# M2D2: Design gRNA for CRISPRi

10/10/19

- 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

#### Today Part 1: Separate DNA by gel electrophoresis Agarose gel electrophoresis

- Driving force:
- activic Jula
- Separates DNA by:



### Today Part 1: Visualize DNA + save a picture!

• DNA Loading dye (6X):

• Sybr-Safe DNA stain:

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.



Dr. Noreen Lyell's lecture M2D1

#### CRISPRi system can block the RNA polymerase



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



#### 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.

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





How do you use these principles to <u>Design the gRNA DNA sequence</u>



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: $5'GTCATG^{3'} \leftarrow \underline{n:n:Himplifte}$ (coding) strand Complementary strand: $\underline{CAGTAC}$ $\leftarrow$ Template (noncoding) strand

**Scenario 1 gRNA (DNA sequence) is the same as the nontemplate (NT) strand:** <sup>5'</sup>GTCATG<sup>3'</sup> The plasmid you generate after inserting the sequence would look something like pgRNA\_target\_1:



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



#### Practically: Using KEGG Database

Position	19376491939091					
	Genome map					
AA seq	480 aa AA seq DB search MSRRLRRTKIVTTLGPATDRDNNLEKVIAAGANVVRMNFSHGSPEDHKMRADKVREIAAK LGRHVAILGDLQGPKIRVSTFKEGKVFLNIGDKFLLDANLGKGEGDKEKVGIDYKGLPAD VVPGDILLLDDGRVQLKVLEVQGMKVFTEVTVGGPLSNNKGINKLGGGLSAEALTEKDKA DIKTAALIGVDYLAVSFPRCGEDLNYARRLARDAGCDAKIVAKVERAEAVCSQDAMDDII LASDVVMVARGDLGVEIGDPELVGIQKALIRRARQLNRAVITATQMMESMITNPMPTRAE VMDVANAVLDGTDAVMLSAETAAAGQYPSETVAAMARVCLGAEKIPSINVSKHRLDVQFDN VEEAIAMSAMYAANHLKGVTAIITMTESGRTALMTSRISSGLPIFAMSRHERTLNLTALY RGVTPVHFDSANDGVAAASEAVNLLRDKGYLMSGDLVIVTOGDVMSTVGSTNTTRILTVE					
NT seq A UevAid	1443 nt NT seq +upstream 0 nt +downstream 0 nt atgtccagaaggcttcgcagaacaaaatcgttaccacgttaggccagcaacagatcgc gataataatcttgaaaaagttatcgcggcggtgccaacgttgtacgtatgaactttct cacggctcgcctgaagatcacaaaatcgtggcgggtgccaacgttgtacgtatgaactttct cacggctcgcctgaagatcacaaaatggcgcgggtacaaagtcgtgagattgccgcaaa ctggggcgtcatgtggctattctgggtgacctccaggggcccaaaatccgtgtatccacc ttaaagaaggcaaagttttcctcaatattggggataaattcctgctcgacgccaacctg gtcgtgcctggtgacatcctgctgtggcgtagcgtcggtcaagtaaagtactggaa gttcagggcatgaaagtgttcaccgaagtcaccgtcggtggtcccctctccaacaataaa ggtatcaacaaacttggcggcgttgtgcggtgggtggtcgctgctgatgacgaag gacattaagactggcggtgatggcgctggcggatggcggatgaaggagacaaggaact gtacggcgcaggatcagggcgttggcgcgcgggtggggtgggaaattggcggaaat gtggcaagatctgaacgtgggagcgttgcggcggcggtggggaaattggaggaaatt gtggcaaggttgaacgtggggagcgttggcggcggcgggggggg					

#### 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: <u>CP014348.1</u> Length: 4657541 Number of Matches: 163

Range 1: 3891267 to 3891292 GenBank Graphics Vext Match 🔺 Previo						us 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	2: 392405	to 392417 GenBank	Graphics	🔻 Next Match	🔺 Previous Match	🛕 First Match
Score		Expect	Identities	Gaps	Strand	
26.3 bi	its(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				
Sbict	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 (next week)

#### 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 *Synechcocystis* 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.



## In lab 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

Note:

- Journal Club I is in two weeks, print your paper and start reading
  - Come to office hours and ask questions!
- Prof. Engelward office hours 8am-12pm tomorrow
- Instructors office hours 10am-4:30pm Saturday Oct. 12<sup>th</sup>
- Data summary draft due Monday Oct. 14<sup>th</sup> at 10pm
- Blog post due Tuesday Oct. 15<sup>th</sup> at 10pm

# Fa19 20.109 Blog Post

- You will receive an email invitation to join the class blog
- Suggested topics listed on the blog
- For full credit, you only need to complete 3 of the 4 posts. Late posts will <u>not</u> be accepted for credit
- Details about use:
  - Do not publish MIT logo
  - Do not post photographs with names tagged
  - Do not write malicious comments
  - Do not plagiarize





