

M2D7:  
Check pgRNA sequences  
Induce CRISPRi system

11/04/2016



## Key assignments of M2

- ✓ Journal club presentation
  - participation points for asking questions
- Research article (20%)
  - due 5pm on Sunday, November 20
  - graded by Dr. Noreen Lyell
  - **use office hours to get input of your draft**
- Blog post(s)
  - due 5pm on Monday, November 21
  - about journal club? about second manuscript?
- Lab notebook



# Extra office hours

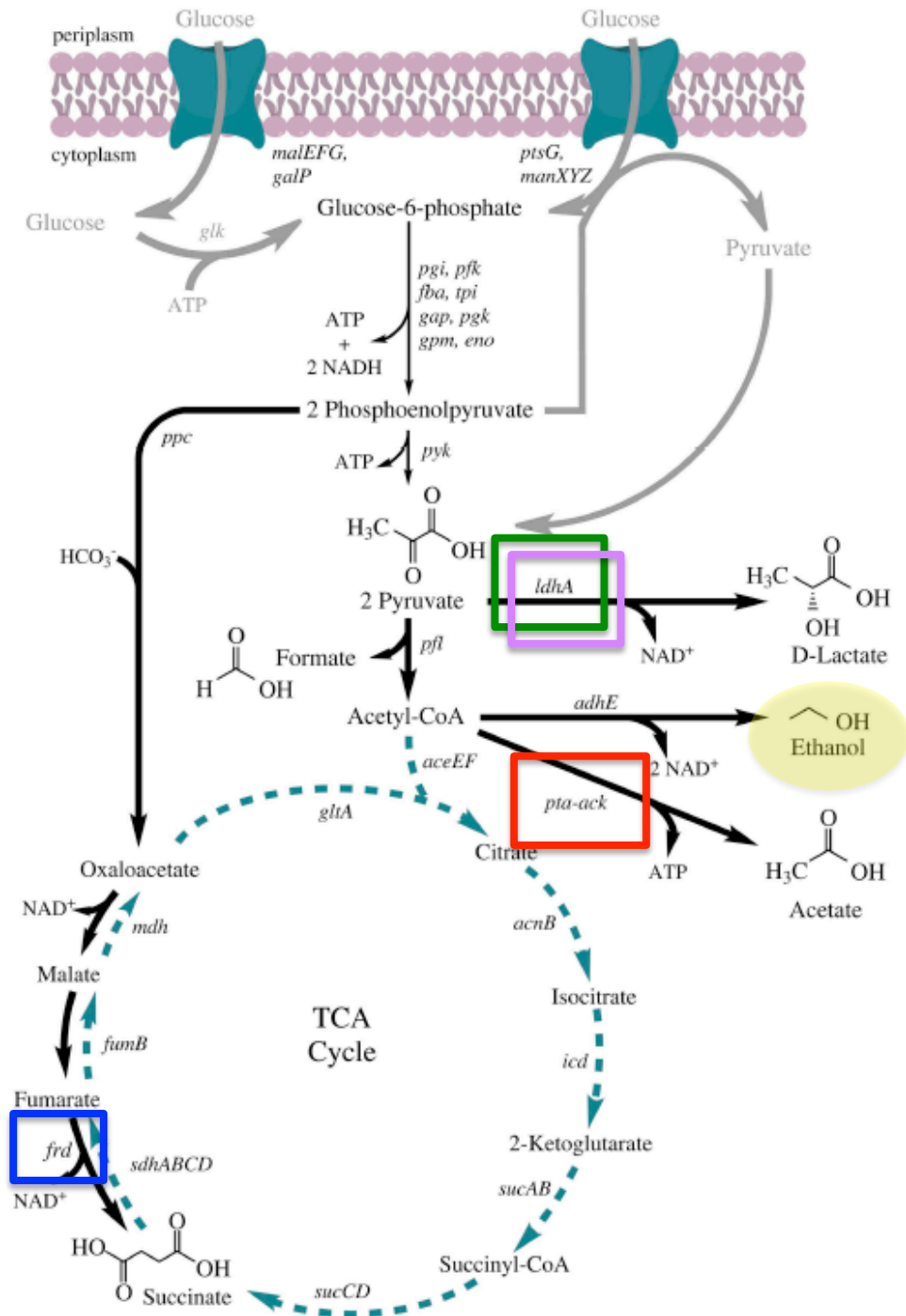


- Mondays
  - Noreen 1pm + 5pm in 16-317
  - Maxine 2pm in 16-239
  - Leslie 4pm in 16-429b
- Wednesdays
  - Leslie 9am in 16-429b
- Fridays
  - Maxine 9am in 16-239
- Saturday, **11/19**
  - **10am – 5pm in 56-302**
- [email](#) us for other times!

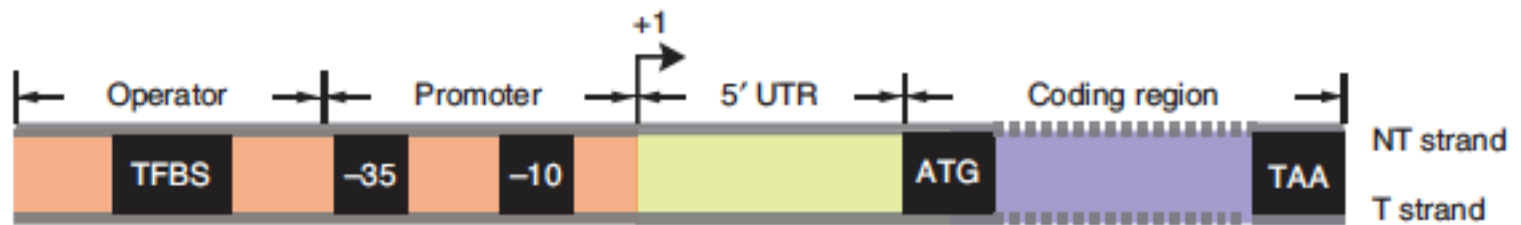
# M2: used CRISPRi

Hypothesis:

- ethanol production by *E. coli* fermentation can be increased by blocking transcription of one enzyme involved in generating alternate products

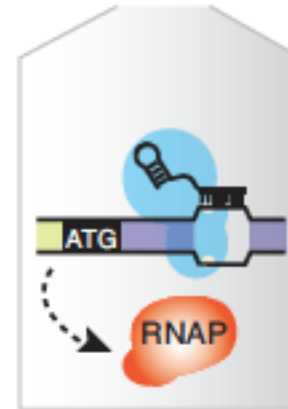
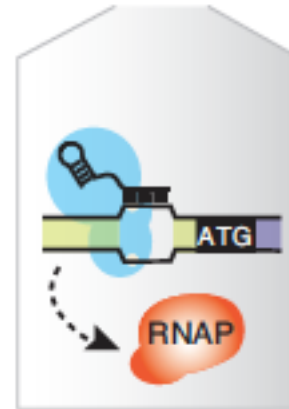
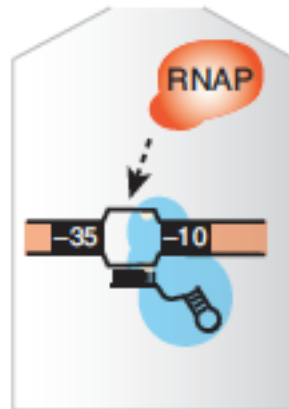
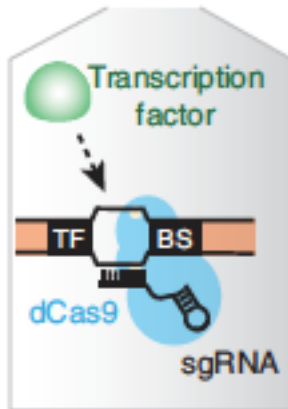


# M2D2: Designed gRNA for CRISPRi system



Block transcription initiation

Block transcription elongation



Effective for both NT and T strands

Effective only for the NT strand

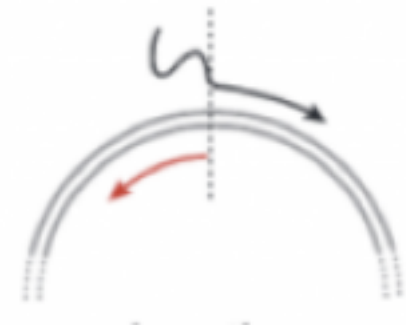
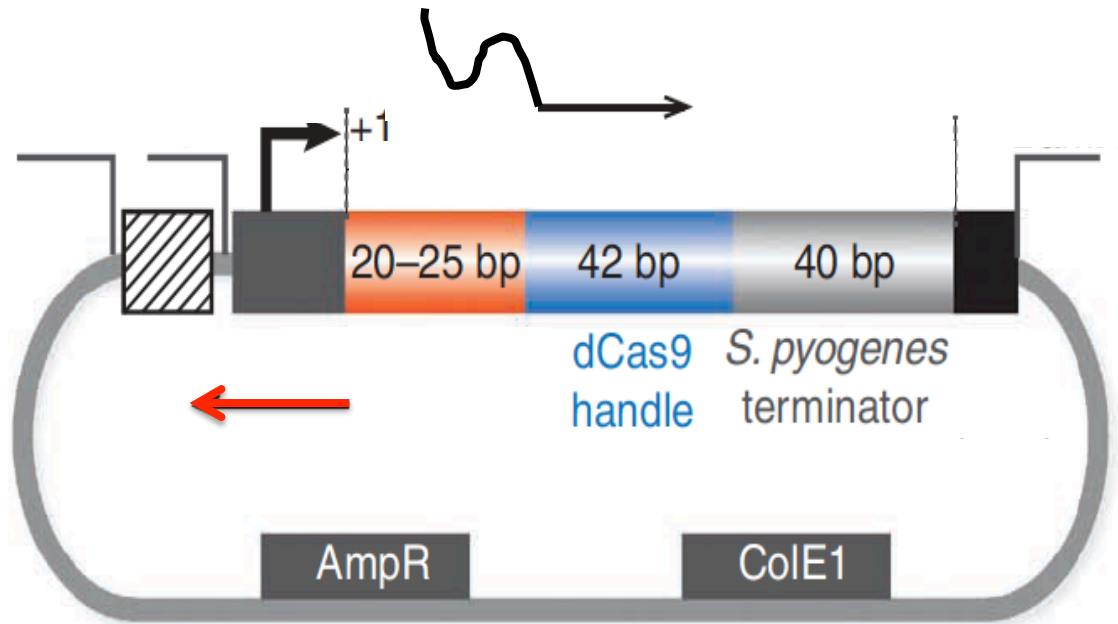
# Please add your targeting info to the wiki today

- M2 overview page, [discussion tab](#)  
[http://engineerbiology.org/wiki/Talk:20.109\(F16\):Module\\_2](http://engineerbiology.org/wiki/Talk:20.109(F16):Module_2)

**W/F: ethanol** [\[edit\]](#)

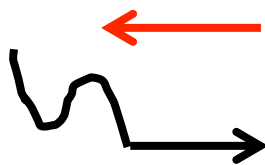
Team	Gene targeted by CRISPRi gRNA	gRNA sequence (without tag at 3' end)	Locus targeted (e.g. beginning of gene, putative promoter, -35 region)
red	pta-ack	GCC ACG TAT CAA TTA TAG GTA C	
green	ldhA	GTAGCTTAAATGTGATTCAACATC	
blue	fdrA	CAAGATCGGCTTGAAAGGTTTGCA	
purple	ldhA	TCGTACTGTTTTGTGCTATAAA	

# M2D3: Generated pgRNA\_target by SDM



pgRNA\_template

insertion (NEB5α kit)



CRISPRi universal *amplification* reverse primer

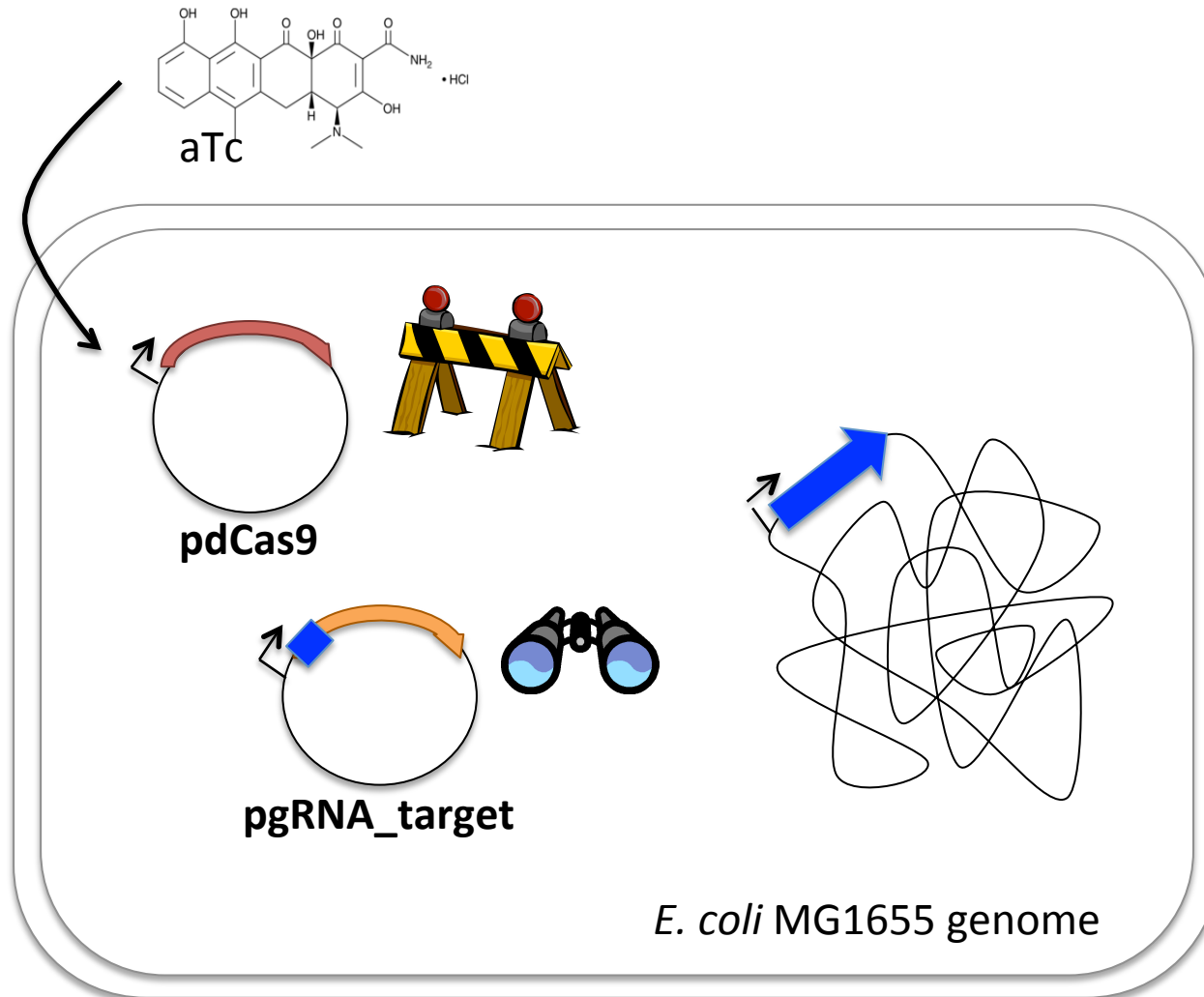
forward primer including crRNA to be inserted (  )

dCas9 handle (  )



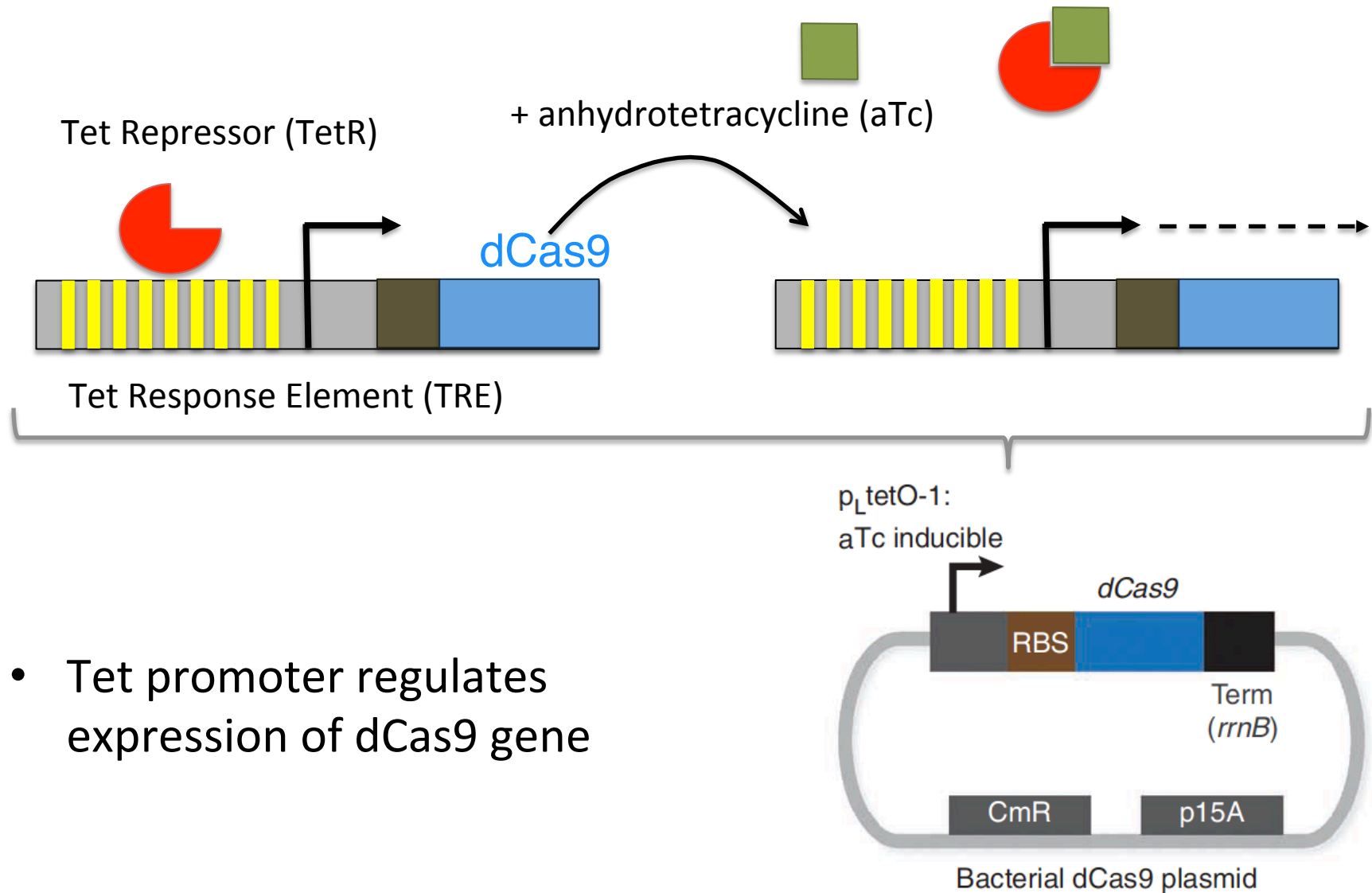


# CRISPRi system overview



- target gene
- pgRNA\_target
  - promoter
  - constitutively ON
- pdCas9
  - promoter
  - aTc inducible

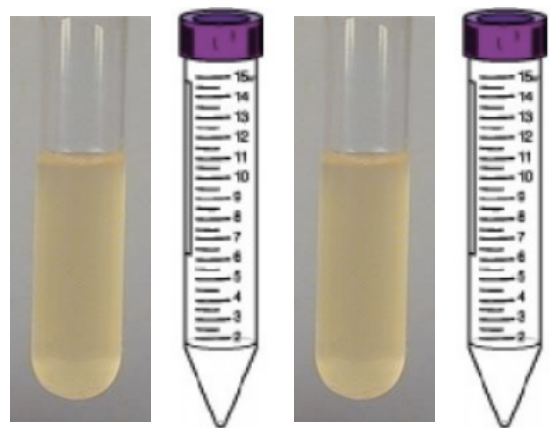
# aTc induction of pdCas9



- Tet promoter regulates expression of dCas9 gene

# Media for mixed-acid fermentation and pdCas9 induction

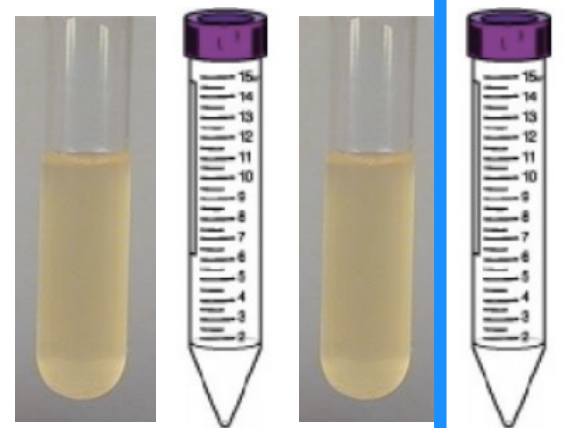
- What are the necessary components?
- Where do we expect most ethanol if hypothesis confirmed?



+ O<sub>2</sub>   - O<sub>2</sub>   + O<sub>2</sub>   - O<sub>2</sub>  
- aTc   - aTc   + aTc   + aTc

MG1655

LB



+ O<sub>2</sub>   - O<sub>2</sub>   + O<sub>2</sub>   - O<sub>2</sub>  
- aTc   - aTc   + aTc   + aTc

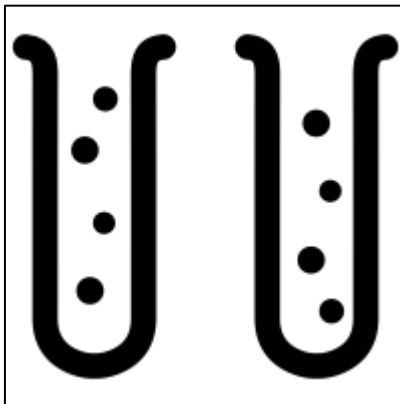
MG1655 with CRISPRi

LB + Amp + Cam

# Today in lab...



- Download your sequencing data and align using ApE software



- Prepare media for mixed-acid fermentation inoculations