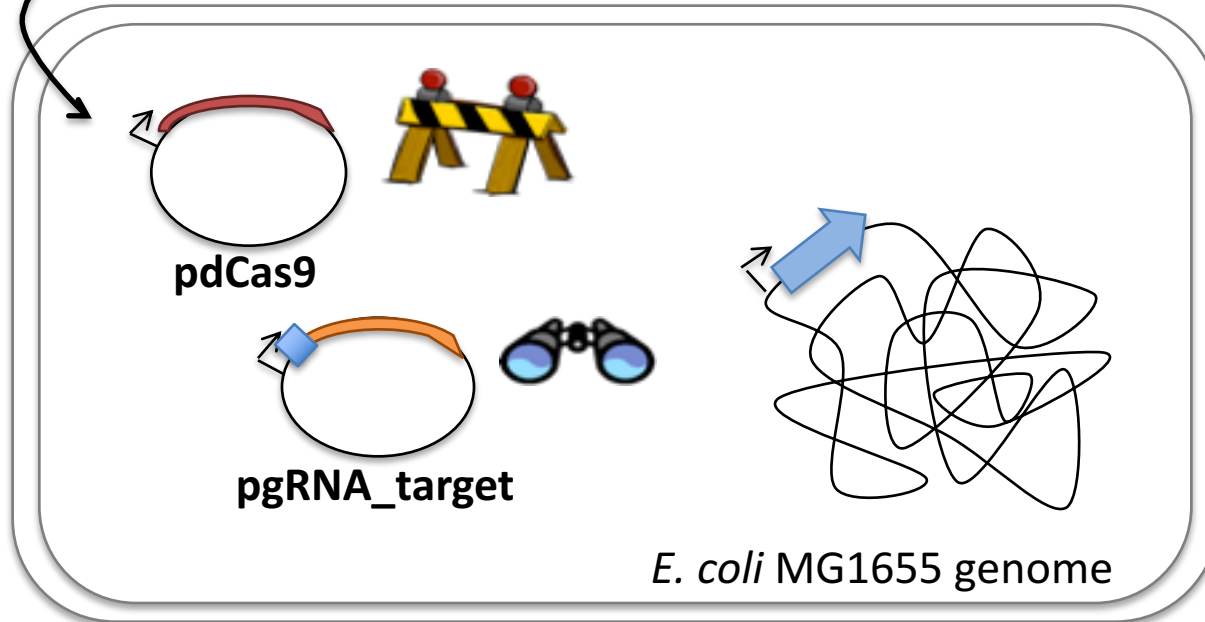
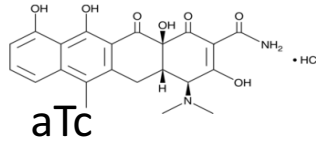
# Let's do this now...

1. Pick up digest from the front bench and prepare samples to be loaded on the agarose gels
2. Two groups at a time can load their samples and we will start gels after 2 groups per gel are loaded
3. Regroup for another short discussion before moving onto Part 2

Today's goal:

**Design gRNA sequence to repress a gene, such that the production of ethanol or acetate will increase.**

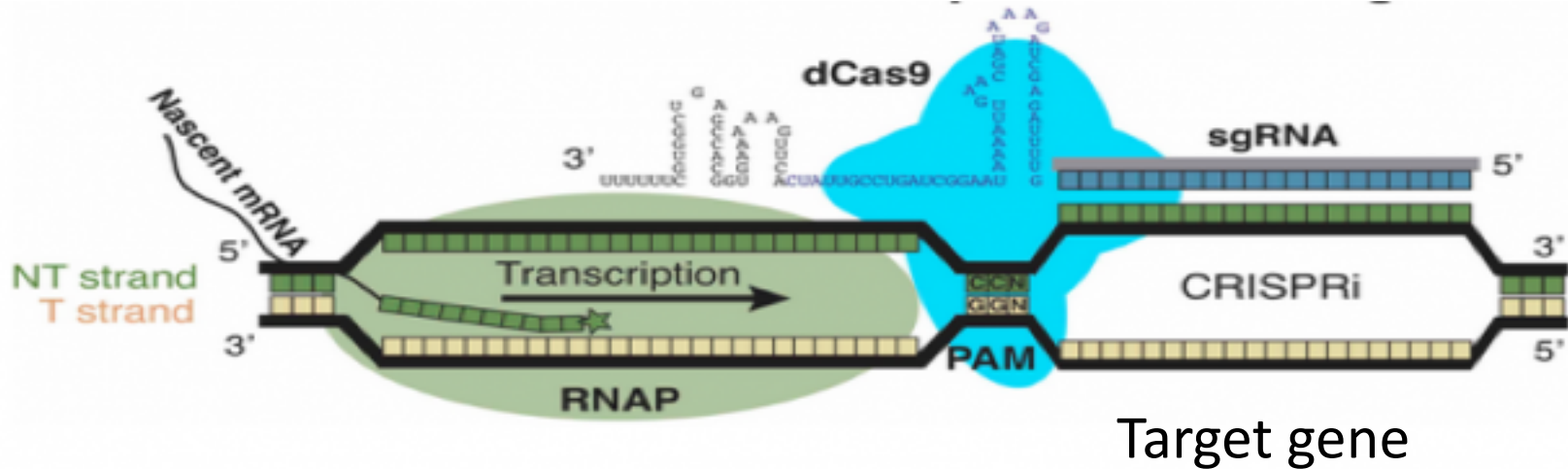
# CRISPRi system overview



gRNA = sgRNA

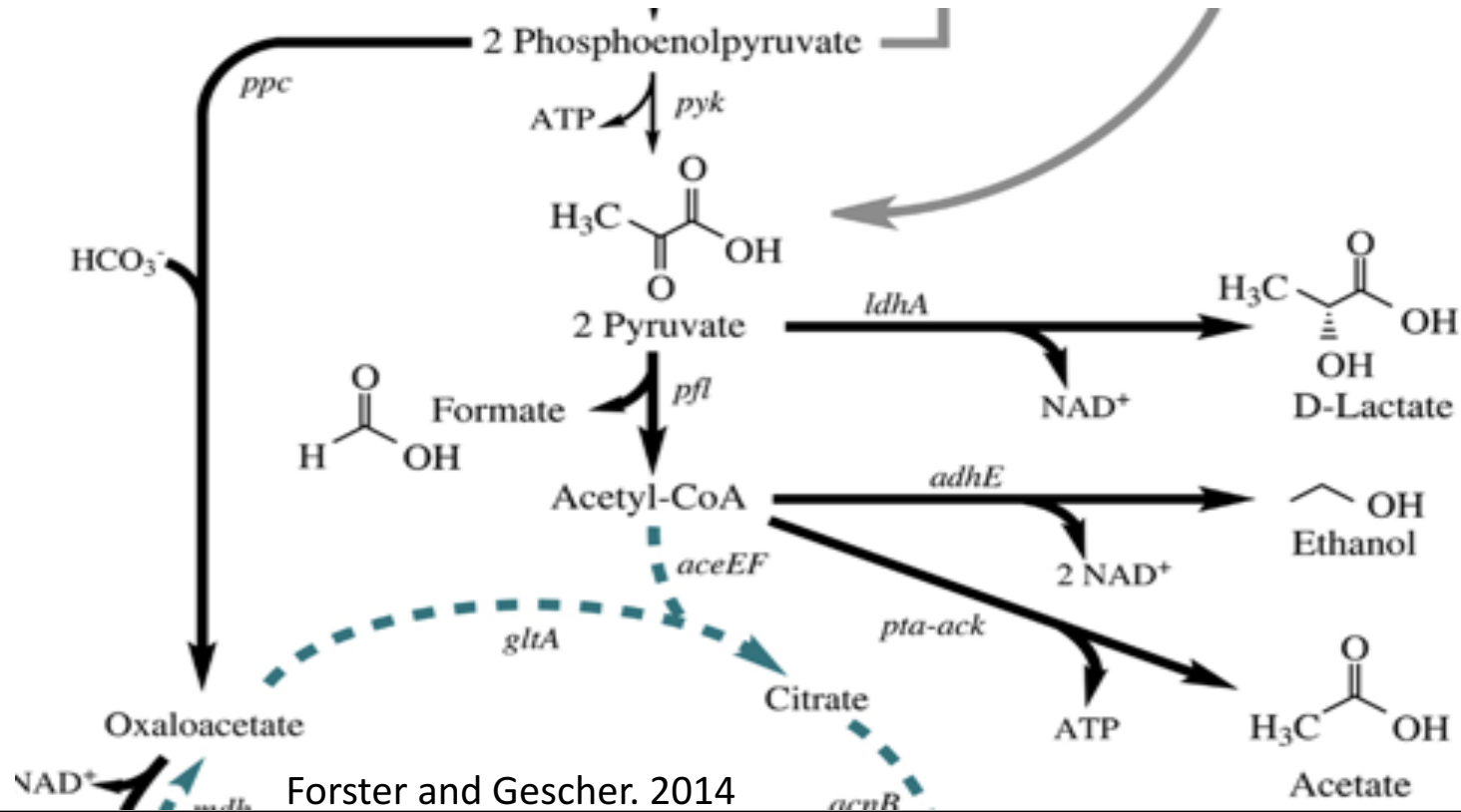
- Target gene
  - increase ethanol or acetate production
- pgRNA\_target
  - express gRNA to specific gene
- pdCas9
  - block expression

# CRISPRi system can block the RNA polymerase

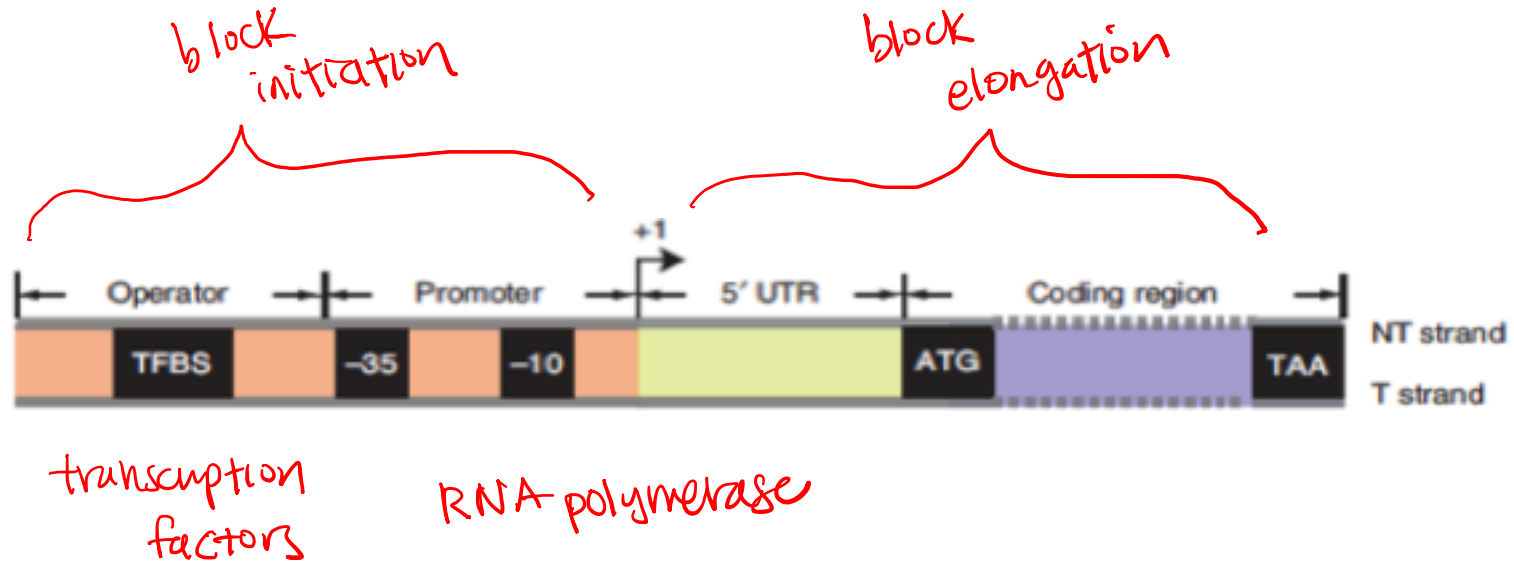




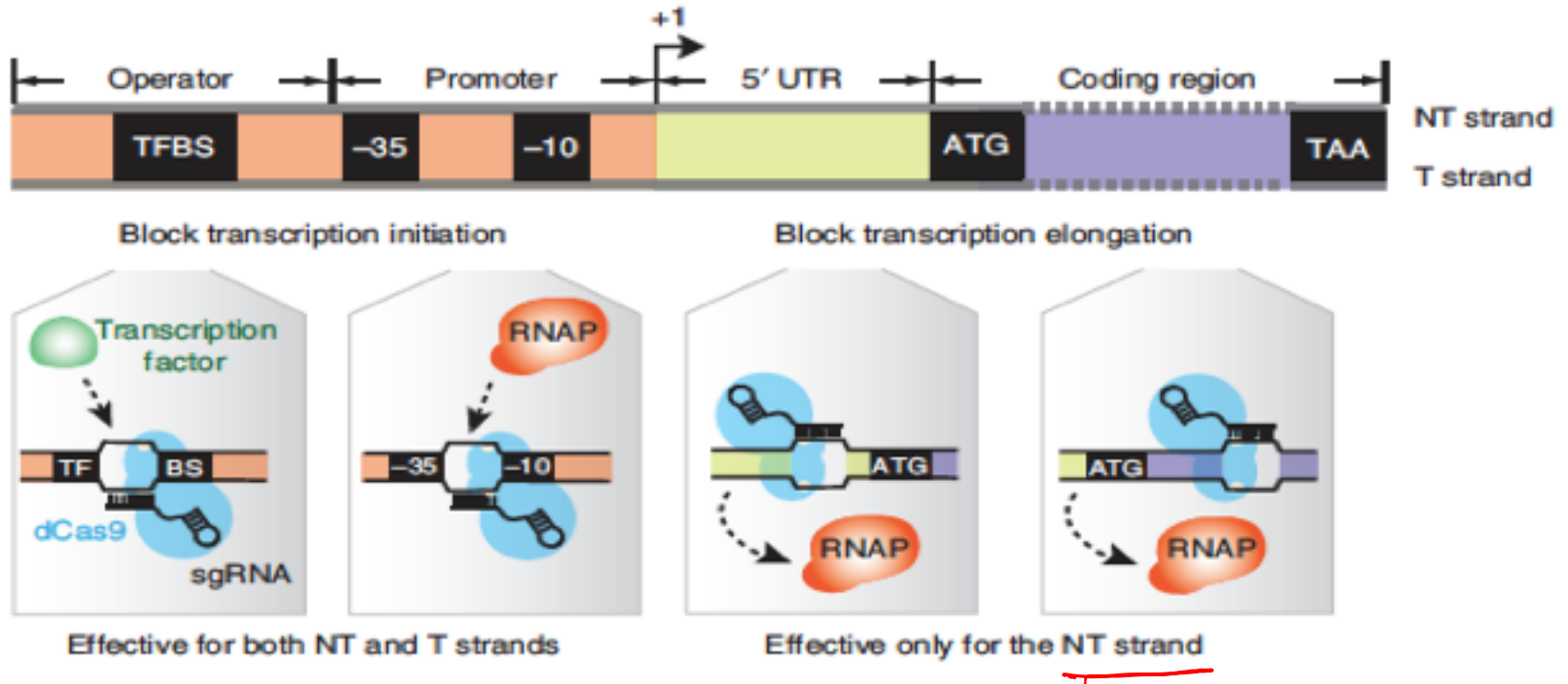
# How would you use CRISPRi to increase yield of the desired product?



# Let's review binding partners:



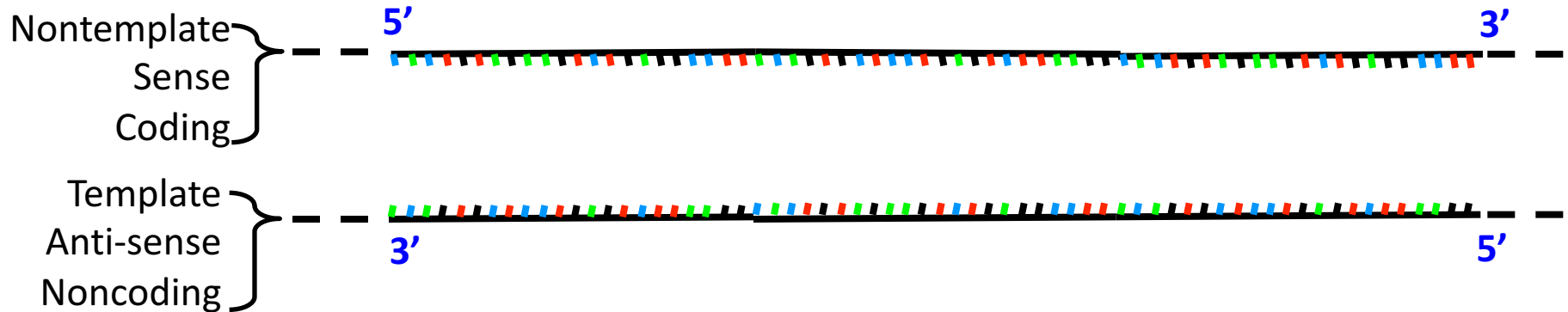
# Which region of the gene will you target?



# Design of gRNA for CRISPRi system

**(1) If you target the template DNA strand, the gRNA sequence will be the same as the transcribed (nontemplate) sequence.**

**(1) If you target the nontemplate strand, the gRNA sequence will be the reverse-complement of the transcribed sequence.**



# Design of gRNA for CRISPRi system

How do you use these principles to  
Design the gRNA DNA sequence



Create a plasmid (pgRNA)



Generate the desired gRNA  
when transcribed



Target gene of interest

## Handout:

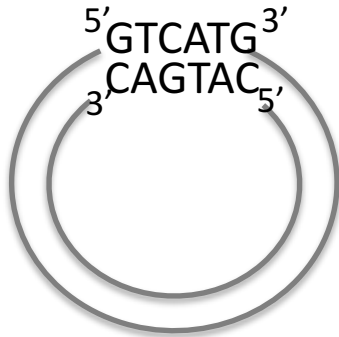
### gRNA (DNA sequence) Design for CRISPRi (fill in the blanks)

Target gene sequence: 5' GTCATG 3' ← Non template (coding) strand  
Complementary strand: 3' CAGTAC 5' ← Template (noncoding) strand

#### Scenario 1

**gRNA (DNA sequence) is the same as the nontemplate (NT) strand:** 5' GTCATG 3'

The plasmid you generate after inserting the sequence would look something like pgRNA\_target\_1:



pgRNA\_target\_1

transcription



gRNA produced: 5' 3'

GTCATG

This gRNA binds to the nontemplate/template (circle one) strand of the target gene.

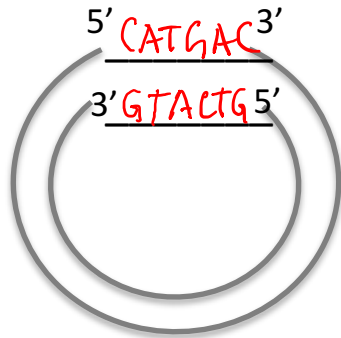
## Handout:

### gRNA (DNA sequence) Design for CRISPRi (fill in the blanks)

#### Scenario 2

gRNA (DNA sequence) is the same as the template (T) strand: 5' CATGAC 3'

The plasmid you generate after inserting the sequence would look something like pgRNA\_target\_2:



pgRNA\_target\_2

transcription



gRNA produced:  
5' CAUGAC 3'

This gRNA binds to the nontemplate template (circle one) strand of the target gene.

# Practically: Using KEGG Database

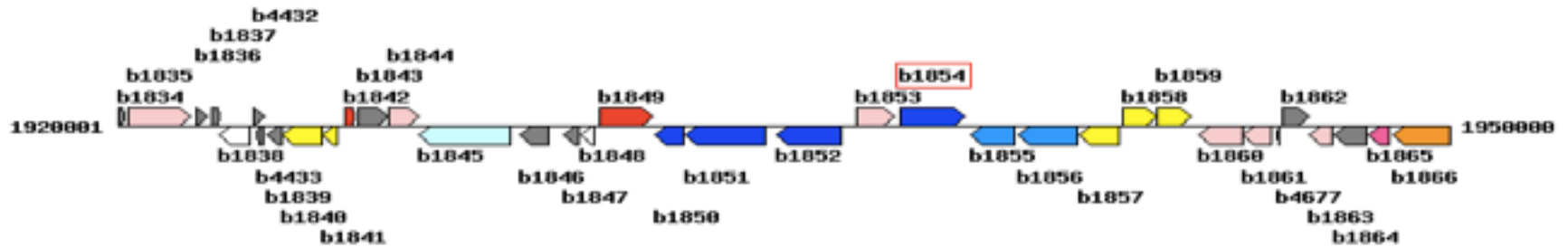
Position	1937649..1939091 <a href="#">Genome map</a>
AA seq	480 aa <a href="#">AA seq</a> <a href="#">DB search</a> MSRRLRRRTKIVTTTLGPATDRDNNLEKVIANGANVVMNFSHGSPEDHKMRADKVRERIAAK LGRHVAAILGDLQGGFKIRVSTFKEGKVFLNIGDKFLLDANLKGEGGDKKRVGIDYFGLPAD VVPFGDILLDDGDRVOLKVLVEVQGMKVFTTEVTVGGLPNSNKGINKLGGGLSAEALTEKDKA DIKTAALIGVDYLAVSFPRCGEDLNYARRLARDAGCDKIVAKVERAEAVCSQDAMDII LASDVVMVARGDLQVEIGDPELVGIGKALIRRARQLNRAVITATQMESHMITNPMPTRAE VMDVANAVLDGTDVHLSAETAAGQYPSETVAAMARVCLGAEKIPSNVFSKRLDVOQFDN VEEAIAHSAHYAANLKGVTAIITMTESGRTALMTSRISGLPIFAMSRHERTLNLTALY RGVTPVHFDSANDGVAAASEAVNLLRDKGYLMSGDLVIVTQGDVMSIVGSTNTTRILTVE
NT seq	1443 nt <a href="#">NT seq</a> <input type="text" value="+upstream 0"/> nt <input type="text" value="+downstream 0"/> nt atgtccagaaggcttcgcagaaacaaaaatcgttaccacggttagggcccagcaacagatcgc gataaataatcttgaaaaaagttatcgcgccgggtgcacaacggttgtaacgtatgaacttttct cacggctcgocctgaagatcacaaaaatgcgcgcgggataaagttcgtgagattgccgcacaaa ctggggcgctcatgtggctattctgggtgacctccaggggcccacaaatccgtgtatccacc tttaaaagaaggcacaagttttctcaatatgggggataaattcgtcgcgcagcccaacctg ggtaaaagggtgaaggcgacaaaagaaaaagtcgggtatcgactacaaaaggcctgcctgctgac gtcgtgcctgggtgacatcctgctgctggagcagatggtgcgcgtccagttaaaagtaactggaa gttcaggggcatgaaaagtgttcacccgaagtcacccgtcgggtgggtccctctccacacataaa gggtatcaacaaacttggcgccgggtttgtcggctgaagcgctgacccgaaaagacaaagca gacattaagactgcggcggttgattggcgtagattacctggctgtctccttccacacgctgt ggcgaaagatctgaactatgcocgtgcgcctggcagcgcgatgcaggatgtgatgcgaaaaatt gttgccaaagggtgaacgtgcgggaagccgtttgcagccaggatgcaatggatgacatcatc ctcgcctctgaactggtaaatggttgacagctggcgacccctcgggtgtggaattggcgacccg gaactggctcggcattcagaaaagcgttgatccgtcgtgcgcgctcagctaaaaccgagcggta atcacggcgacccagatgatggagtcaatgattactaacccgatgccgacgcgctgcagaa gtcatggacgtagcaaacgcggttctggatggtaactgacgcctgtgatgctgtctgcagaa actgcgcgtcgggcagtatccgtcagaaaaccgttgacgccatggcgccgctttgcctgggt gcggaaaaaaatcccagcgcataaacgtttctaaacacccgtctggacggttcagttcgacaat gtggaagaagctattgccatgtcagcaatgtacgcagctaaccaacctgaaggccgttacg gcgatacatcccatgaaccgaatcggggtcgtaaccgcgctgatgacctccgctatcagctct ggctctgcacattttcgcctatgtcgcgcctatgaacgtacgctgaacctgactgctctctat cgtggcggttaacgcgggtgcactttgatagcgctaataagcggcgtagcagctgccagcgaa gcggttaactctcgtcgcgcgataaaagggttaacttgatgtctggtagacctggtgattgtcacc caggcgacgctgatgagtagacctgggttctactaataaccacgcgtattttaacggtagag taa

NT here means nucleotide  
sequence



# Practically: Using KEGG Database

## Genome Map



# Practically: Using BLAST (Basic local alignment search tool) to assess off-target binding

Sequence ID: [CP014348.1](#) Length: 4657541 Number of Matches: 163

Range 1: 3891267 to 3891292 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
52.0 bits(26)	1e-07	26/26(100%)	0/26(0%)	Plus/Plus

Query 1 ATGAAACTCGCCGTTTATAGCACAAA 26  
Sbjct 3891267 ATGAAACTCGCCGTTTATAGCACAAA 3891292

Range 2: 392405 to 392417 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	5.8	13/13(100%)	0/13(0%)	Plus/Minus

Query 4 AAAC TCGCCGTTT 16  
Sbjct 392417 AAAC TCGCCGTTT 392405

Range 3: 1595715 to 1595727 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match ▲ First Match

Score	Expect	Identities	Gaps	Strand
26.3 bits(13)	5.8	13/13(100%)	0/13(0%)	Plus/Minus

Query 1 ATGAAACTCGCCG 13  
Sbjct 1595727 ATGAAACTCGCCG 1595715

# M2D3 HW: Figure/Caption/Results

- Figure = agarose gel image with title and caption
  - don't add drawings/modification on top of image, next to image is sufficient
- Results **paragraph** should follow below figure and results subsection title (take-home message)
- Minimize interpretation in the results section in Mod2 report (separate discussion section; *M2D5HW*)
  - State the result of the experiment without discussing conclusions drawn from experiment
- Figure/text expectations same as Mod1 report, formatting major difference

# In lecture discussion Thursday

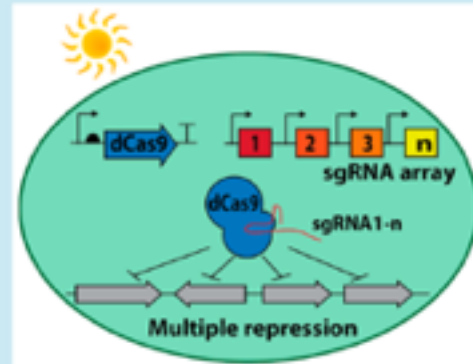
## Multiple Gene Repression in Cyanobacteria Using CRISPRi

Lun Yao, Ivana Cengic, Josefine Anfelt, and Elton P. Hudson\*

KTH—Royal Institute of Technology, Division of Proteomics and Nanobiotechnology, Science for Life Laboratory, Stockholm SE-171 21 Sweden

### Supporting Information

**ABSTRACT:** We describe the application of clustered regularly interspaced short palindromic repeats interference (CRISPRi) for gene repression in the model cyanobacterium *Synechocystis* sp. PCC 6803. The nuclease-deficient Cas9 from the type-II CRISPR/Cas of *Streptococcus pyogenes* was used to repress green fluorescent protein (GFP) to negligible levels. CRISPRi was also used to repress formation of carbon storage compounds polyhydroxybutyrate (PHB) and glycogen during nitrogen starvation. As an example of the potential of CRISPRi for basic and applied cyanobacteria research, we simultaneously knocked down 4 putative aldehyde reductases and dehydrogenases at 50–95% repression. This work also demonstrates that tightly repressed promoters allow for inducible and reversible CRISPRi in cyanobacteria.



# Today

- Sign up for ethanol or acetate at front bench
- Write your gRNA (DNA) sequence on wiki and let me know so I can order them today!

Note: (your sequence + 35 bp tag)  $\leq$  60 bp