M2D2: Design gRNA for CRISPRi

10/17/18

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

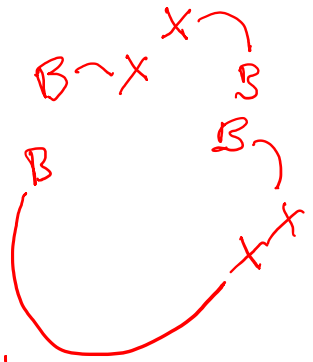
Announcements

- 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)
- My office hour tomorrow (Oct 18th) is canceled.
- Bring presentation slide homework for Comm Lab workshop at 2:00pm on Friday
- BE career fair is Friday. Talk to me if you have a serious conflict; consider going early

From Last Time: Ligation Calculation

Optimal backbone-to-insert ratio

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

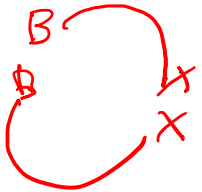


- What if too much insert?

multiple inserts daisy-chained, inserted into backbone

- What if too much backbone?

multiple backbones ligated



Today Part 1:

DNA negatively charged

Separate DNA by gel electrophoresis

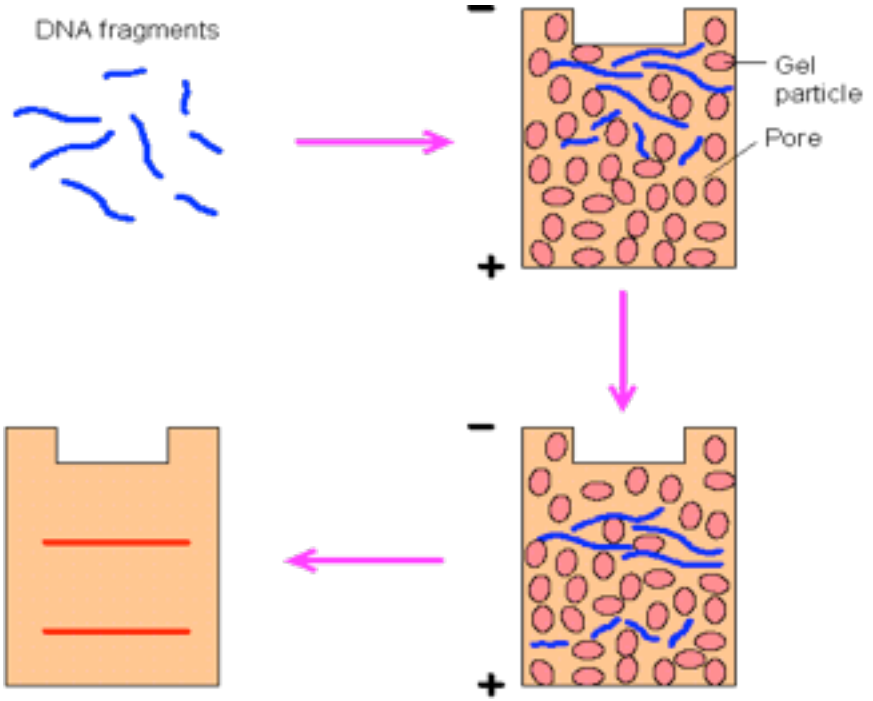
Agarose gel electrophoresis

– Driving force:

charge, electric field

– Separates DNA by:

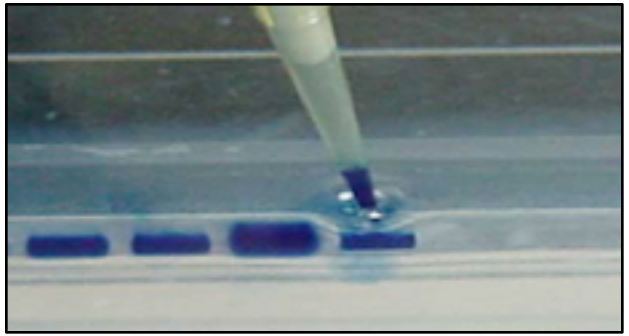
size

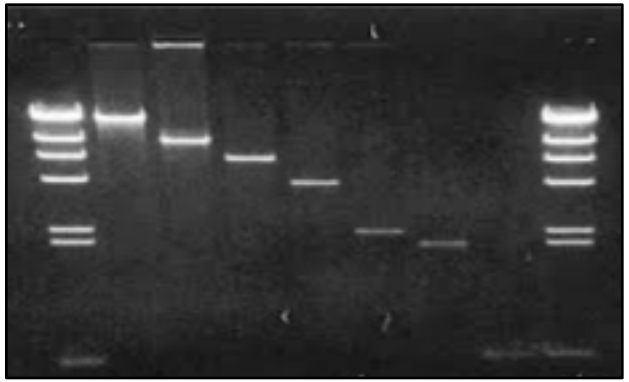


Today Part 1:

Visualize DNA + save a picture!

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





➢ Safety : wear nitrile gloves

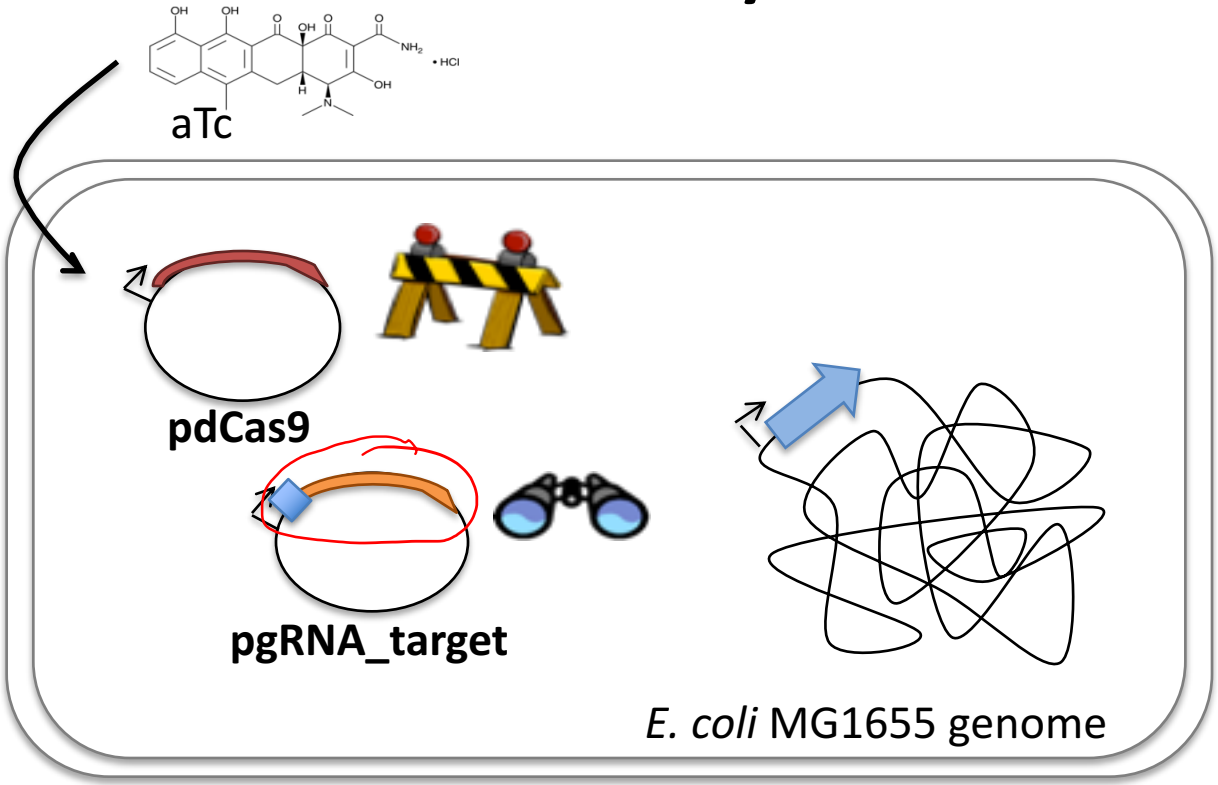
Let’s do this now…

1. Pick up digest from the front bench and prepare samples to be loaded on the agarose gels
2. Each group will load their samples and we’ll start gels after all 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

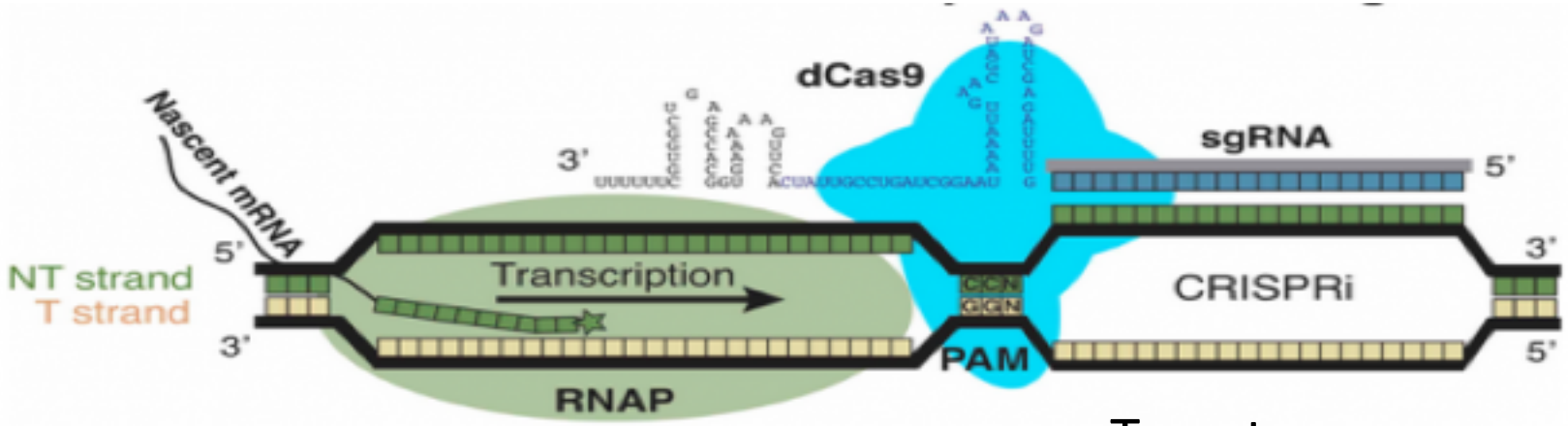


- Target gene
  - increase ethanol or acetate production
- pgRNA_target
  - express gRNA to specific gene
- pdCas9
  - blocks expression

gRNA = sgRNA

Dr. Noreen Lyell's lecture M2D1

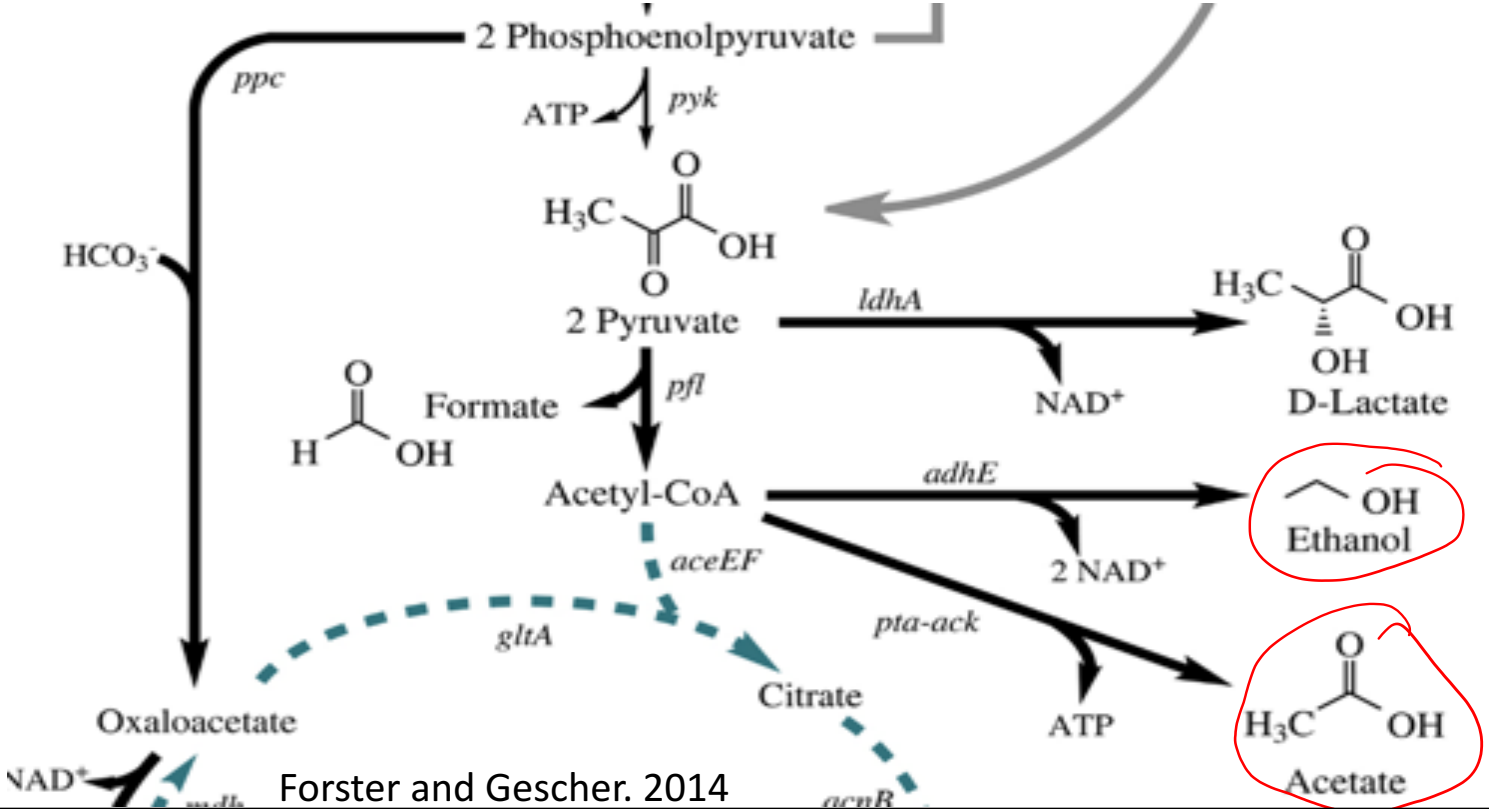
CRISPRi system can block the RNA polymerase



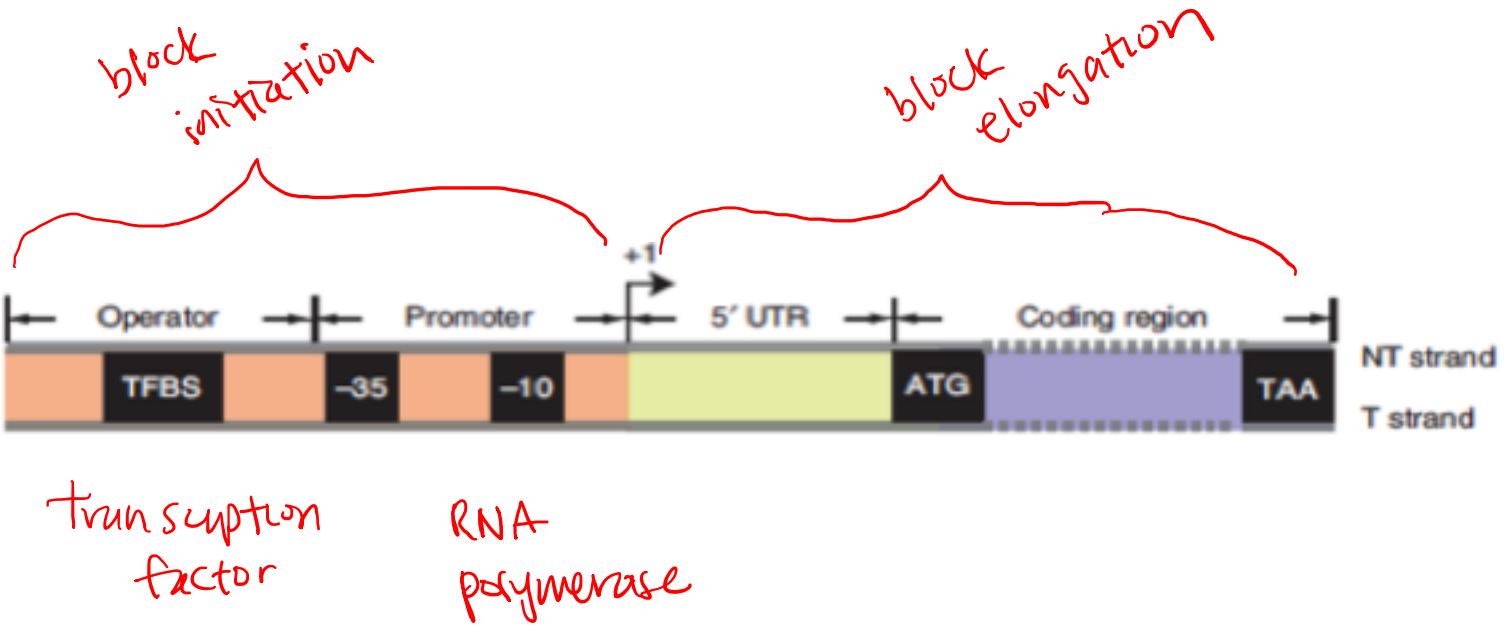
Target gene

http://qi.ucsf.edu/CRISPR_transcription

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

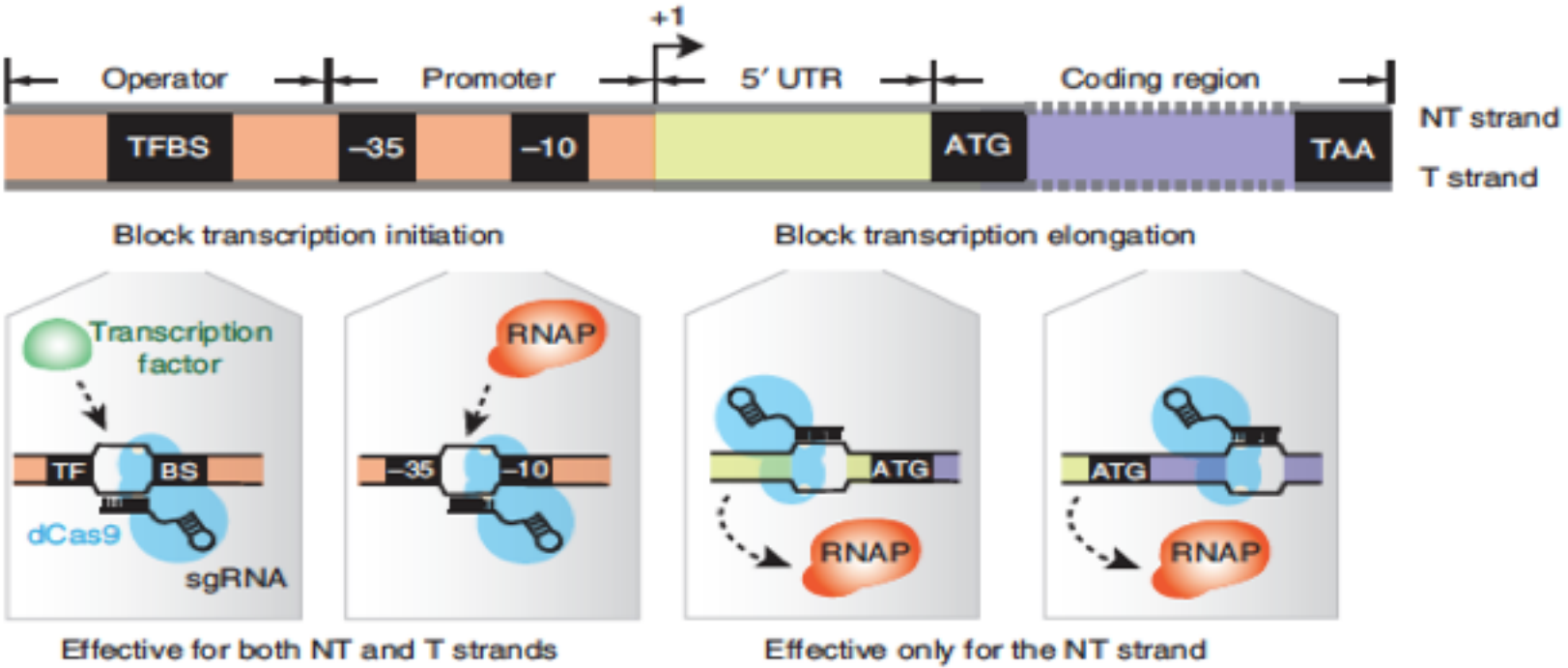


Let's review binding partners:



Larson, et al. CRISPR interference for sequence-specific control of gene expression. *Nature Protocols.* 2013.

Which region of the gene will you target?

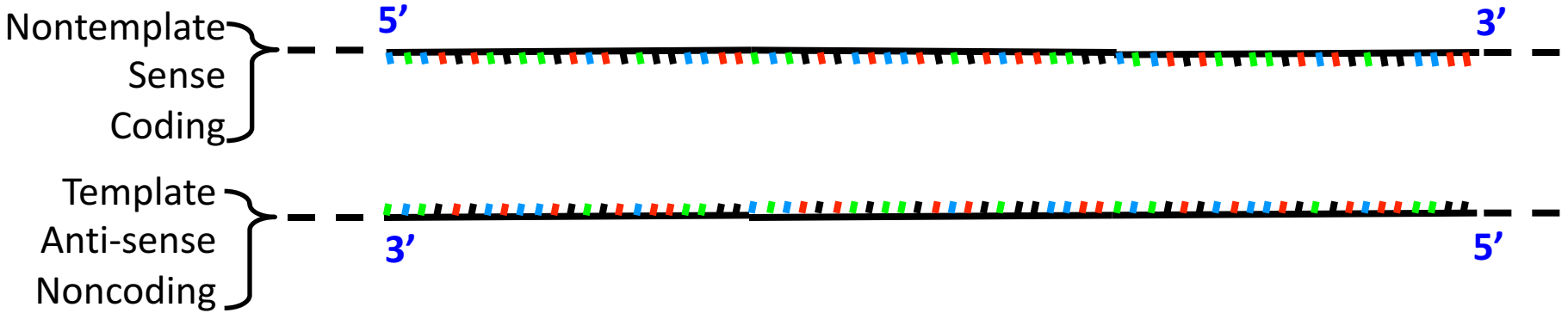


Larson, et al. CRISPR interference for sequence-specific control of gene expression. *Nature Protocols.* 2013.

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

See Handout

Handout:

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

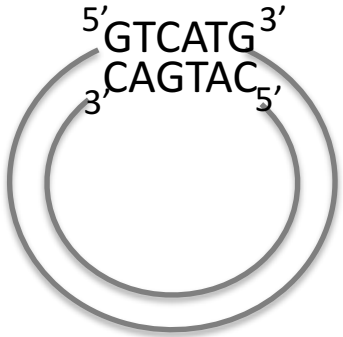
Target gene sequence: 5′ GTCATG 3′ ← Nontemplate (coding) strand

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′ GUCAUG 3′

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

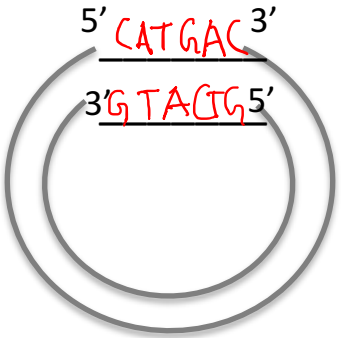
Handout:

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’ CAU GAC 3’

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

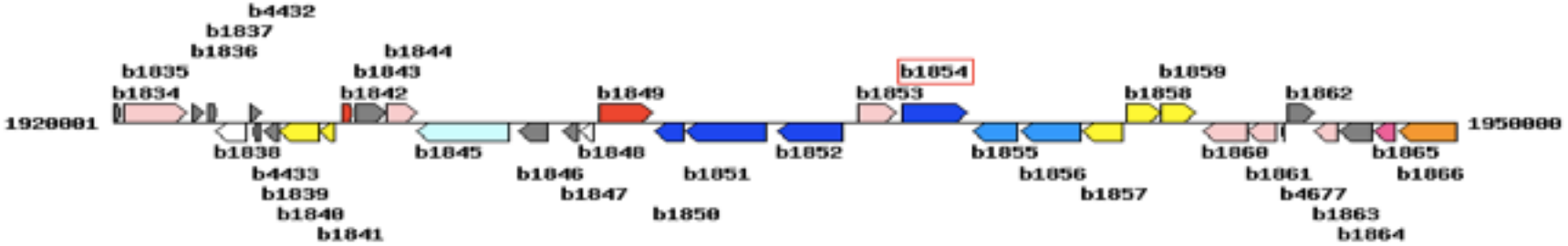
Practically: Using KEGG Database

Position	1937649..1939091 Genome map
AA seq	480 aa AA seq DB search MSRRLRRTKIVTTLGPATDRDNNLEKVIAAGANVVRMNFSHGSPEDHKMRADKVREIAAK LGRHVAILGDLQGPKIRVSTFKEGKVFLNIGDKFLLDANLGKGEGDKEKVGIDYKGLPAD VVFGDILLLDDGRVQLKVLEVQGMKVFTEVTVGGPLSNNKGINKLGGGLSAEALTEKDKA DIKTAALIGVDYLAVSFPRCGEDLNYARRLARDAGCDAKIVAKVERAEAVCSQDAMDDII LASDVVMVARGDLGVEIGDPELVGIQKALIRRARQLNRAVITATQMMESMITNPMPTRAE VMDVANAVLDGTDAVMLSAETAAGQYPSETVAAMARVCLGAEKIPSINVSKHRLDVQFDN VEEAIAMSAMYAANHLKGVTAIITMTESGRTALMTSRISSGLPIFAMSRHERTLNLTALY RGVTPVHFDSANDGVAAASEAVNLLRDKGYLMSGDLVIVTQGDVMSTVGSTNTTRILTVE
NT seq	1443 nt NT seq +upstream 0 nt +downstream 0 nt atgtccagaaggcttcgcagaaacaaaaatcgttaccacgttaggcccagcaacagatcgc gataataatcttgaaaaagttatcgcggcgggtgccaacgttgtacgtatgaacttttct cacggctcgcctgaagatcacaaaatgcgcgcggataaagttcgtgagattgccgcaaaa ctgggcgtcatgtggctattctgggtgacctccagggcccaaaatccgtgtatccacc tttaaagaaggcaaagttttcctcaatattggggataaattcctgctcgacgccaacctg ggtaaaggtgaaggcgacaaagaaaaagtcggtatcgactacaaaggcctgcctgctgac gtcgtgcctggtgacatcctgctgtggacgatggtcgcgtccagttaaaagtactggaa gttcagggcatgaaagtgttcaccgaagtcaccgtcggtggtcccctctccaacaataaa ggtatcaacaaacttggcggcggtttgtcggctgaagcgctgaccgaaaaagacaaagca gacattaagactgcggcgttgattggcgtagattacctggctgtctccttcccacgctgt ggcgaagatctgaactatgcccgtcgcctggcacgcgatgcaggatgtgatgcgaaaatt gttgccaaggttgaacgtgcggaagccgtttgcagccaggatgcaatggatgacatcatc ctcgcctctgacgtggtaatggttgcacgtggcgacctcggtgtggaaattggcgacccg gaactggtcggcattcagaaagcgttgatccgtcgtgcgcgtcagctaaaccgagcggta atcacggcgacccagatgatggagtcaatgattactaaccgatgccgacgcgtgcagaa gtcatggacgtagcaaacgccgttctggatggtactgacgctgtgatgctgtctgcagaa actgccgctgggcagtatccgtcagaaaccgttgcagccatggcgcgcgtttgcctgggt gcggaaaaaatcccgagcatcaacgtttctaaacaccgtctggacgttcagttcgacaat gtggaagaagctattgccatgtcagcaatgtacgcagctaaccacctgaaaggcgttacg gcgatcatcaccatgaccgaatcgggtcgtaccgcgctgatgacctcccgtatcagctct ggtctgccaatttccgccatgtcgcgccatgaacgtacgctgaacctgactgctctctat cgtggcgttacgccggtgcactttgatagcgctaatgacggcgtagcagctgccagcgaa gcggttaatctgctgcgcgataaaggttacttgatgtctggtgacctggtgattgtcacc cagggcgacgtgatgagtaccgtgggttctactaataccacgcgtattttaacggtagag taa

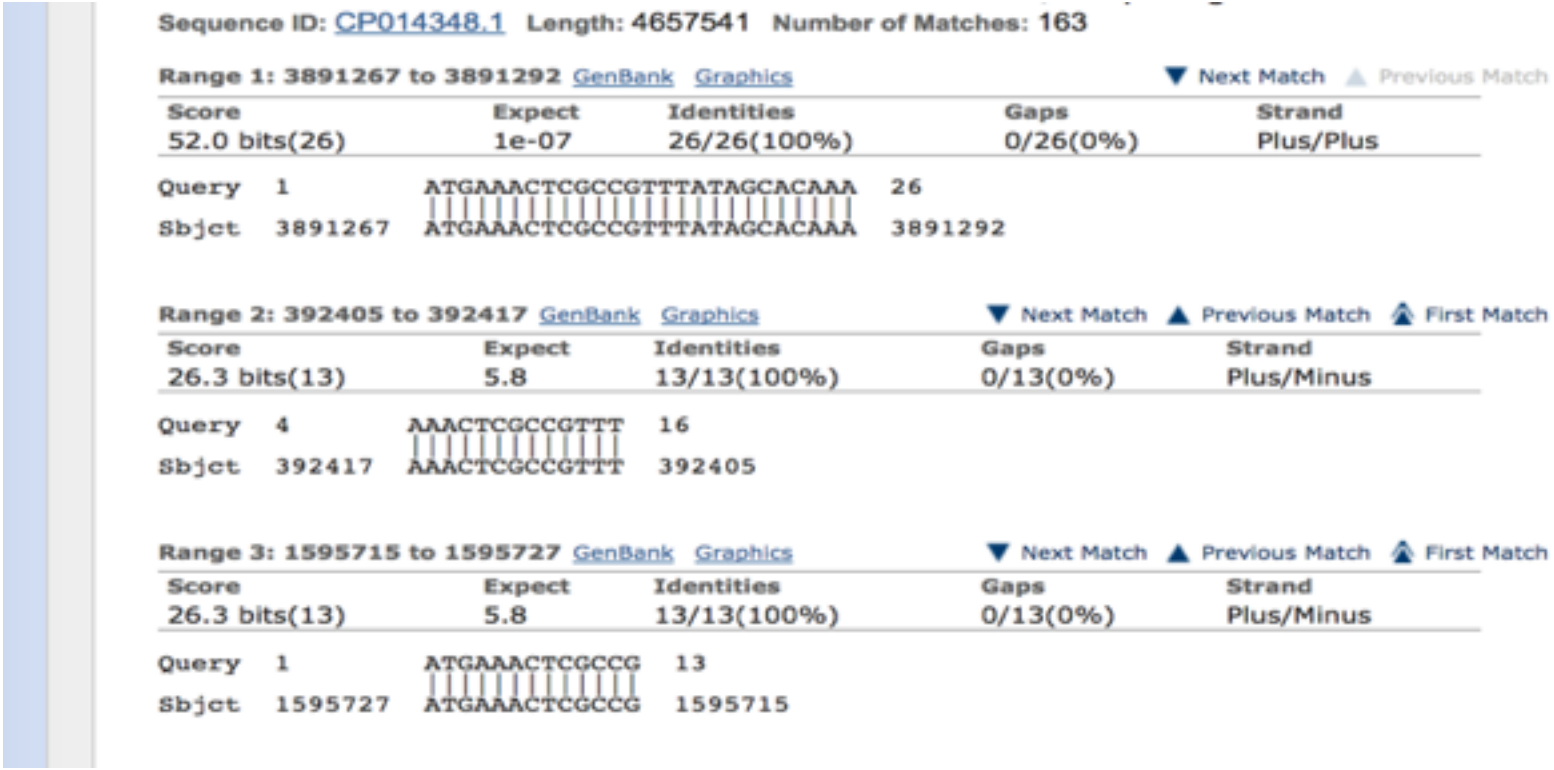
NT here means nucleotide sequence

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       AAACTCGCCGTTT  16
              |||||||||||||
Sbjct  392417  AAACTCGCCGTTT  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 (tomorrow)

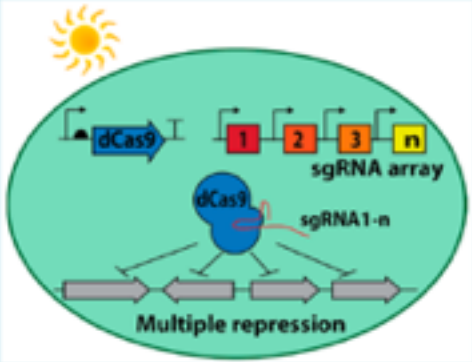
Multiple Gene Repression in Cyanobacteria Using CRISPRi

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 *Streptrococcus pyogenes* was used to repress green fluorescent protein (GFP) to negligible levels. CRISPRi was also used to repress formation of carbon storage compounds polyhydroxybutryate (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) ≤ 60 bp