M2D7: Induce CRISPRi system

11/3/17

1. BE Communication workshop
2. Pre-lab
3. Analyze sequencing results
4. Prep for dCas9 induction and mixed-acid fermentation

Major assignments for M2

- **Research Article**
  - Due by 10pm on Mon., November 20th

Research Article content

1. Title
2. Abstract
3. Introduction
4. Materials and Methods
5. Figures and Results
6. Discussion
7. References

- **Blog post for M2** due by 10pm on Tues., Nov. 21st

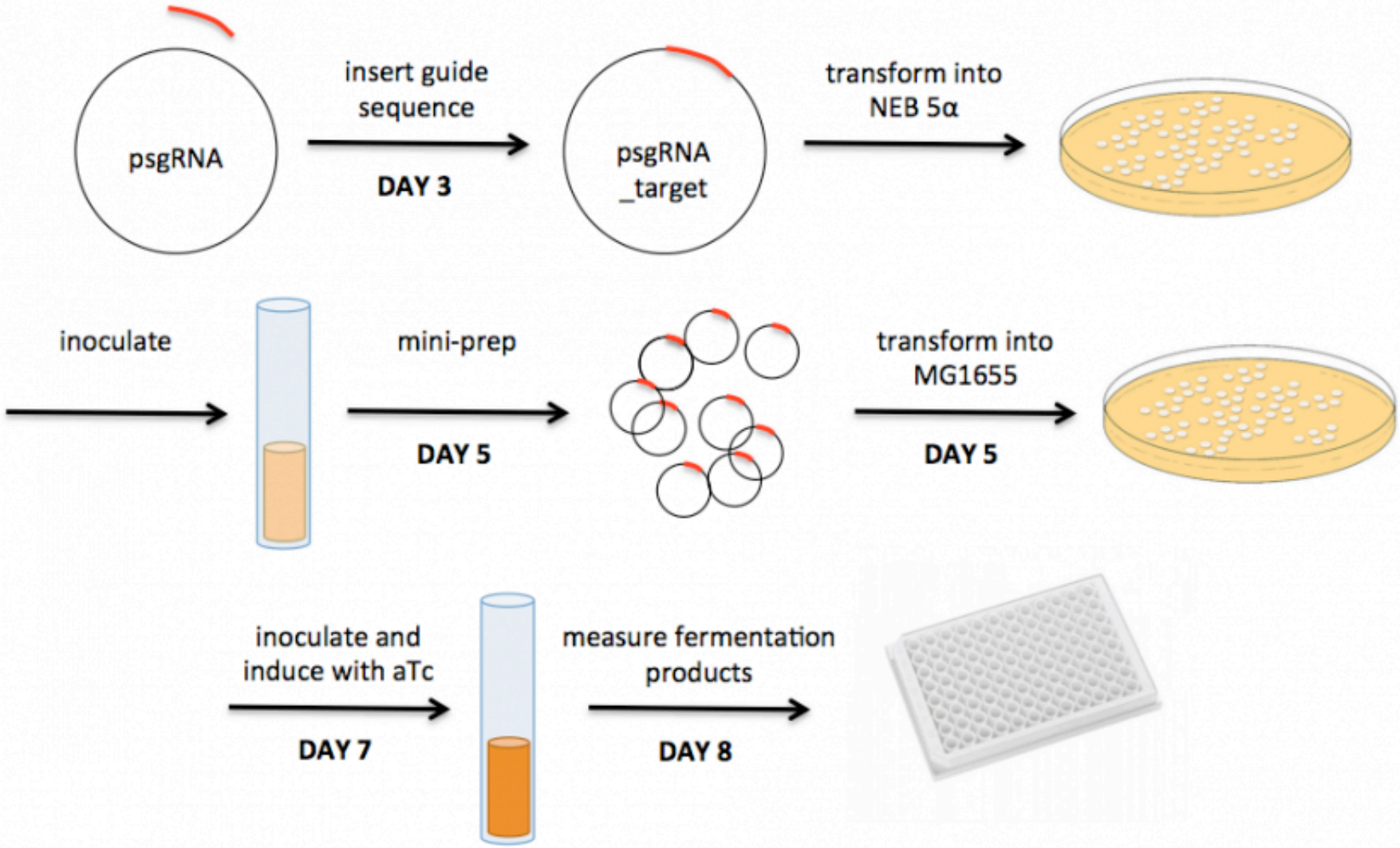
Extra office hours

- Saturday Nov. 18th
  12pm-5pm (56-302)
- Monday Nov. 20th
  11am-2pm (56-341c)
  2-5pm (16-317)

Regular office hours

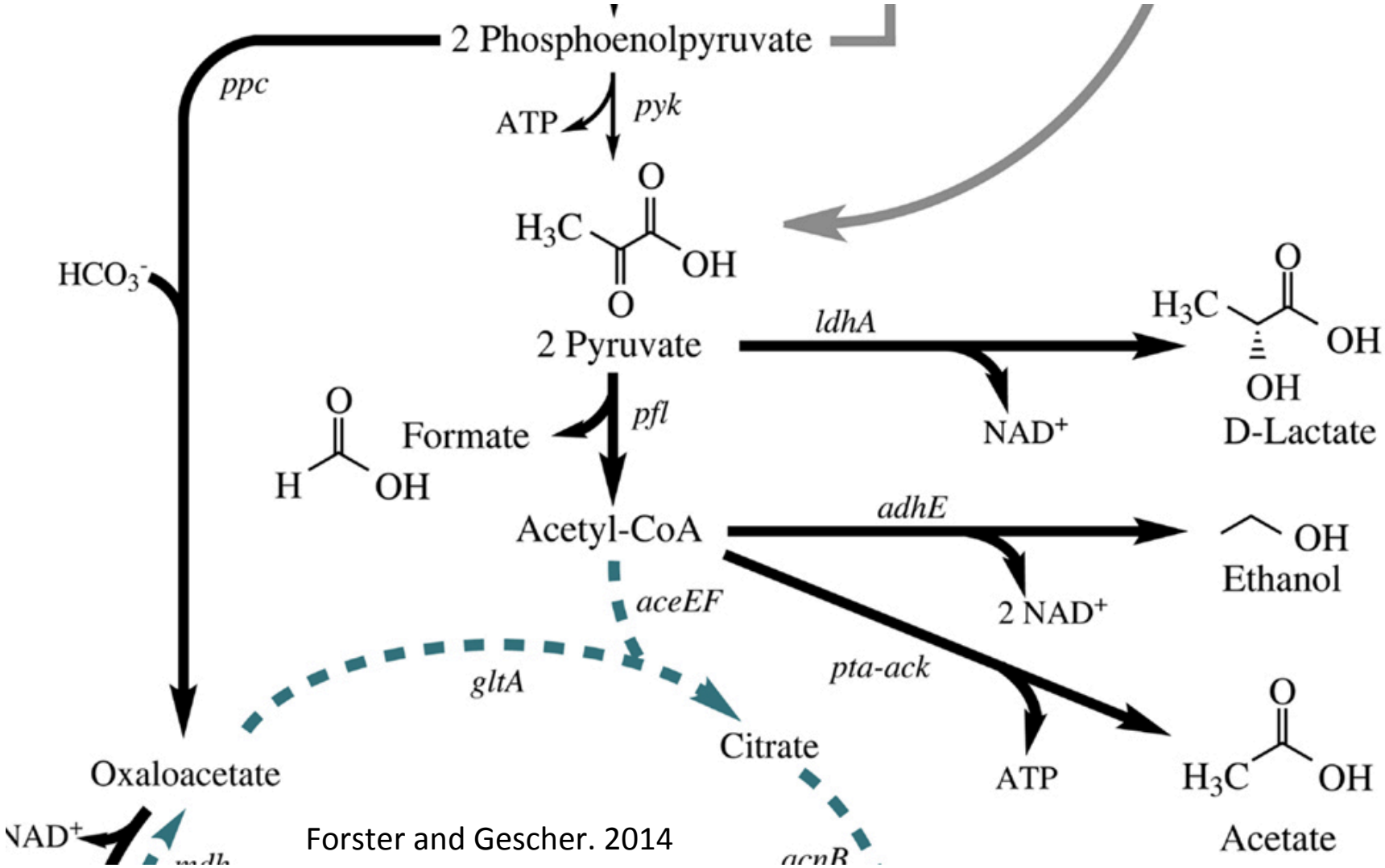
- Noreen: Mon. 2-5pm (16-317)
- Leslie: Fri. 9am and 3pm (56-341c)
- Josephine: Mon. 1pm, Thurs. 2pm (56-341c)

M2 experimental overview



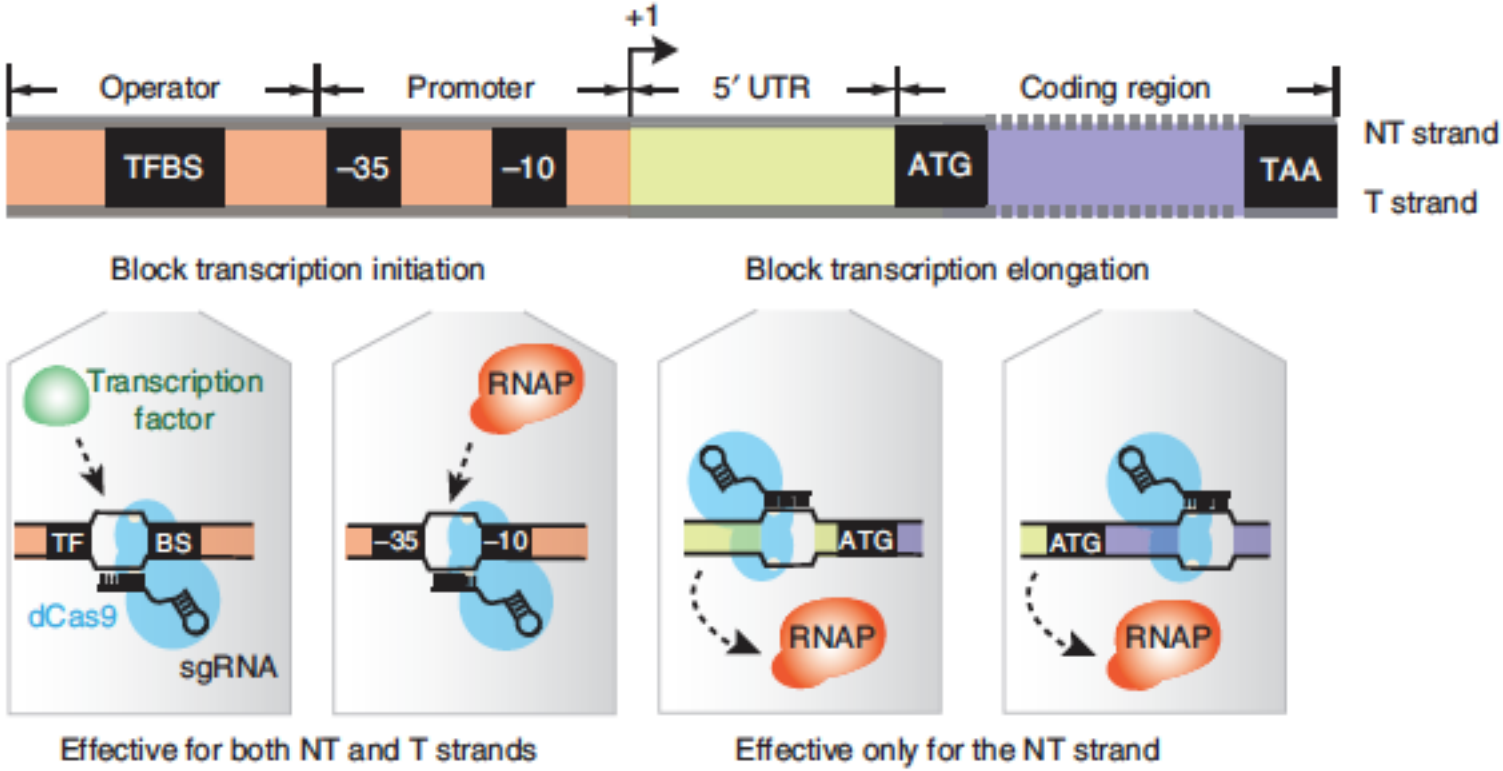
Note: sgRNA = gRNA

Using CRISPRi to manipulate the *E. coli* fermentation pathway



Forster and Gescher. 2014

Design of gRNA for CRISPRi system

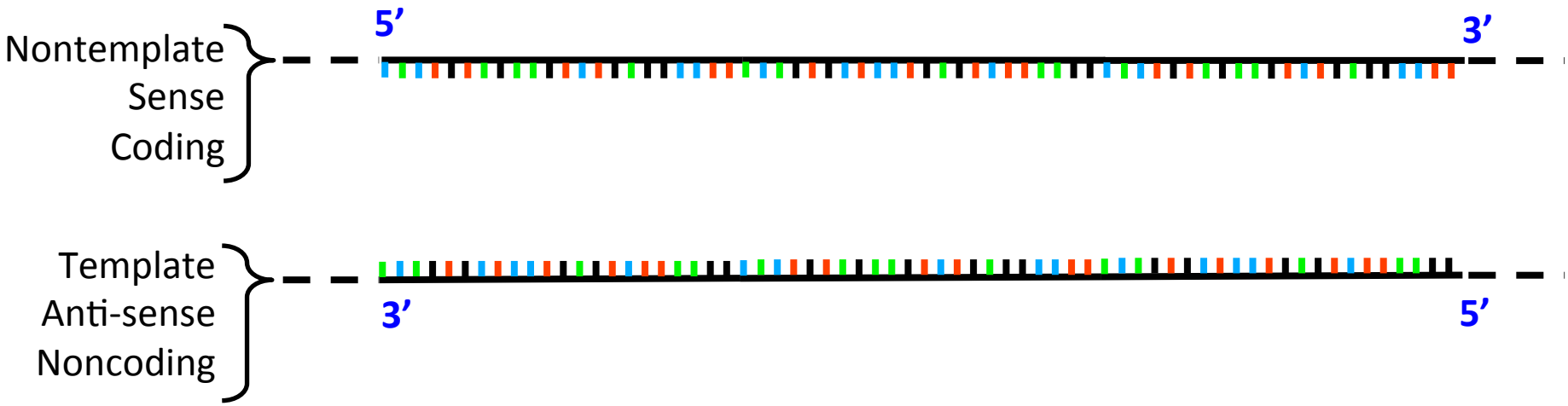


Larson, et al. CRISPR interference for sequence-specific control of gene expression. *Nature Protocols.* 2013.

Design of gRNA for CRISPRi system

(1) If you target the template DNA strand, the gRNA (DNA) sequence will be the same as the transcribed (nontemplate) sequence.

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



Confirm gRNA design rules (1)

(1) If you target the template DNA strand, the gRNA (DNA) sequence will be the same as the transcribed (nontemplate) sequence.

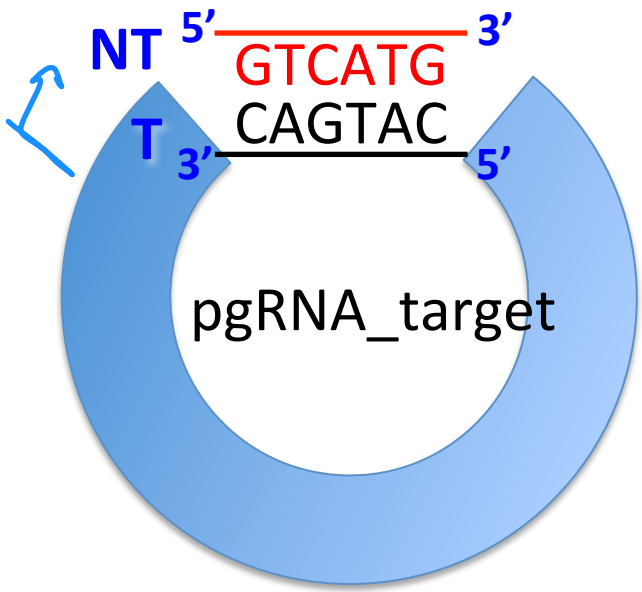
Gene of interest

Nontemplate (NT) 5' GTCATG 3'

Template (T) 3' CAGTAC 5'

binds to T

If gRNA (DNA) is same as NT strand: 5' GTCATG 3'



transcribe

5' GUCAUG 3'

gRNA strand

Confirm gRNA design rules (2)

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

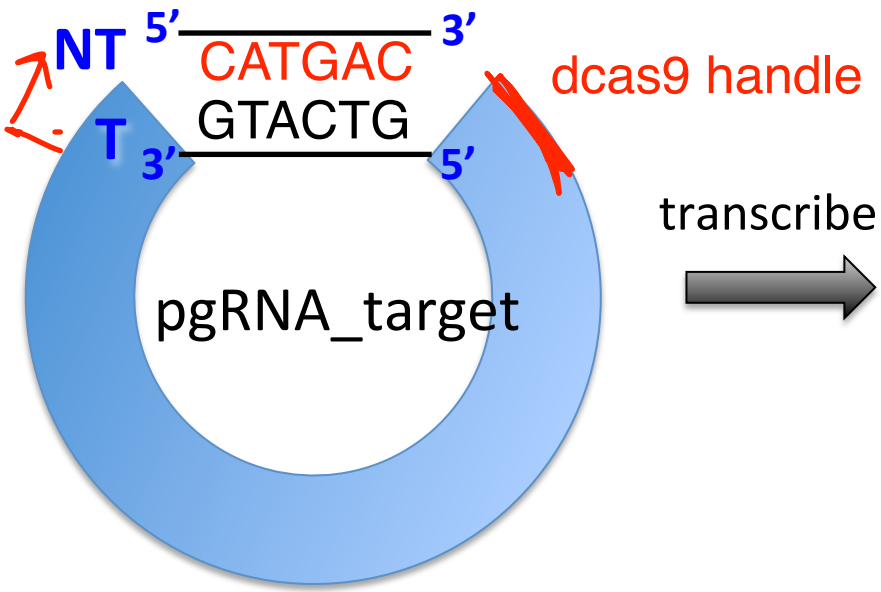
Gene of interest

Nontemplate (NT) 5' GTCATG 3'

Template (T) 3' CAGTAC 5'

binds NT

If gRNA (DNA) is same as T strand: 3' CAGTAC 5'



transcribe

5' CAUGAC 3'

gRNA strand

Please add your targeting info to the wiki today

On the Mod2 Overview page, discussion tab

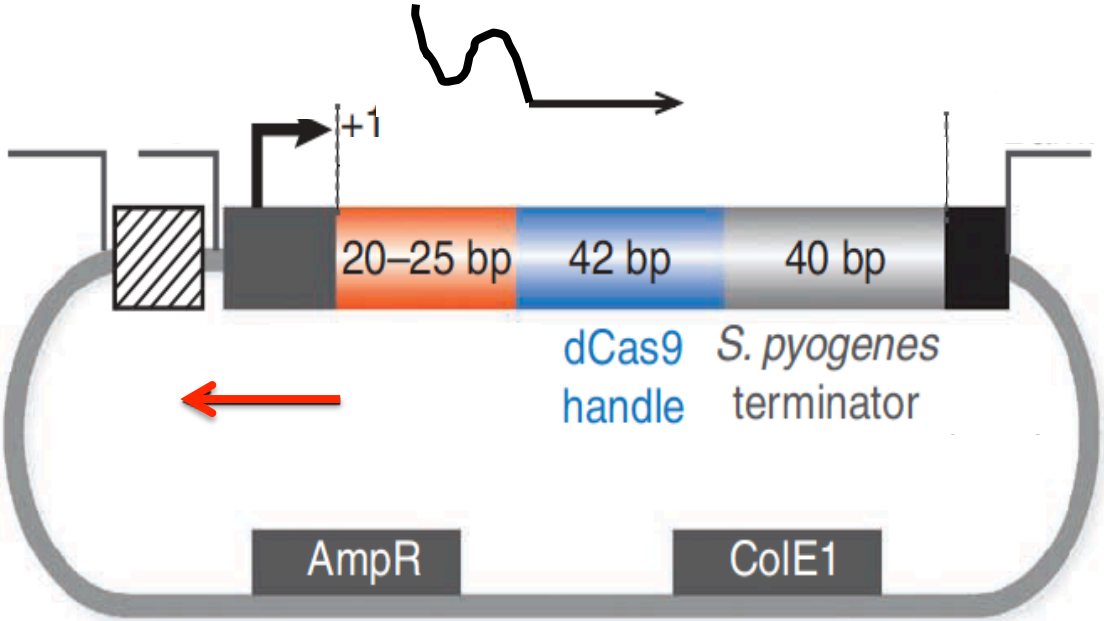
W/F

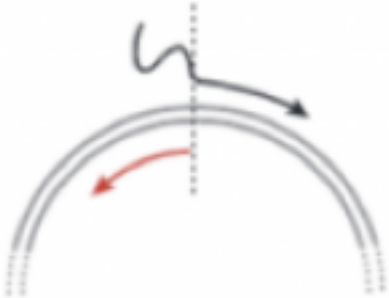
Team	Ethanol (E) or Acetate (A)	Gene targeted by CRISPRi gRNA	gRNA sequence end)
red	Ethanol	ack (indirectly, pta)	GTTTTTTTAGCC
orange	Ethanol	ldhA	ATTCAACATCAC
blue	Ethanol	ackA	TTTTTAGCCACG

...

	Locus targeted (eg. beginning of gene, putative promoter, -35 region)	Target template or nontemplate strand
TT		

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 ( )

Analyzing Sequence Information

- Reverse and complement your reverse primer sequence before alignment
- Check whether your target sequence was successfully incorporated into the pgRNA_target plamsid

```
                *         *         *         *         *                                   *         *
  1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt-------------------------gttttagagctagaaatagcaag>73
 73>---GAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>269
 01>CTGGAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>500
  1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt-------------------------gttttagagctagaaatagcaag>73

      *         *         *         *         *         *         *         *         *         *
 74>ttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgaagcttgggcccgaacaaaaactcatctcagaagaggat>173
 70>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGGGCCCGAACAAAAACTCATCTCAGAAGAGGAT>369
 01>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGGGCCCGAACAAAAACTCATCTCAGAAGAGGAT>600
 74>ttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgaagcttgggcccgaacaaaaactcatctcagaagaggat>173
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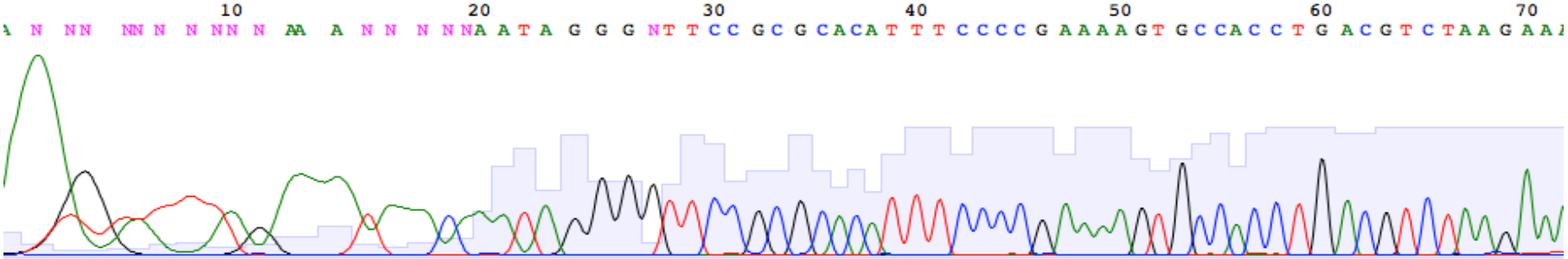
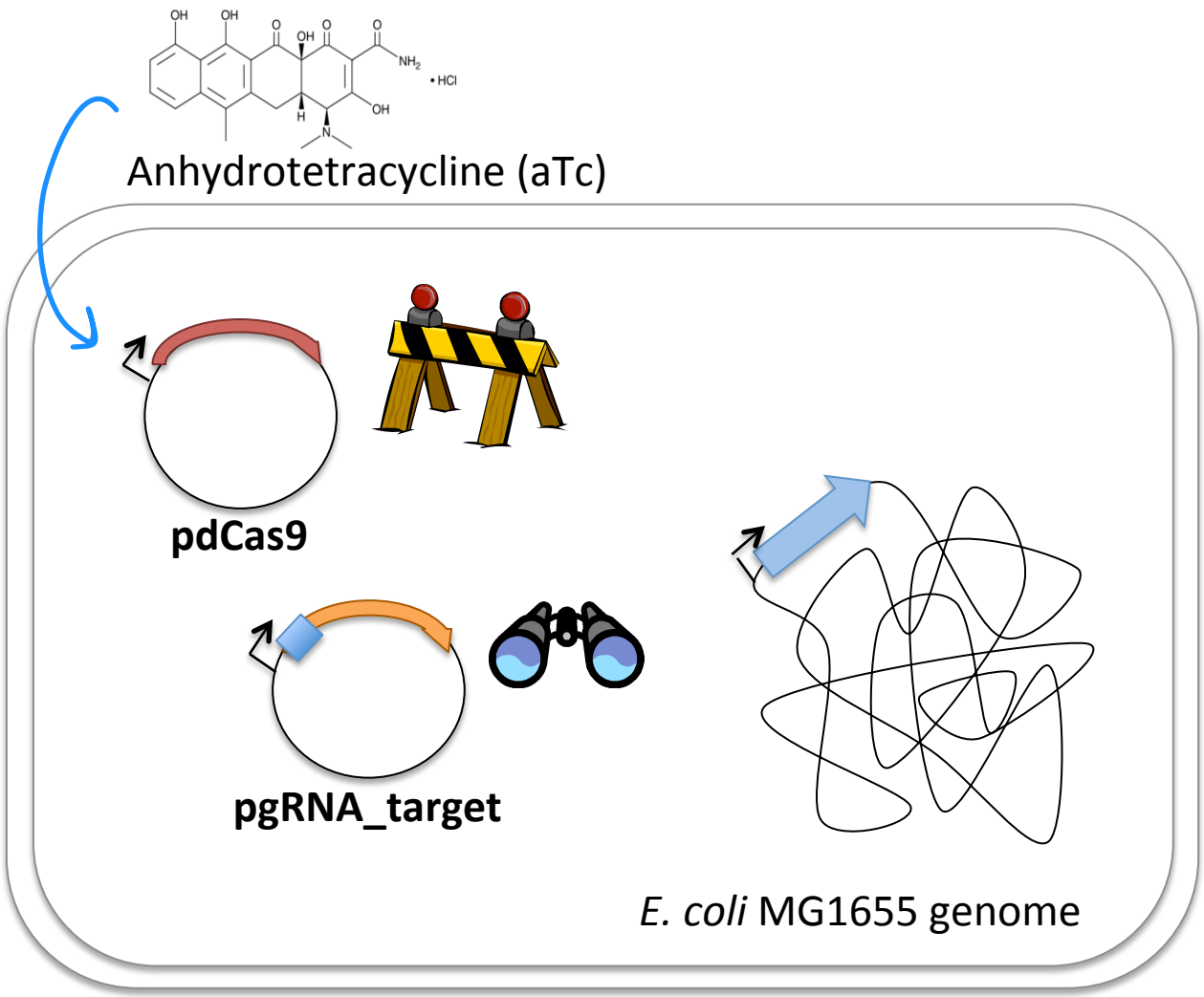
- Sanger sequencing traces are also on wiki for your reference



Induction of CRISPRi system with aTc



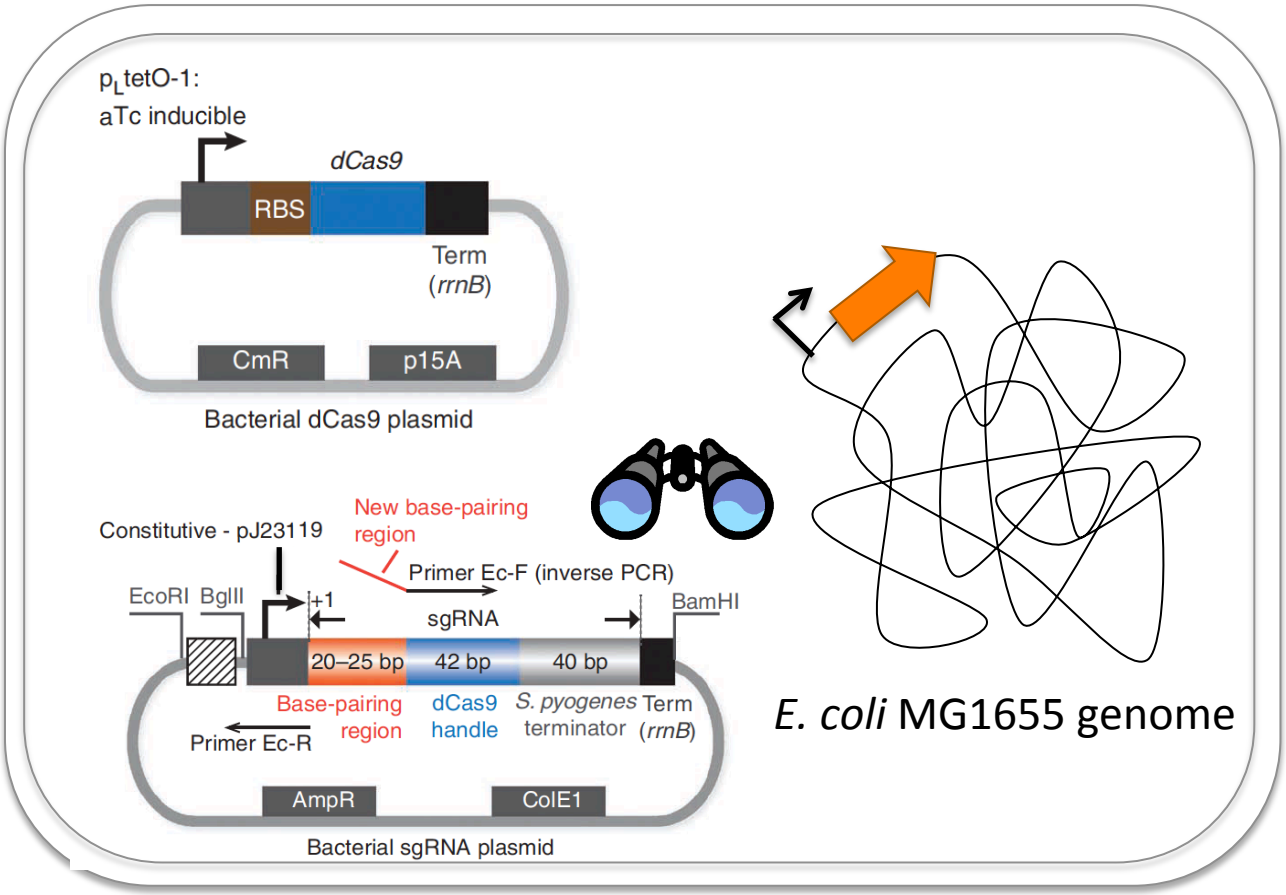
- Expressed constitutively:

  gRNA

- Expression induced with aTc:

  dCas9

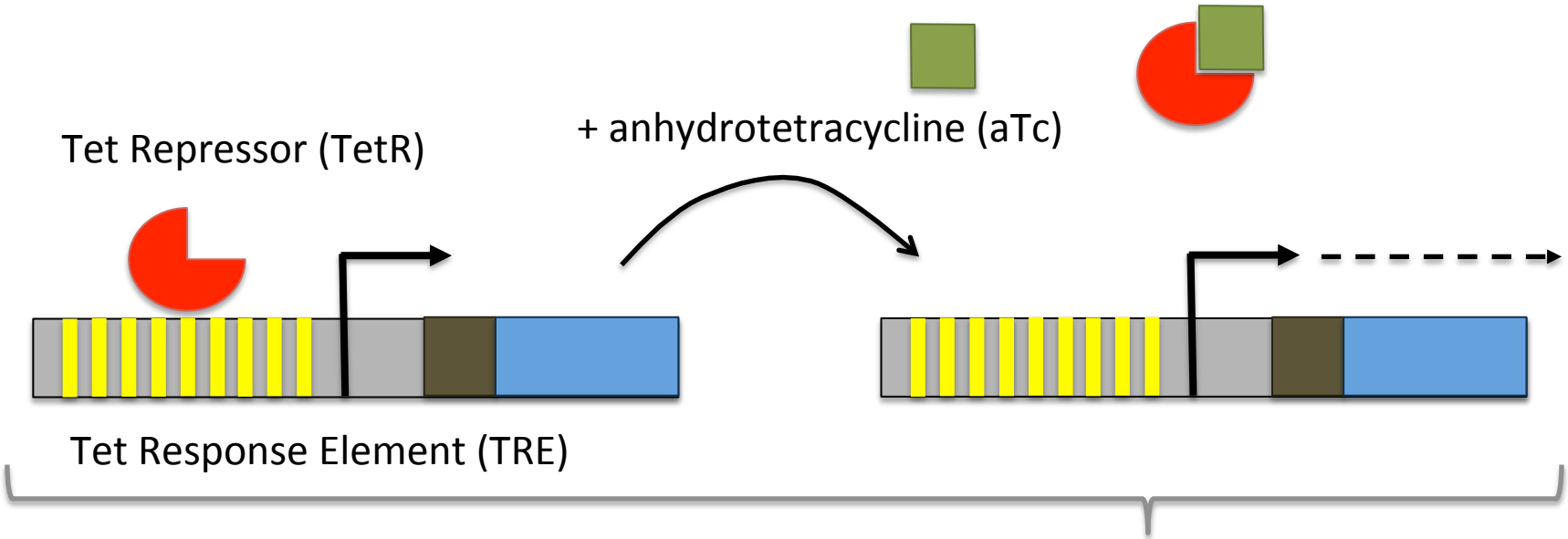
CRISPRi 'inactive' in absence of inducer



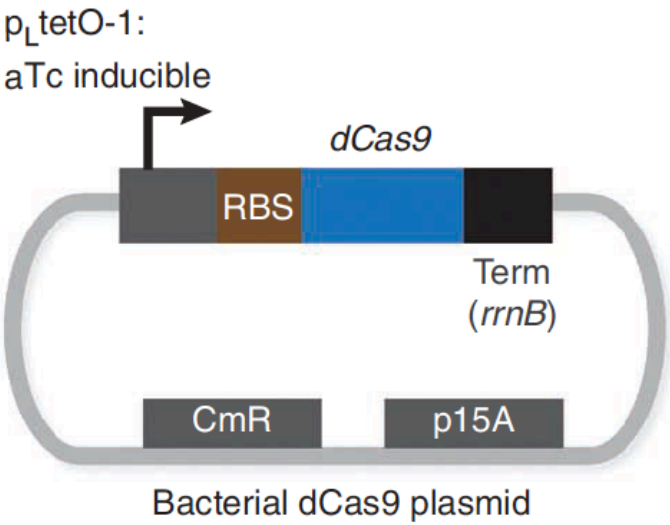
Without aTc

- Only gRNA present
- No (or little) dCas9

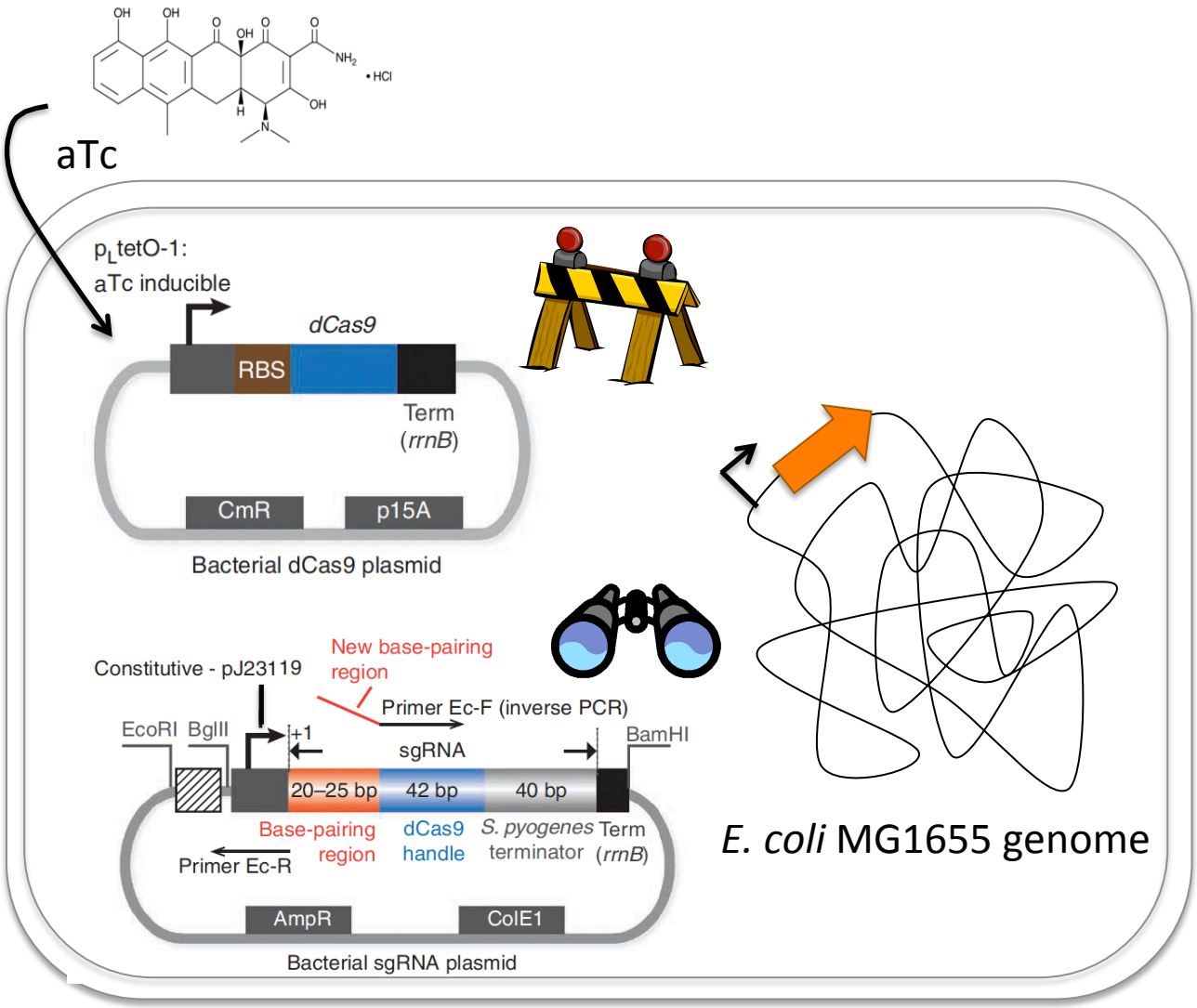
aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene



CRISPRi ‘blocks’ gene expression in presence of inducer



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

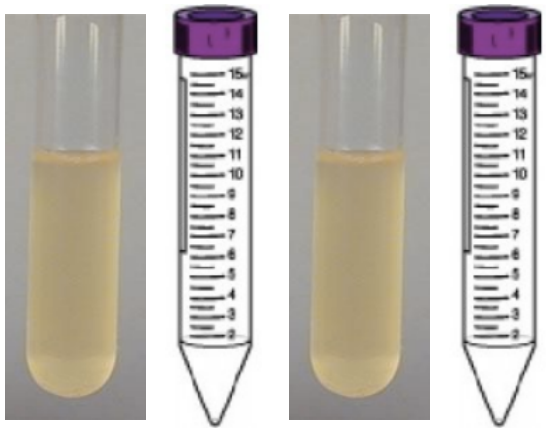
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- What are the necessary components?

LB (tryptone, yeast extract, NaCl)
Antibiotics (Amp, Cam)
aTc (induce dCas9 expression)

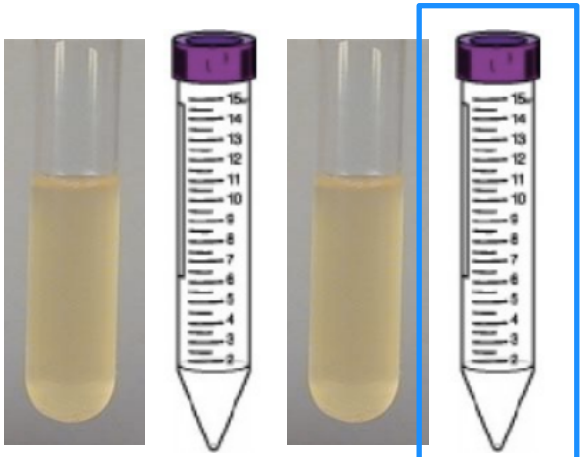
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- Where do we expect most ethanol if hypothesis confirmed?



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

MG1655



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

MG1655 with CRISPRi

Today in lab…

- Upload gRNA design information to wiki
- Download your sequencing data from discussion tab and align (using ApE software)
- Prepare media for mixed-acid fermentation inoculations

M2D8 Assignments

- Quiz on Wed. (M2D8) Nov. 8th
- Peer-review methods (see wiki for details)

  Do not leave today before receiving Methods to peer-review