

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

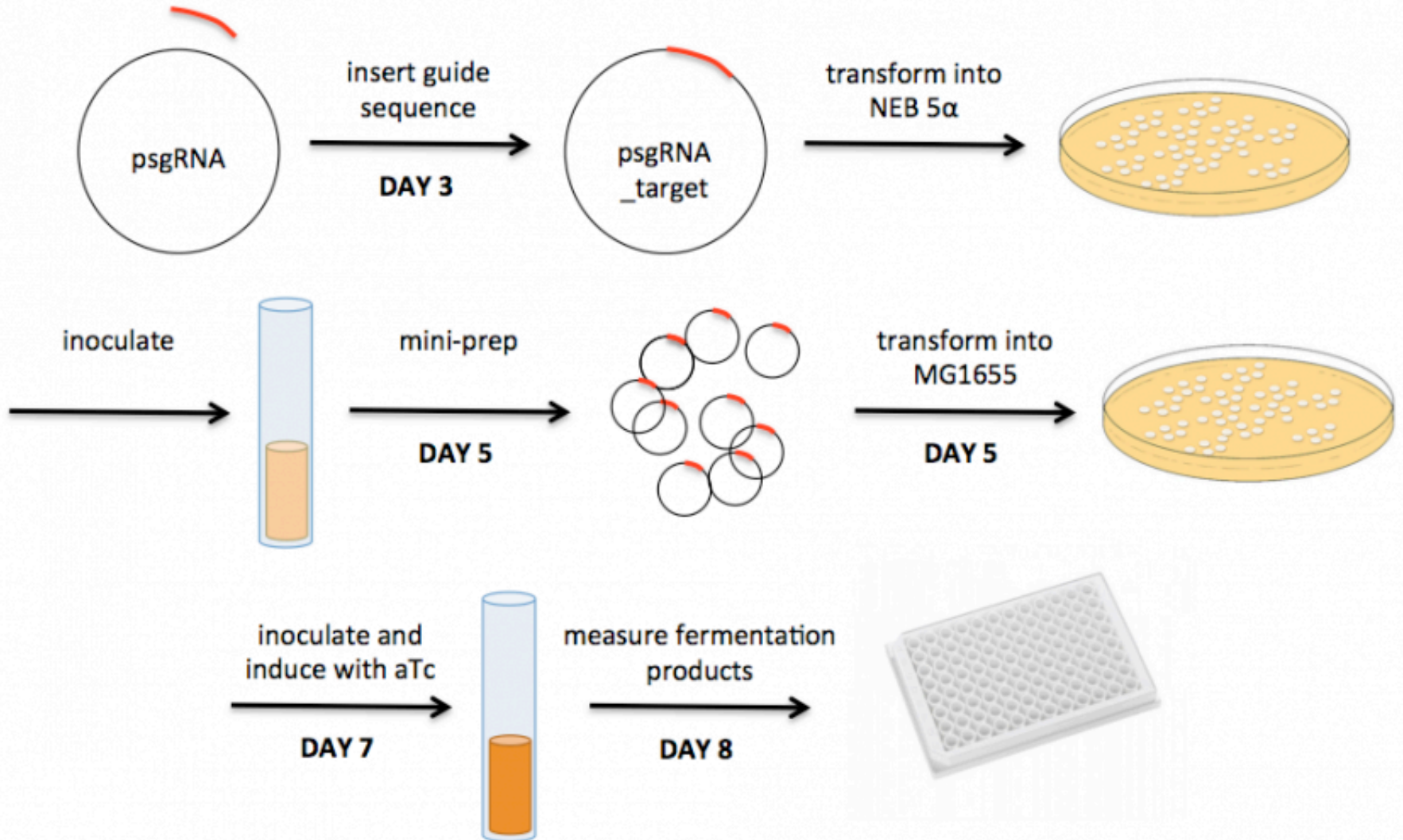
Extra office hours

- Saturday Nov. 18th
12pm-5pm
- Monday Nov. 20th
11am-5pm

Regular office hours

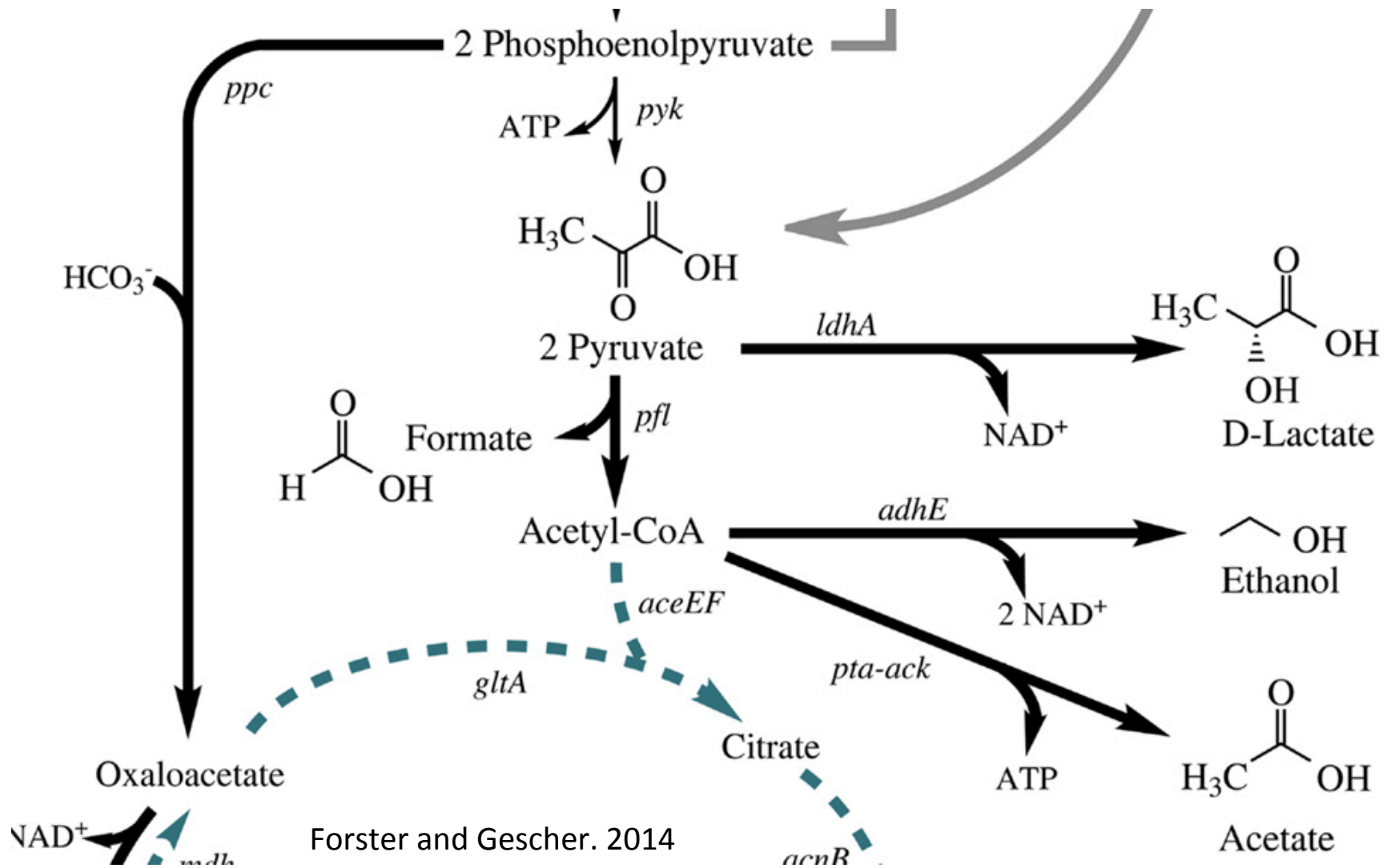
- Noreen: Mon. 2-5pm
(16-317)
- Leslie: Fri. 9am and 3pm
(56-341c)
- Josephine: Mon. 1pm,
Thurs. 2pm (56-341c)

M2 experimental overview

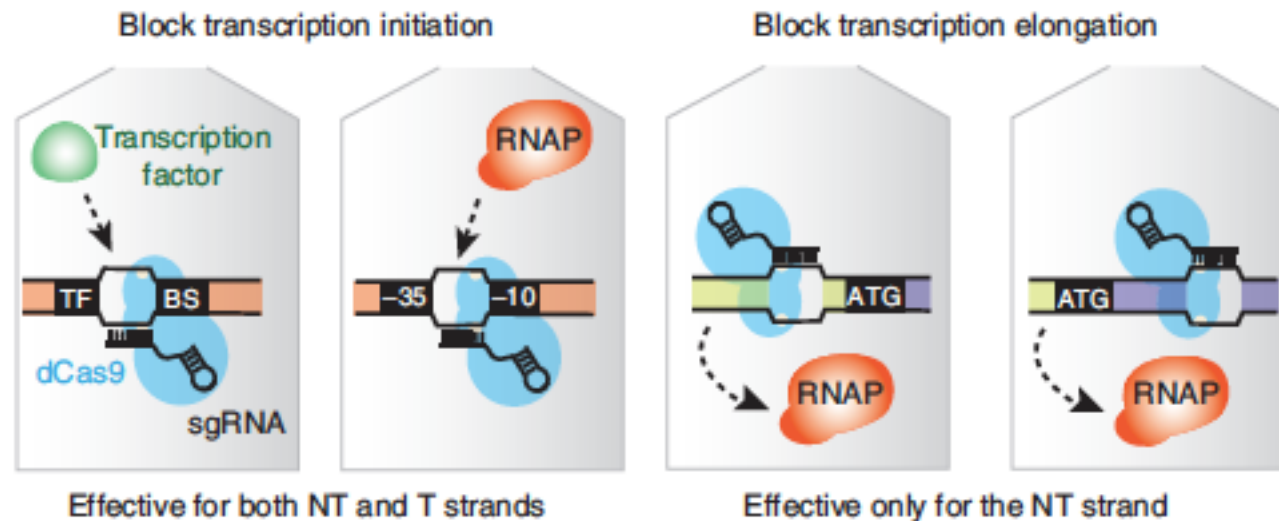
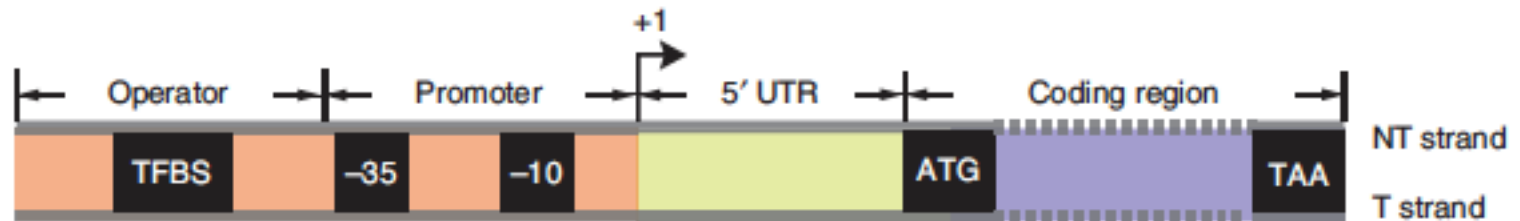


Note: sgRNA = gRNA

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

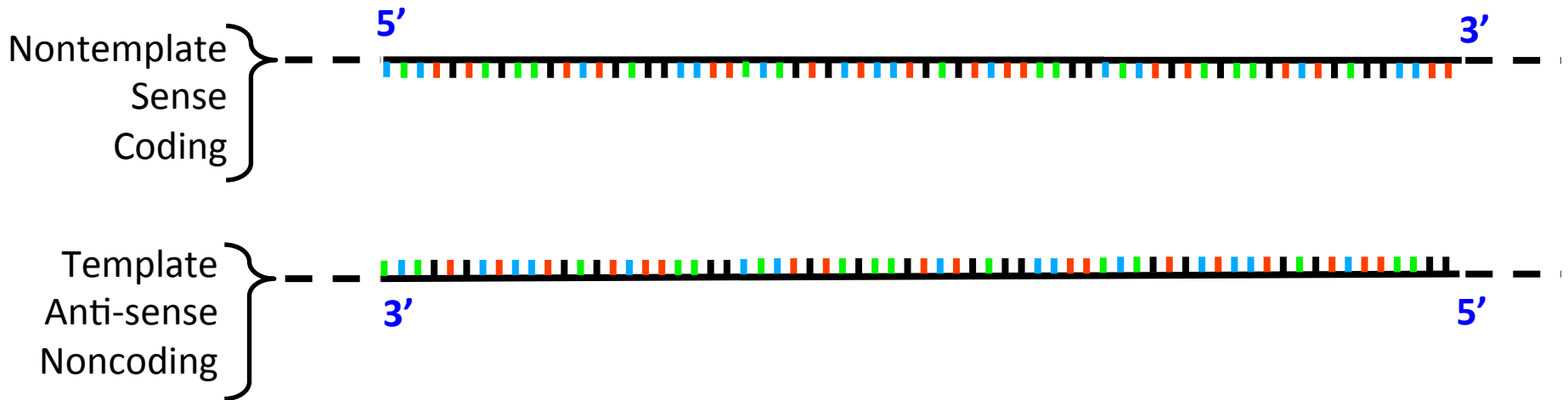


Design of gRNA for CRISPRi system



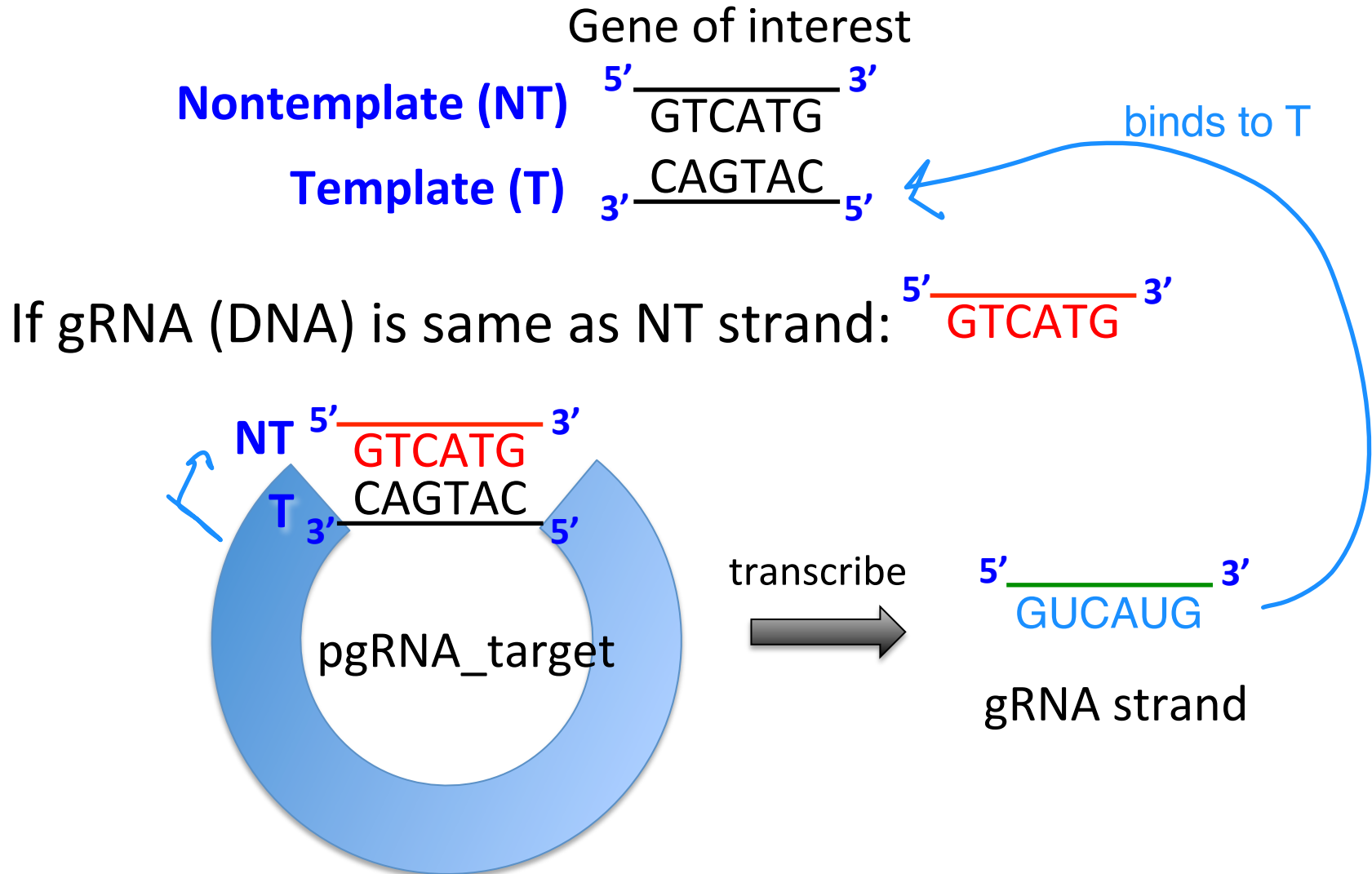
Design of gRNA for CRISPRi system

- (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.**



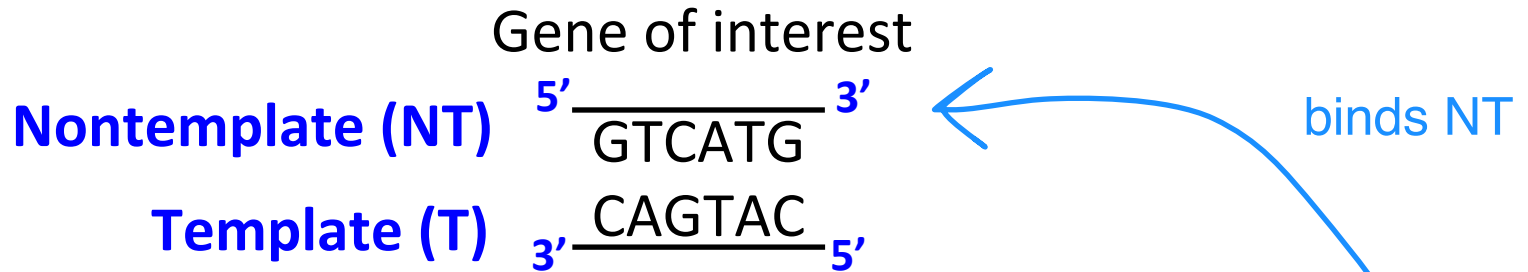
Confirm gRNA design rules (1)

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

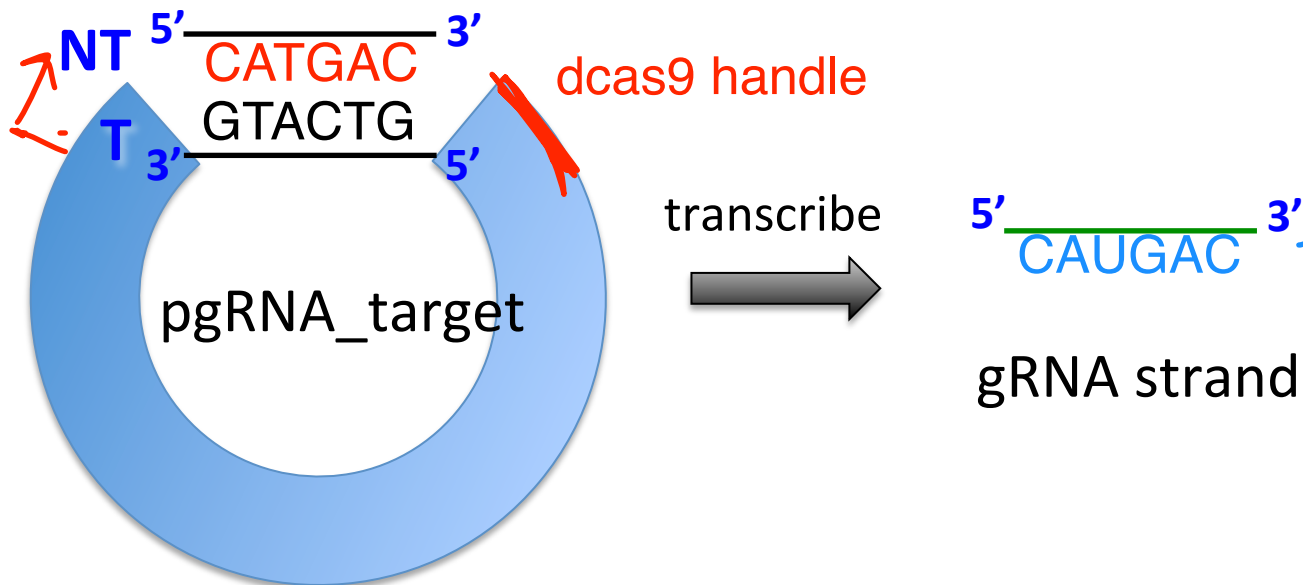


Confirm gRNA design rules (2)

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



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



Please add your targeting info to the wiki today

[On the Mod2 Overview page, discussion tab](#)

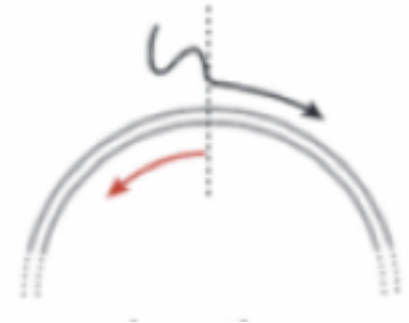
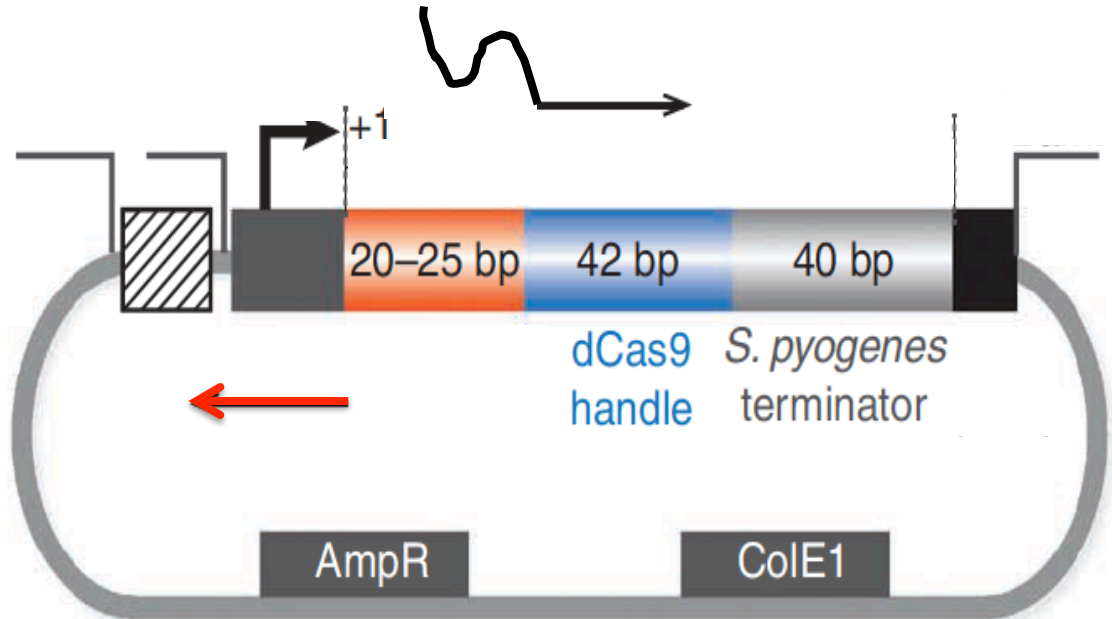
W/F

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
I		
II		

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 (→)

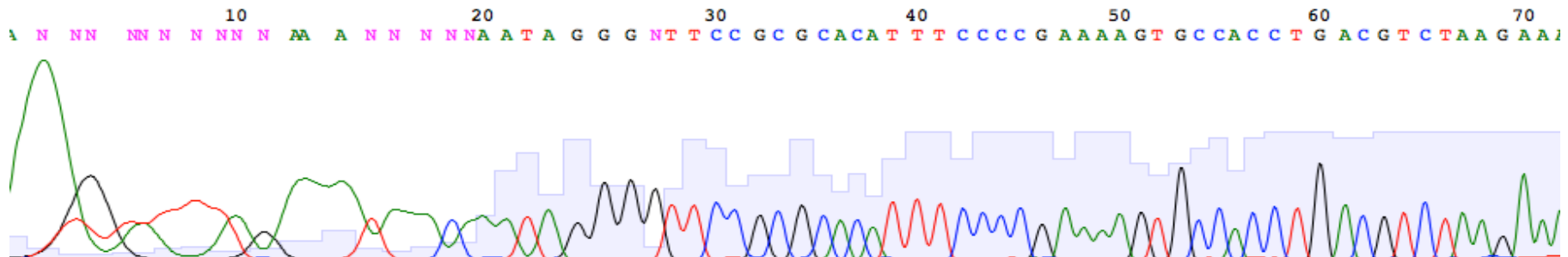
Analyzing Sequence Information

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

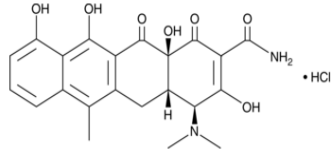
```
1>---gaattc*taagatc*tttgac*agctagctcagtcctaggtataataactag*-----g*ttttagagctagaaatagcaag*73
73>---GAATTC*TAAGATCT*TTGACAGCTAGCTCAGTCCTAGGTATAACTAGT*AAATCCACTT*AAGAAGGTAGGTGT*GTTTTAGAGCTAGAAATAGCAAG>269
01>CTGGAATTC*TAAGATCT*TTGACAGCTAGCTCAGTCCTAGGTATAACTAGT*AAATCCACTT*AAGAAGGTAGGTGT*GTTTTAGAGCTAGAAATAGCAAG>500
1>---gaattc*taagatc*tttgac*agctagctcagtcctaggtataataactag*-----g*ttttagagctagaaatagcaag*73

74>*t*taaaataaggct*tagtccg*ttatcaacttg*aaaaagtggcaccgagtcggtgct*tttttttgaagctt*gggccccaacaaaaactcatctcagaagaggat>173
70>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGGGCCCGAACAAAACTCATCTCAGAAGAGGAT>369
01>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGGGCCCGAACAAAACTCATCTCAGAAGAGGAT>600
74>*t*taaaataaggct*tagtccg*ttatcaacttg*aaaaagtggcaccgagtcggtgct*tttttttgaagctt*gggccccaacaaaaactcatctcagaagaggat>173
```

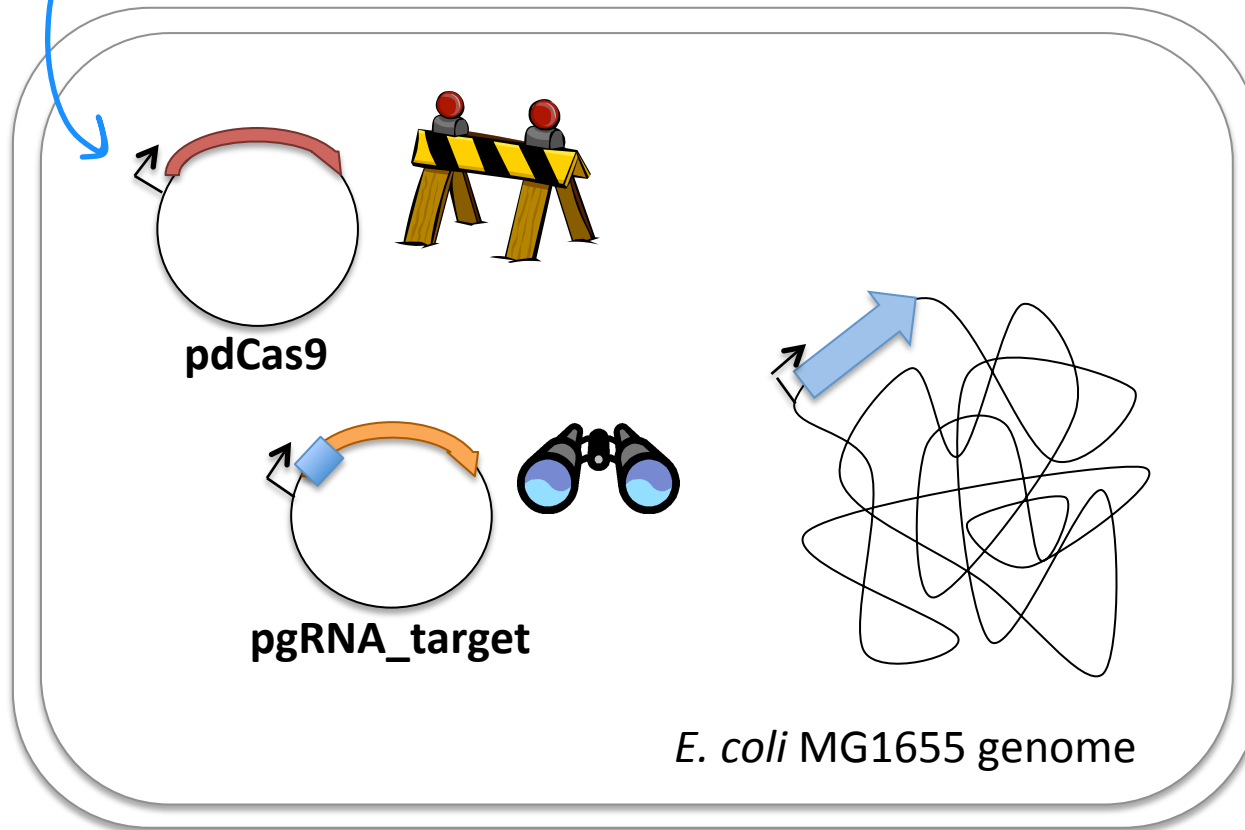
- Sanger sequencing traces are also on wiki for your reference



Induction of CRISPRi system with aTc



Anhydrotetracycline (aTc)



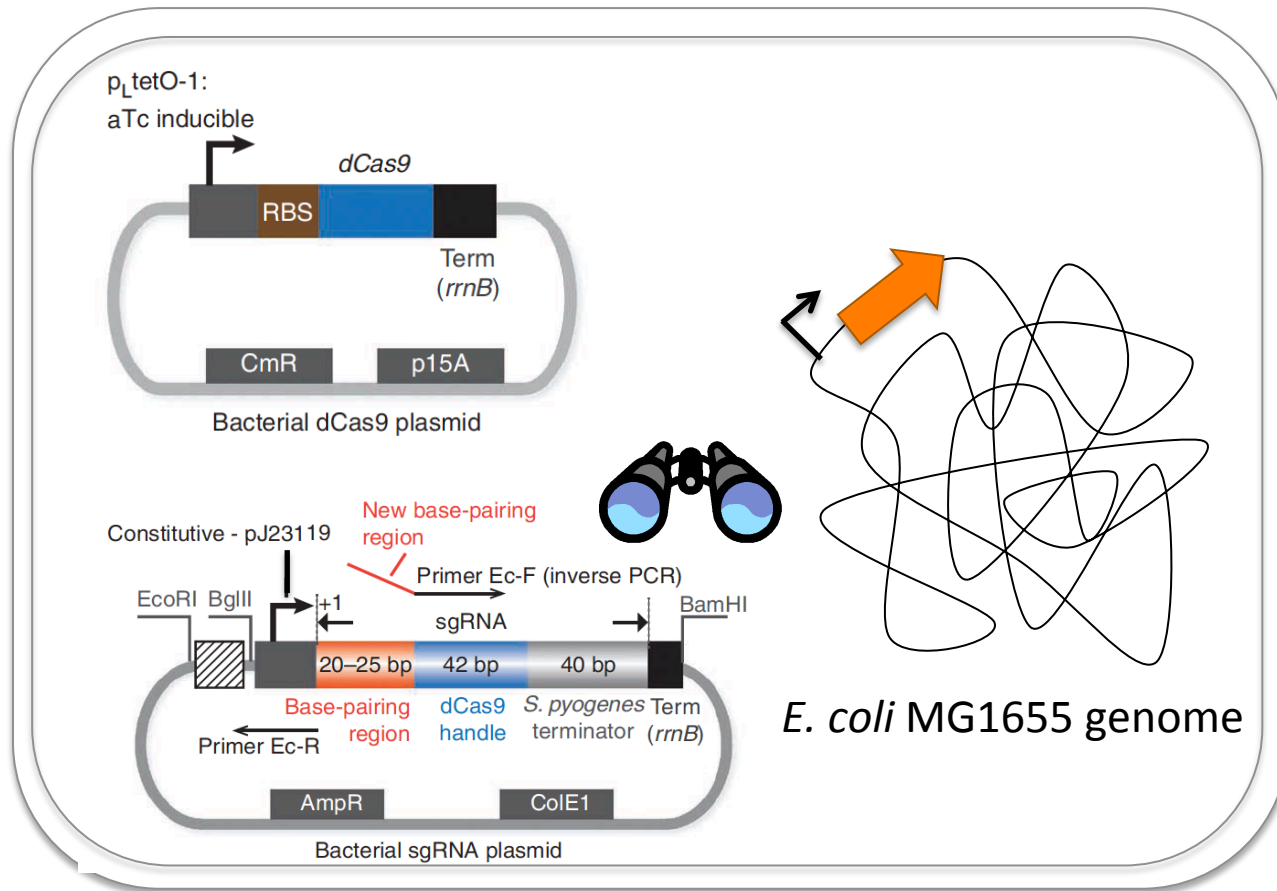
- Expressed constitutively:

gRNA

- Expression induced with aTc:

dCas9

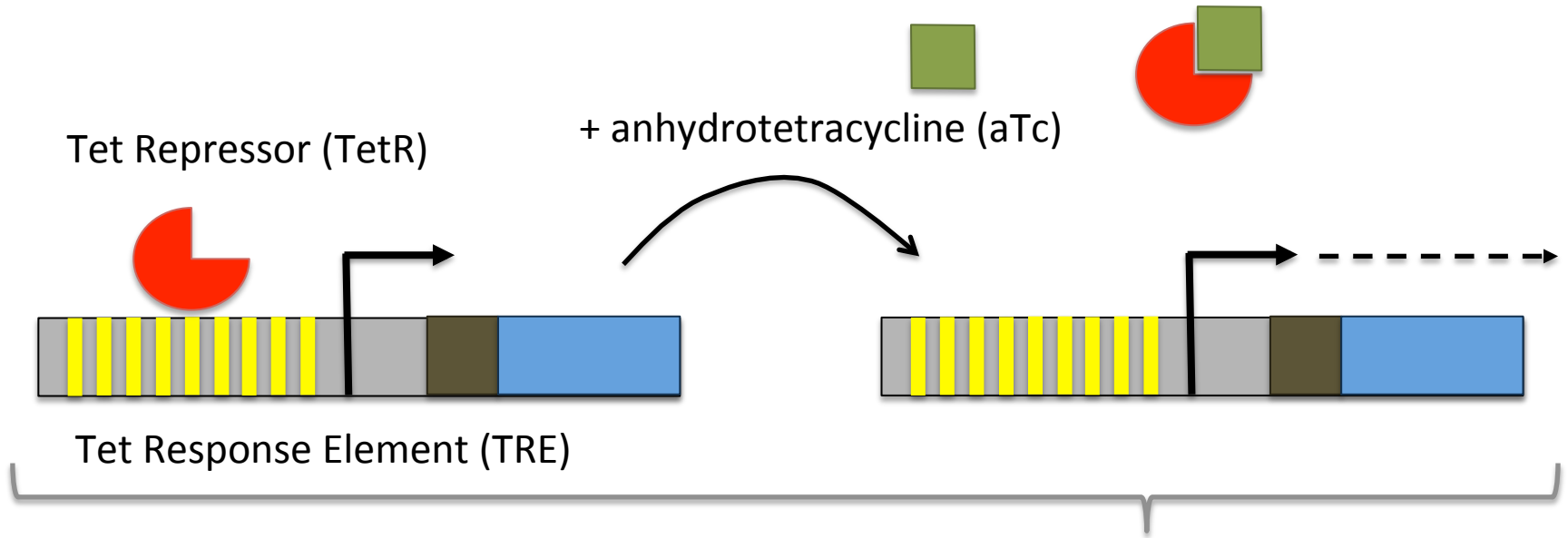
CRISPRi 'inactive' in absence of inducer



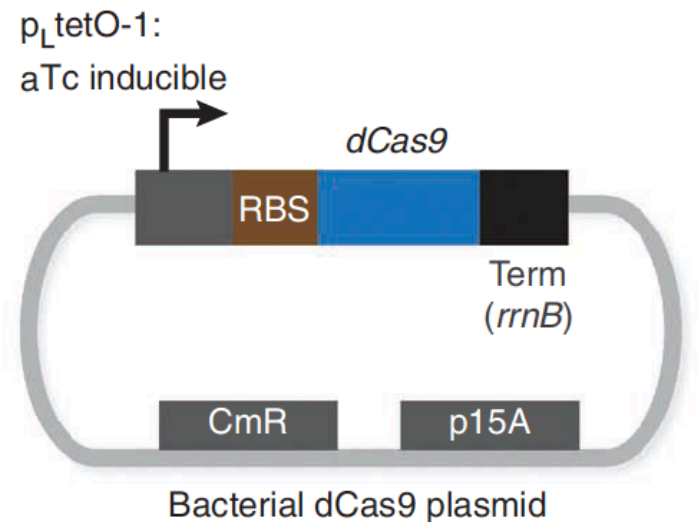
Without aTc

- Only gRNA present
- No (or little) dCas9

aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene



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

- What are the necessary components?

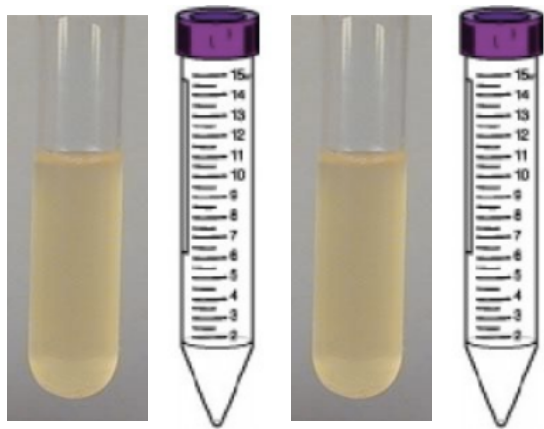
LB (tryptone, yeast extract, NaCl)

Antibiotics (Amp, Cam)

aTc (induce dCas9 expression)

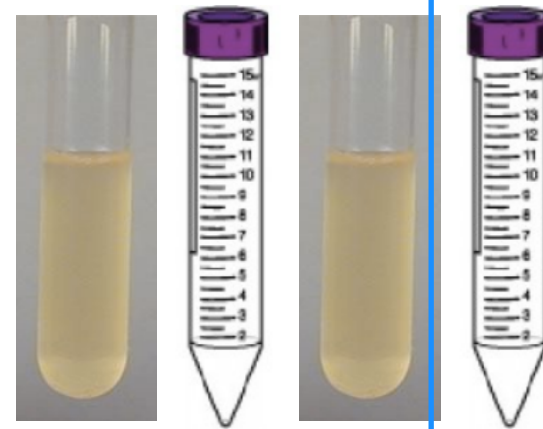
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- Where do we expect most ethanol if hypothesis confirmed?



+ O₂ - aTc - O₂ - aTc + O₂ + aTc - O₂ + aTc

MG1655



+ O₂ - aTc - O₂ - aTc + O₂ + aTc - O₂ + 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