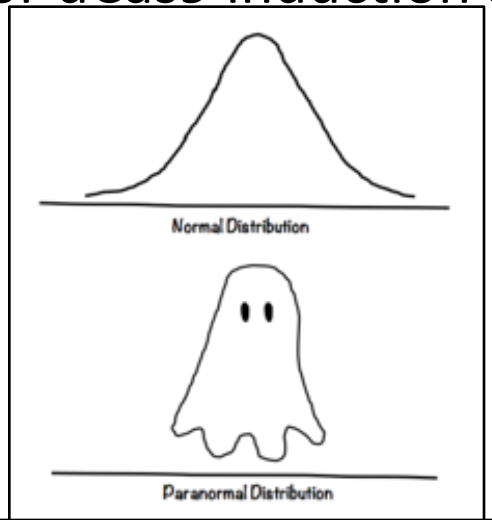
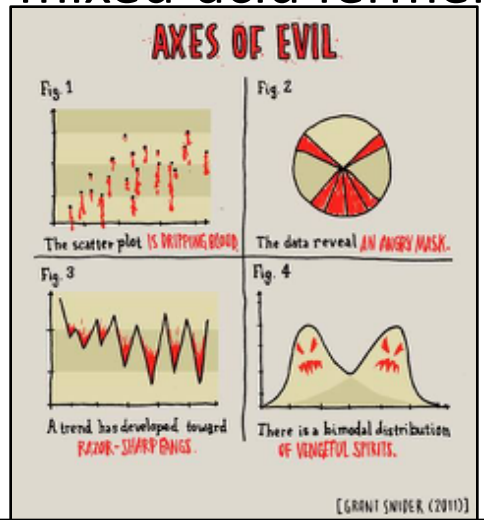
M2D7: Induce CRISPRi system

10/31/19

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





Mod2 Research Report (20% of final grade)

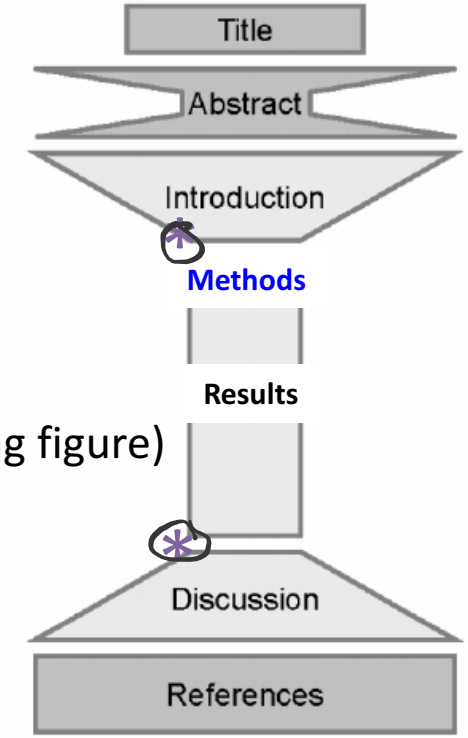
< 14 pages (+ figures, ref.)

2-3 pages

3-4 pages

3-4 pages
(not including figure)

2-3 pages



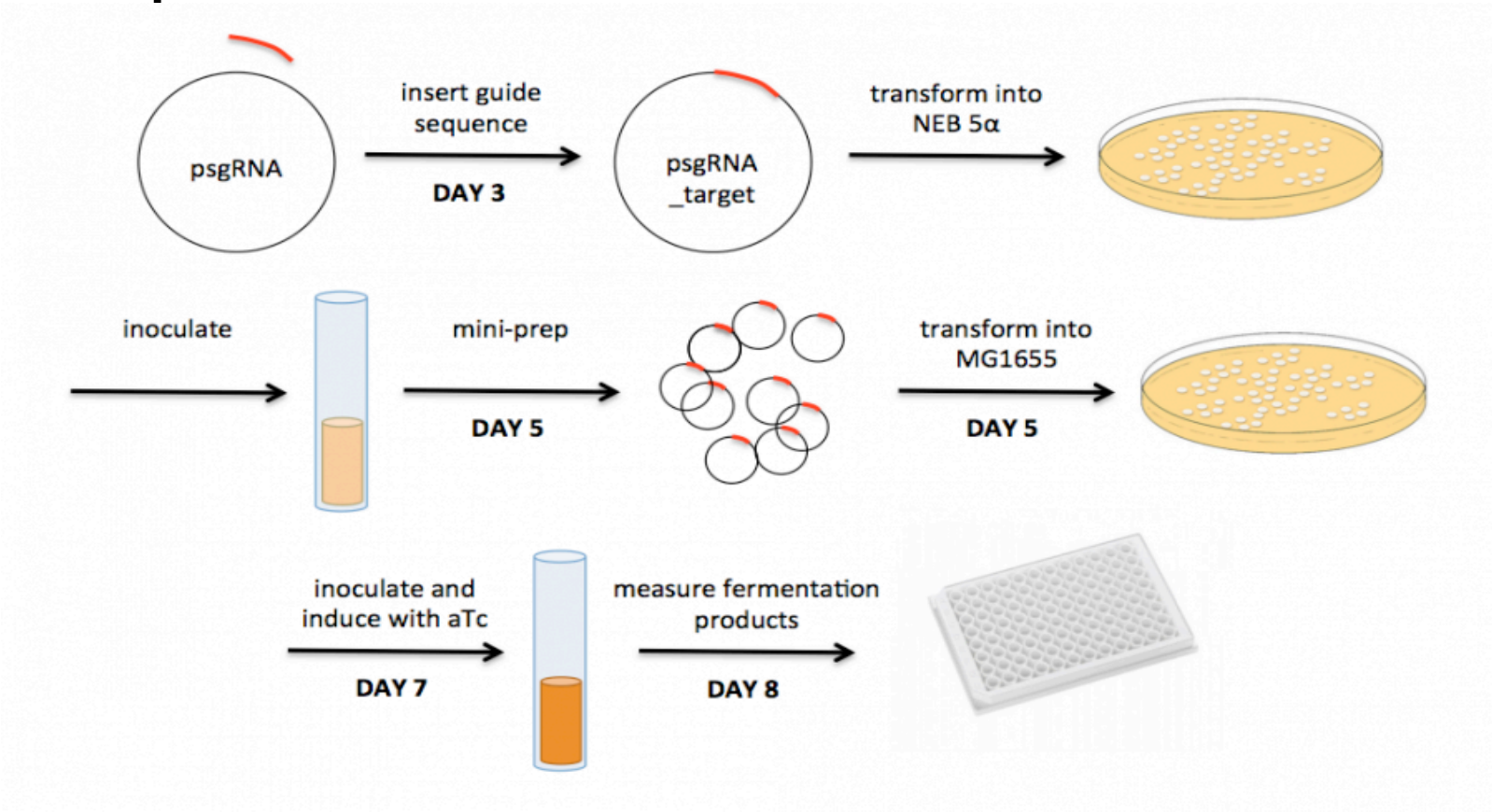
Due Monday 11/11 at 10pm

- Title, Abstract (10%)
- Introduction (10%)
- Methods (20%)
- Results, Figures and captions (50%)
- Discussion (10%)
- References

* similar statements "here we show"

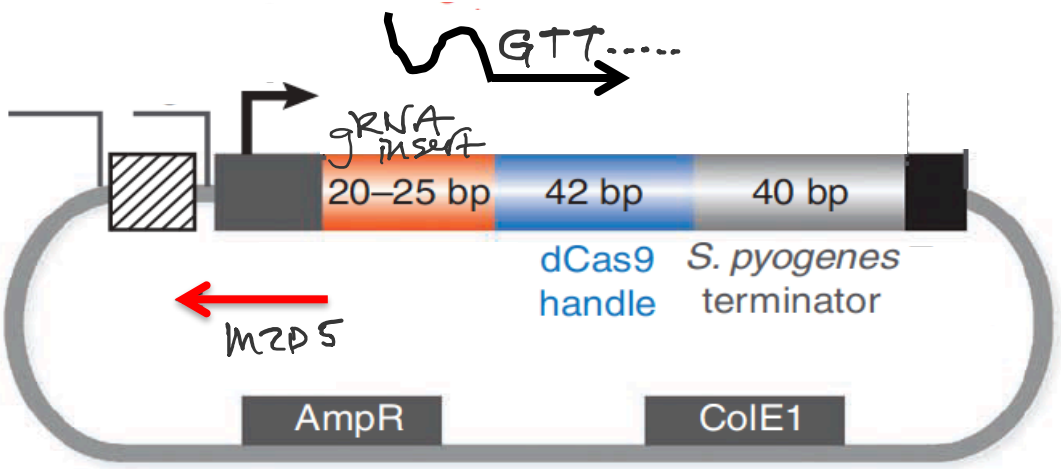
Don't forget:
Blog Post 2, Journal Club due Nov. 1
Blog Post 3, Mod2 due Nov. 12

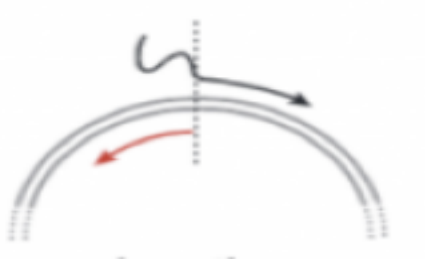
M2 experimental overview



Note: sgRNA = gRNA

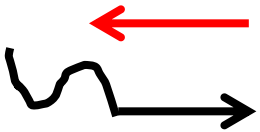
On M2D3: Generated pgRNA_target by SDM





pgRNA_template

insertion (NEB5α kit)



CRISPRi universal *amplification* reverse primer

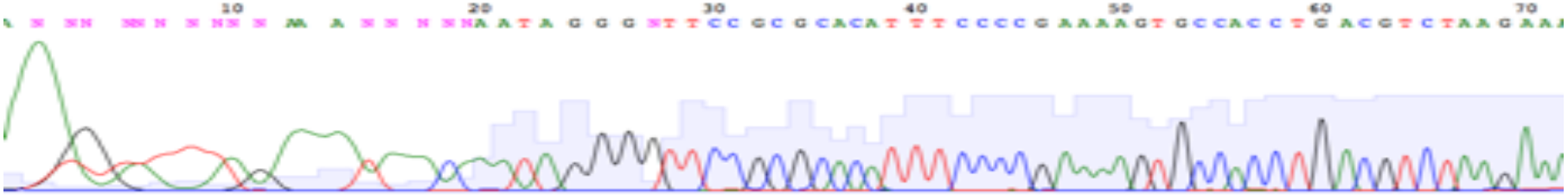
forward primer including crRNA to be inserted ( ) dCas9 handle ( )

Analyzing Sequence Information

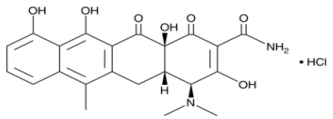
- Check whether your target sequence was successfully incorporated into the pgRNA_target plasmid
- Reverse and complement your reverse primer sequence before alignment

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

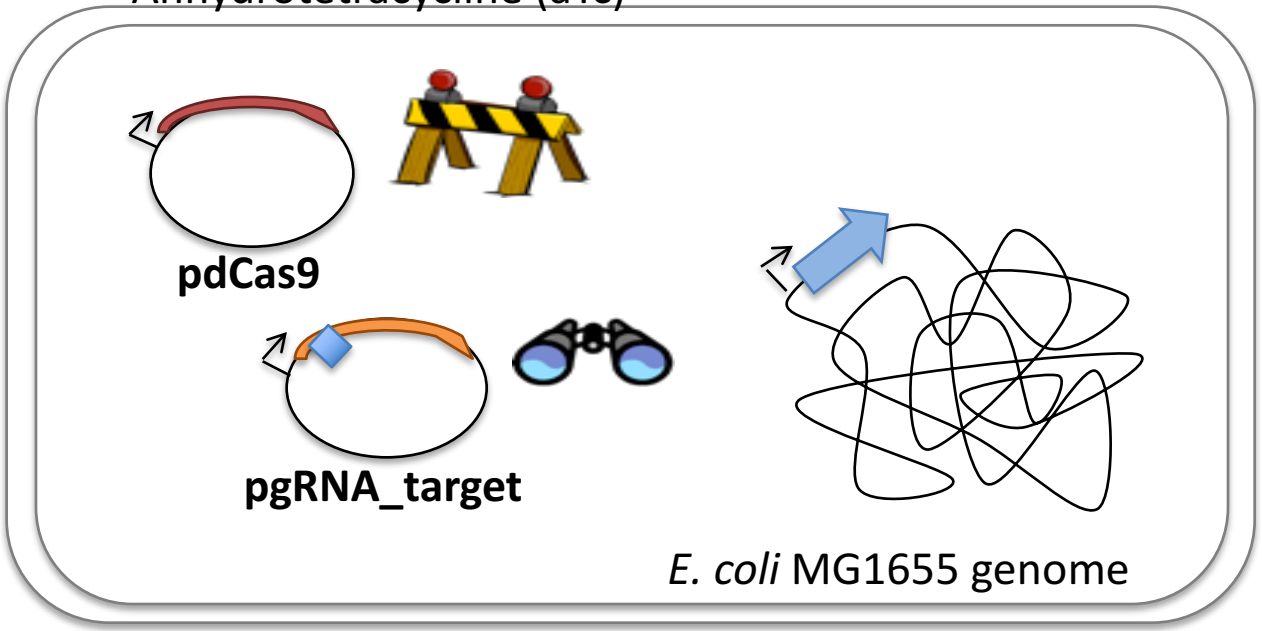
- Sanger sequencing traces for your reference



Induction of CRISPRi system with aTc

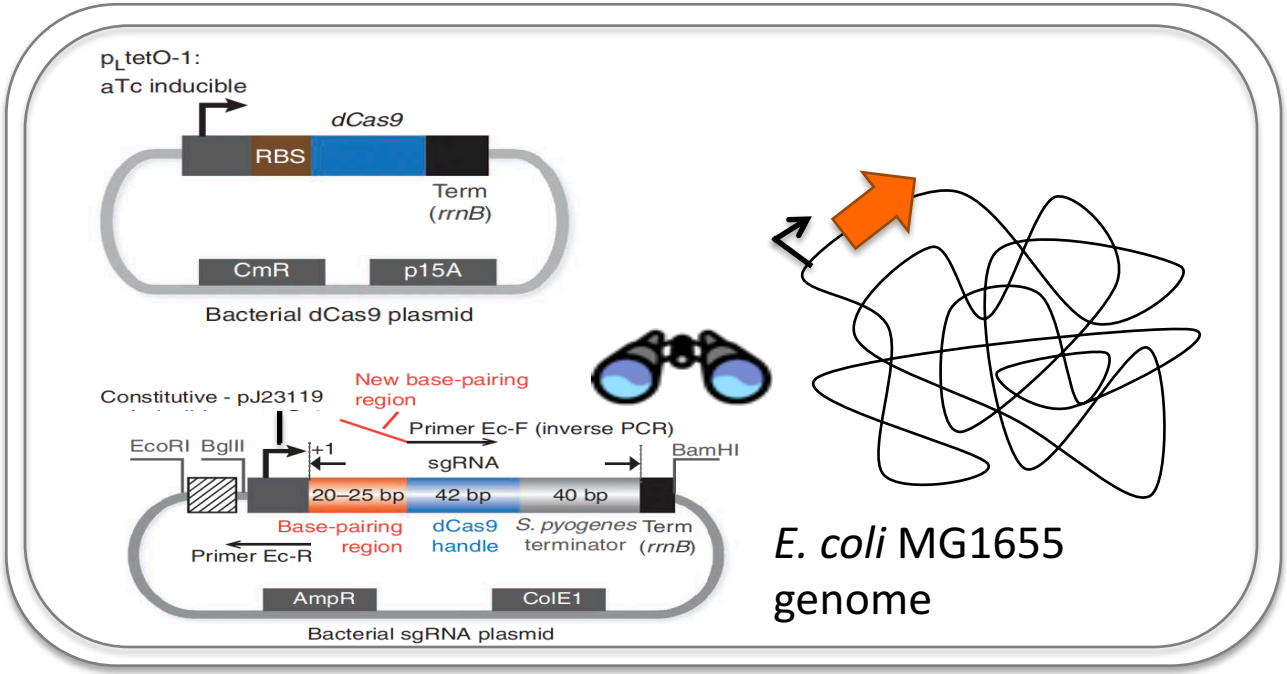


Anhydrotetracycline (aTc)



- Expressed constitutively:
  gRNA
- Expression induced with aTc:
  dCas9

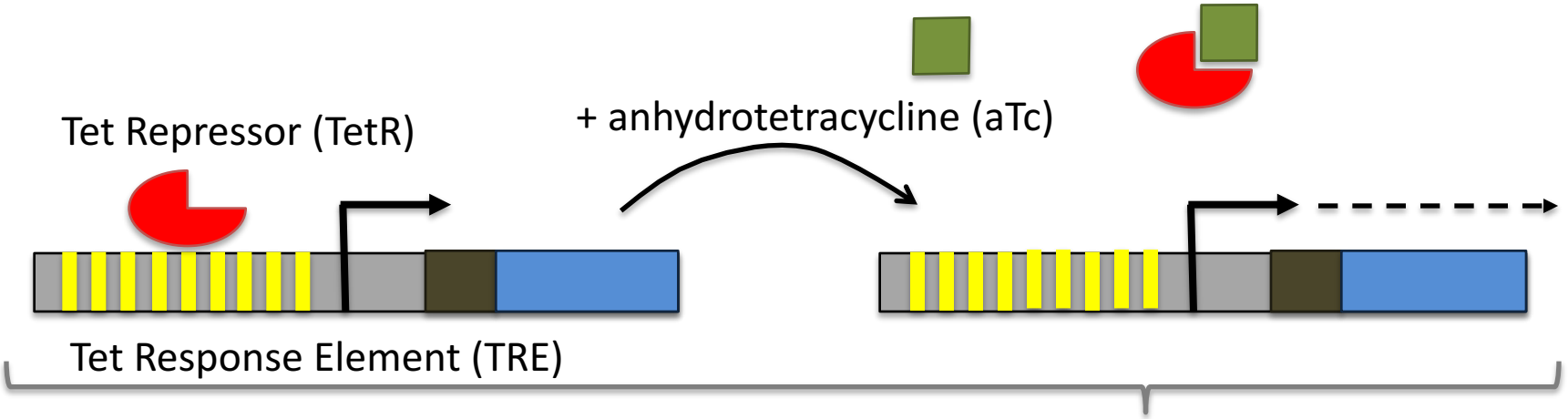
CRISPRi ‘inactive’ in absence of inducer



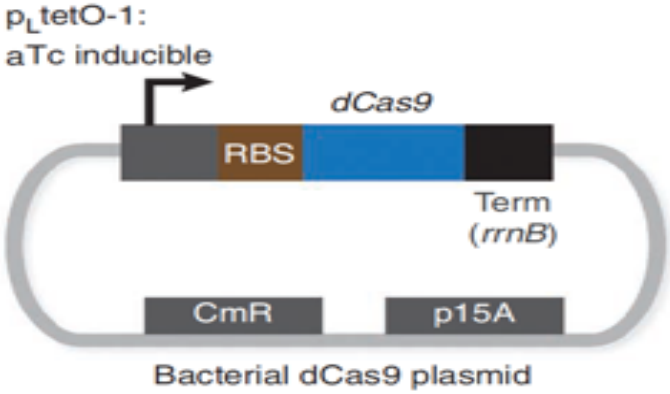
Without aTc

- Only gRNA present
- No (or little) dCas9

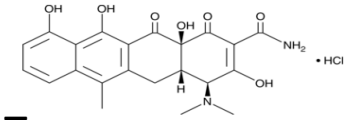
aTc induction of pdCas9



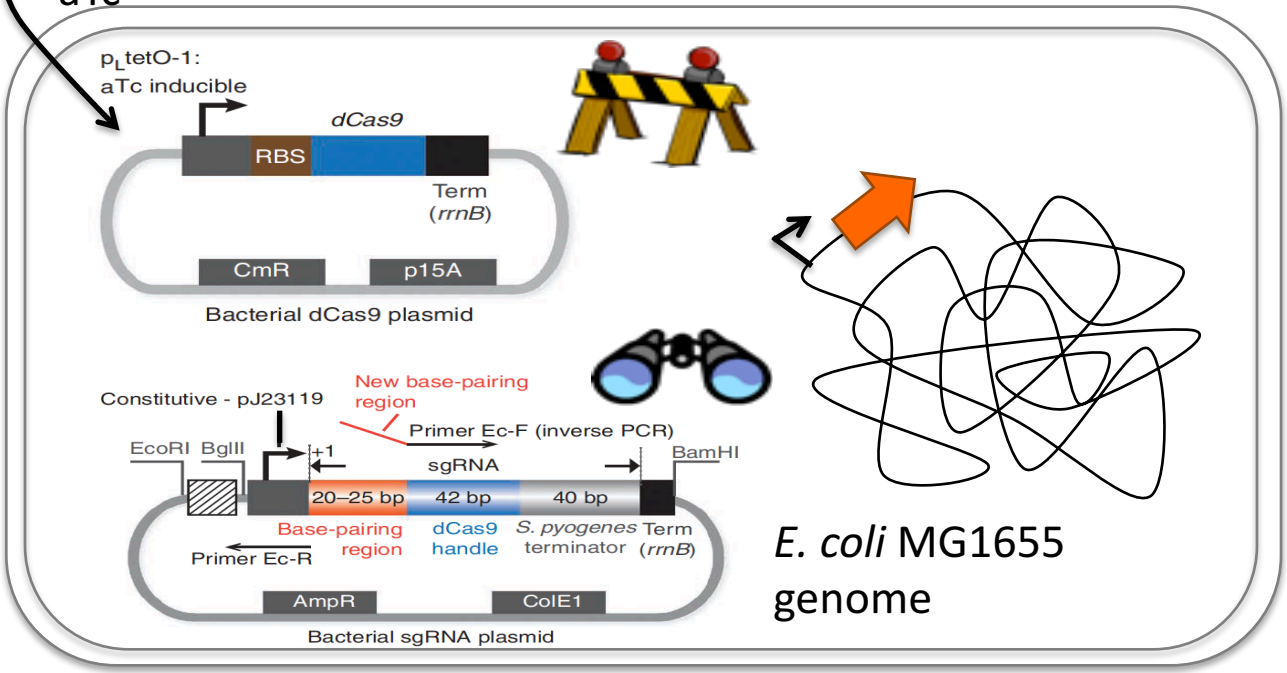
- Tet promoter regulates expression of dCas9 gene



CRISPRi ‘blocks’ gene expression in presence of inducer



aTc



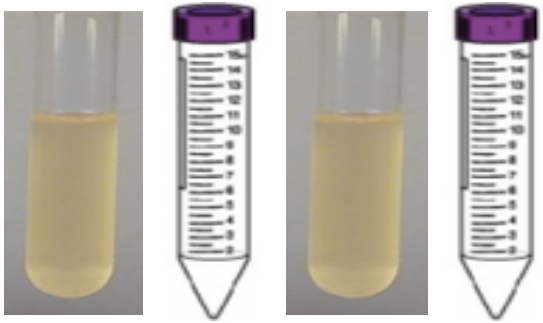
dCas9 protein associates with gRNA/target gene to repress gene expression at your targeted gene

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

- What are the necessary components?
  - LB
  - AMP, CML
  - with O_2, without O_2
  - MG1655 w/ dCas9 + gRNA
  - MG1655 w/no dCas9/gRNA
  - aTC (+/-)
- What control conditions will we have?
  - anaerobic vs. aerobic
  - + atc induced vs - atc uninduced
  - MG1655 alone vs MG1655 + dCas9 + gRNA

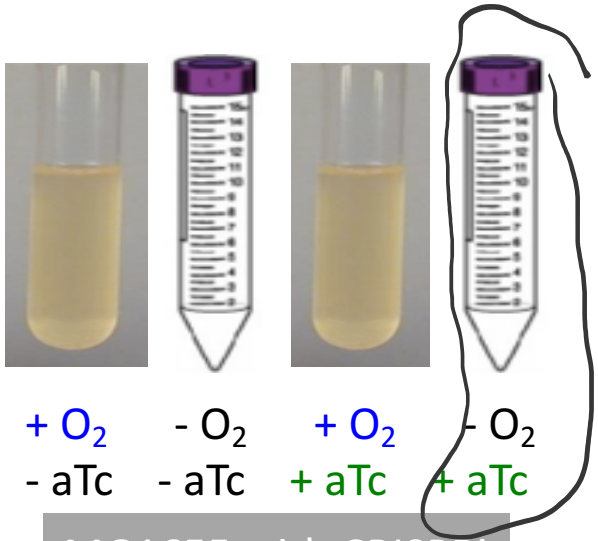
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- Where do we expect most (ethanol or 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

M2D8 Assignments

- Quiz on M2D8, lab notebook due the next day
- HW is Peer-review methods
  - Do not leave today before receiving Methods to peer-review
  - Indicate which part of the methods each comment refers to, type out comments. Scan to submit online or hand in hard copy.

Today in lab…

- Review sequencing information you received via email and align to gRNA plasmid sequence you prepared on M2D5
- Prepare media for mixed-acid fermentation inoculations
- Happy Halloween!