M2D7: Induce CRISPRi system

11/3/16

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

Major assignments for M2

- Research Article (20% of grade)
  - due by 5pm on Sun., 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 M1 due by 10pm on Mon., Nov. 21st

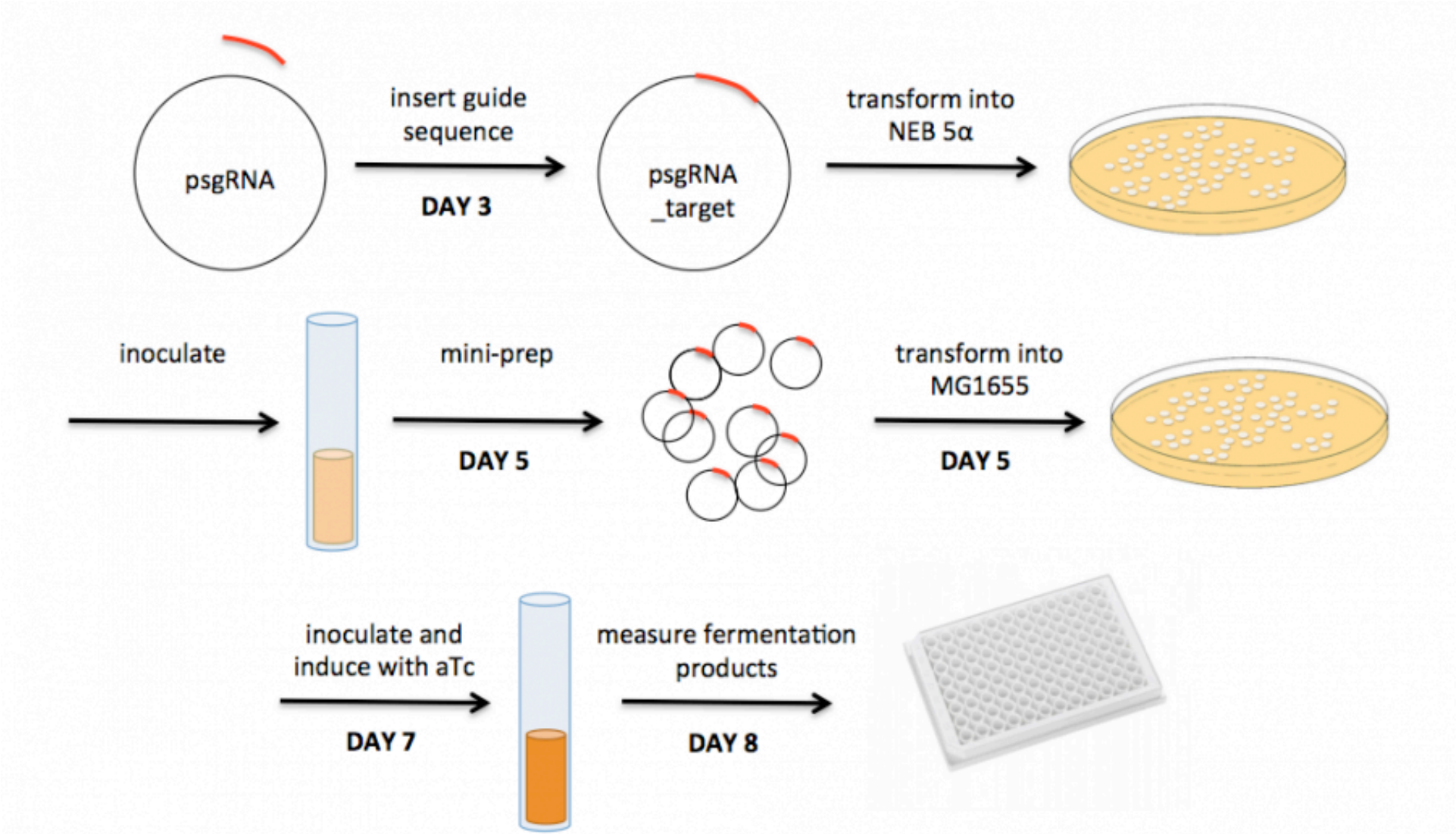
Extra office hours

- Saturday Nov. 19th 10am-5pm

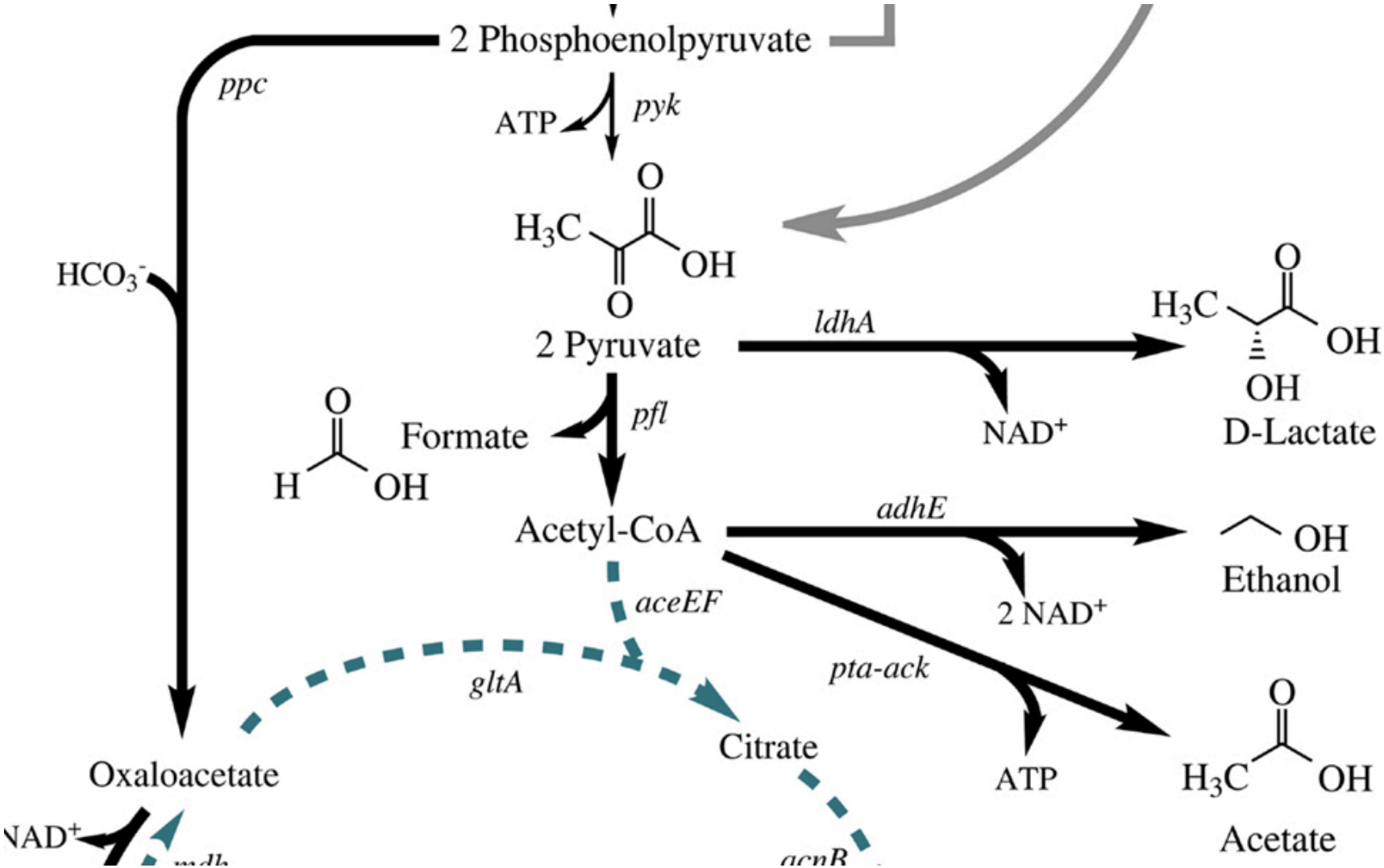
Regular office hours

- Noreen: Monday 1pm and 5pm in 16-317
- Leslie: Monday 4pm, Wednesday 9am in 16-429b
- Maxine: Monday 2pm, Friday 9am in 16-239

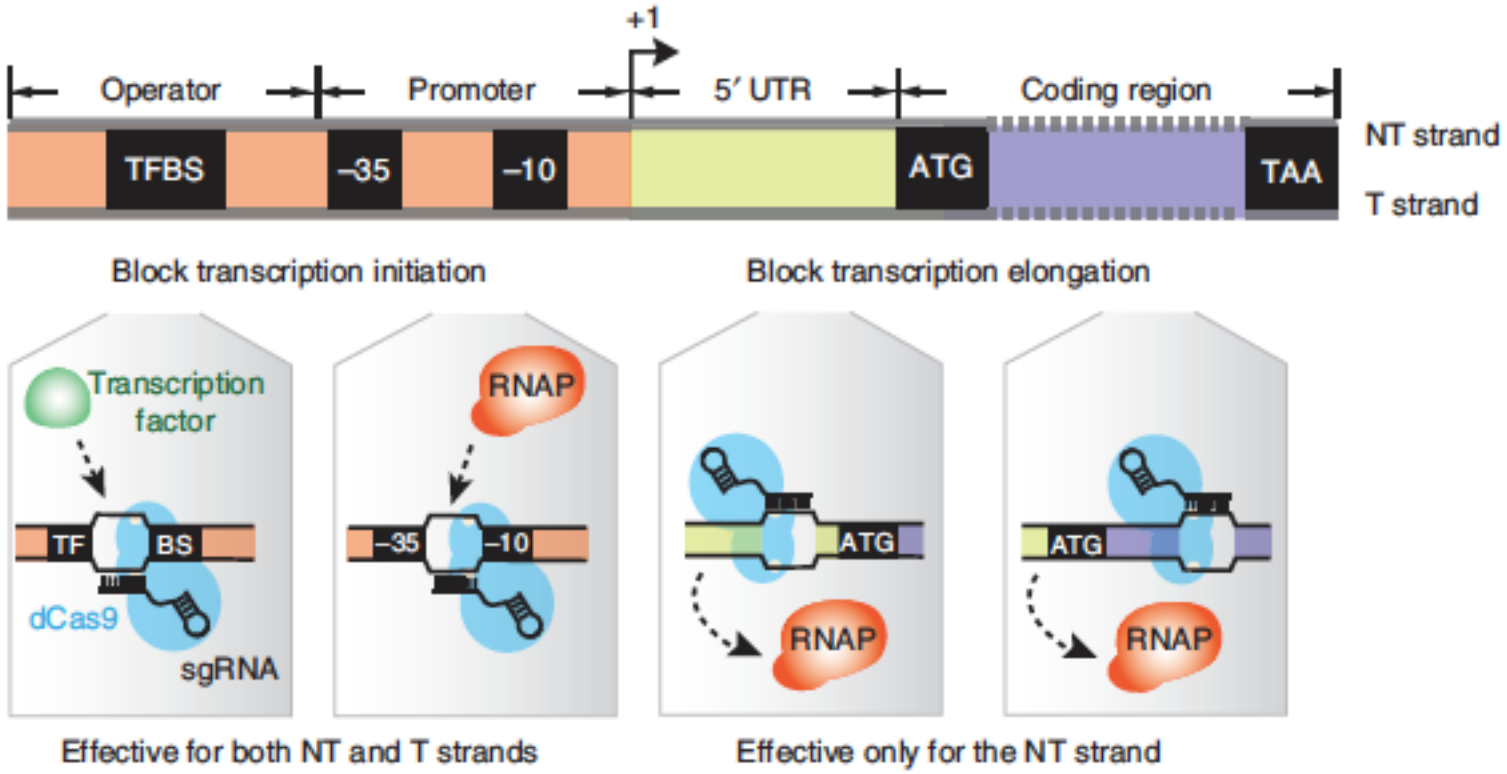
M2 experimental overview



Using CRISPRi manipulate the *E. coli* fermentation pathway



Design of gRNA for CRISPRi system



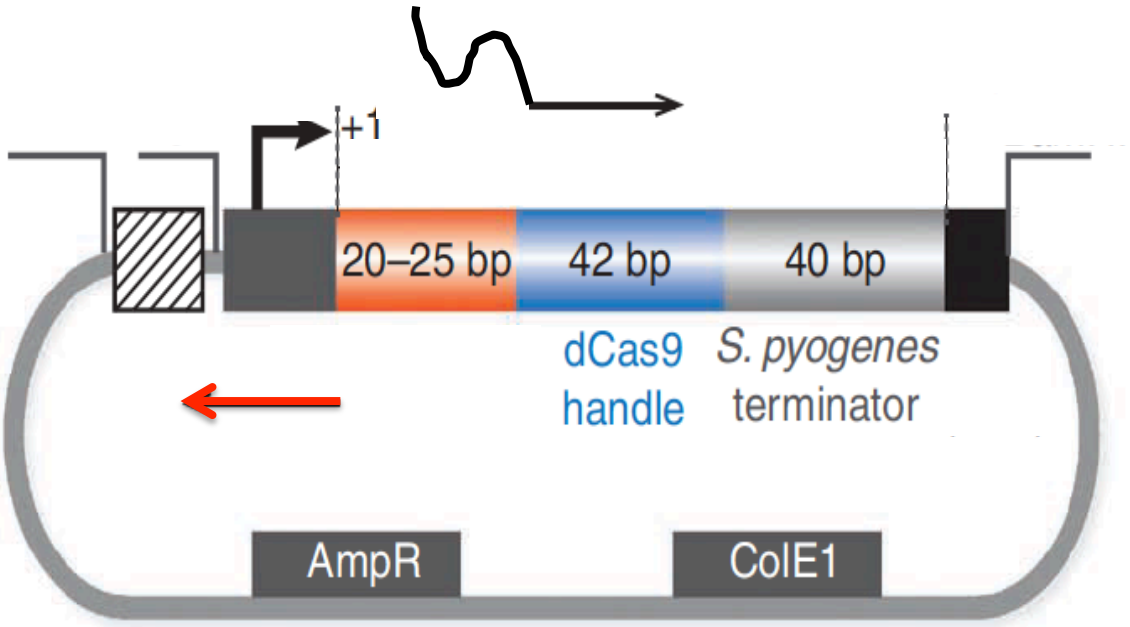
Please add your targeting info to the wiki today

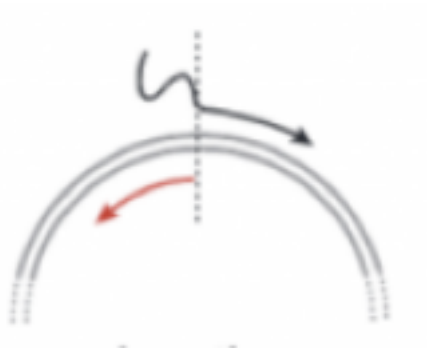
-> On the Mod2 Overview page, discussion tab

T/R: lactate [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)
yellow	pflB	TGTCGAAGTACGCAGTAAAT	
green	pflB	ATAAAAAATCCACTTAAGAAGGTA	
blue	pflB	TTCATTAAGCTCGGACATGTAACA	
pink	pflB	AAATAAAAAATCCACTTAAGAAGGT	
purple	pflB	AAATCCACTTAAGAAGGTAGGTGT	

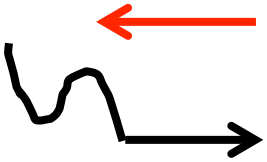
M2D3: Generated pgRNA_target by SDM





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
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  1>~~~gaattctaaagatctttgacagctagctcagtcctaggtataatactagt------------------------gttttagagctagaaatagcaag>73

        *         *         *         *         *         *         *         *         *         *
 74>ttaaaataaggctagtccgttatcaacttgaaaaagtggcaccgagtcggtgcttttttttgaagcttgggcccgaacaaaaactcatctcagaagaggat>173
 70>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTGAAGCTTGGGCCCGAACAAAAACTCATCTCAGAAGAGGAT>369
 01>TTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTGAAGCTTGGGCCCGAACAAAAACTCATCTCAGAAGAGGAT>600
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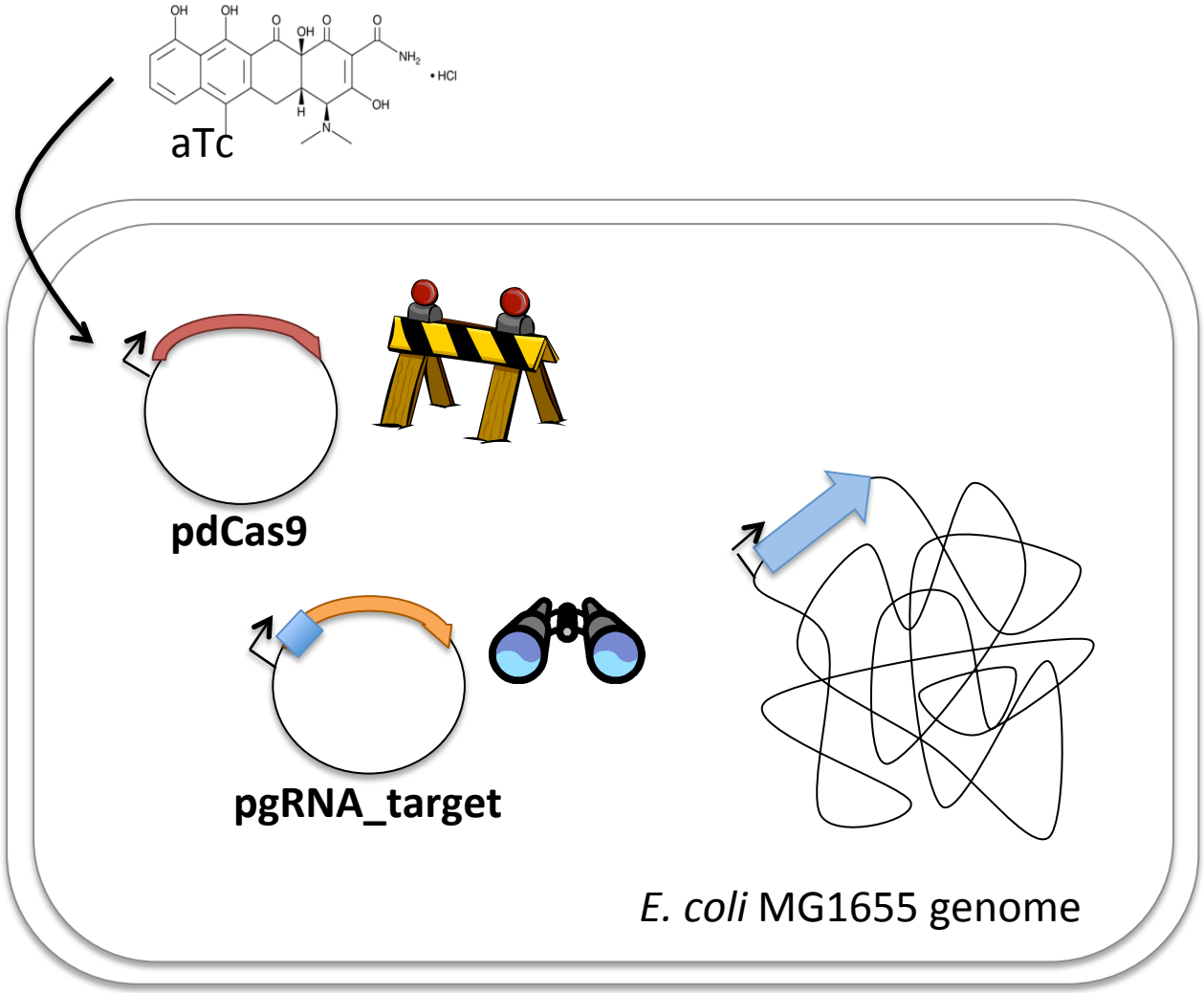
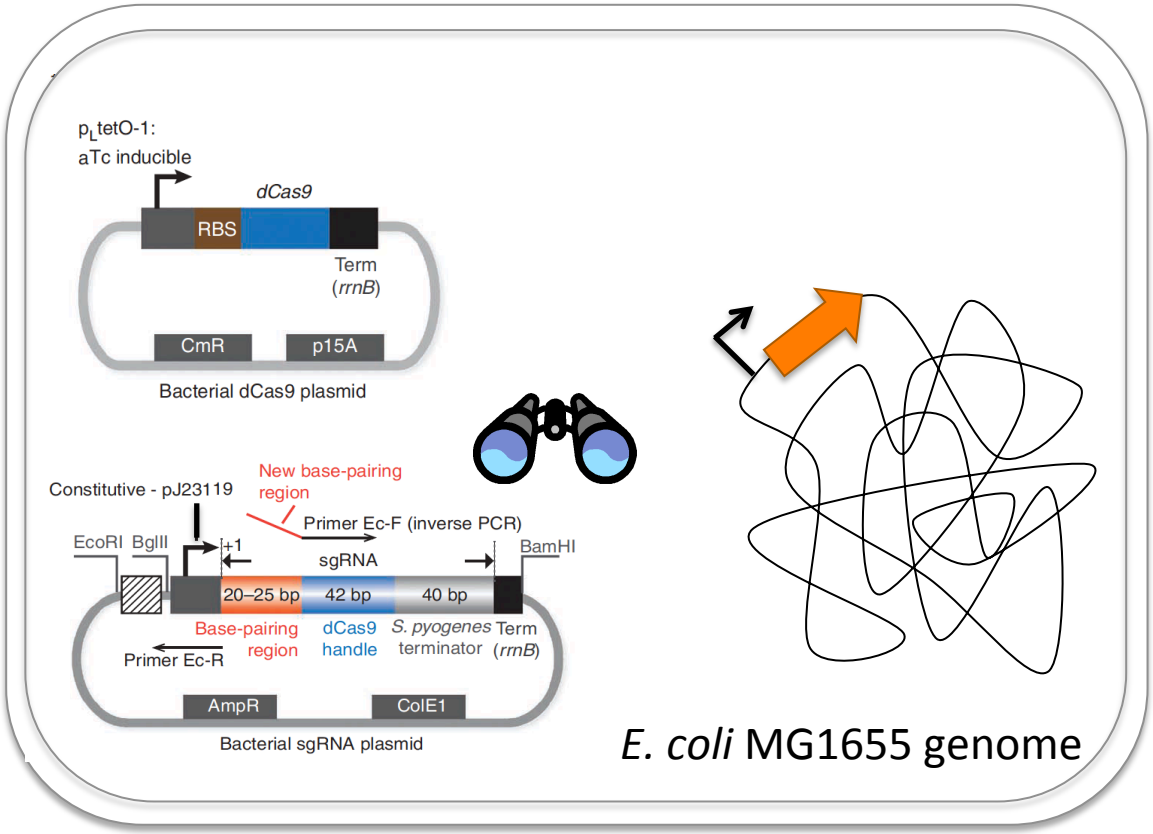
CRISPRi system overview



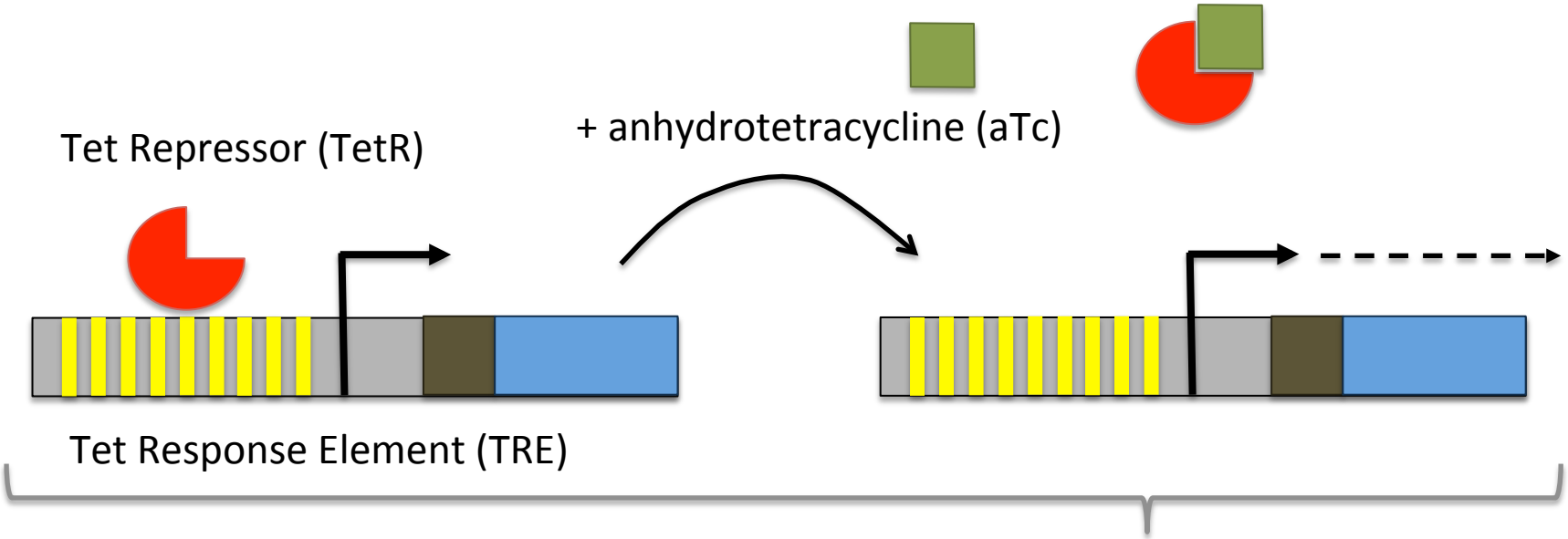
- Target gene
- pgRNA_target
- pdCas9

CRISPRi 'inactive' in absence of inducer

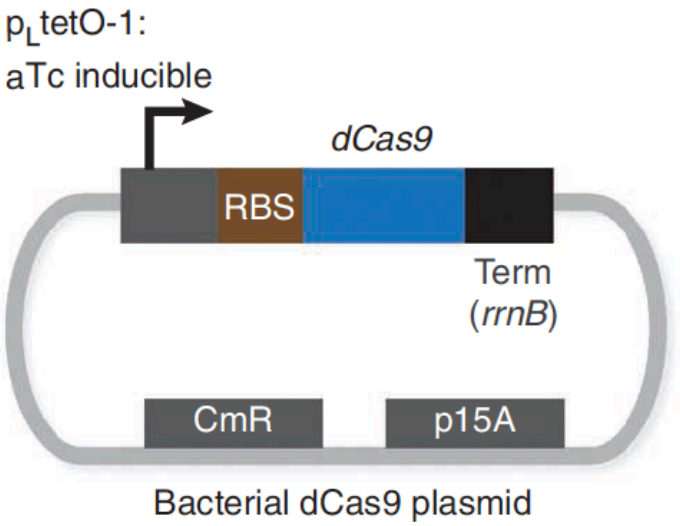


- pgRNA_target expressed constitutively
  - Always transcribed and binding to target gene

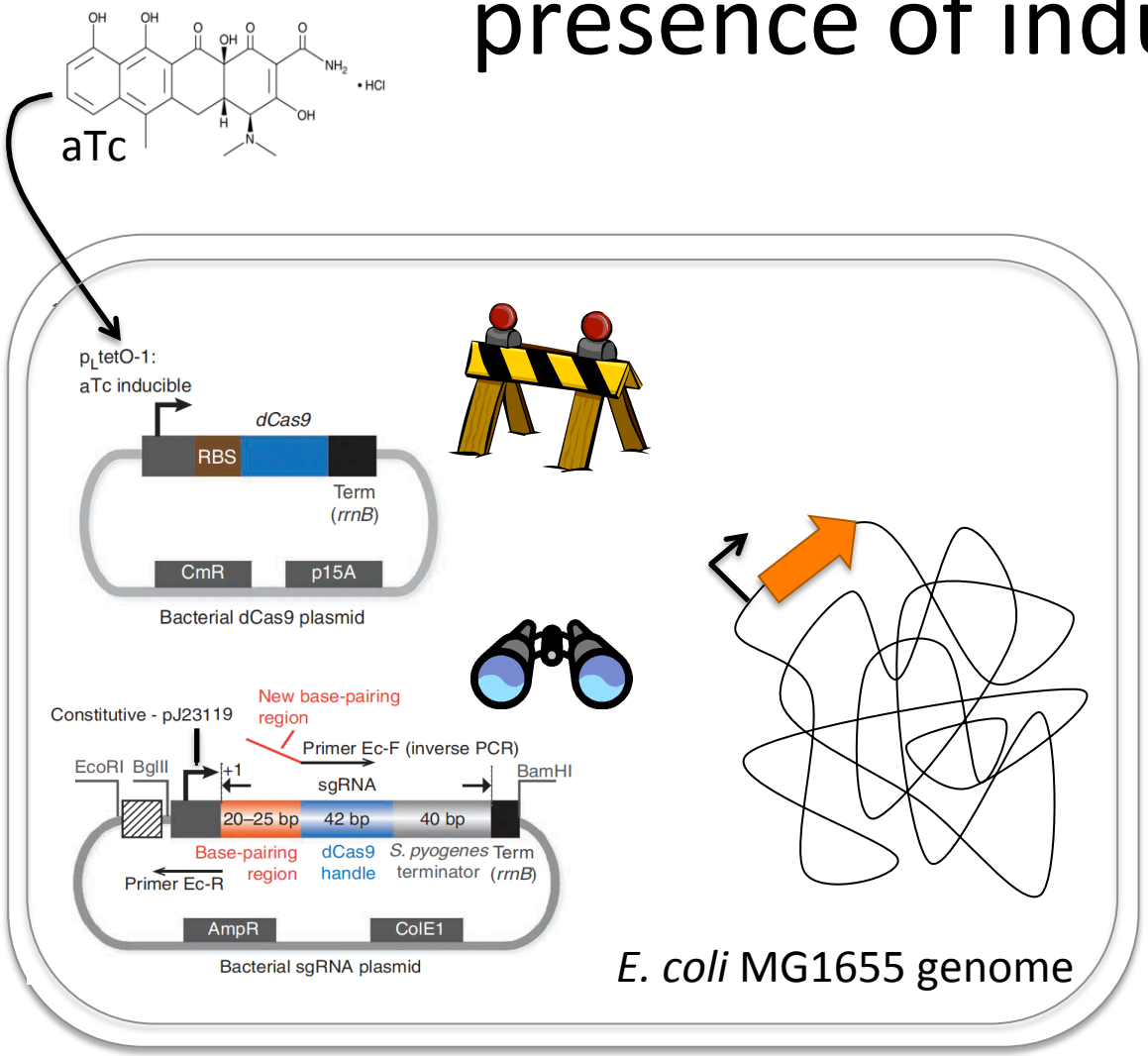
aTc induction of pdCas9



- 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?

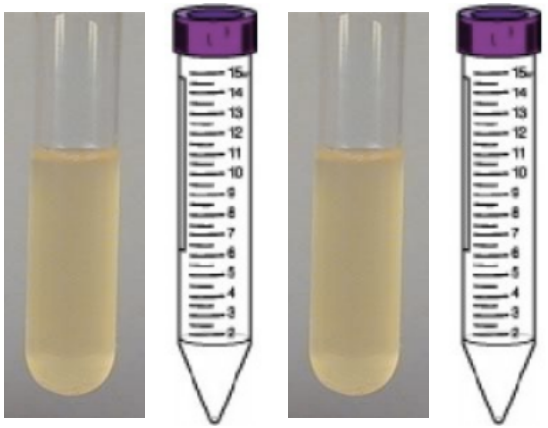
LB (E coli media)

AMP, CML ← antibiotics

ATC ← induce dCas9 expression

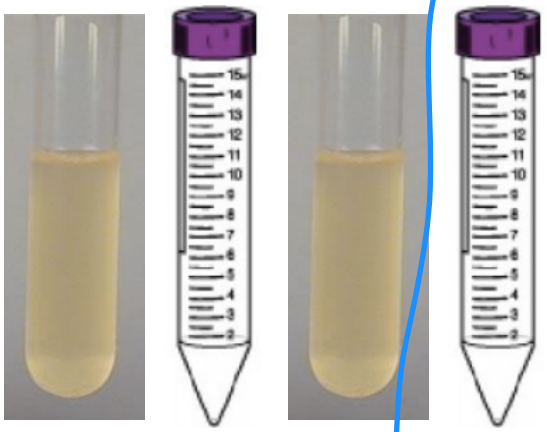
Media for mixed-acid fermentation and pdCas9 induction

- 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

Today in lab…

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