M2D7: Induce CRISPRi system

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

Extra Office Hours

Leslie: 5-7pm
Thurs Nov.7, 16-469

Noreen & Becky: TBA
Sat Nov. 9, 56-302

Additional: Email!

Mod2 Research Report (20% of final grade)

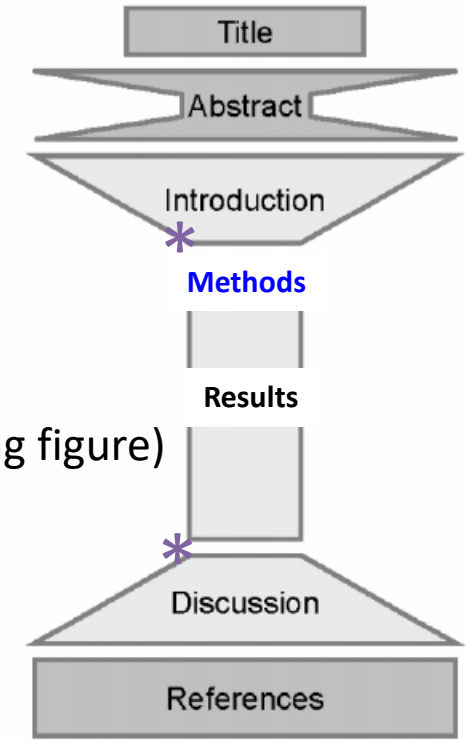
< 14 pages (+ figures, ref.)

2-3 pages

3-4 pages

3-4 pages
(not including figure)

2-3 pages

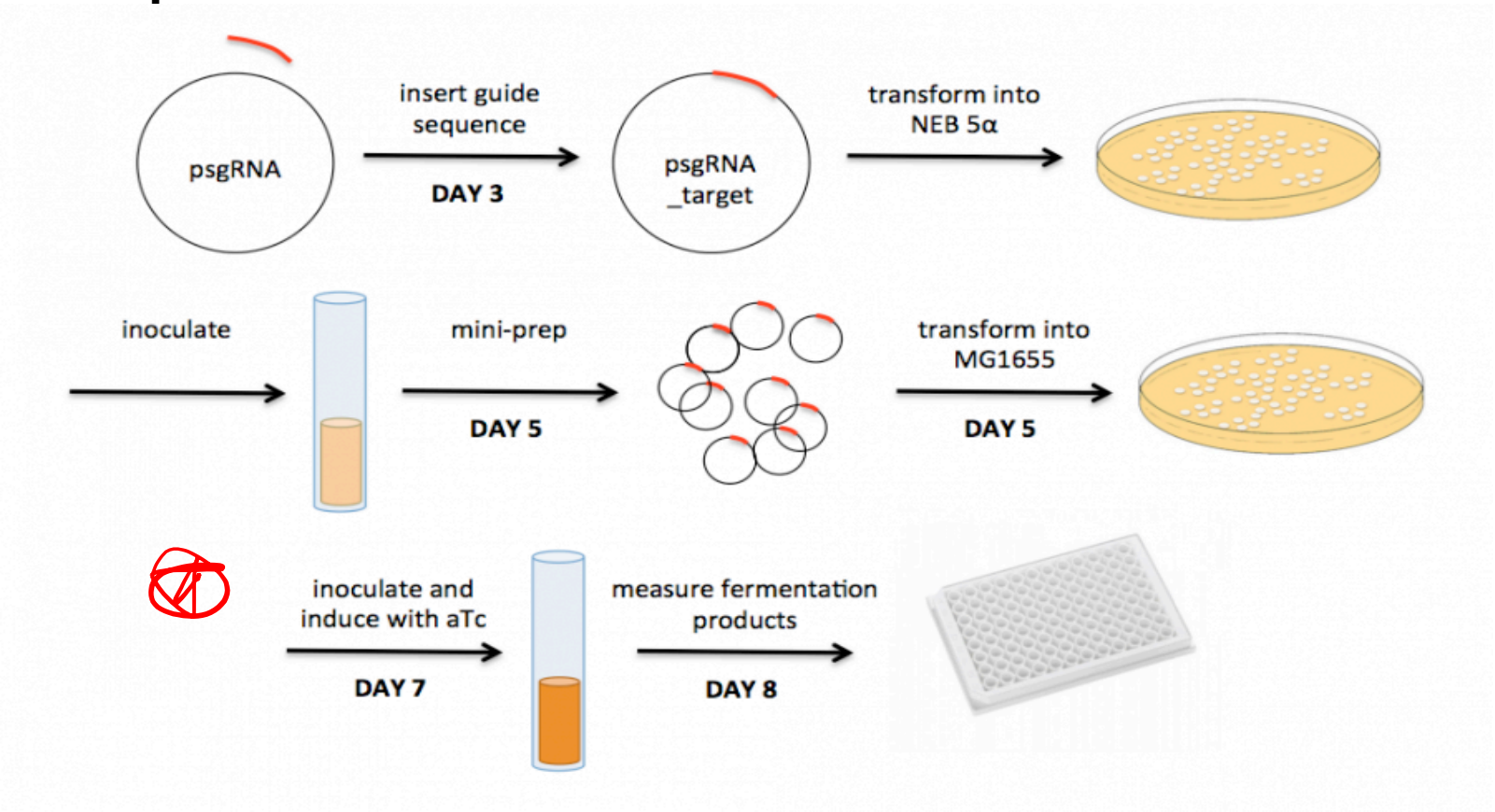


Due Monday 11/11 at 10pm

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

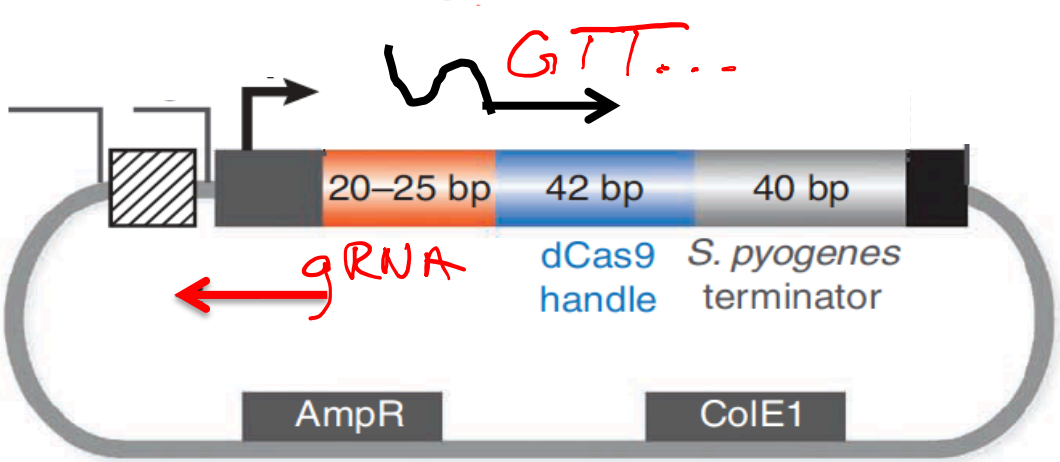
Don't forget:
Blog Post 2, Journal Club due Nov. 1
Blog Post 3, Mod2 due Nov. 12

M2 experimental overview

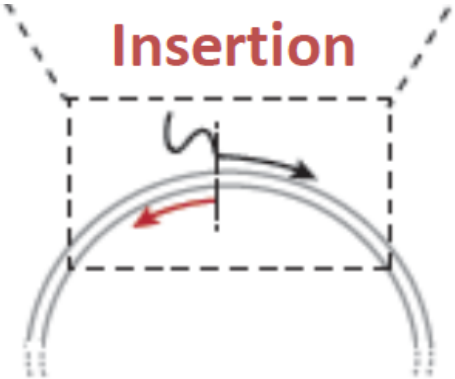


Note: sgRNA = gRNA

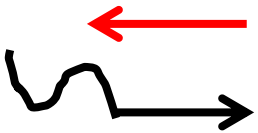
M2D3: Generated pgRNA_target by SDM



pgRNA_template



insertion (NEB5α kit)



CRISPRi universal *amplification* reverse primer

forward primer including crRNA to be inserted ()

dCas9 handle (⟶)

Analyzing Sequence Information

Can show picture of gene sequence & where different gRNA targets

- Remember:
  - You will need to take the reverse complement of you primer to align it with the Reverse Sequencing file
  - Think about where the 5' end is…

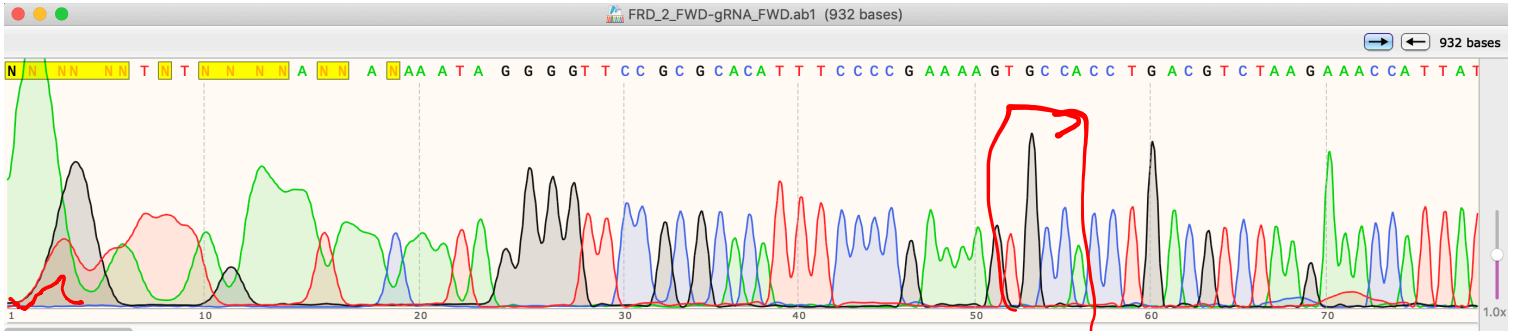
Snapgene does automatically

- Check whether your target sequence was successfully incorporated into the pgRNA_target plamsid

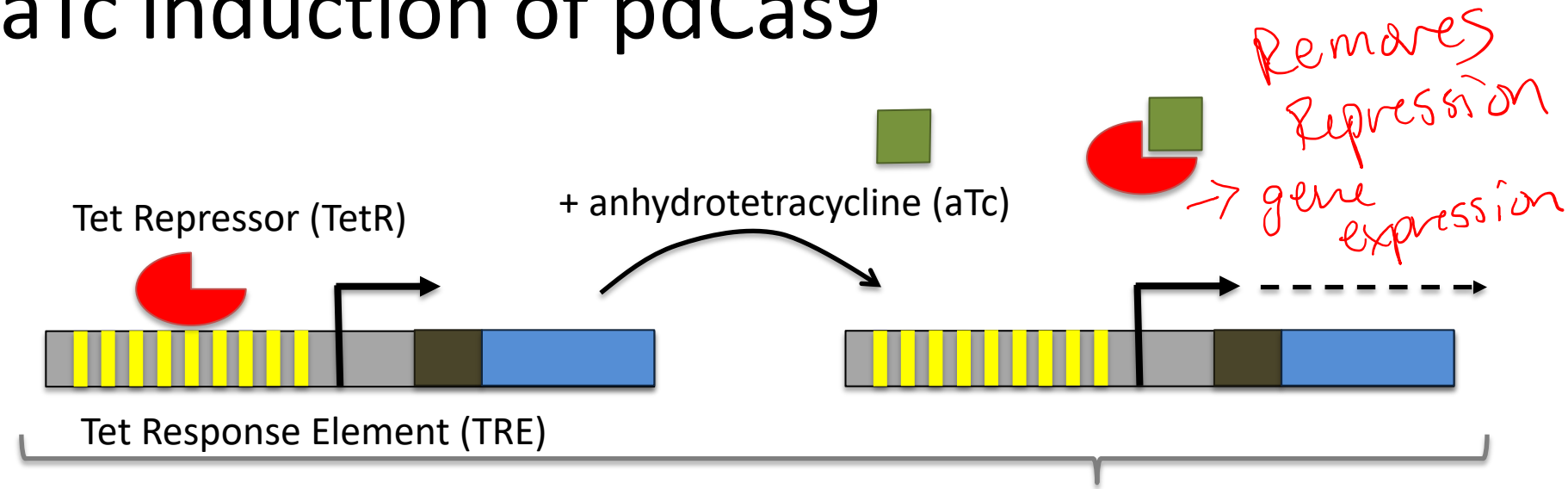
```
 1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt----------gtttagagctagaaatagcaag>73
73>---GAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>269
01>CTGGAATTCTAAAGATCTTTGACAGCTAGCTCAGTCCTAGGTATAATACTAGTAAATCCACTTAAGAAGGTAGGTGTGTTTTAGAGCTAGAAATAGCAAG>500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

- Sanger sequencing traces are also on Dropbox

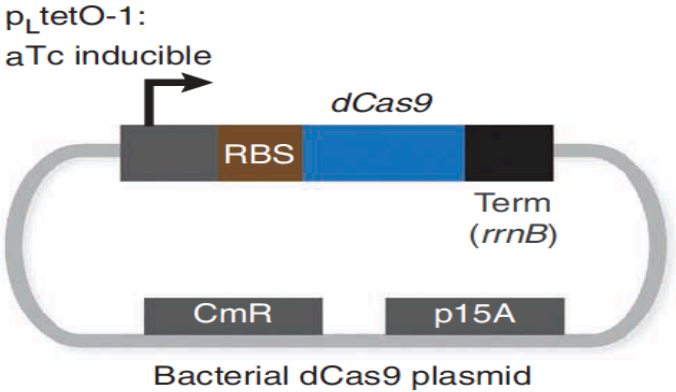
.ab1



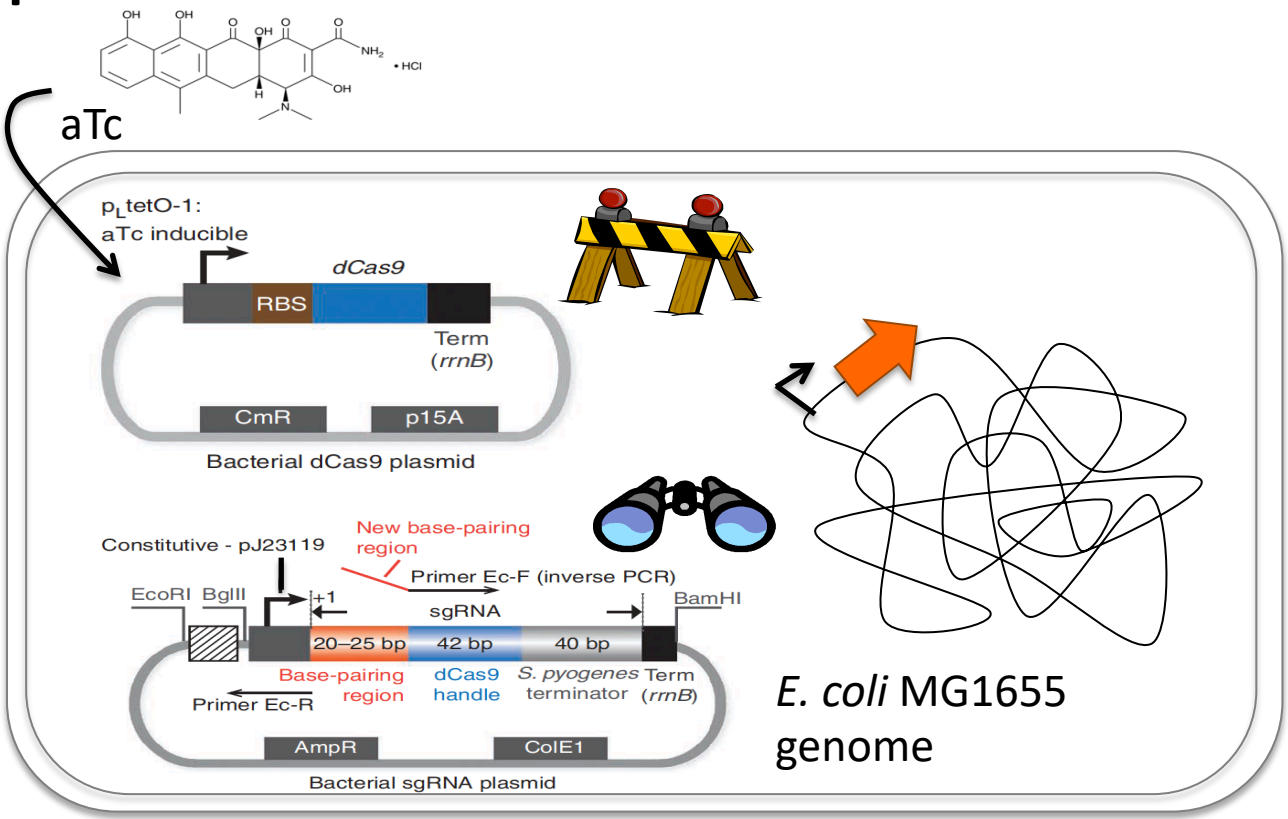
aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene



CRISPRi ‘blocks’ gene expression in presence of inducer



- Expressed constitutively: gRNA
- Expression induced with aTc: pdCas9

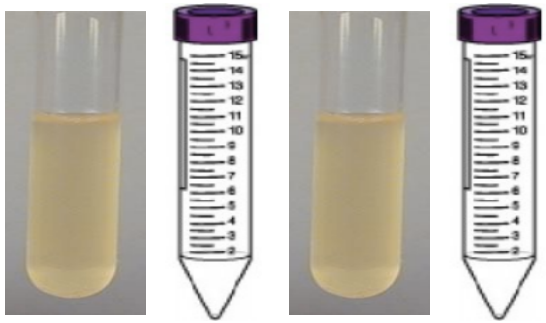
dCas9 protein associates with gRNA/target gene to repress target gene expression

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

- What are the necessary components?
  - gRNA & dCas9
  - Amp, Cmp (Antibiotics)
  - aTc (-/+)
  - MG1655
  - LB
  - +/- O2
- What control conditions will we have?
  - O2
  - aTc
  - CRISPR components

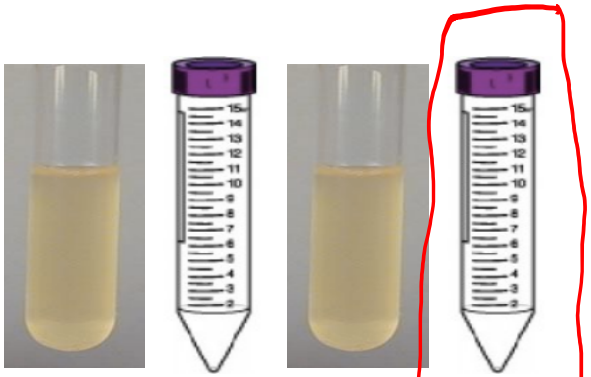
Set up liquid cultures for mixed-acid fermentation and pdCas9 induction

- Where do we expect most ethanol 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 notebooks due that Thursday (next day)
- Peer-review methods If you submitted by 1:05pm today
  - Do not leave today before receiving Methods to peer-review (hard copy)
  - Turn in to Becky– not the student you have reviewed
  - Indicate which part each comment refers to, type out comments, and scan to submit online, or hand in hard copy.

Today in lab…

- Download your sequencing data from the Dropbox folder and align (using Snapgene software) or Benchling with Colin
- Prepare media for mixed-acid fermentation inoculations