M1D4:Prepare Expression System

02/18/16

1. Mod1 Quiz 1
2. Prelab
3. Prep BL21 competent cells
4. Mini-prep SDM product from NEB5alpha
5. Prep DNA for sequencing
6. Transform BL21 cells with mini-prep DNA
7. Count colonies on SDM plates

Protein Engineering Summary

- due by 5pm on Saturday, March 12
- revision due by 5pm on Monday, March 28

Summary Content

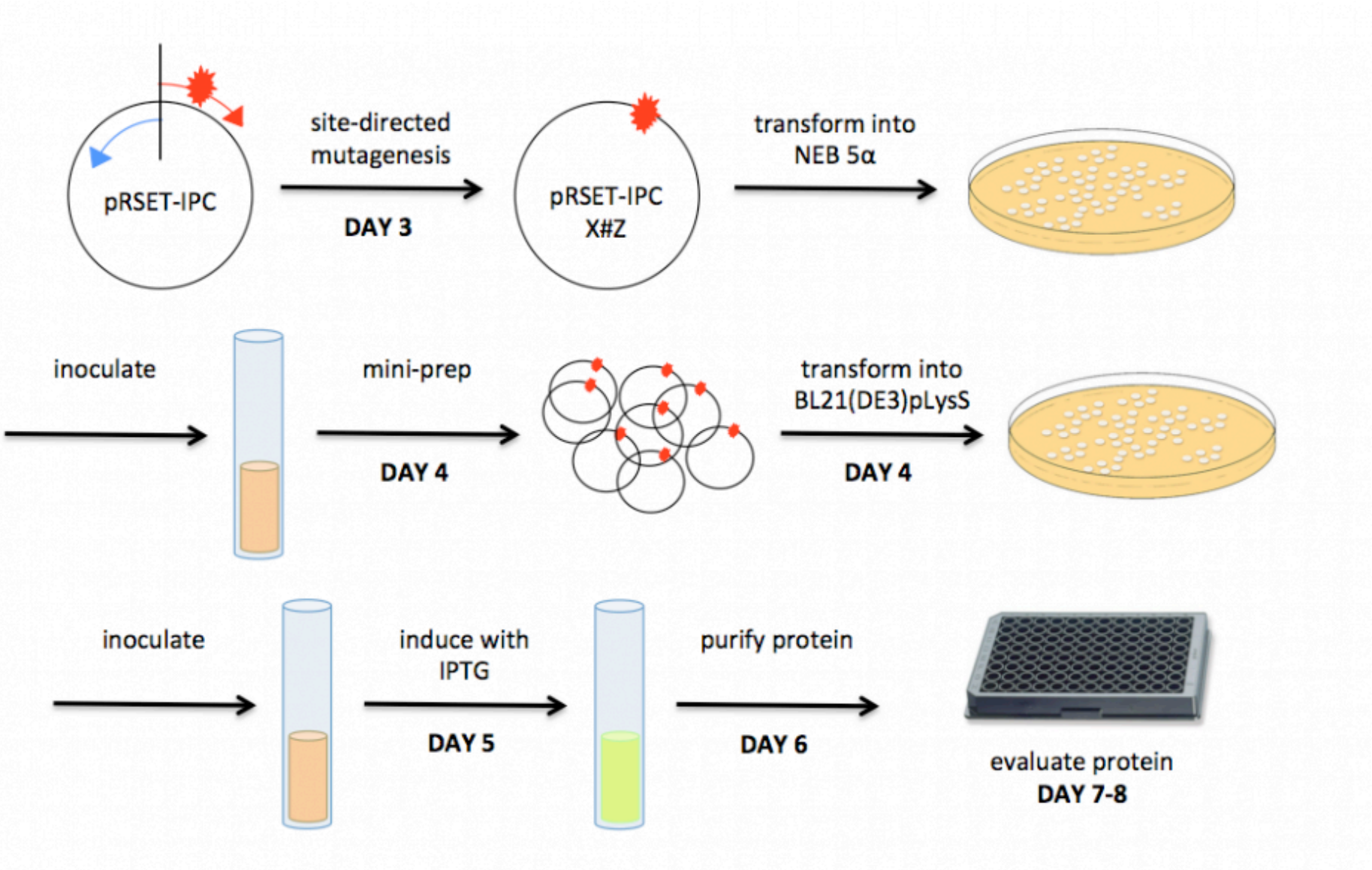
1. Title
2. Abstract
3. Background, Motivation
4. Figures, Results, Interpretation
5. (Discussion) Implications, Future Work

Results section bullets for M1 summary

State concisely in bullet point:

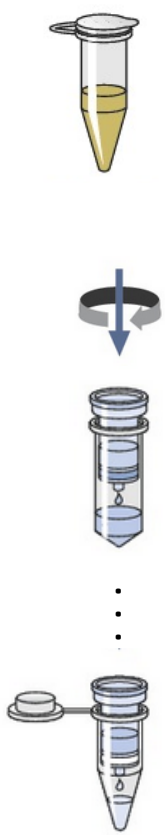
1. What was the overall goal of the experiment? (introductory sentence)
2. What was your expected result?
    - What are the expected band sizes on your gel?
3. What evidence do you have that your result is correct or incorrect?
    - What controls did you perform and did they work as expected?
4. What was the result?
    - Were bands of the expected size present? Why or why not?
5. In sum, what do these data suggest or indicate? Think about how the data were used.
6. What does this motivate you to do next?

Since last week…



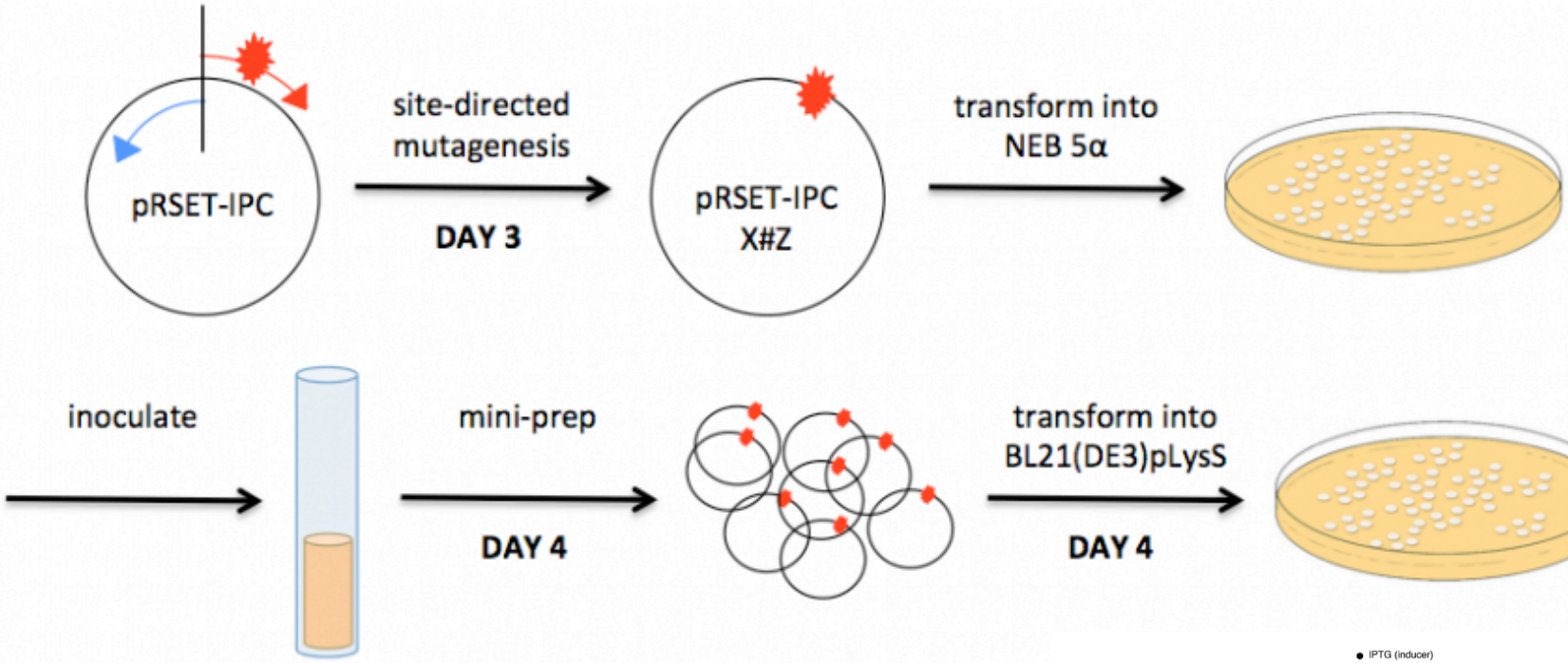
Mini-prep to isolate plasmid DNA from *E. coli*

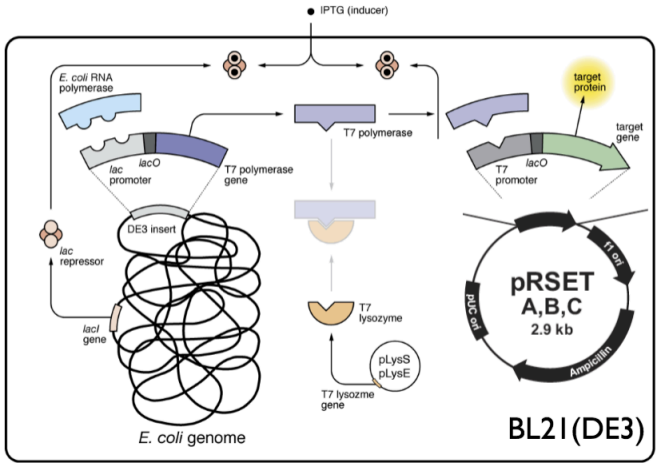
alkaline lysis protocol



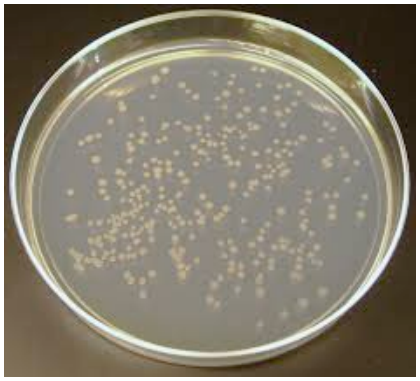
steps	contents	purpose
prepare	Tris/EDTA buffer RNase	weaken the membrane resuspend pellet
lyse	SDS NaOH	alkaline solubilize proteins, denature DNA
neutralize	acetic acid, chaotropic salt, potassium acetate	renatures short DNA, precipitates long DNA salt-DNA bind column
spin		separates soluble and insoluble
bind	silica column	concentrate DNA
wash	ethanol	wash away contaminates, protein ** get rid of *all* ethanol
elute	water, pH 8.0	Elute *all* DNA from column

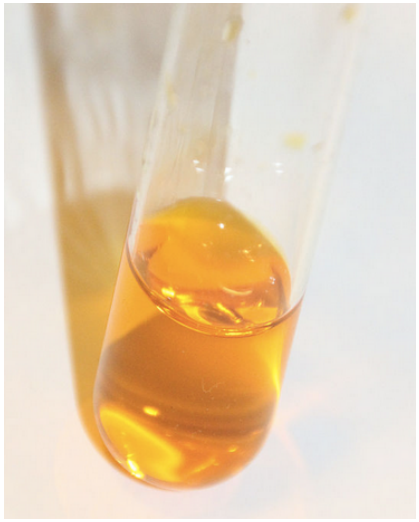
DNA vs. protein amplification in NEB 5α vs. BL21





Transforming BL21(DE3)pLysS competent cells





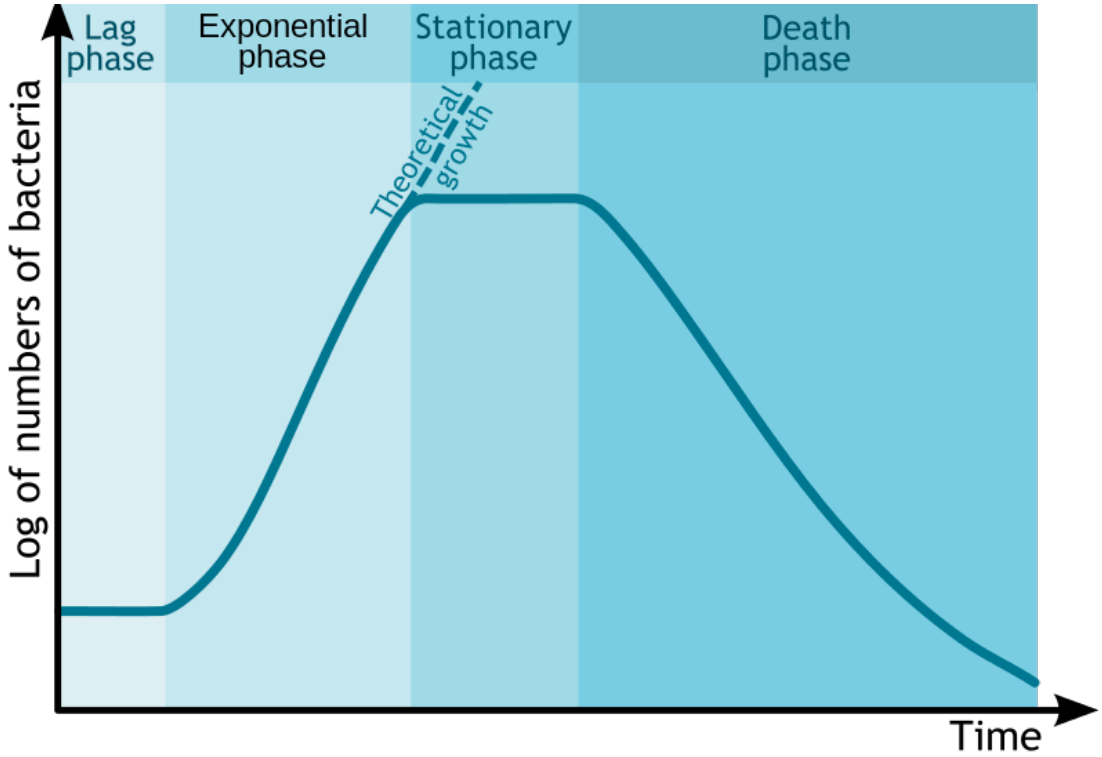
- can express IPC protein
  - when induced by lactose analog…
  - …details on M1D5!
- made competent by $CaCl_2$
  - Ca^{2+} ions attract both DNA and bacterial cell wall LPS
  - heat shock
- in exponential growth phase, OD_{600} = 0.4-0.8
- handle very gently, or will lyse
  - *on ice* all the time, and with chilled solutions
  - not vortexed
- Cam (chloramphenicol) resistant *E. coli* strain
  - Amp (ampicillin) resistant if transformed with pRSET-IPC

A few brief notes on *E. coli* growth curve

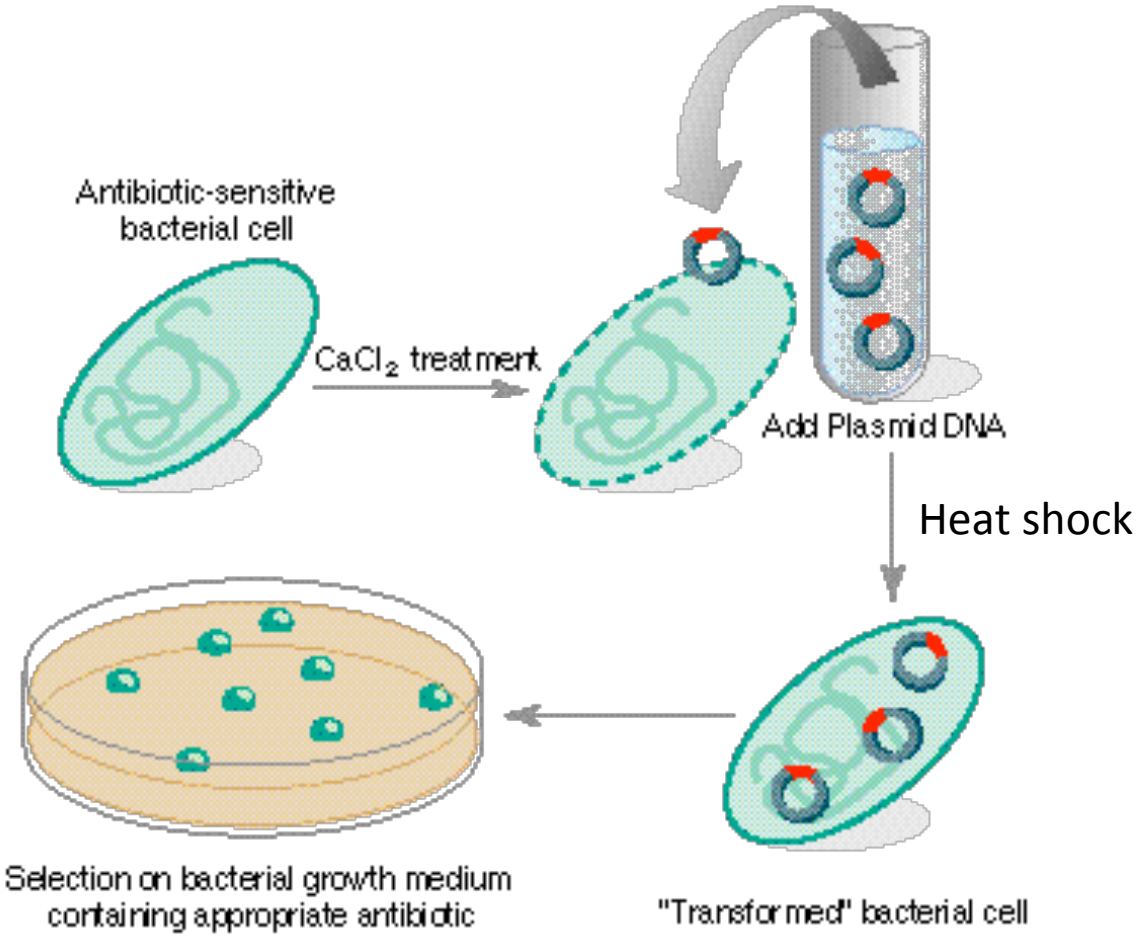
- exponential phase
  - binary fission
  - OD600 ~ 0.4 - 0.8
  - machinery ready

- OD ≠ absorbance
  - -turbidity, light scatter
  -
  - -E. coli, don't absorb at 600nm

    -600nm safe wavelength
    UV damages DNA



Bacterial Transformation



AMP
CAM ⇒ uptake pRSET-IPC

Transformation controls & expected outcomes

BL21+plasmid=AMP+CAM

sample	expectation / what if?	role
no DNA	no colony. What if many? -contamination -given LB agar plate with wrong antibiotics	negative
control	many. What if none/few? -transformation efficiency -killed bacteria -wrong antibiotic -poor DNA quality or concentration	positive
your X#Z or wt IPC	some. What if X#Z << control? -transformation efficiency -killed bacteria -wrong antibiotic -poor DNA quality or concentration, mini prep DNA	experimental

Today in lab:

- Obtain BL21(DE3)pLysS in mid-log phase and make them prepare them for transformation
- Isolate mutant DNA by Qiagen mini-prep X#Z #1, X#Z #2
- Transform your competent cells with:

  X#Z #1, X#Z #2, wt IPC, or no DNA
- Prepare X#Z #1 and X#Z #2 for sequencing
- Count colonies from X#Z plate

Methods section tips

- Use subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence
- Use clear and concise full sentences
  - Avoid tables and lists
- Use the most flexible units
  - Write concentration rather than volume

Homework due M1D6

- Methods M1D3-M1D5: SDM, Prep of expression system, protein induction
  - Eliminate 109 specific details
  - Report concentrations (NOT volumes)
  - Do not include details about tubes and water
  - Avoid repeating information
  - Use sub-section titles
  - Include topic sentences in each section

UNDERGRAD & GRAD STUDENT UROP MIXER

Course 20 | Course 7 | Course 5 | Course 10B | Course 6-7

2/25 4:30-5:30
56-614

Present your research to a variety of undergrads and get a UROP!

Refreshments will be served!

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biotech-undergrad-officers@mit.edu

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You are invited to a BE / "Course 20"

Spring Term Open House

for faculty, staff & undergraduate students.

Friday, February 26, 2016
***Between 2:30pm and 4:30pm**–stop by anytime, stay as long as you like*
Room 16-341

Come for refreshments, good company and BE swag!

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