M2D7: Induce CRISPRi system

11/2/17

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

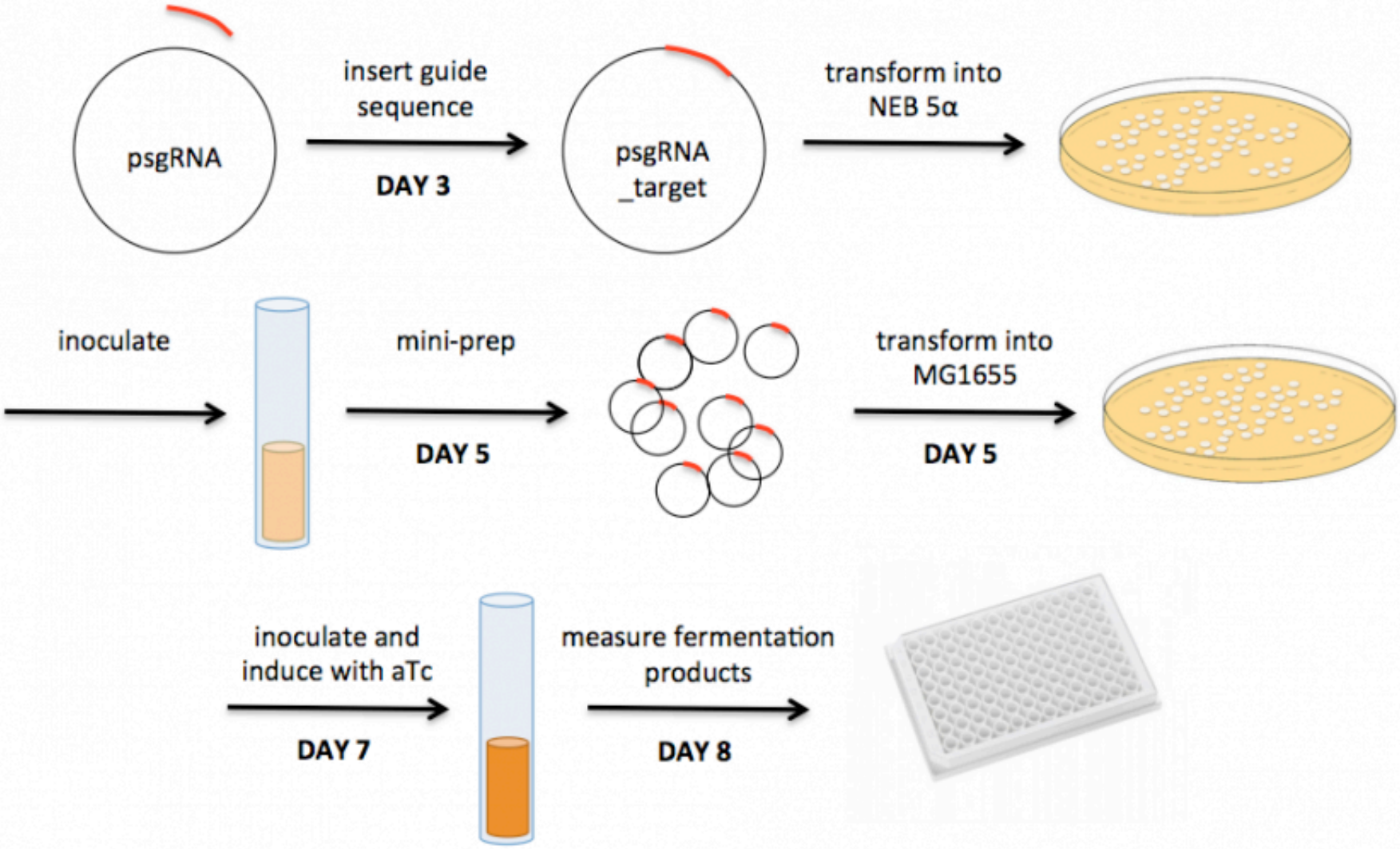
Extra office hours

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

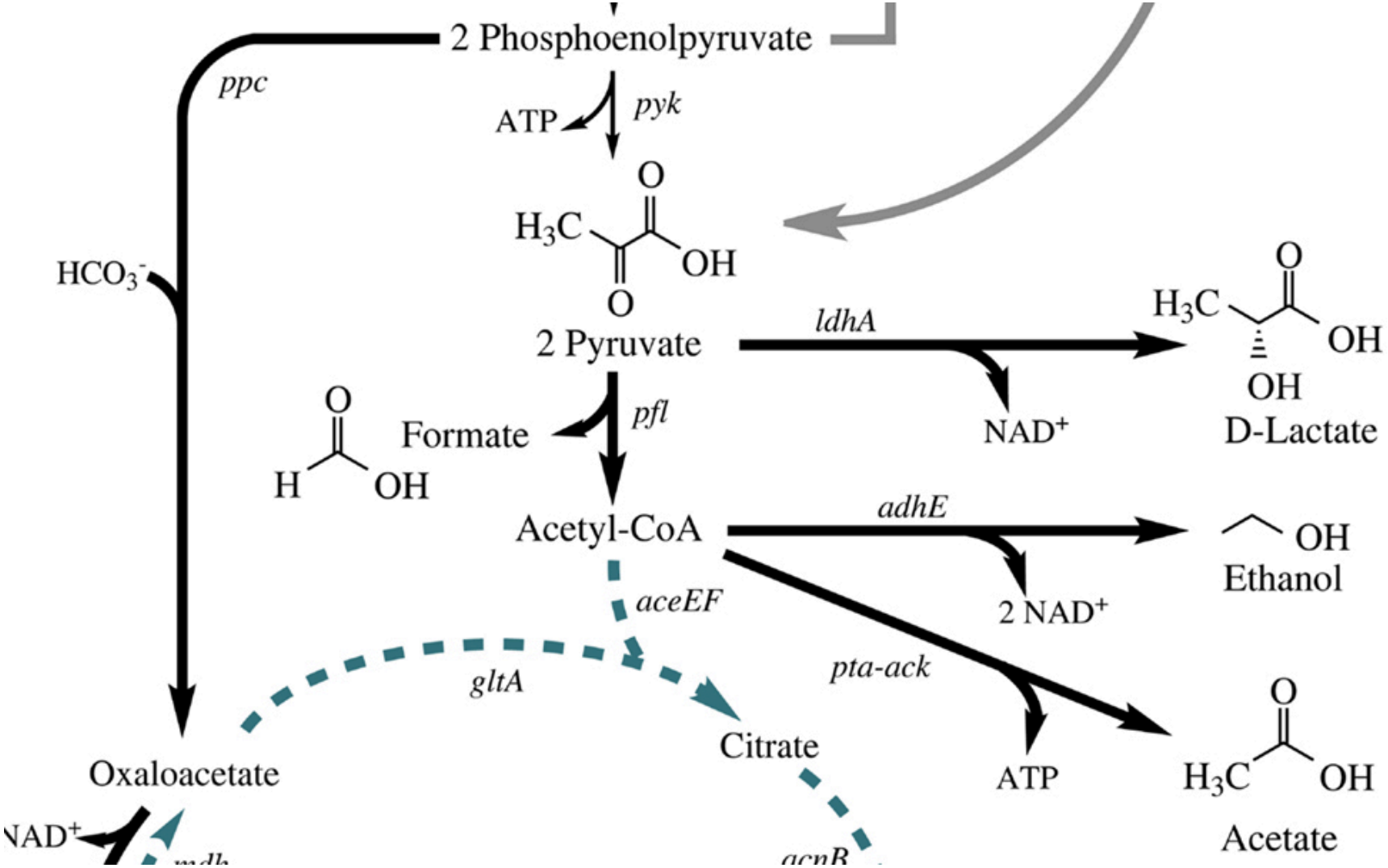
Regular office hours

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

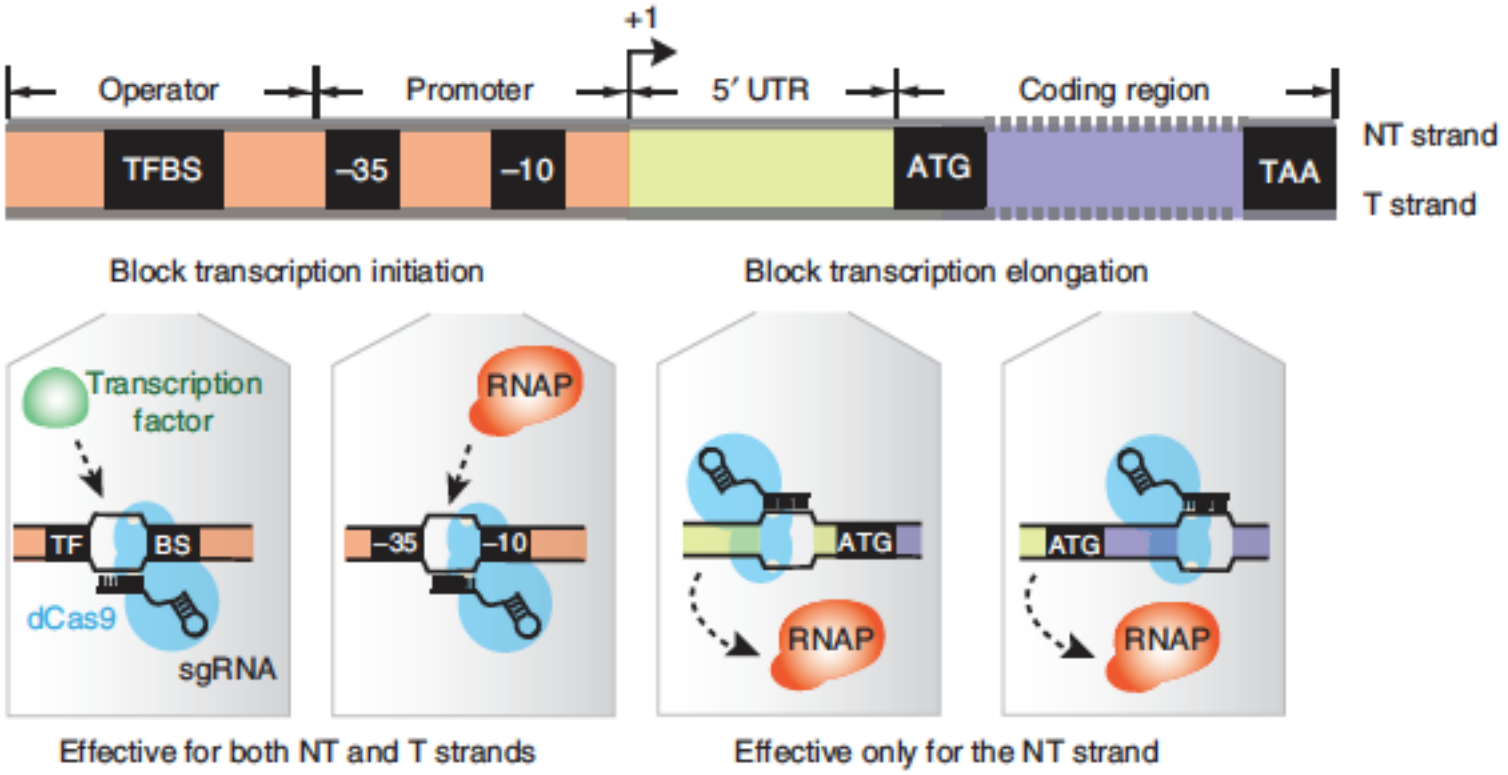
M2 experimental overview



Using CRISPRi manipulate the *E. coli* fermentation pathway



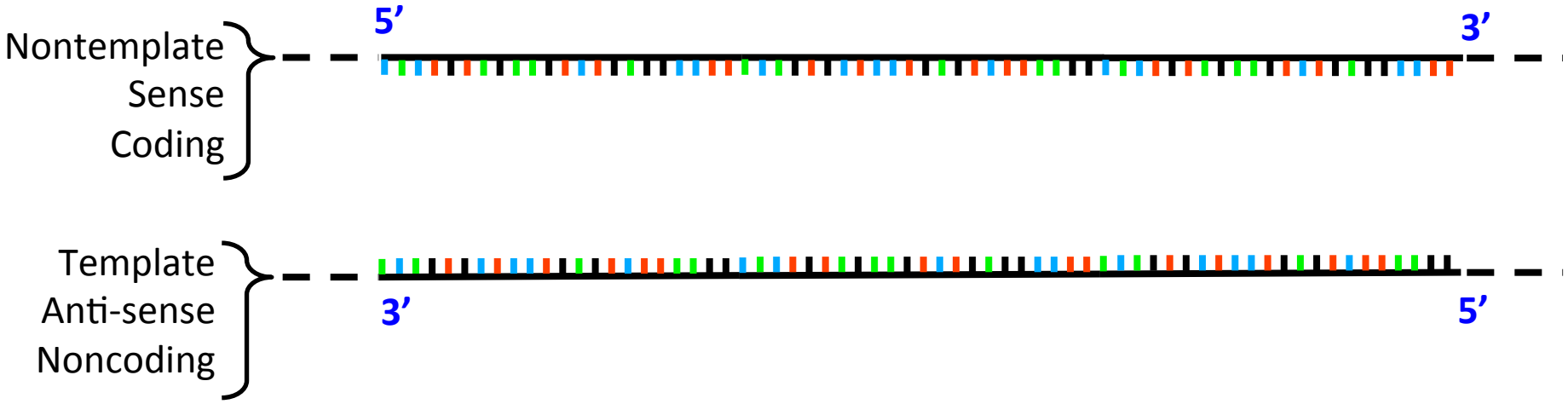
Design of gRNA for CRISPRi system



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.



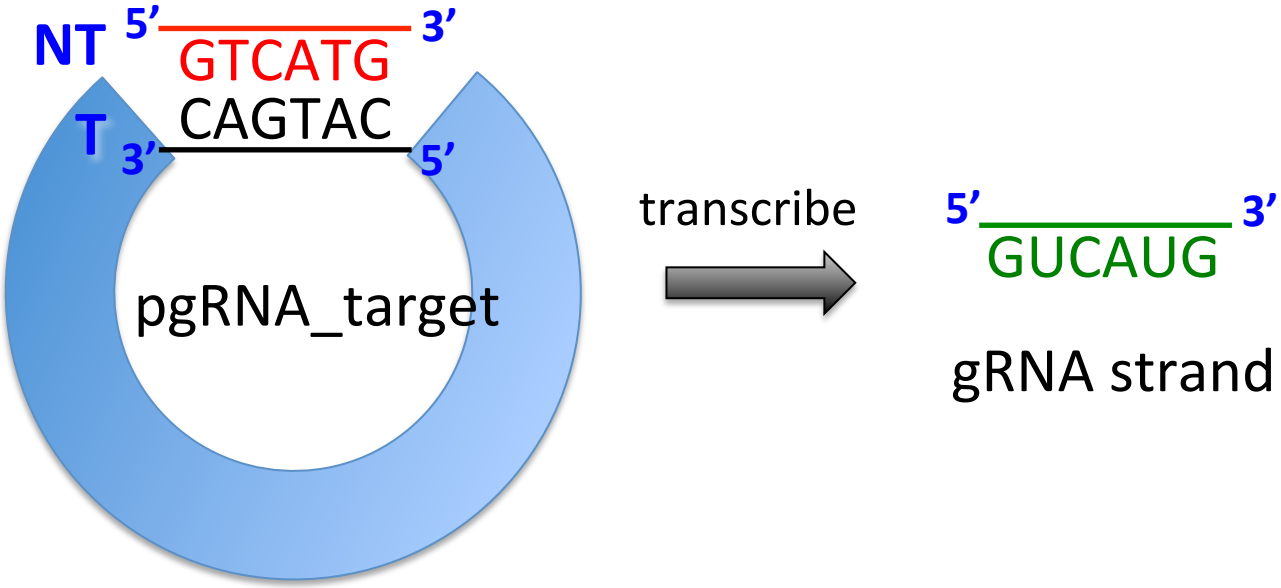
Confirm gRNA design rules (1)

Gene of interest

Nontemplate (NT) 5’ GTCATG 3’

Template (T) 3’ CAGTAC 5’

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



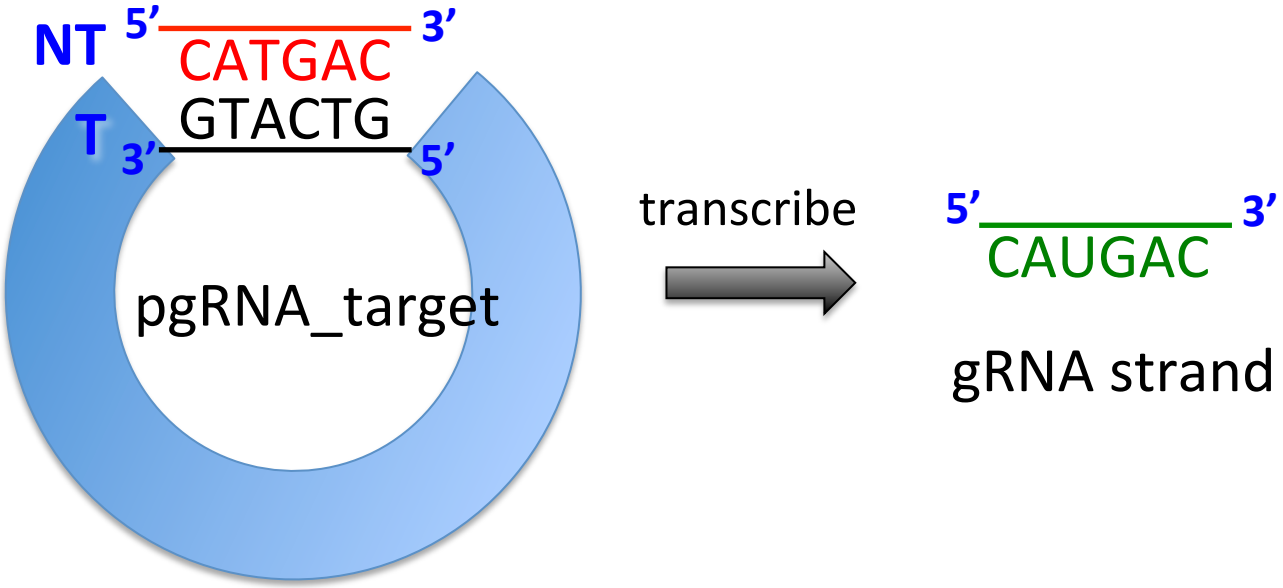
Confirm gRNA design rules (2)

Gene of interest

Nontemplate (NT) 5’ GTCATG 3’

Template (T) 3’ CAGTAC 5’

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

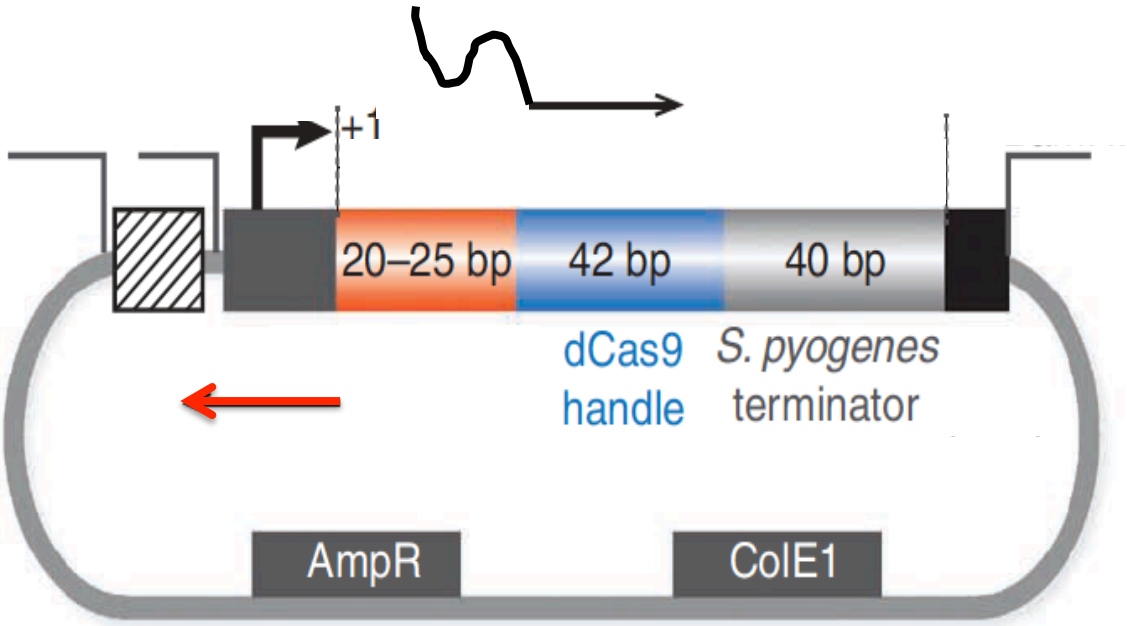
W/F

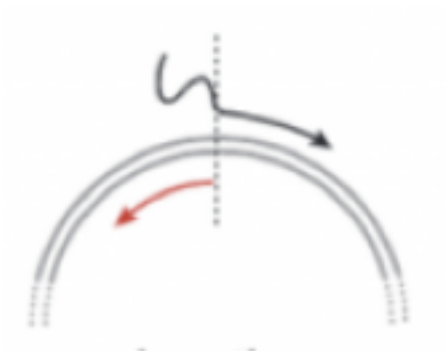
Team	Ethanol (E) or Acetate (A)	Gene targeted by CRISPRi gRNA	gRNA sequence end)
red	Ethanol	ack (indirectly, pta)	GTTTTTTTAGCC
orange	Ethanol	ldhA	ATTCAACATCAC
blue	Ethanol	ackA	TTTTTAGCCACG

...

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

- Remember to reverse and complement your reverse primer sequence before alignment
- Consider importing your entire gRNA expression vector seq. with your target sequence if alignment is not working well

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                 *         *         *         *         *         *         *         *         *         *
   0>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~>0
  72>---------------------------------------------------------------------------------------------------->172
  01>AAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGATTT>400
   0>~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~>0

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

           *         *         *         *         *         *         *         *         *         *
  74>ttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgaagcttgggcccgaacaaaaactcatctcagaagaggat>173
  70>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTGAAGCTTGGGCCCGAACAAAAACTCATCTCAGAAGAGGAT>369
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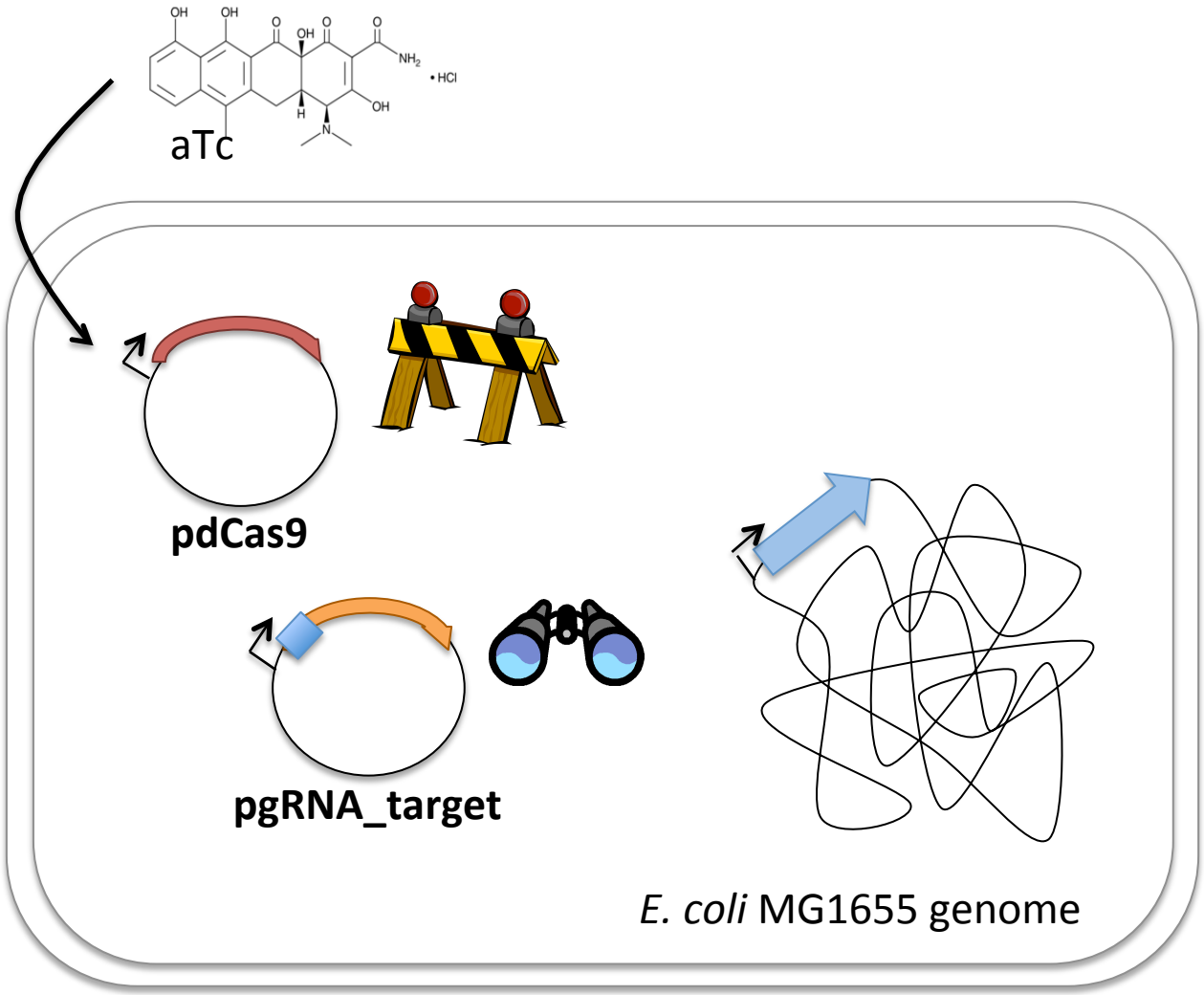
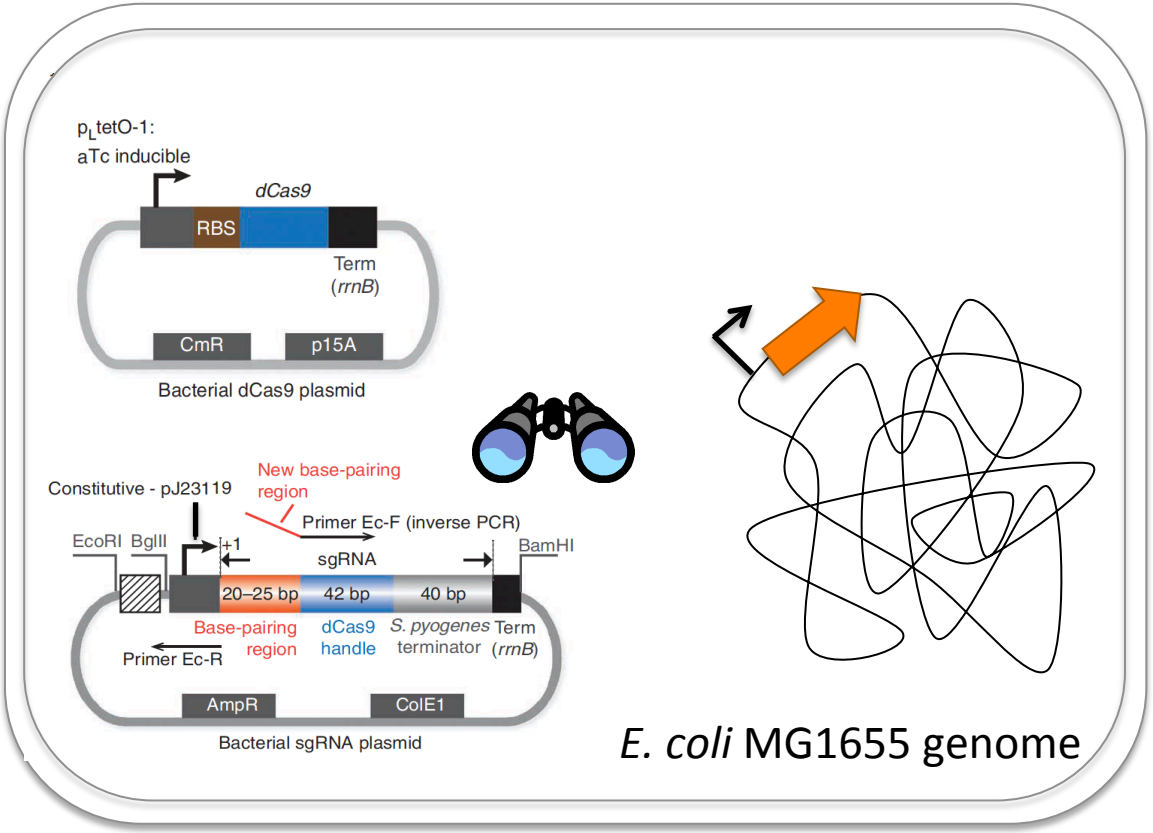
  74>ttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgctttttttgaagcttgggcccgaacaaaaactcatctcagaagaggat>173
```

CRISPRi system overview



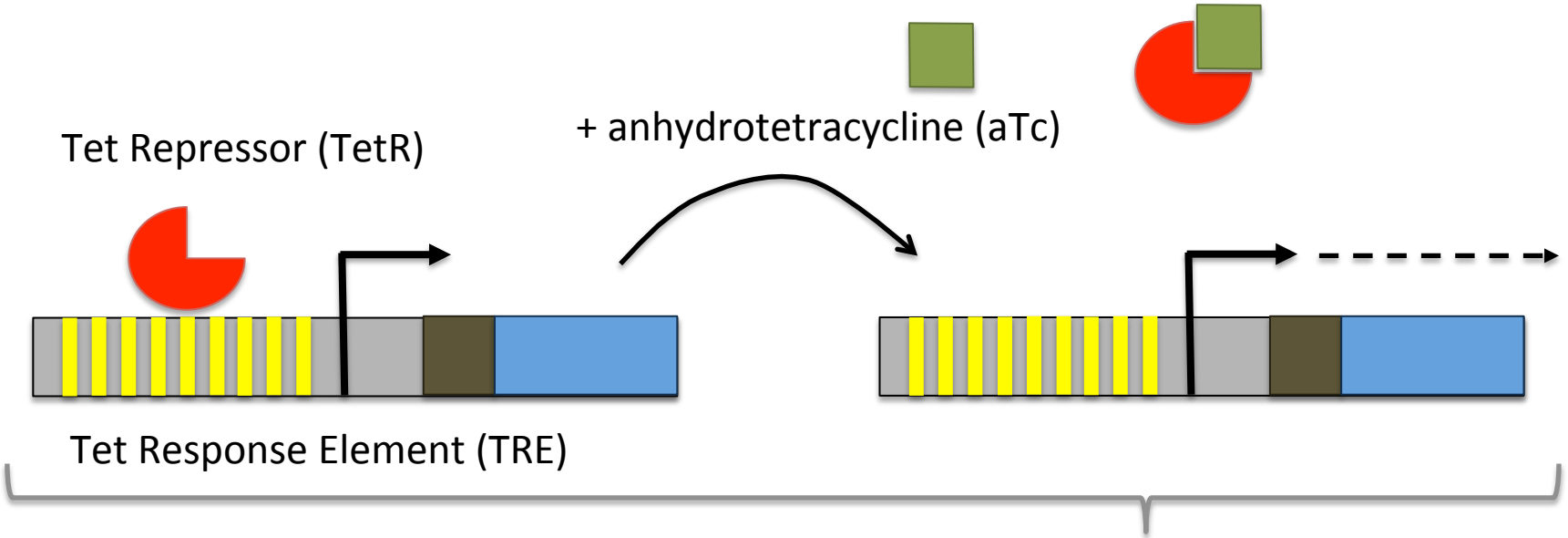
- Target gene
- pgRNA_target
- pdCas9

CRISPRi 'inactive' in absence of inducer

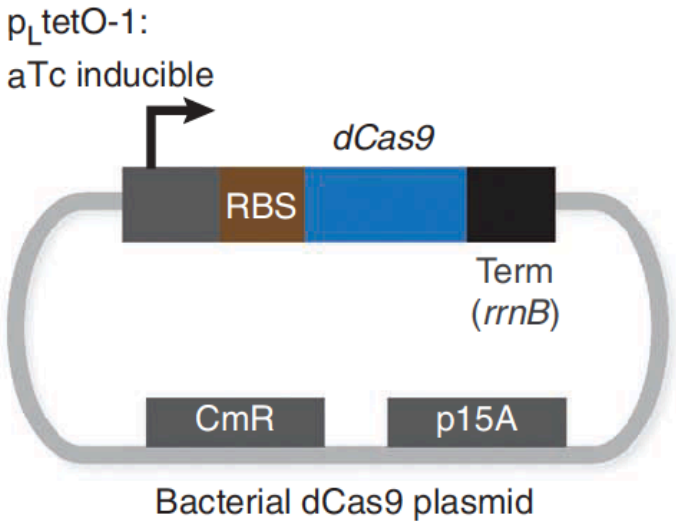


- pgRNA_target expressed constitutively
  - Always transcribed and binding to target gene

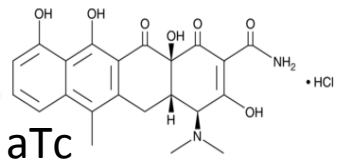
aTc induction of pdCas9

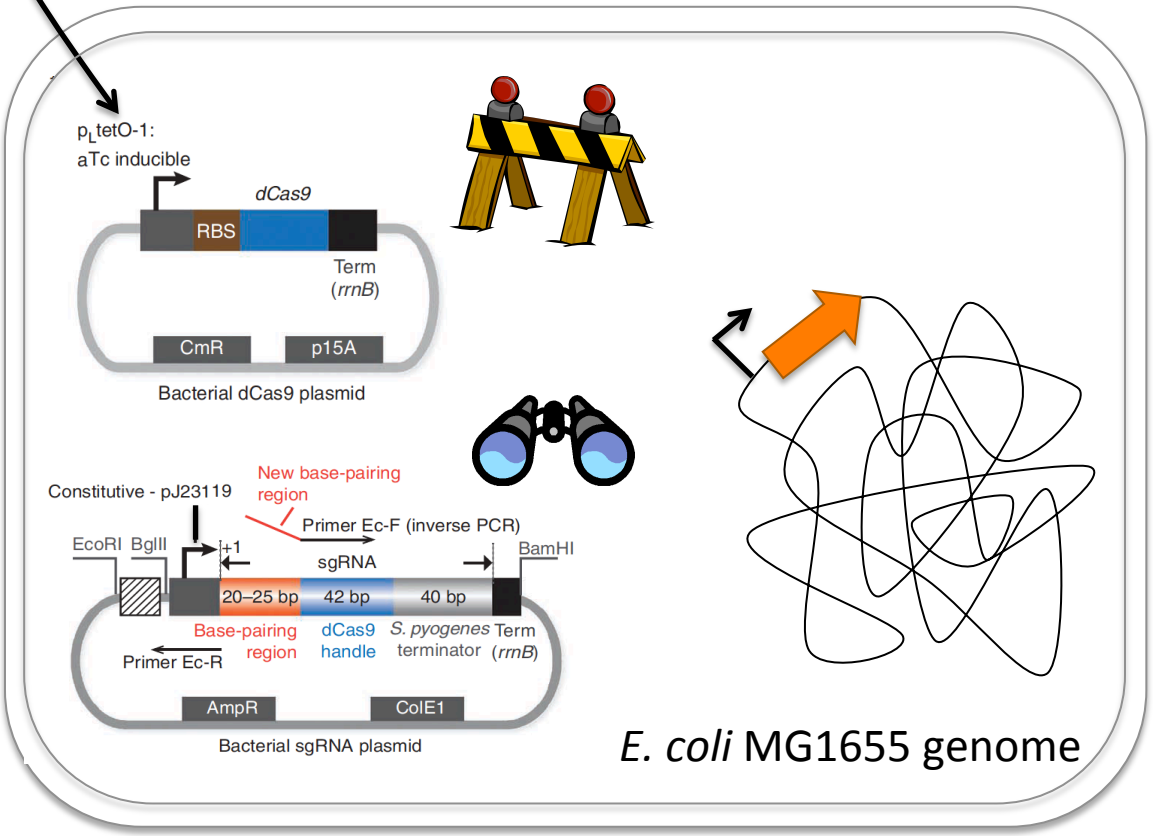


- Tet promoter regulates expression of dCas9 gene



CRISPRi ‘blocks’ gene expression in presence of inducer





- pdCas9 expressed when aTc added
  - When transcribed associates with pgRNA_target / target gene

Media for mixed-acid fermentation and pdCas9 induction

- What are the necessary components?

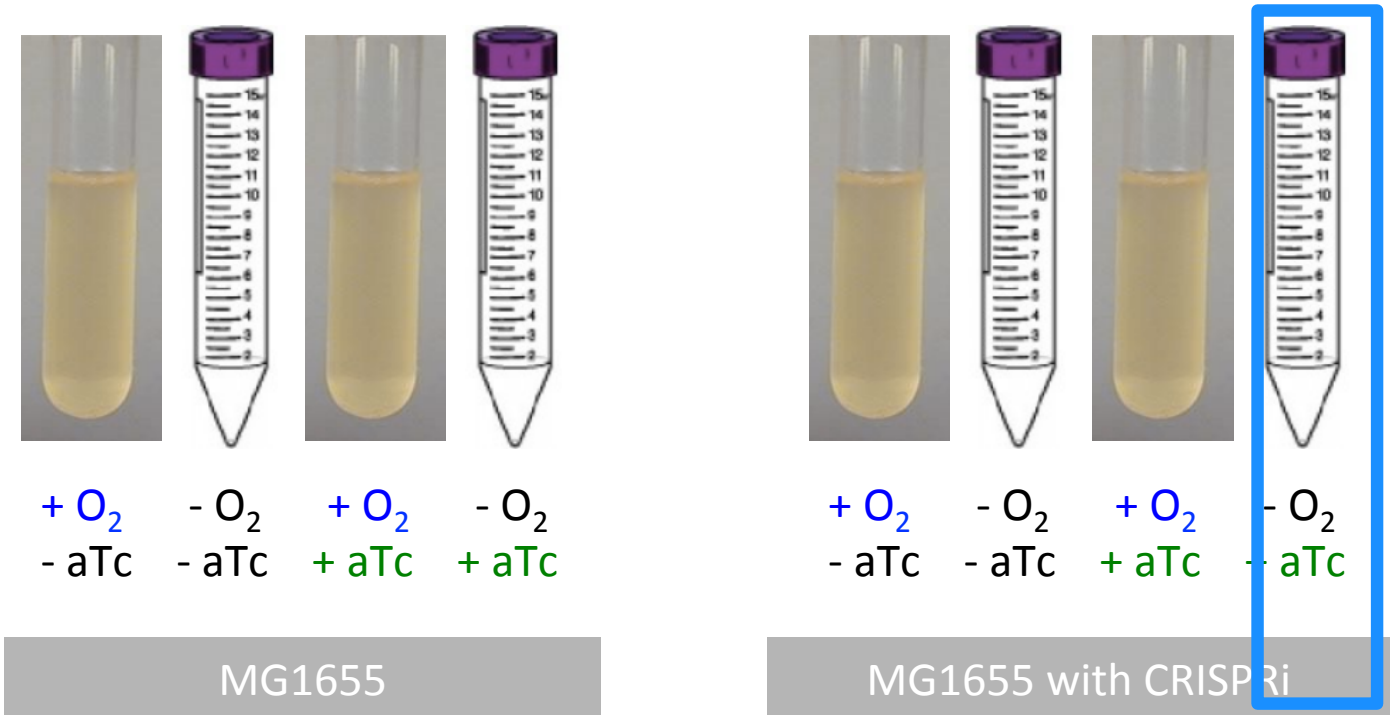
LB

Antibiotics - AMP, CAM

aTc

Media for mixed-acid fermentation and pdCas9 induction

- Where do we expect most ethanol if hypothesis confirmed?



Today in lab…

- Download your sequencing data from discussion tab and align using ApE software
- Prepare media for mixed-acid fermentation inoculations

M2D8 Assignments

- Quiz on Tues (M2D8) Nov. 7th
- Peer-review (see wiki for details)