M2D7: Examine features in gRNA-targeted genomic sequences

QUIZ → Thursday. Sorry!

- Prelab discussion
- Perform computational analysis of MG1655 regulatory elements potentially affected by gRNA binding
- Work on research article with extra time

T/R OH - go to offices instead: T = Becky 16-317
R = Nireet 16-319
either = Jamie 16-469

Mod2 Overview

Research goal: Increase the yield of commercially valuable byproducts in *E.coli* using CRIPSRi technology to target genes involved in mixed-acid fermentation pathway.

Last Lab:

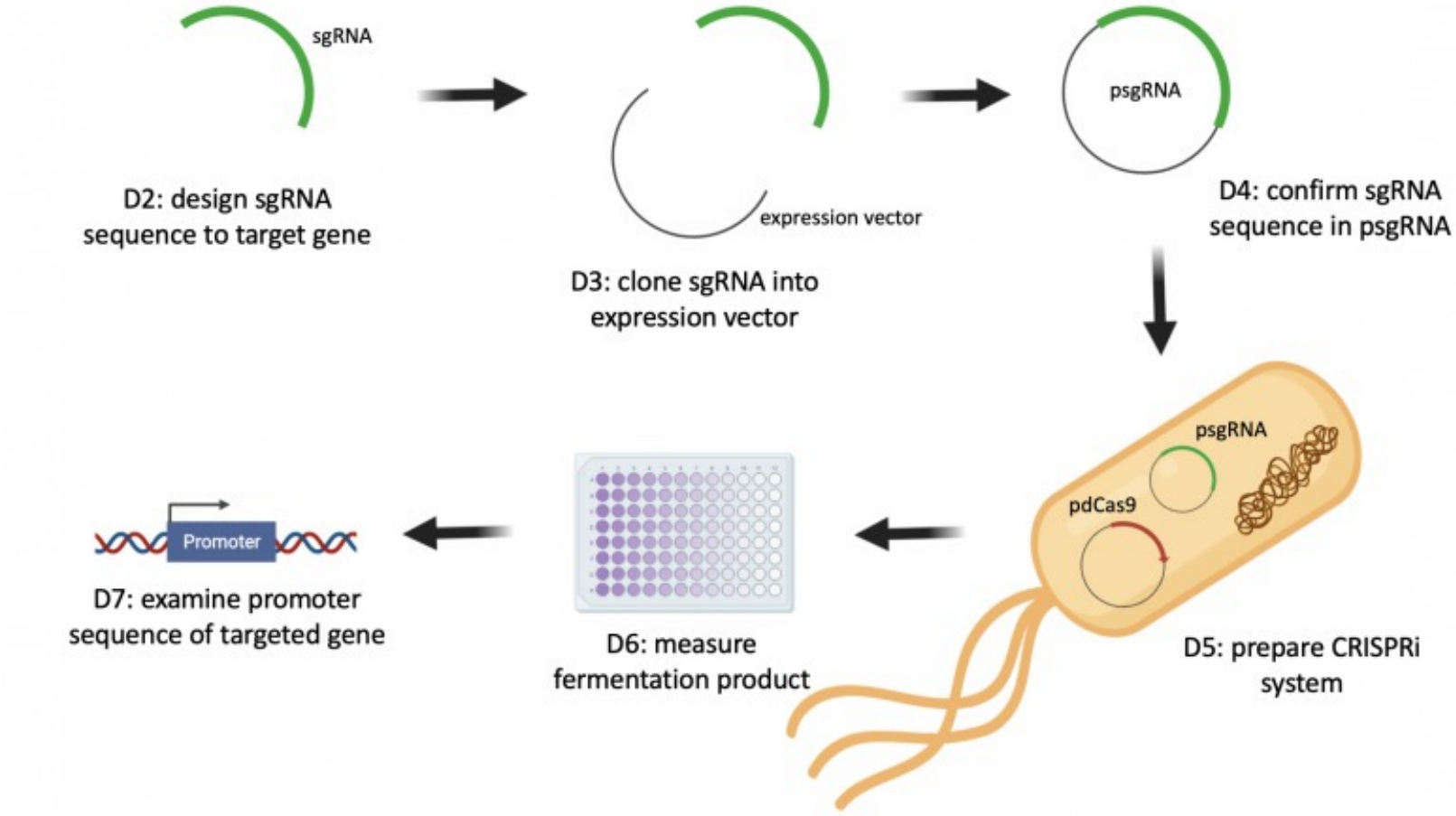
Measure bacteria O.D. and fermentation products

This Lab:

Examine DNA regulatory elements that may impact the efficacy of your CRISPRi system

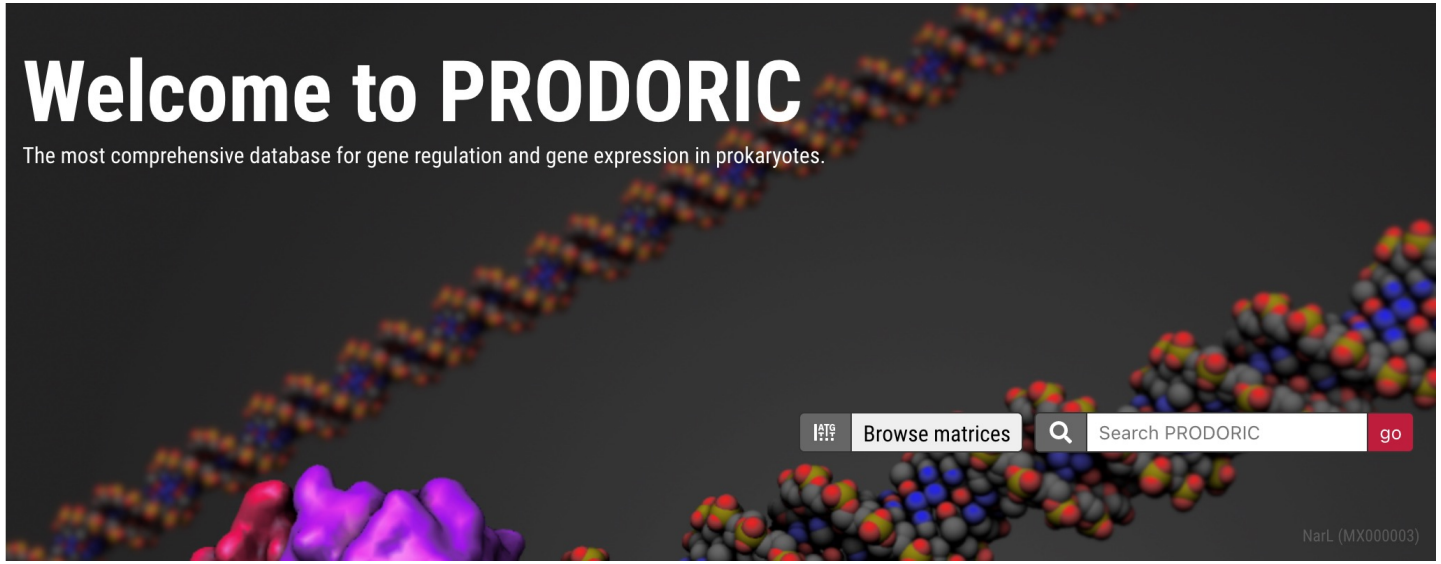
Next Lab:

Organize figures and outline text for the research article



Determine the potential for your gRNA to inhibit transcription factor binding

- Even if you did not specifically target a regulatory element of the gene
- Use the online computational tool Virtual Footprint in the PRODORIC database to examine the potential interference of sgRNA to the binding of relevant transcription factors
- Examine the conserved motifs that can promote transcription factor binding



Sequence logo - graphical rep. of consensus sequence

Position Weight Matrix

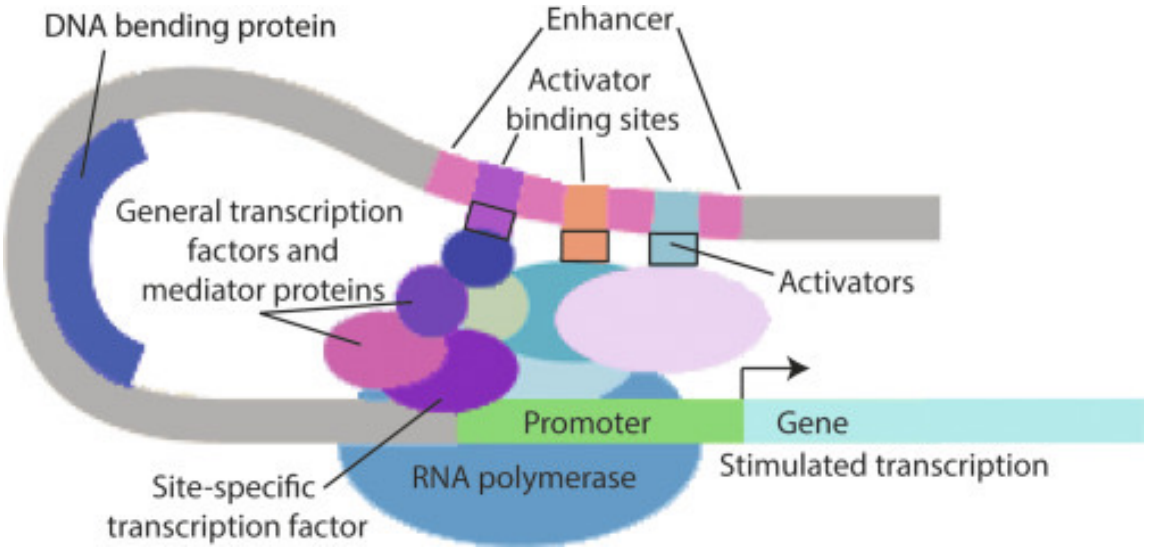
PWM Score

- Bioinformatics approach used to predict transcription factor binding based on consensus sequences and probability
  - Predicts sequence motifs in DNA that will interact with protein TFs

- One way to determine a PWM is to align known TFBS and count the frequency at which each base occurs in each position
  - Use the base frequency table you generate here to create a matrix of the log probability of each base frequency at each position
  - Can then use this PWM to calculate the probability of each base occurring at each position in the "true binding sites" based on what is seen in the known sites
  - Calculate a "score" for any particular sequence by taking the sum of the weights in the sequence

Why would you care about transcription factor binding if you didn't target a TFBS?

- We have been focused on using our CRISPRi system to block RNAP
- Other regulatory elements, including transcription factors, can be affected by the binding of your CRISPRi complex
- Your project involved engineering a gene regulation complex
  - When considering the efficacy of your designed system, you also need to consider any potential unexpected consequences



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For Today

- Complete the wiki exercises on regulatory elements
- With extra time, work on:
  - Data analysis
  - Research article outline
  - Homework for M2D8

For M2D8…

- Outline the discussion section for the Research Article

Discussion section

→ Implications & Future Works + discussion of experiments

- Start with 'here we showed…'
  - Restate major results and broad implications
  - Follow same order as in Figures/Results
- Describe your conclusions from your data
  - If necessary, describe caveats of experiment and suggest improvements
- Identify unknowns and speculate (within reason)
  - Don't make huge generalizations or overreach
- Propose future experiments, identify new questions that arise
- Come back to the big picture / impact statement topic introduced in background