Important notes:

- Submission information for major assignments

Logistics [edit]

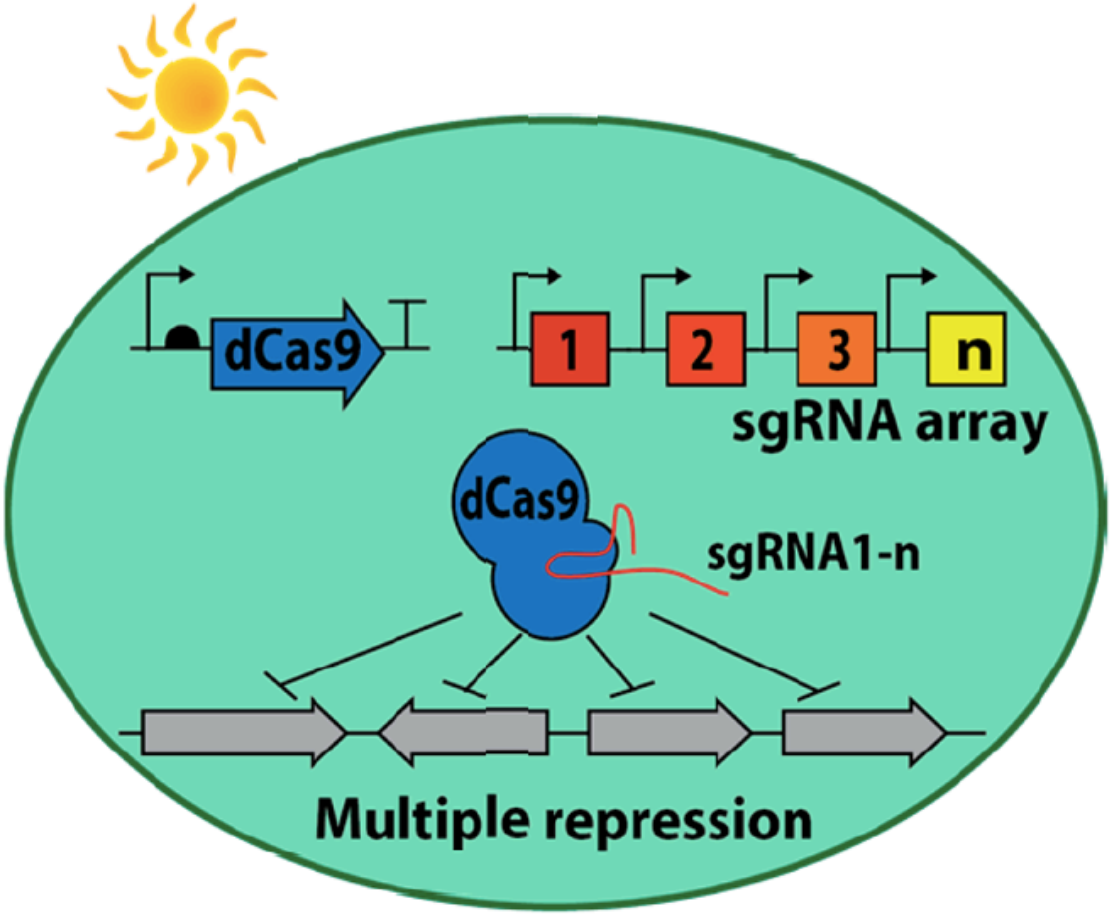
You will complete this assignment individually.

As you prepare your assignment be sure to review the resources provided on the Communication tab.

Please submit your completed Mini-presentation **due by Saturday, October 13 at 10 pm** to bioeng20.109@gmail.com, with filename **Name_LabSection_MP.extension** (for example, NoreenLyell_TR_MP.mov).

- Include your name(s) on ALL assignments

What is the research question?



Section	Minutes	Number of slides	DO	DON'T
Introduction	~2	2-3	• Introduce the key concepts that the audience will need to follow your presentation. • Briefly state the overall scope and significance of the study -- what is the central question and why is it interesting? • Try to summarize background material with a model slide rather than lines of text. If text is needed, bring in the details as you speak using PowerPoint animation.	• Don't assume you are addressing an expert audience. • Don't give more information than is absolutely needed to understand the rest of your talk. • Don't put too much information on each slide.
Data	~7	4-6	• Present the data in a logical sequence, letting each slide build upon the previous ones. • Include a title for each slide. The title should be the conclusion and should be unique to the information on the slide. • Make every element of your slide visible to the entire room. This means 20-point font or greater. • Interpret each slide thoroughly and carefully. • Point out strengths and weaknesses of the data along the way.	• Don't read your talk. Similarly, do not read lists from slides. • Don't put much information on each slide. Each slide should make only one point. • Never say, "I know you can't read this, but...". Everything on each slide should be legible. • Don't be afraid to remind the audience how the data fits into the overall question
Summary	~1	1	• Review each of your main messages. • Clearly state what the study contributed to the field.	• Don't repeat experimental details.
Question & Answer	?	0	• Answer the question being asked. If you are unclear about the question, ask for clarification. • Respect every question and questioner.	• Don't take too long with one question. If the discussion is involved, suggest meeting after the talk to discuss it more.

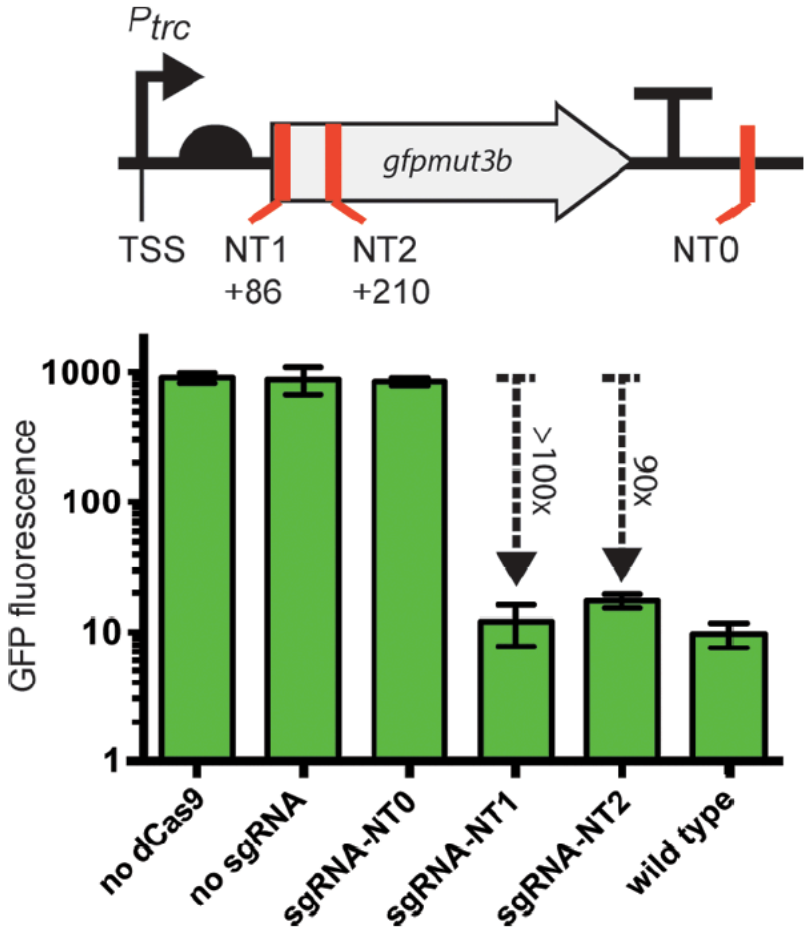
Multiple Gene Repression in Cyanobacteria Using CRISPRi

Lun Yao, Ivana Cengic, Josefine Anfeldt, and Elton P. Hudson

Presented by Fa18 20.109

ACS Synthetic Biology. 2016. 5:207-212

Successful targeted GFP repression in *gfpmut3b* gene



- Top: Gene map depicting sgRNA protospacer on target gene
- Bottom: Corresponding GFP fluorescence upon insertion of sgRNA protospacer
- sgRNA-NT1 and sgRNA-NT2 = significant GFP fluorescence decrease
- No sgRNA and sgRNA-NT0 = no GFP repression
- WT has weak auto fluorescence
- Found target site to repress GFP gene
- Next: Test to identify best promoter to optimize gene repression

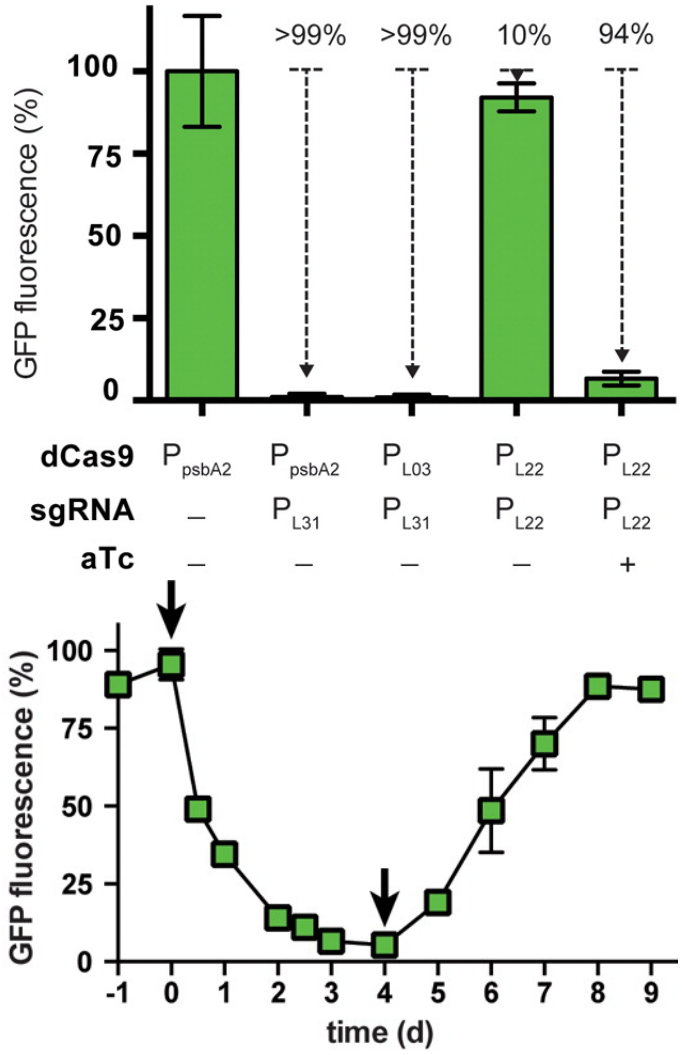
GFP Repression is Reversible With CRISPRi

dCas9, sgRNA-NT1 repression of GFP

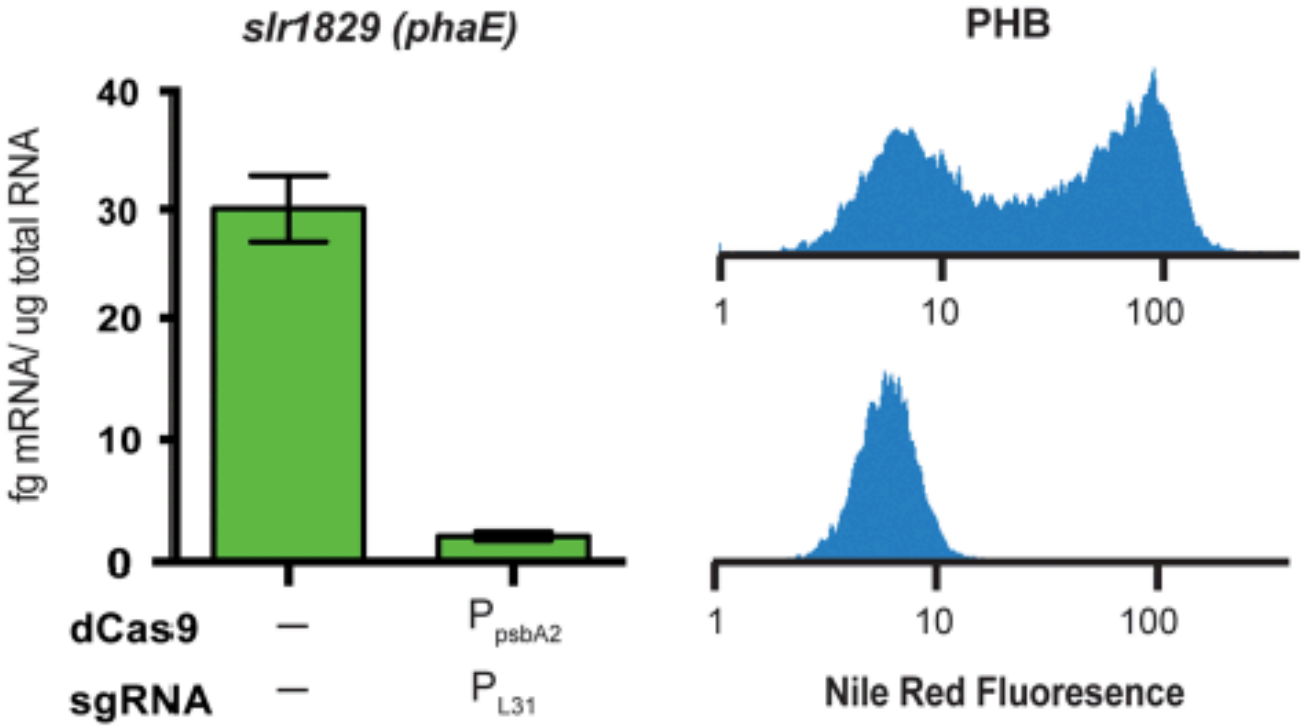
Little repression with TetR-P_{L22}

Induced repression with added aTc

aTc removal restores GFP expression

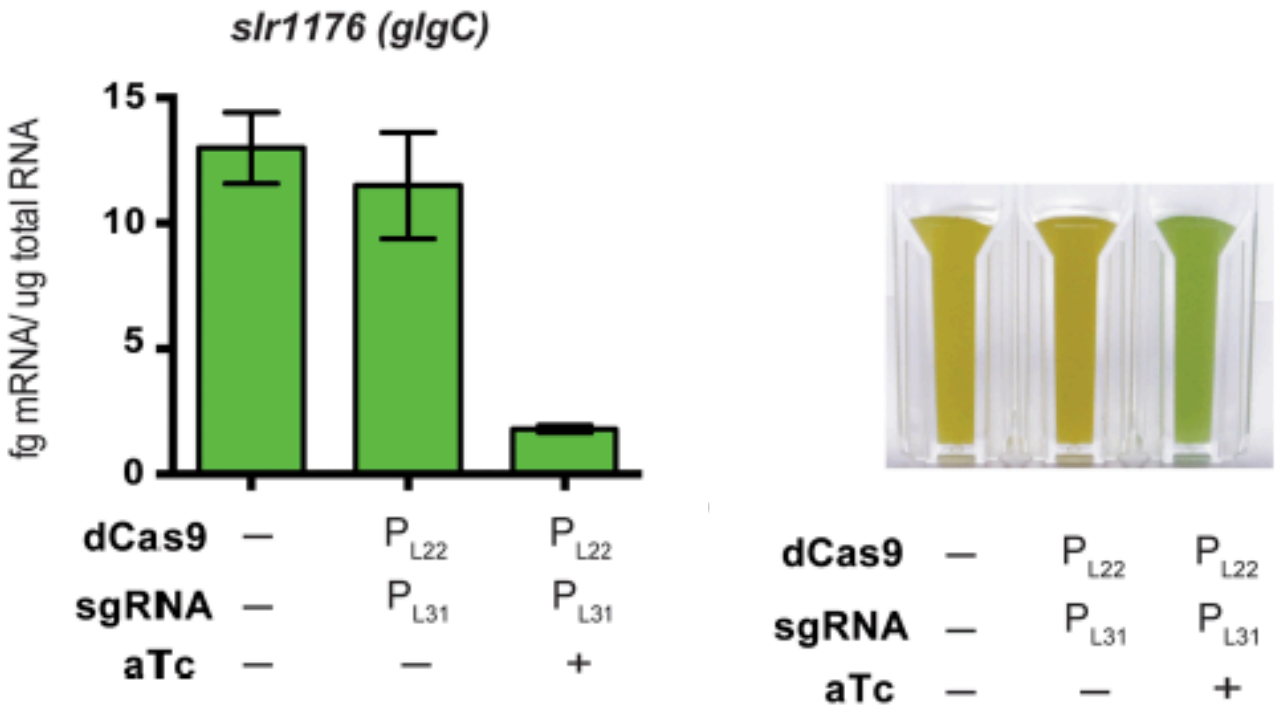


Enzymes involved in glycogen synthesis knocked down by CRISPRi

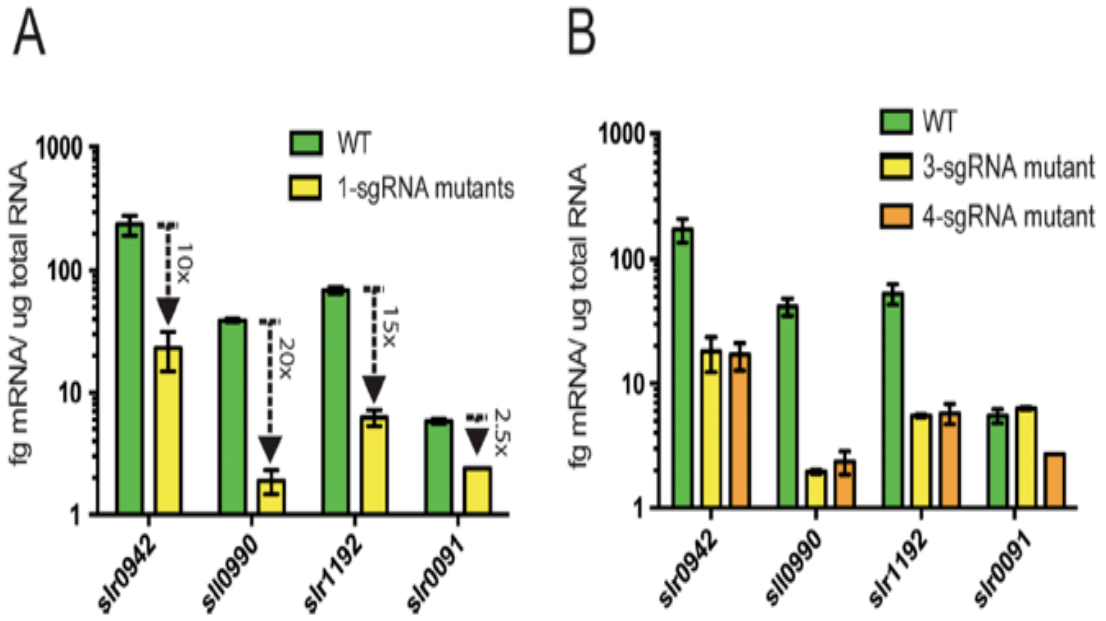


- CRISPRi survives nitrogen starvation.
- Mutant strain showed 10x repression of phaE.
- PHB biosynthesis was not detected in mutant strain.

glgC knockdown achieved through CRISPRi construct



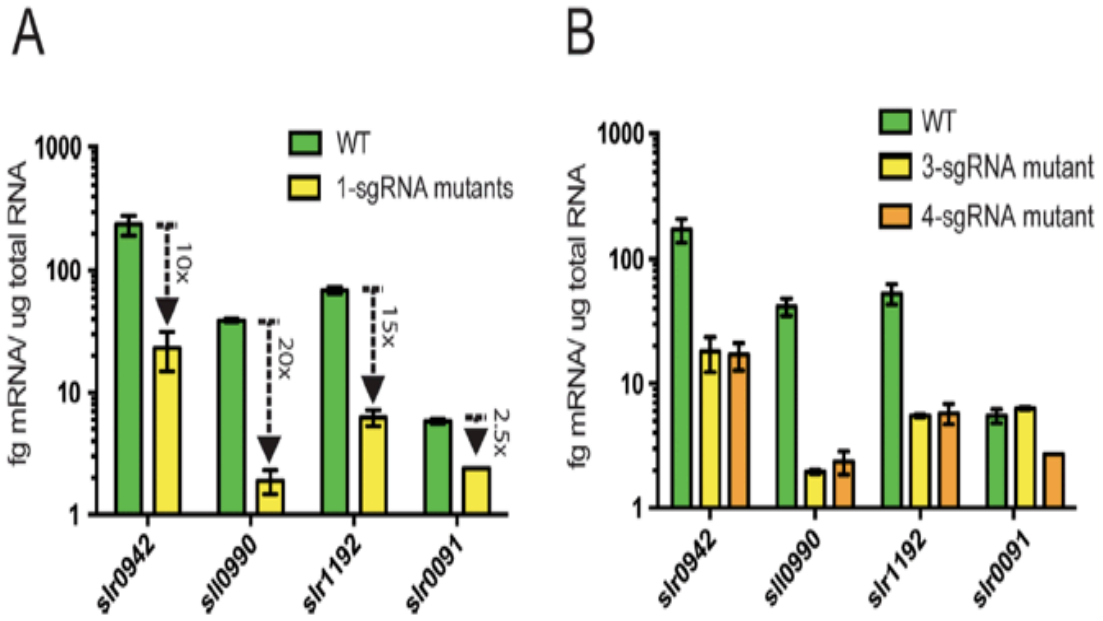
- Induction with aTc repressed glgC transcript levels to 10% after 24 hours.
- After two days of nitrogen starvation, mutated line accumulated 25% of glycogen that wild type accumulated.
- Non-bleached phenotype is typical of glycogen deficient strains.
- Glycogen quantification showed 80% less glycogen when glgC was knocked down.



CRISPi enables multiplex repression abilities

A. Single knockdown, capability of repression for one knockdown (baseline)

- All knockdowns experienced ≥ 10x repression except for slr0091 (2.5x), which may be due to a distant TSS (blocking of transcription elongation inefficient)



B. Simultaneous knockdowns (multiplexing)

- Multiplexing 3 and 4 are as efficient as single-gene knockdown in repression of genes
- 3-sgRNA did not knock down slr0091, so it makes sense that levels of expression are comparable to WT

Take Away: Multiplexing is successful although multiple sgRNAs targeting the gene of interest may be necessary to achieve full knockdown