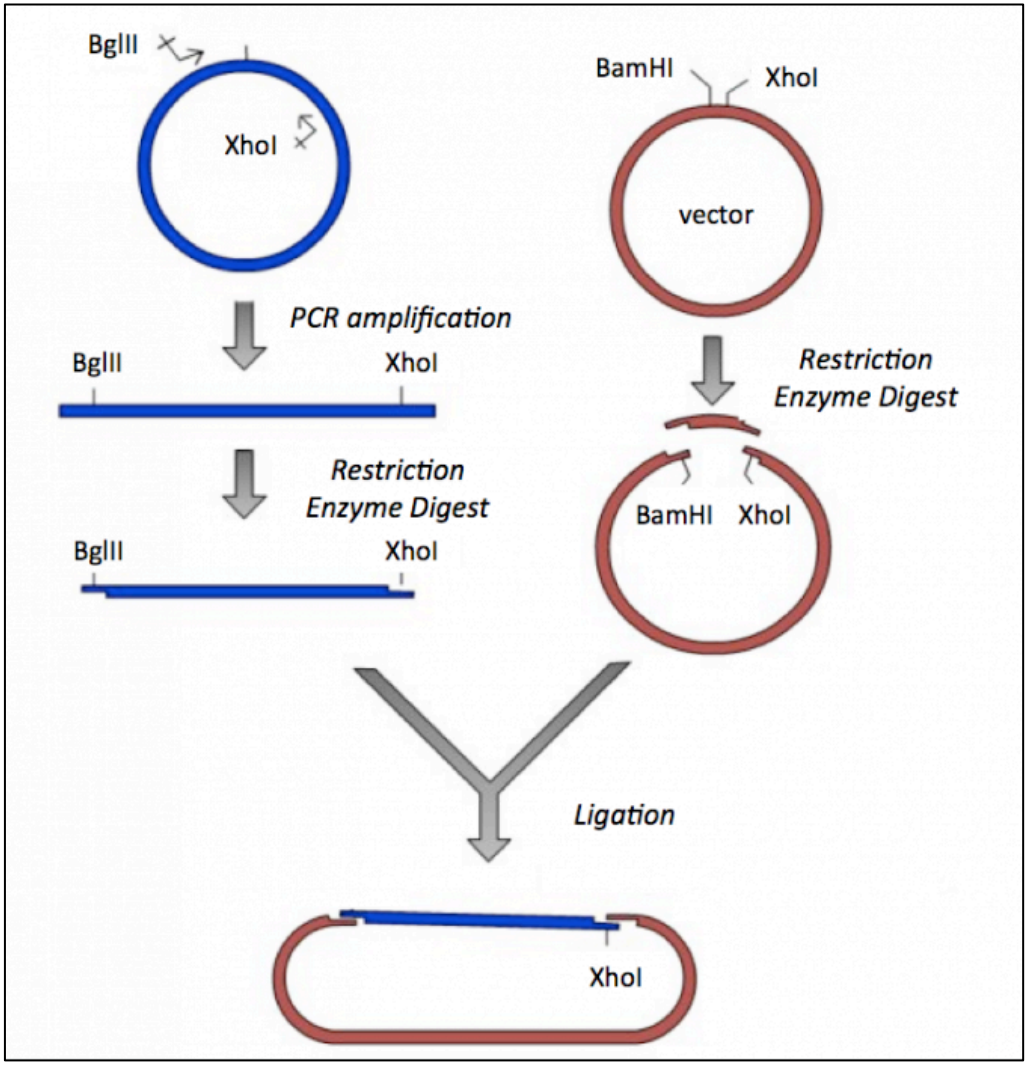
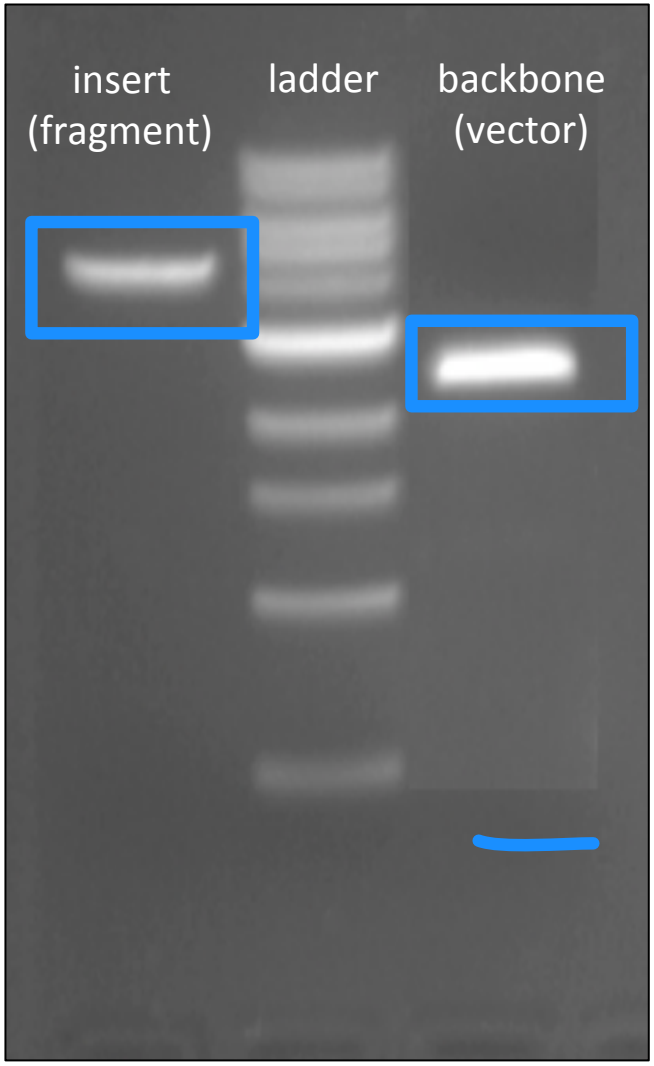
M2D2:
Design gRNA for CRISPRi

10/19/2016

pdCas9 was constructed by ligation

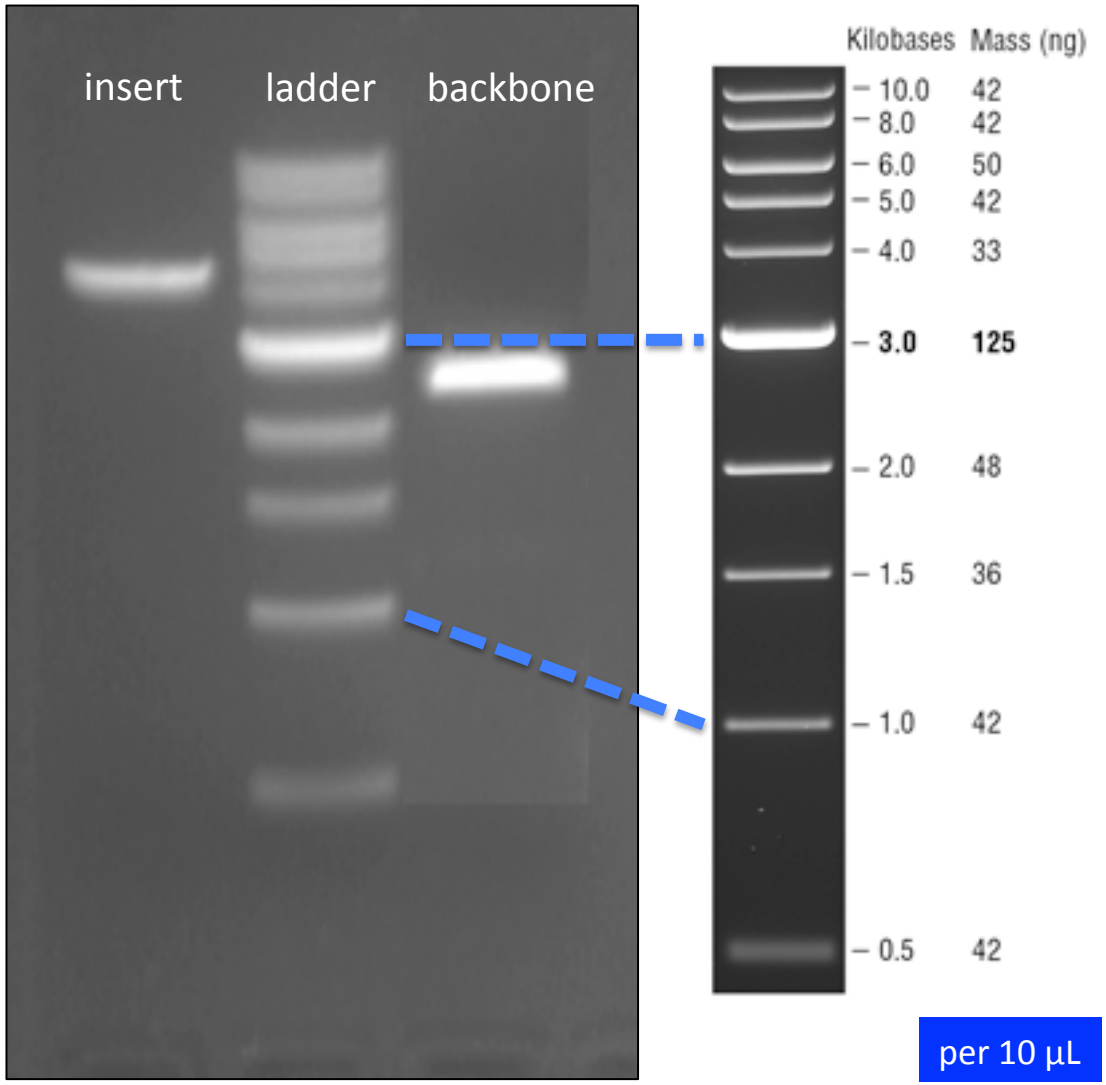


pdCas9 cloning strategy



recovery gel

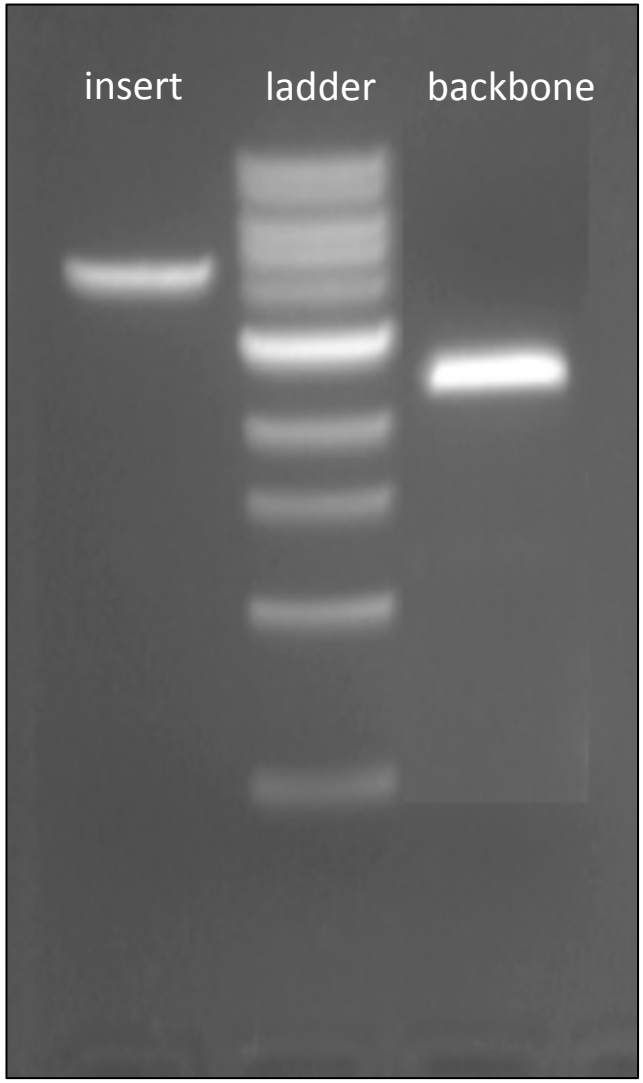
For ligation, mix 1:4 *molar* backbone : insert



- Assuming
  - 10 µL of ladder loaded,
  - 5 µL of double digests,
- amount of backbone = 250 ng
- amount of insert = 100 ng

➢ but mass of DNA ≠ molar amount of DNA

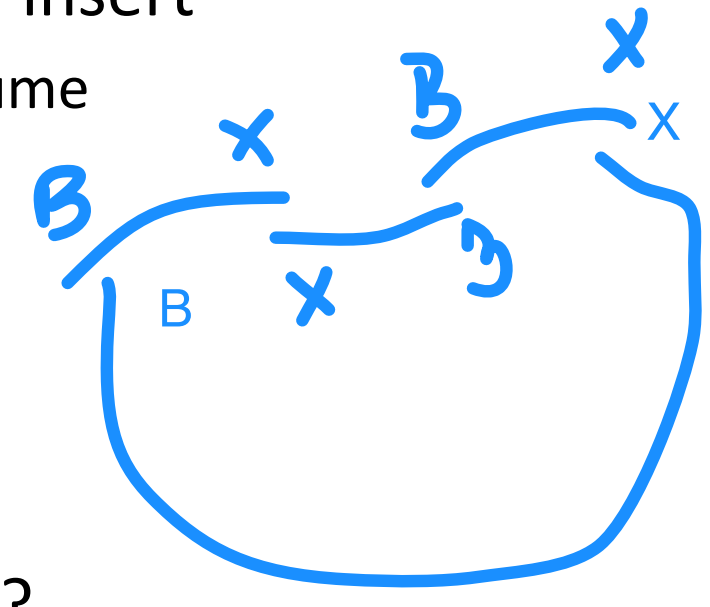
Calculate the 1:4 *molar* amounts for ligation



1. From recovery gel, estimate
   - backbone: 250 ng / 5 µL = 50 ng/µL
   - insert: 100 ng / 5 µL = 20 ng/µL
2. Determine volume of backbone needed
   - 50-100 ng, choose 50 ng, *i.e.* 1.0 µL
3. Calculate moles of backbone
   - 2636 bp * (660 g / (mol*bp)) = 1.74 x 10^6 g/mol
   - so 50 ng / (1.74 x 10^6 g/mol) = 2.87 x10^-14 mol
4. Determine moles of insert needed (4x bkbn)
   - 4 x 2.87 x10^-14 ~ 1.15 x 10^{-13} mol
   - with 4107 bp * (660 g / (mol*bp)) = 2.7 x 10^6 g/mol
   - so use 1.15 x 10^{-13} mol * 2.7 x 10^6 g/mol ~ 310 ng
5. Calculate volume of insert needed
   - 310 ng / (20 ng/µL) = 15 µL

Optimal backbone-to-insert ratio

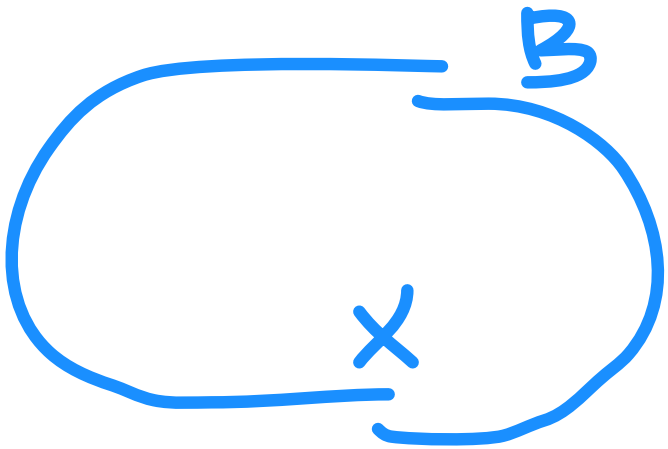
- ideally, want 1:4 backbone : insert
  - molar ratio, ***not*** mass or volume



- What if too much insert?

  tandem insertions

- What if too much backbone?



Separate DNA by gel electrophoresis

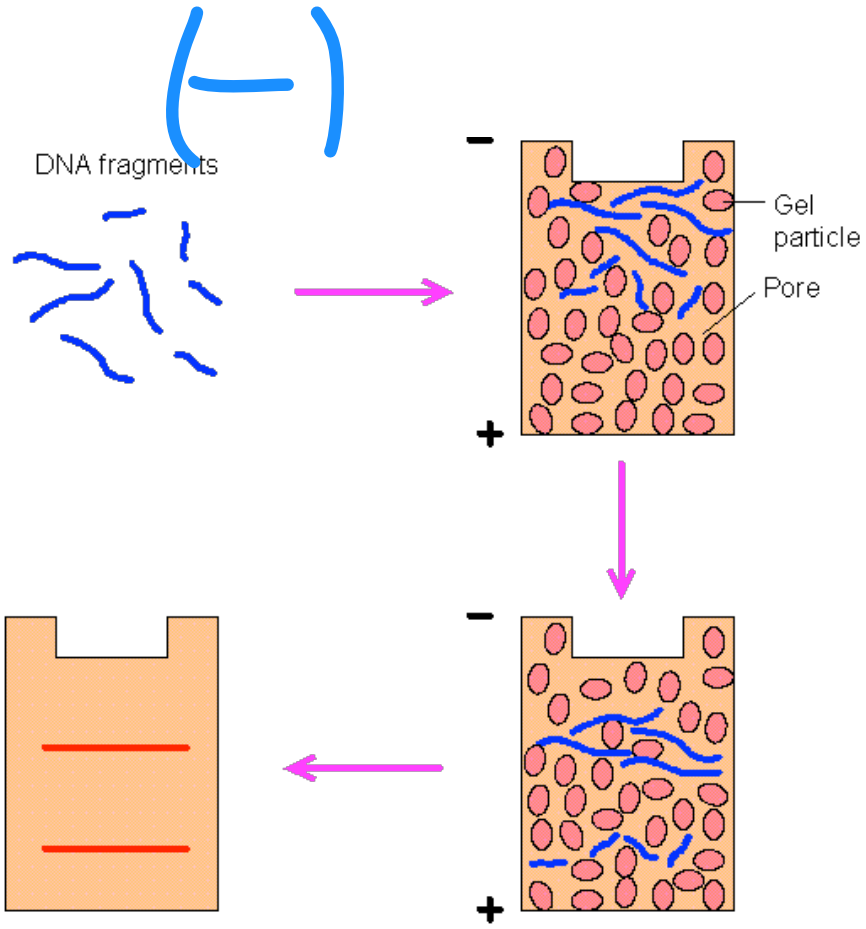
- Agarose gel electrophoresis

  – driving force:

  charge (electrical)

  – separates DNA by:

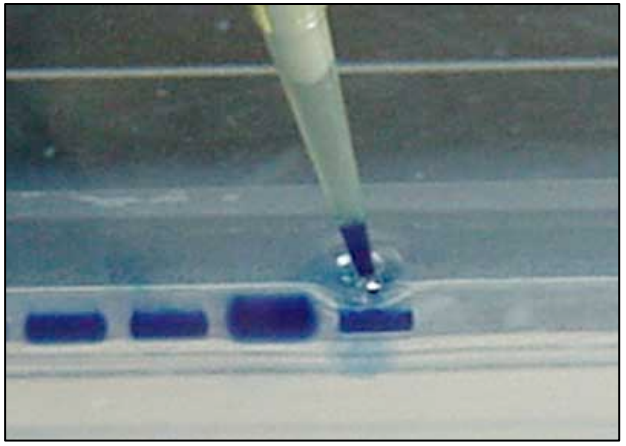
  size



Visualize DNA + save a picture!

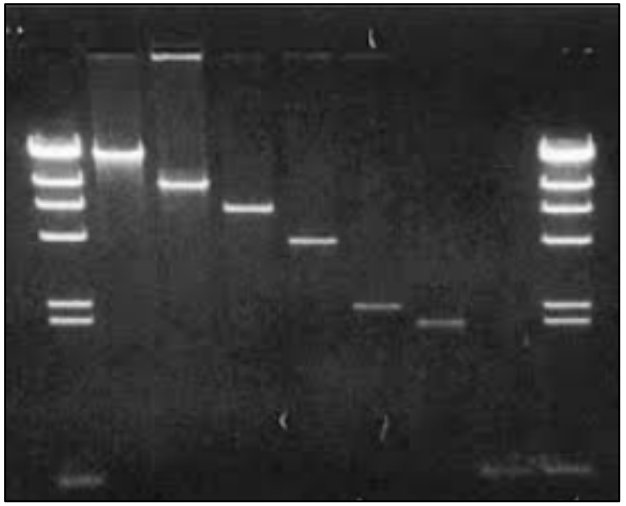
- Loading dye during the migration

  bromophenol blue
  - glycerol (density helps sink)
  - travels as ~ 500 bp



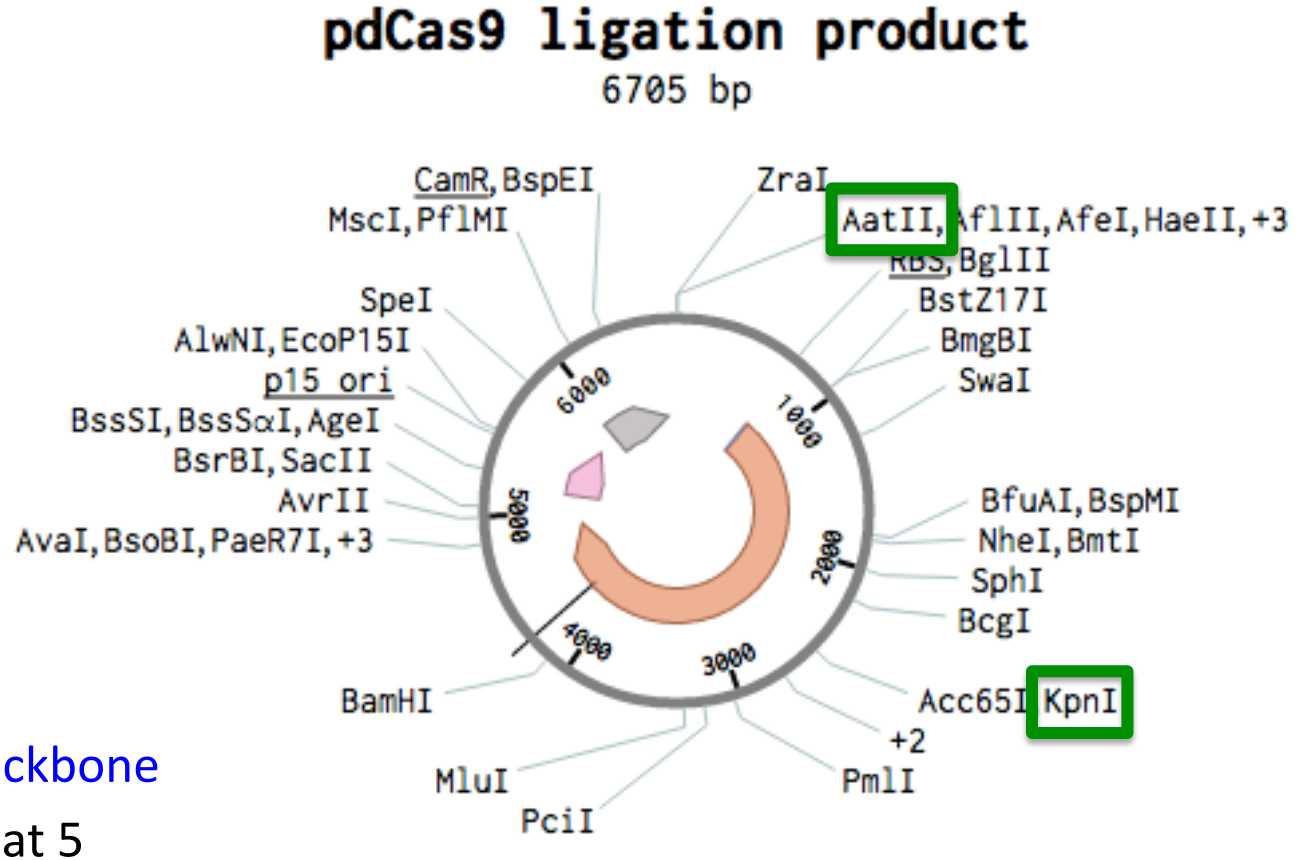
- DNA stain

  DNA intercalator
  SYBR Safe



➢ Safety : wear nitrile gloves

Confirmation digest example



- Goal:
  - 1 cut only in backbone

    AatII cuts at 5
  - 1 cut only in insert

    KpnI-HF cuts at 2517
- Fragments distinguishable? yes: 2512 bp *vs.* 4193 bp
- Compatible? yes: in CutSmart buffer

Confirmation digest example

- Goal:
  - 1 cut only in backbone
    AatII cuts at 5
  - 1 cut only in insert
    KpnI-HF cuts at 2517
- Fragments distinguishable?
- Compatible?

- Expectation:

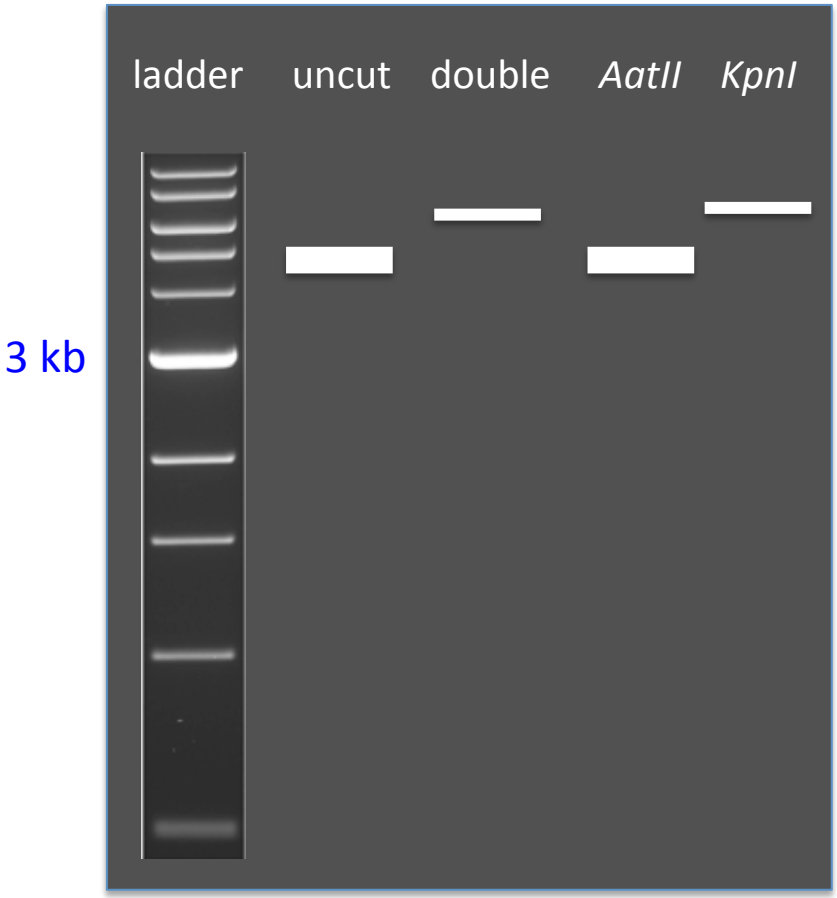
yes: 2512 bp *vs.* 4193 bp

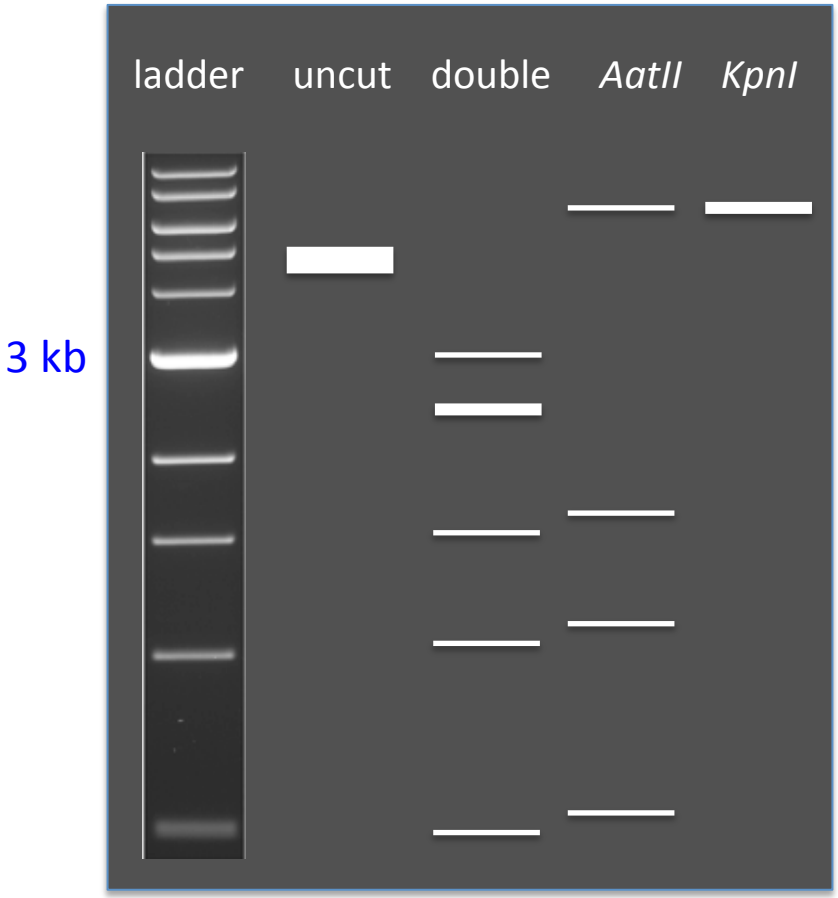
yes: in CutSmart buffer

Confirmation digest – Why load 4 lanes?

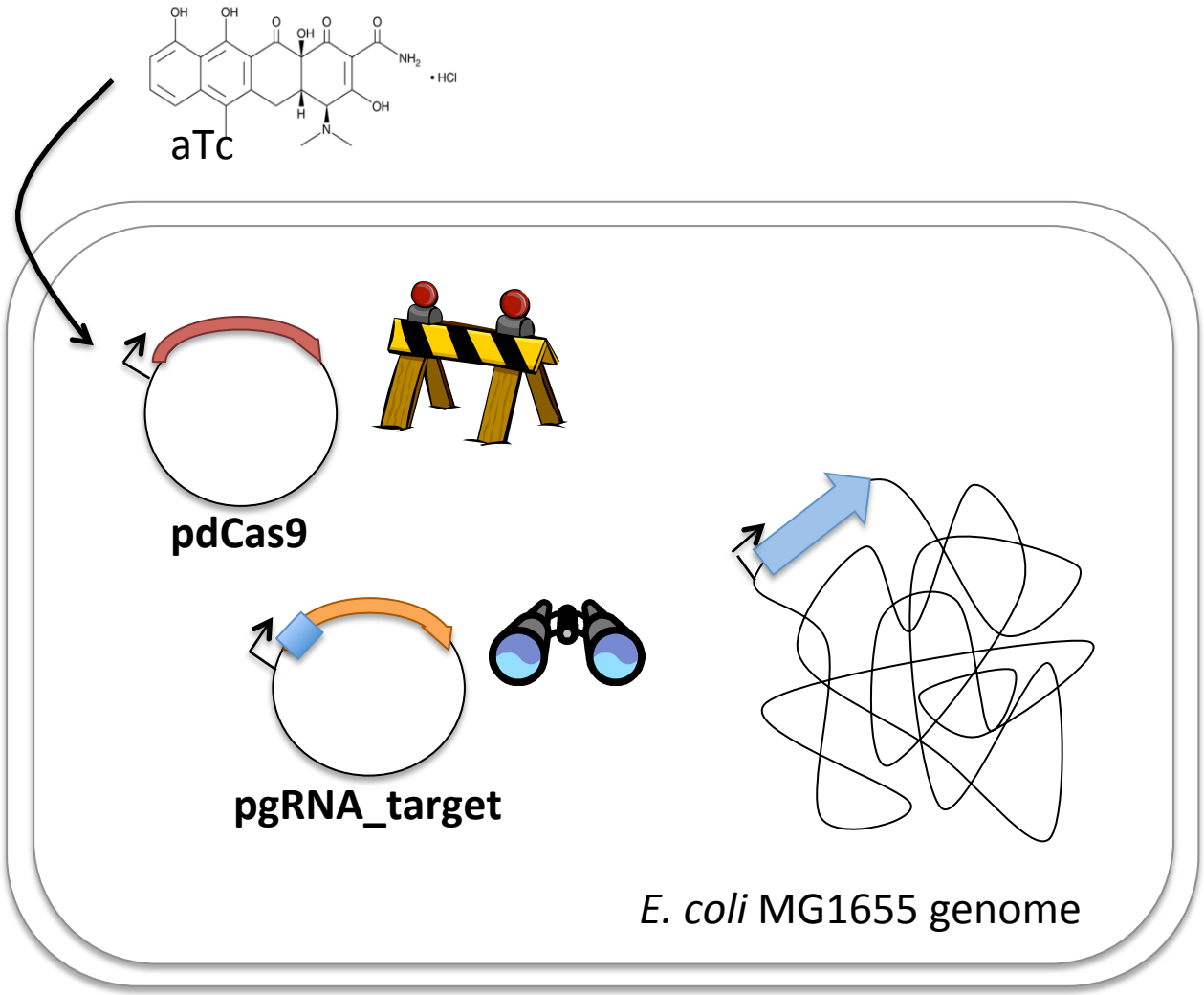
Uncut and singly-cut controls

- What if we observe:





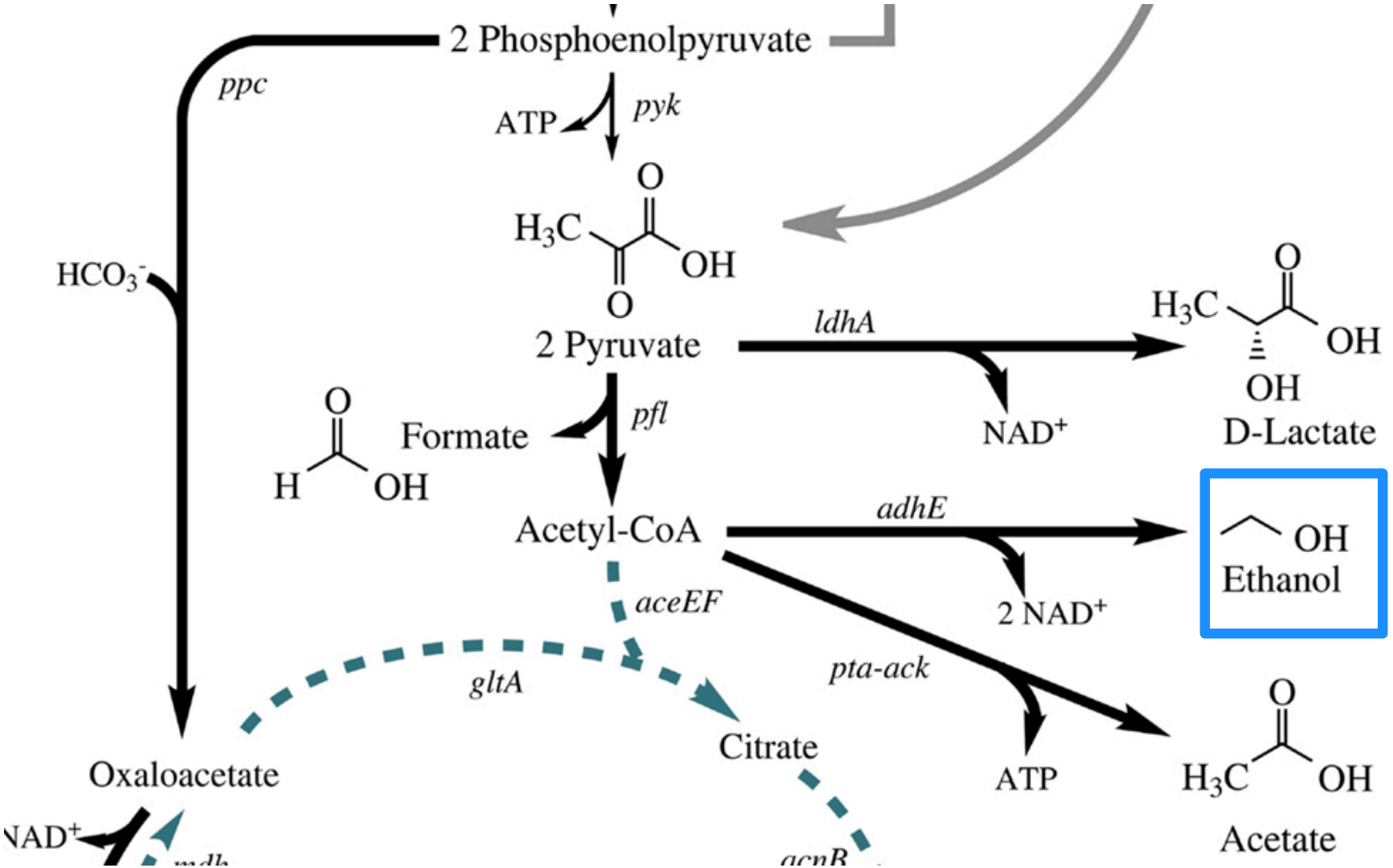
CRISPRi system overview



- target gene
one in the fermentation pathway
- pgRNA_target

- pdCas9

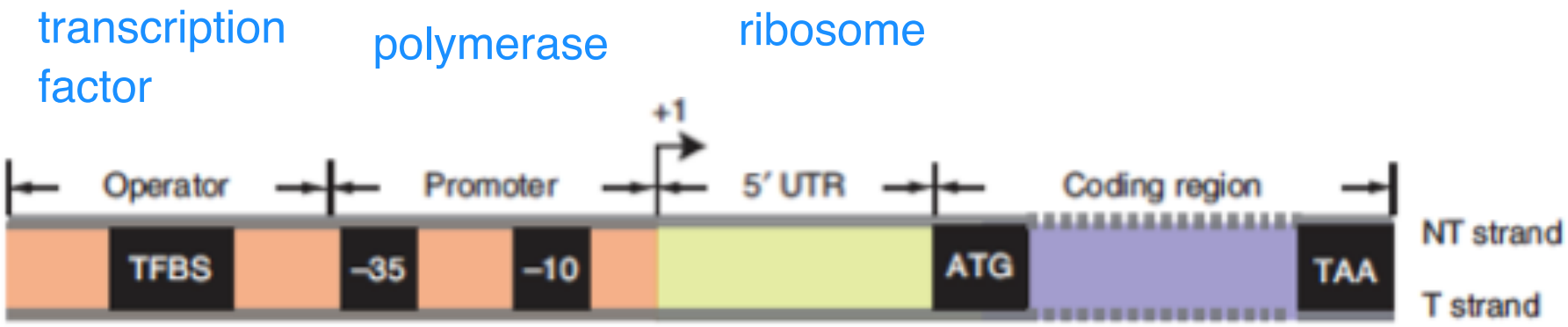
In lab today: Use CRISPRi to manipulate the *E. coli* fermentation pathway



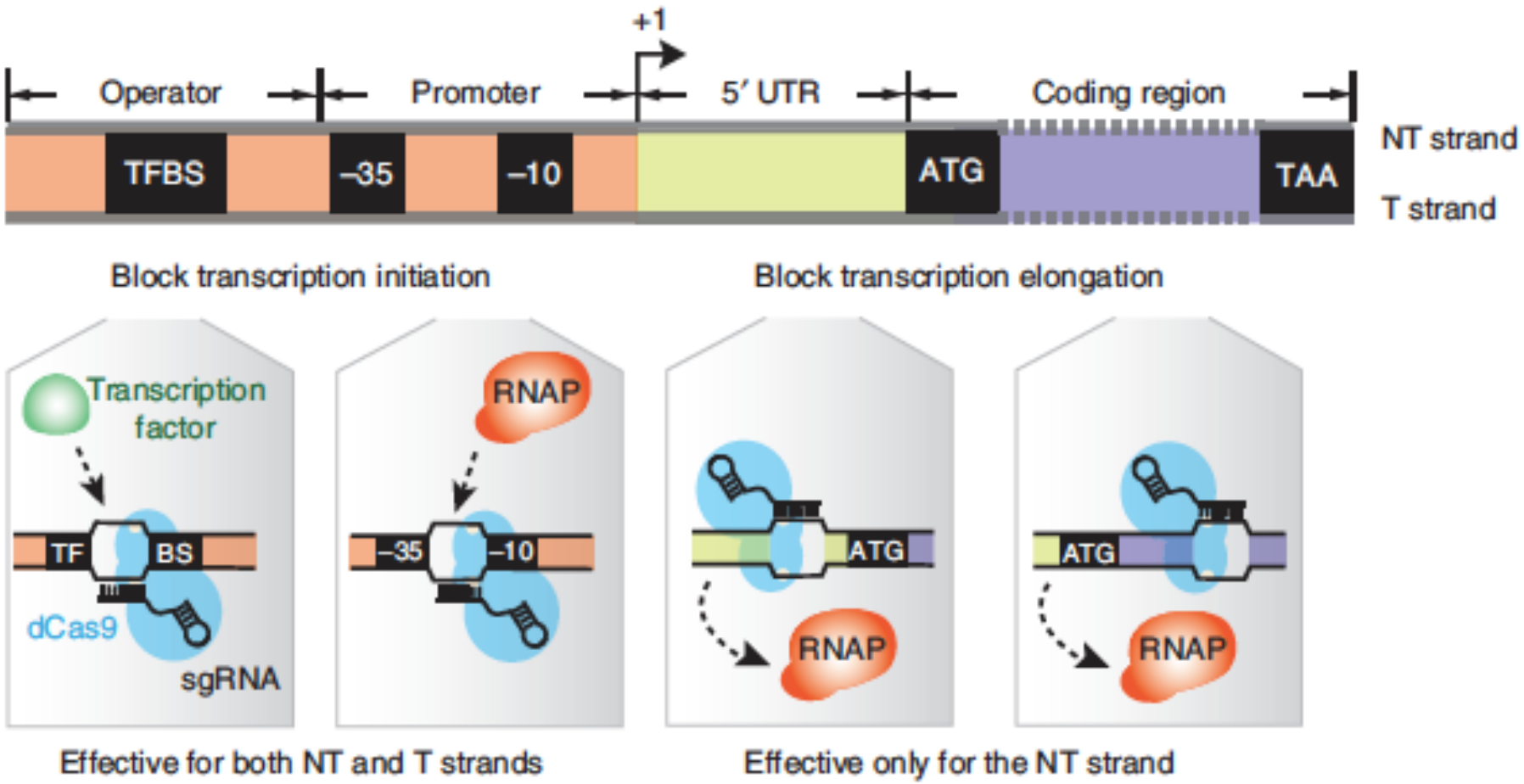
Hypothesis?

- Explicitly state your hypothesis in your M2D2 lab notebook.

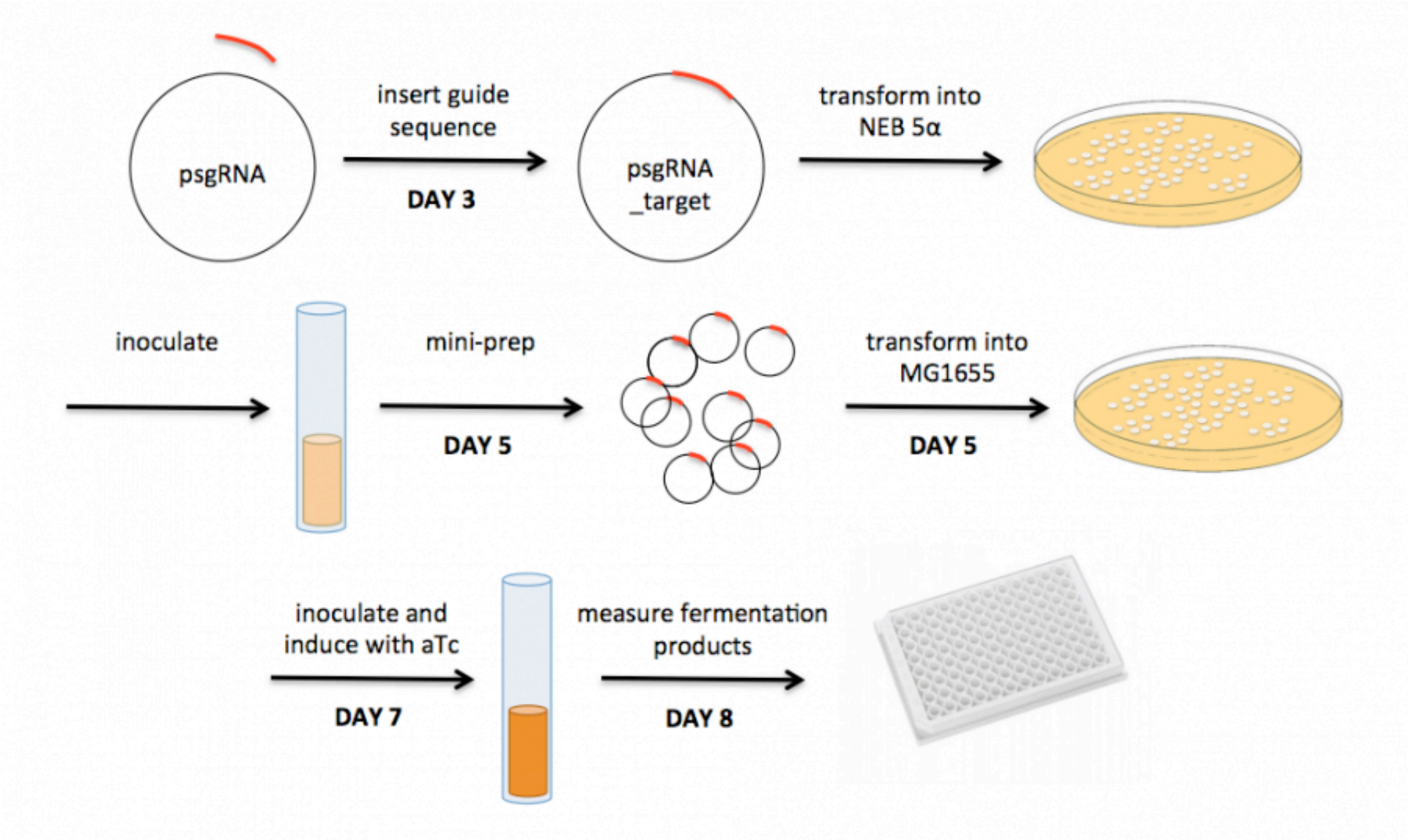
Let's review binding partners:



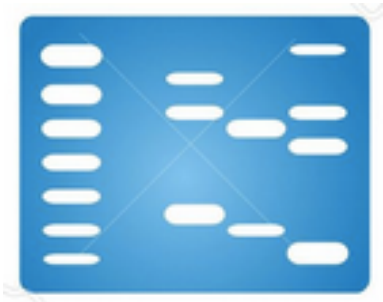
Which region will you target?



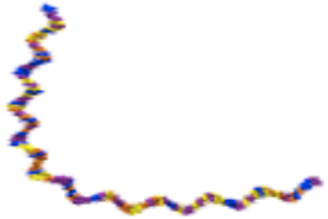
M2 experimental overview



Today in lab



- Run pdCas9 confirmation digest



- Design gRNA
  - articulate a hypothesis
  - sequences submitted to IDT *tonight*

- Read before M2D3

Looking ahead: homework due M2D3

- Figure / caption / results
  - no more bullet points
  - result subsections need titles (take-home message)
  - minimize interpretation in the results section in M2
  - Unbiased
- Read for in-class discussion

ACS SyntheticBiology

Research Article

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CRISPR Perturbation of Gene Expression Alters Bacterial Fitness under Stress and Reveals Underlying Epistatic Constraints

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