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:

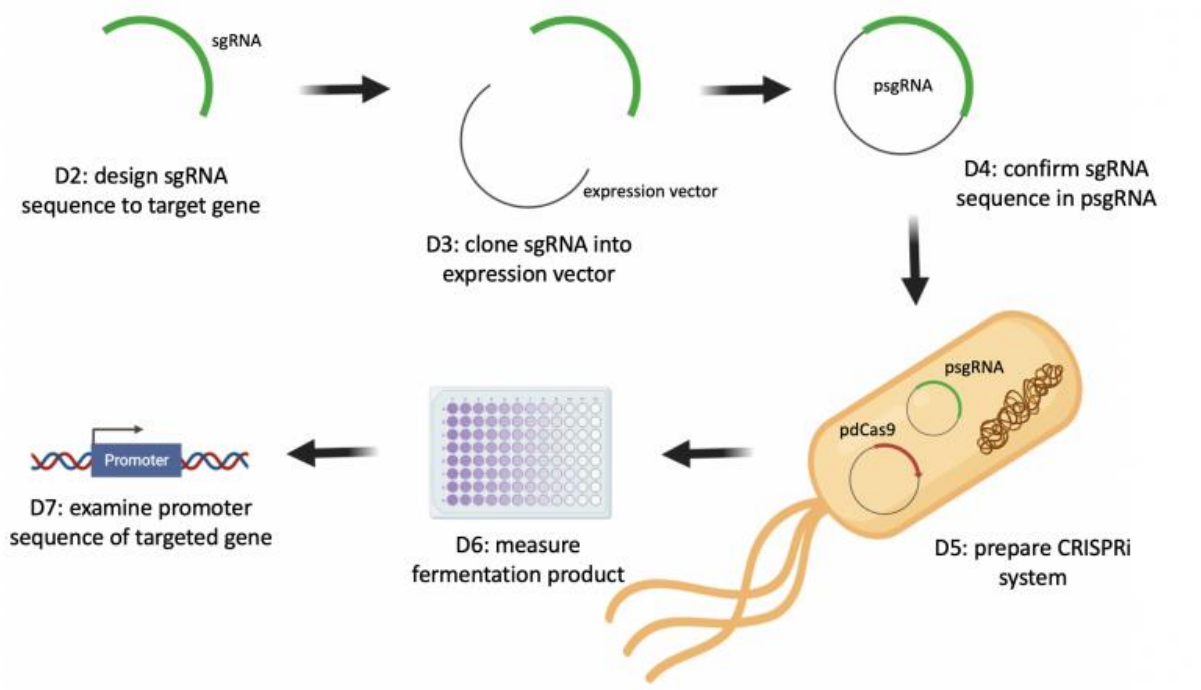
Clone sgRNA into vector to create plasmid that targets gene of interest

This Lab:

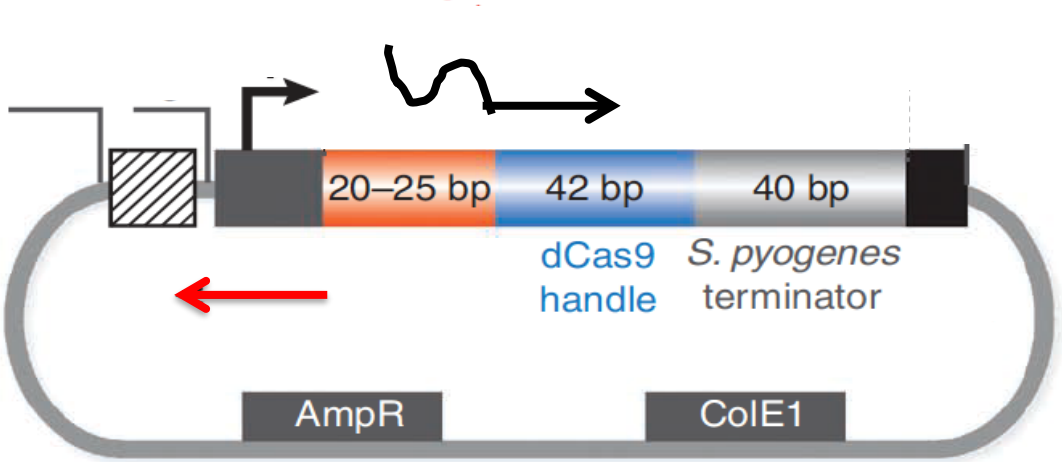
Confirm correct sgRNA cloning and do preliminary CRISPRi system preparations

Next Lab:

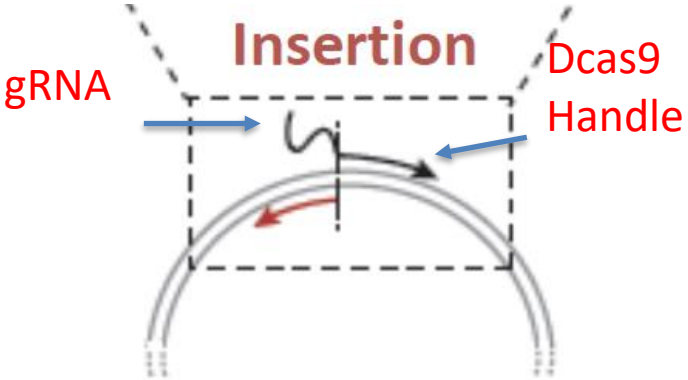
Measure fermentation products



M2D3: Generated pgRNA_target by SDM



pgRNA_template



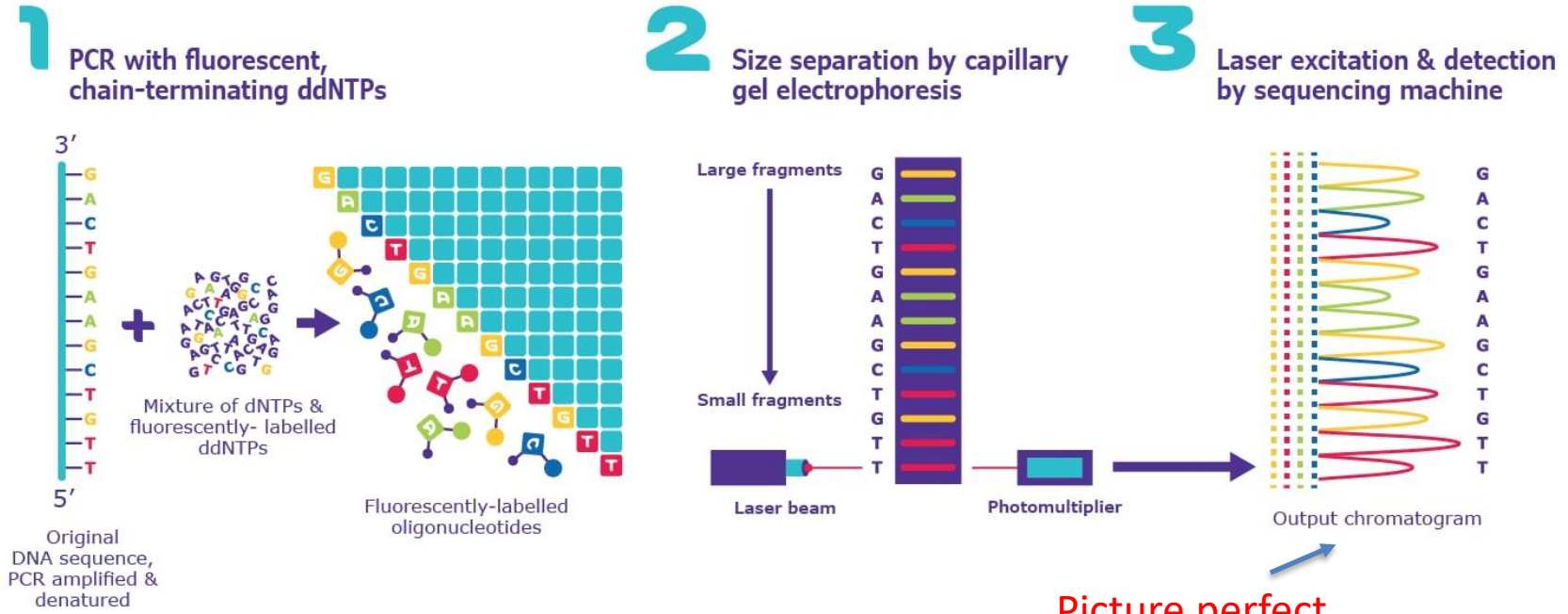
insertion (NEB5α kit)

CRISPRi universal *amplification* reverse primer

forward primer including crRNA to be inserted ( )

dCas9 handle ( )

Sanger Sequencing review

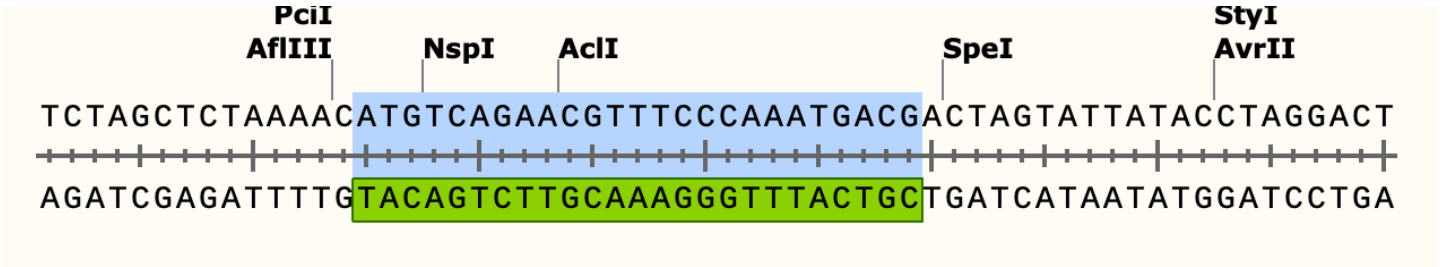


Picture perfect chromatogram

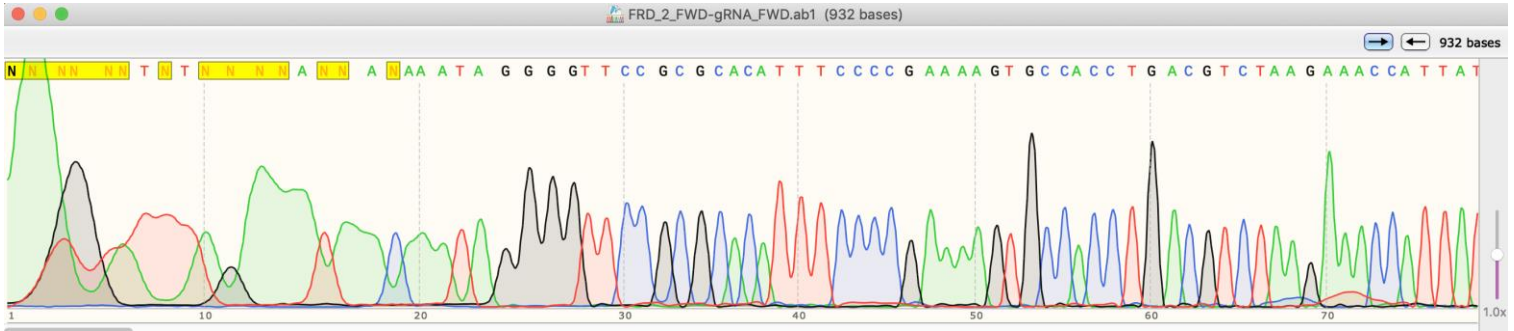
Sigma Aldrich

Analyzing Sequence Information

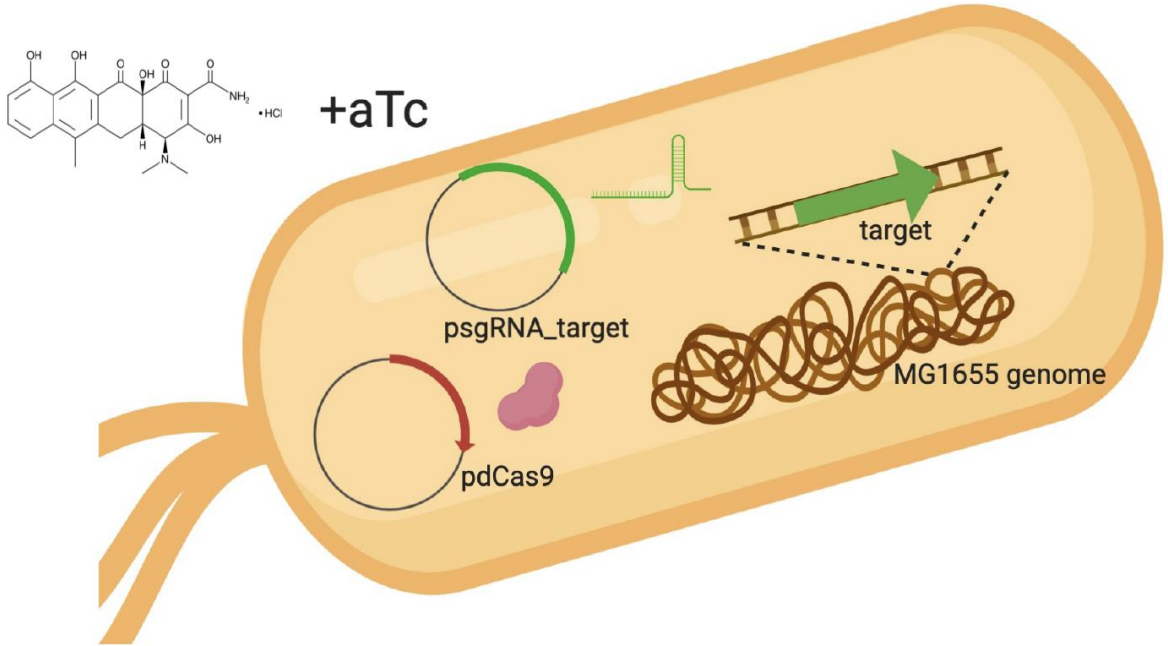
- Was your target sequence successfully incorporated into the pgRNA_target plasmid?
  - Open the Seq file in Snapgene and search for your gRNA sequence



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



CRISPRi blocks gene expression in presence of inducer



- Expressed constitutively:

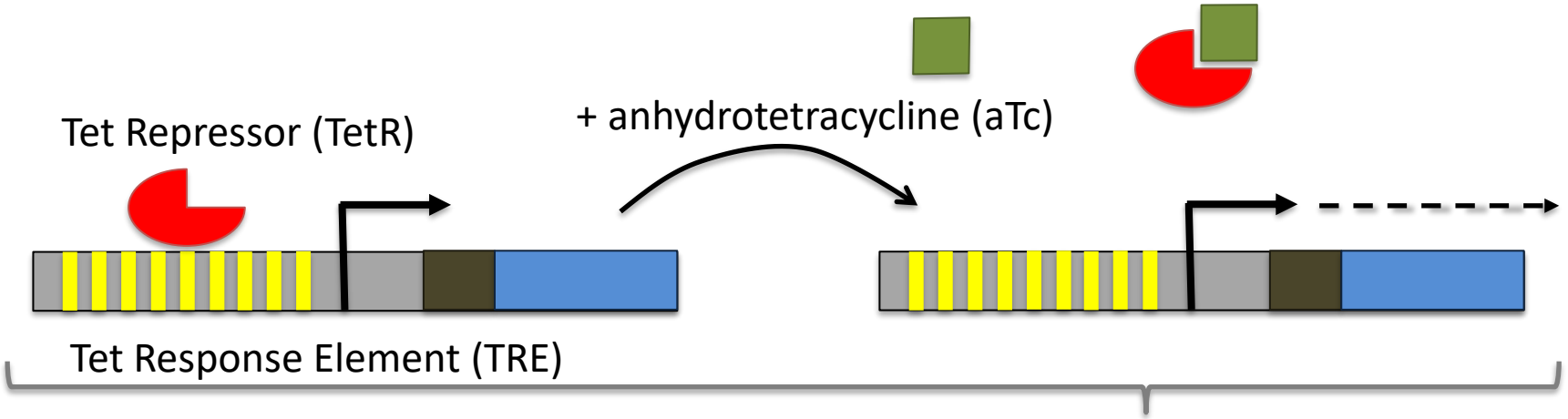
  sgRNA

- Expression induced with aTc:

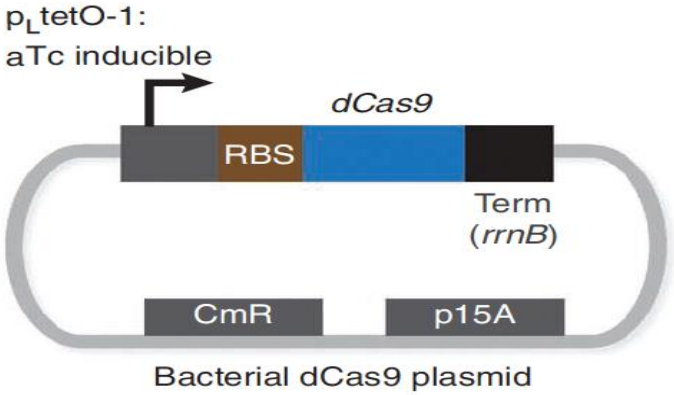
  dcas9

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

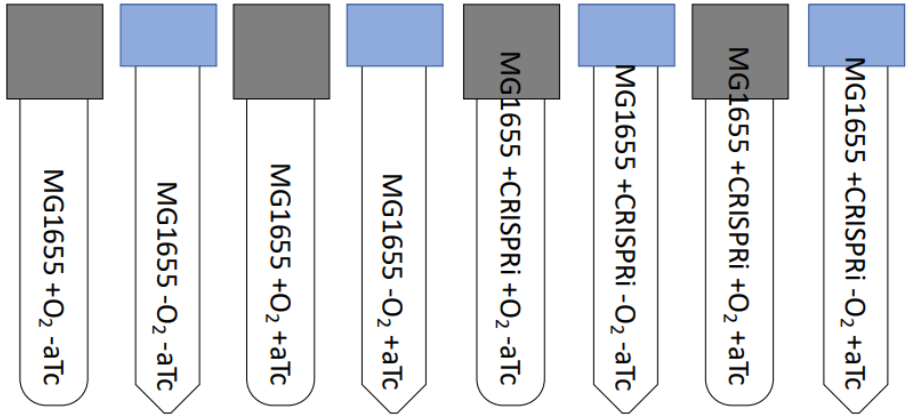
aTc induction of pdCas9



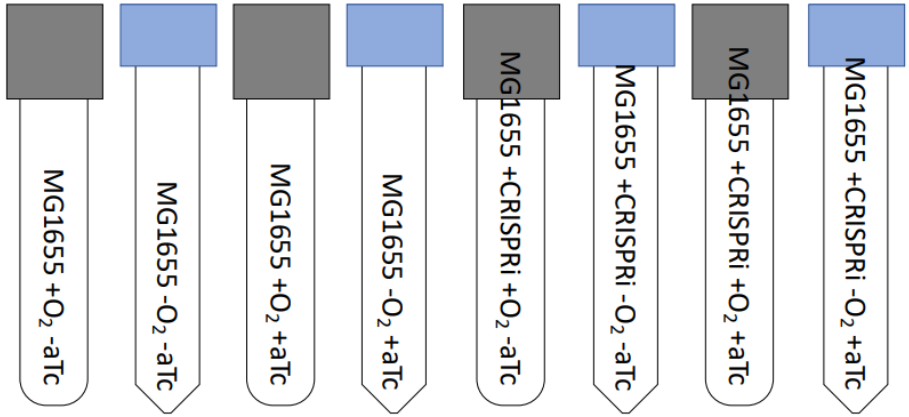
- Tet promoter regulates expression of dCas9 gene



Experimental question/ hypothesis?



Condition	Control Question / Purpose
Glass Tube vs Screw cap	
MG1655 vs MG1655+CRISPRi	
Amp+CM vs No antibiotic	
+aTc vs -aTc	



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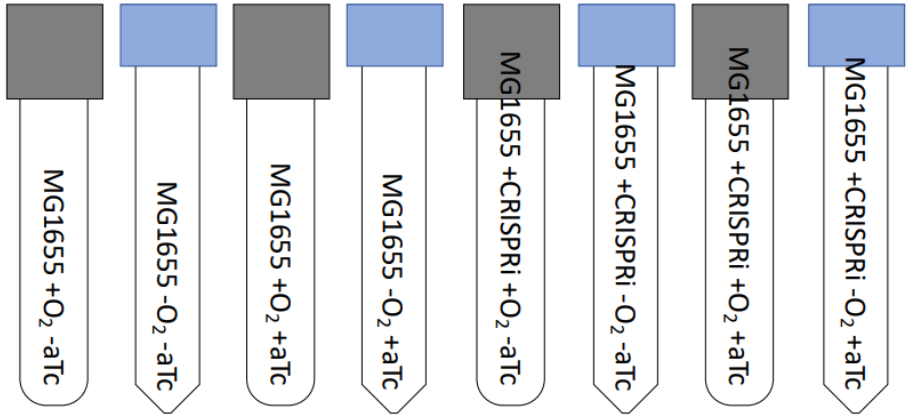
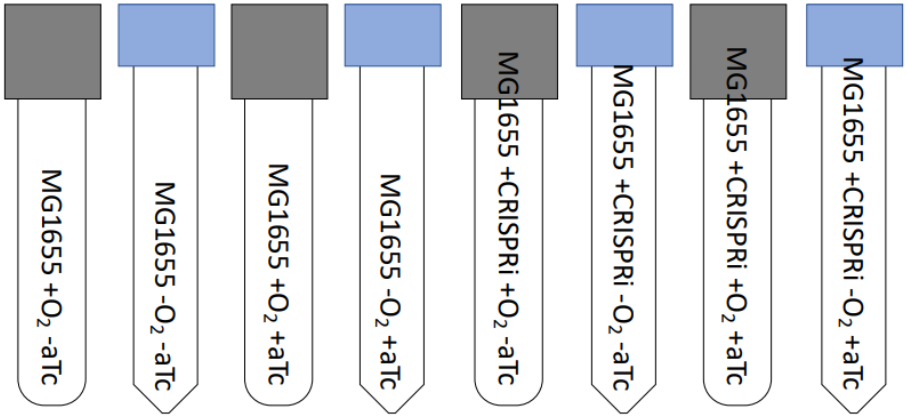
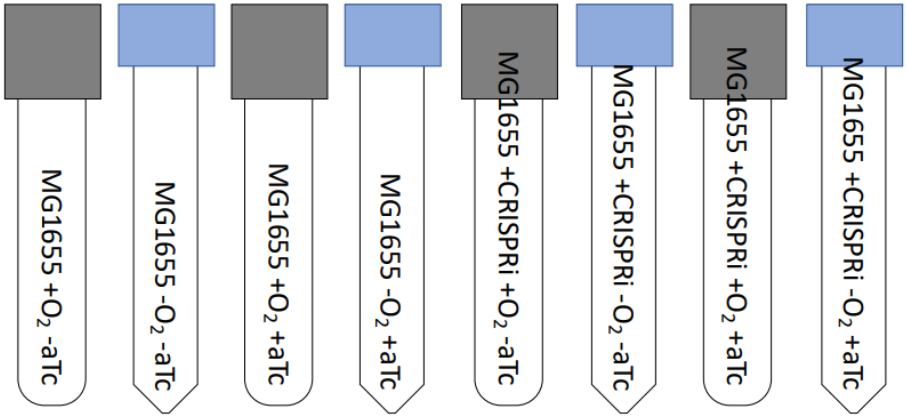


Condition	Control Question / Purpose
Glass Tube vs Screw cap	Can we activate mixed fermentation?
MG1655 vs MG1655+CRISPRi	
Amp+CM vs No antibiotic	
+aTc vs -aTc	



Condition	Control Question / Purpose
Glass Tube vs Screw cap	Can we activate mixed fermentation?
MG1655 vs MG1655+CRISPRi	Does the presence of our constructs help?
Amp+CM vs No antibiotic	
+aTc vs -aTc	



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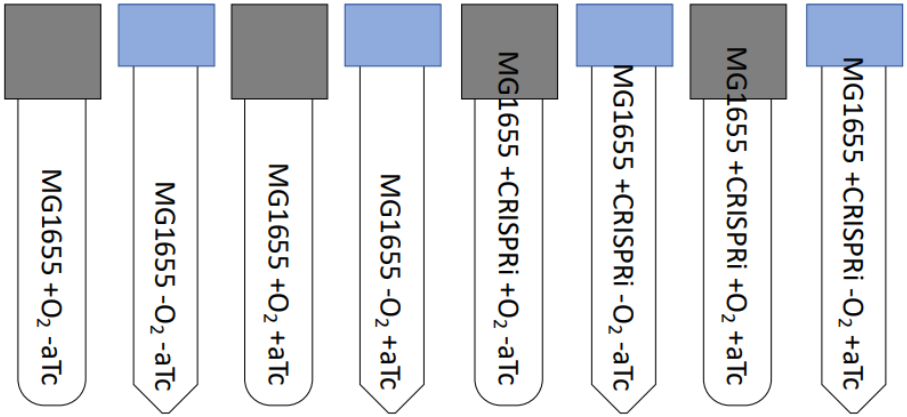
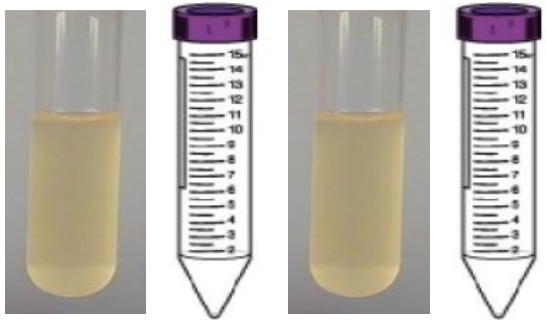
Glass Tube vs Screw cap	Can we activate mixed fermentation?
MG1655 vs MG1655+CRISPRi	Does the presence of our constructs help?
Amp+CM vs No antibiotic	MG1655 & MG1655+CRISPRi need different media to thrive
+aTc vs -aTc	



Condition	Control Question / Purpose
Glass Tube vs Screw cap	Can we activate mixed fermentation?
MG1655 vs MG1655+CRISPRi	Does the presence of our constructs help?
Amp+CM vs No antibiotic	MG1655 & MG1655+CRISPRi need different media to thrive
+aTc vs -aTc	Does the *activation* of our system help? Is aTc *sufficient* to help?

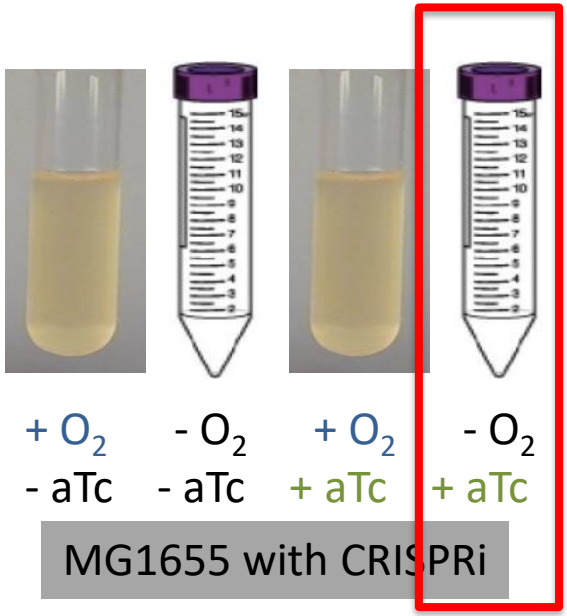
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- 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

1) Anaerobic
2) +aTC (inducing pdcas9)
3) Cons expressing sgRNA

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