M2D5: Prepare for induction of CRISPRi system

1. Pre-lab discussion

2. Examine sequencing data

3. Prepare media conditions

4. Innoculate starter culture

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:

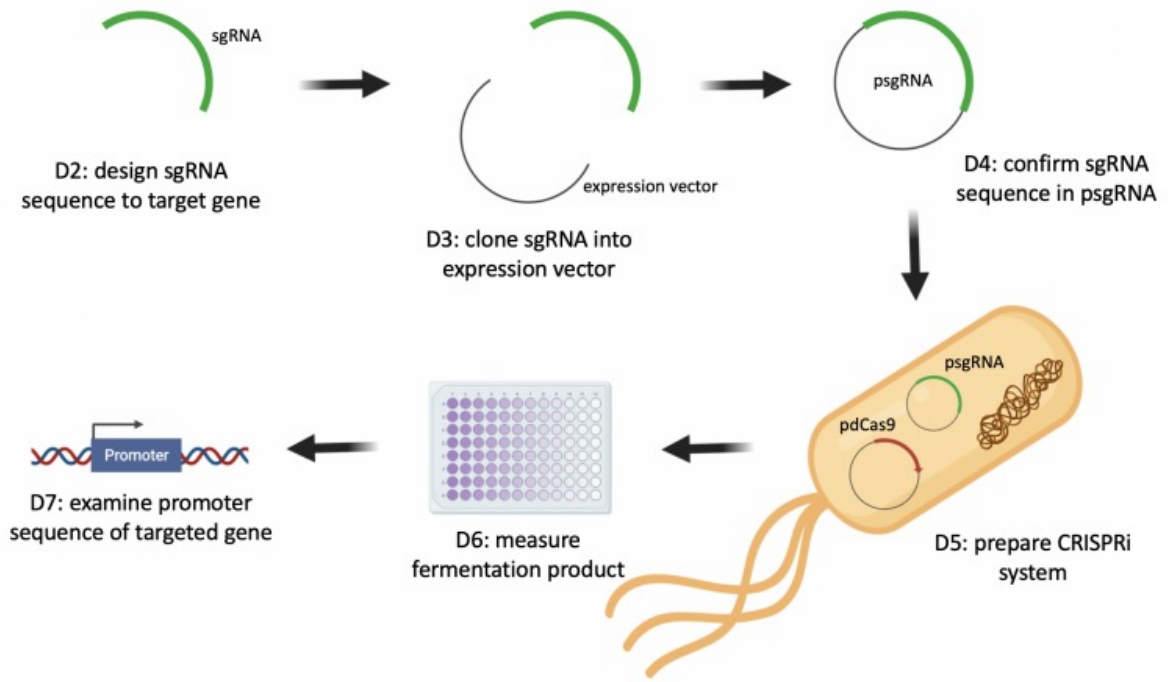
Clone sgRNA into vector to create plasmid that targets gene of interest

This Lab:

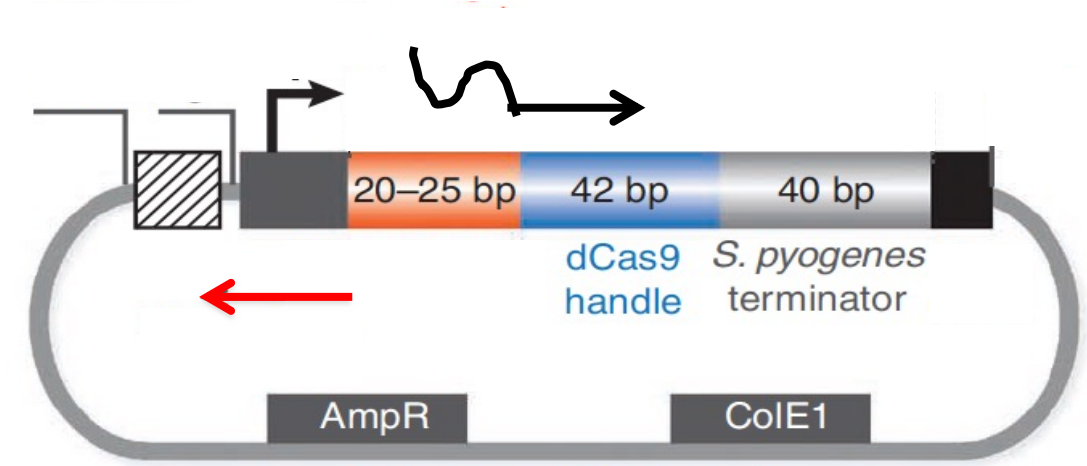
Confirm correct sgRNA cloning and do preliminary CRISPRi system preparations

Next Lab:

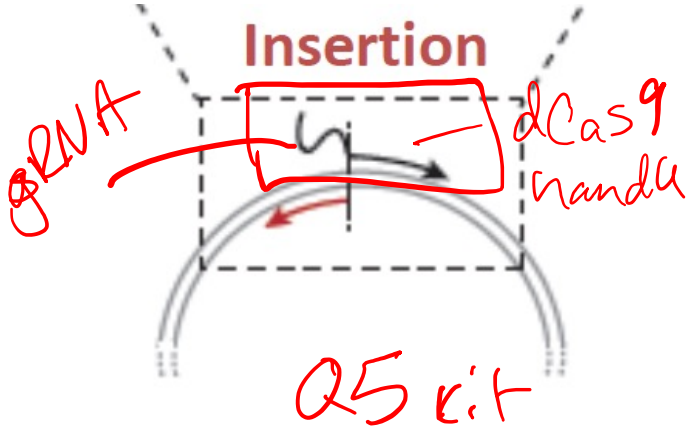
Measure fermentation products



M2D3: Generated pgRNA_target by SDM



pgRNA_template



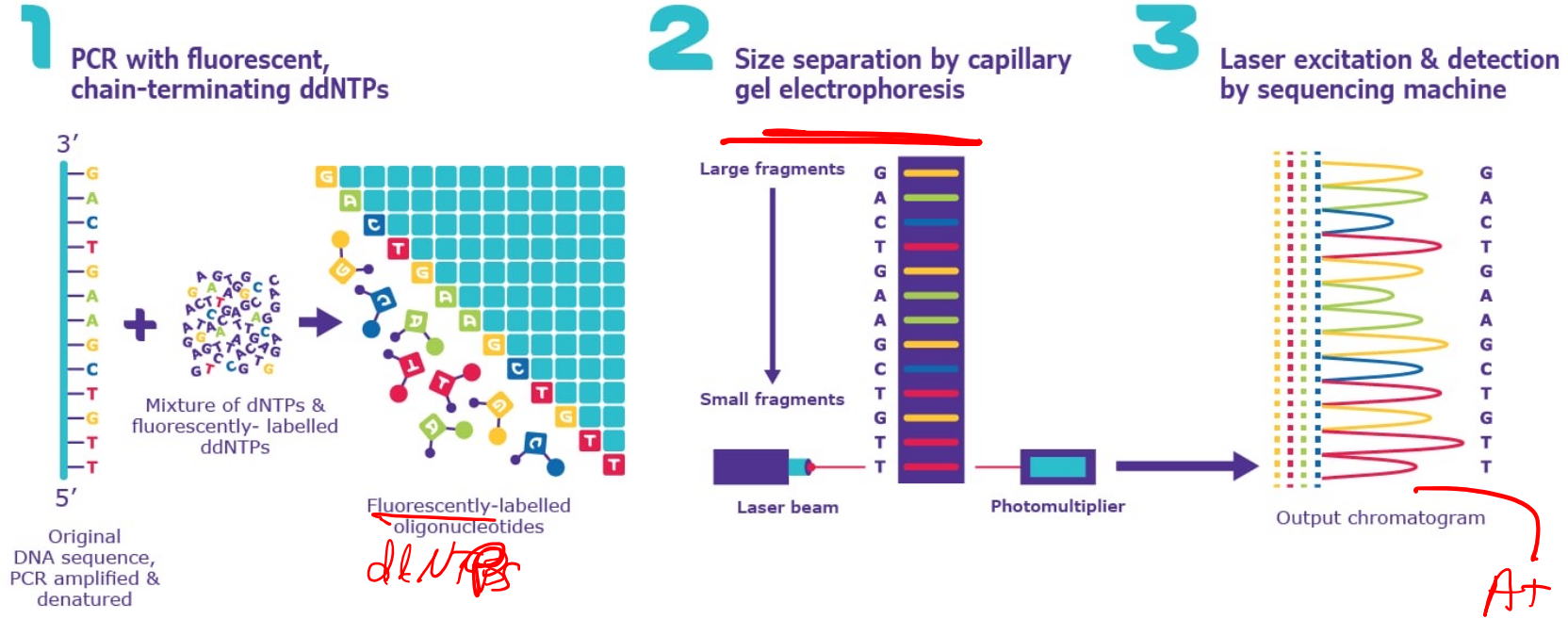
insertion (NEB5α kit)

CRISPRi universal *amplification* reverse primer

forward primer including crRNA to be inserted ( )

dCas9 handle ( )

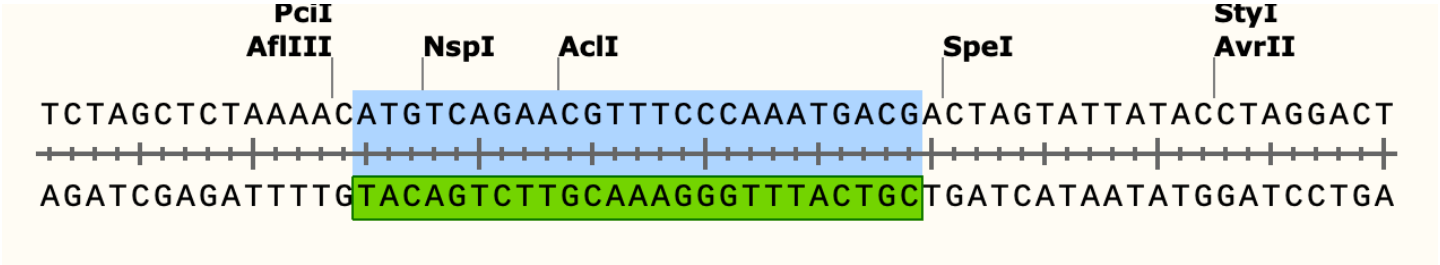
Sanger Sequencing review



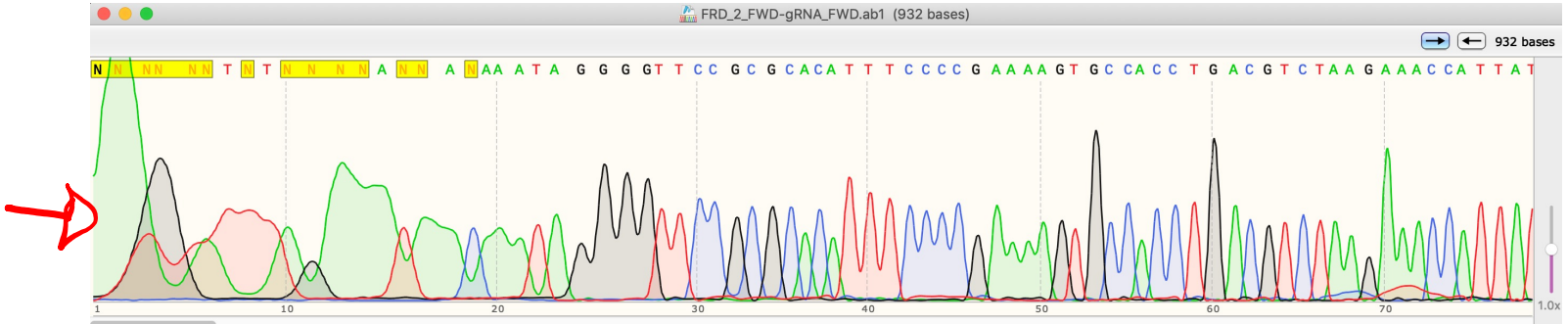
Sigma Aldrich

Analyzing Sequence Information

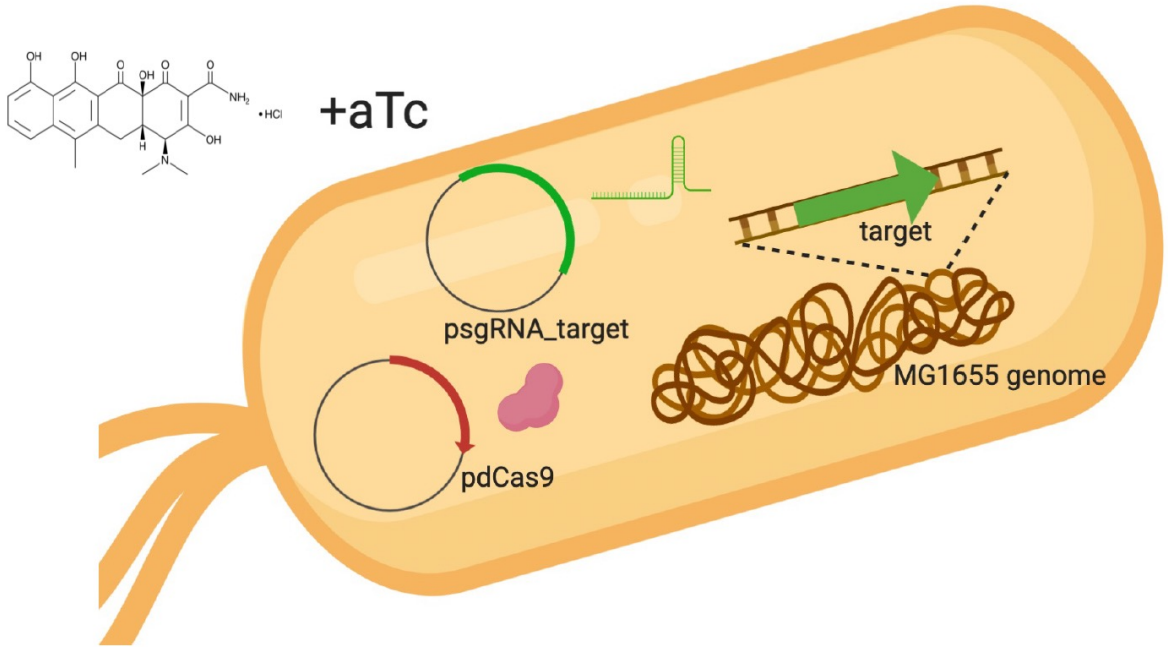
- Was your target sequence successfully incorporated into the pgRNA_target plasmid?
  - Open the Seq file in Snapgene and search for your gRNA sequence



- Sanger sequencing traces are also on Dropbox (ab1 files)



CRISPRi blocks gene expression in presence of inducer



- Expressed constitutively:

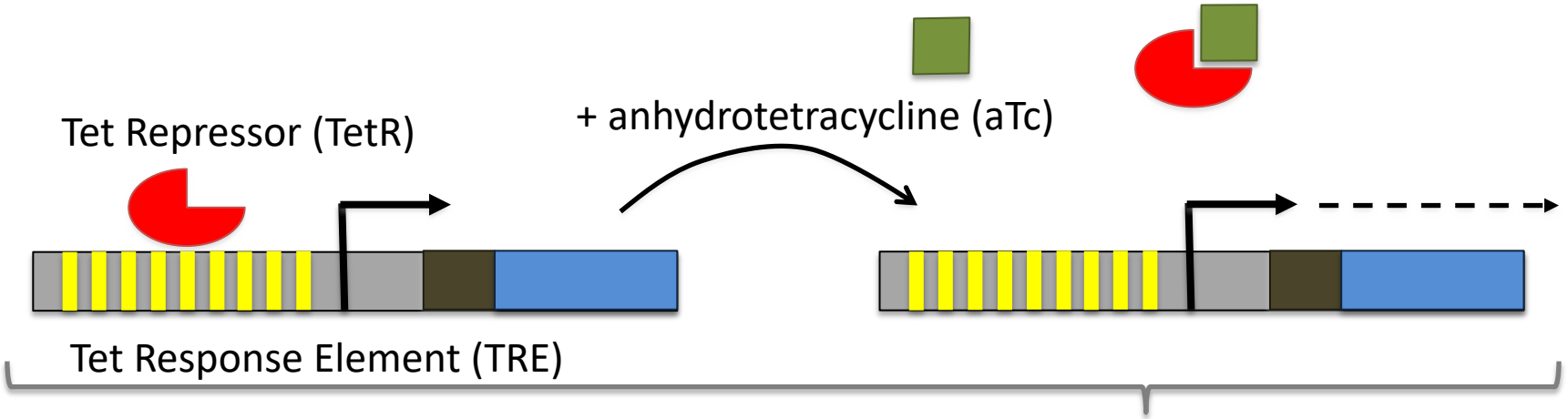
  sgRNA

- Expression induced with aTc:

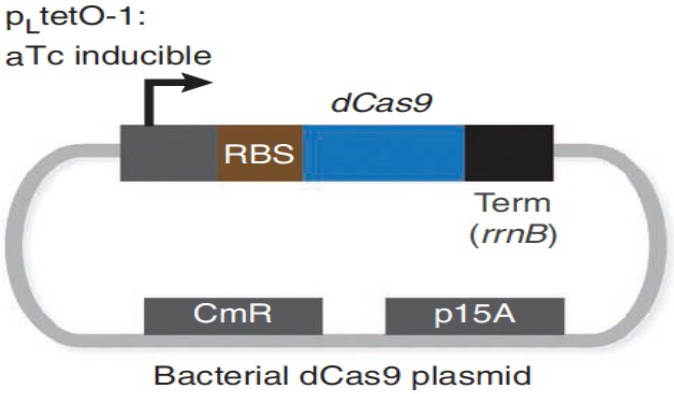
  pdCas9

dCas9 protein associates with gRNA/target gene to repress target gene expression

aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene



Set up culture for mixed-acid fermentation and pdCas9 induction

What components do we need to include for each condition?

- MG1655
- MG1655 + CRISPR

LB media

glass / conical tubes

+/- aTC

-/+ CRISPR

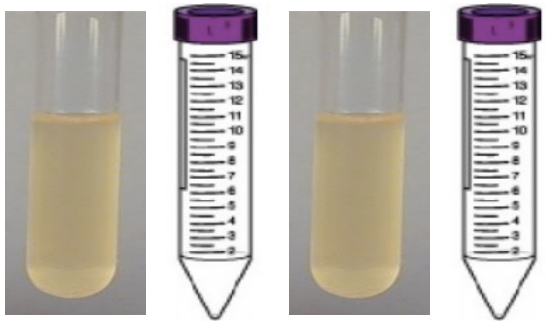
AMP / CM

pSGRNA

pdCas9

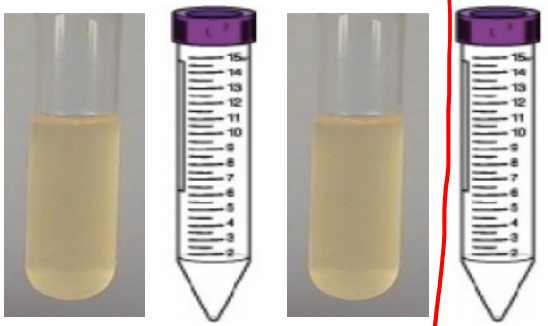
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- Where do we expect most ethanol/acetate if hypothesis confirmed?



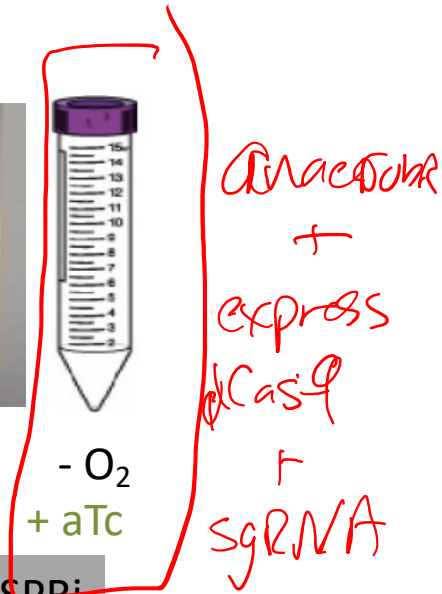
+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655



+ O_2 - aTc | - O_2 - aTc | + O_2 + aTc | - O_2 + aTc

MG1655 with CRISPRi



For today

1. Examine sequencing data
2. Set up media conditions for inoculation
3. Innoculate starter culture of bacteria for experiments

For M2D6…

1. Write a methods section for M2D3-M2D5