M1D4:
Prepare protein expression system

02/19/2016

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!

RSVP at: https://goo.gl/A69WLQ

Email questions to
biotech-undergrad-officers@mit.edu

Sponsored by: MIT Biotech Group | MIT BMES Biomedical Engineering Society | Event sponsored by Graduate Student Council


SPRING IS ALMOST HERE!
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!
MIT BE
BIOLOGICAL ENGINEERING

Today in lab

- 

- Isolate mutants DNA by Qiagen mini-prep
- Obtain BL21(DE3)pLysS in mid-log phase, make them competent, and transform with X#Z #1, X#Z #2, wt IPC, or no DNA
- Prepare mutant DNA for sequencing
- Count colonies from X#Z plate

Results & Discussion section for M1 summary

State concisely in bullet point:

Use this slide as a checklist !

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?

Assignments for M1

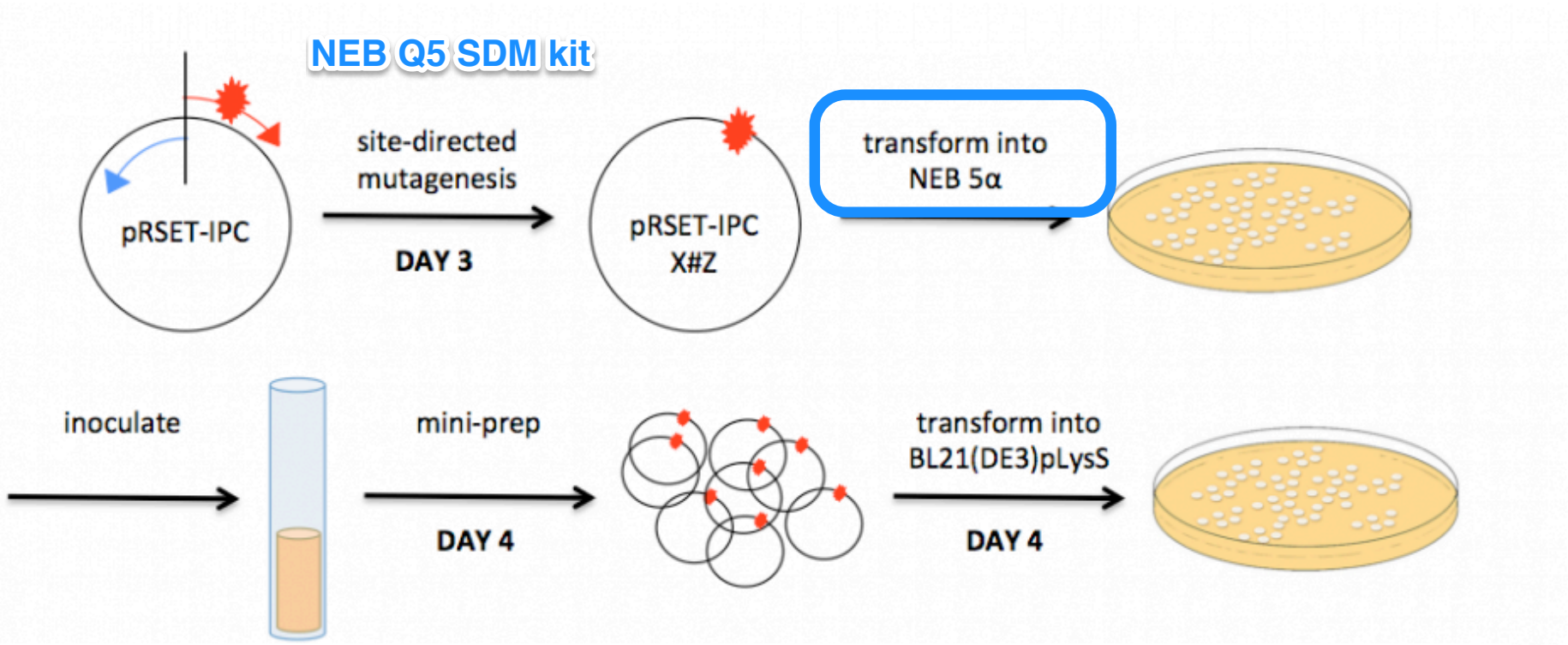
- Protein engineering summary draft
  - due by 5pm on Saturday, March 12
  - revision due by 5pm on Monday, March 28

Summary content

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

- Mini presentation due on March 16
- Blog post for M1 due March 29

Since last time…



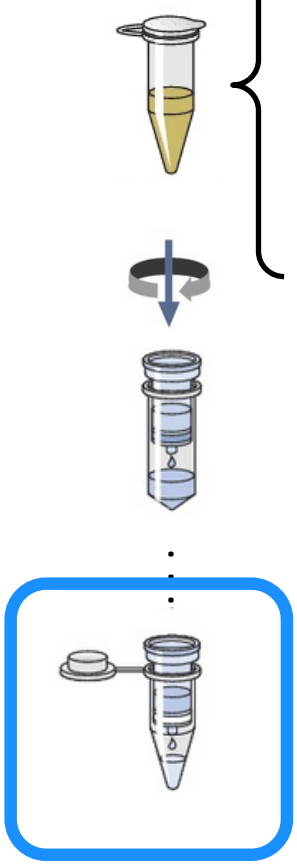
- Only 1 W/F team had colonies. Show me your pipetting skills!

Transformation controls & outcomes

sample	expectation **/ what if?**	**role**
no DNA	no colony. What if many? **ampicillin not working** **wrong antibiotic** **contamination**	**negative control**
control **NEB SDM**	many. What if none/few? **damaged DNA, not Amp resistant anymore** **bad kit** **killed NEB 5alpha cells**	**positive control for reagents, for technique**
your X#Z or wt IPC	some. What if X#Z << control? **damaged plasmid or low transformation** **toxic plasmid** **low [plasmid], poor SDM efficiency**	**experiment**

Mini-prep to isolate DNA

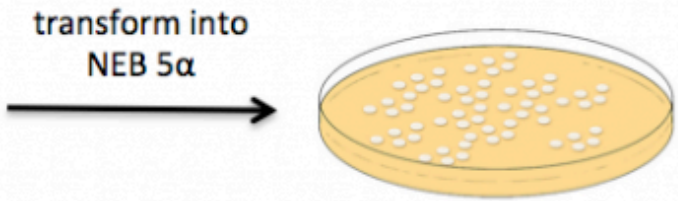
using QIAprep kit by Qiagen

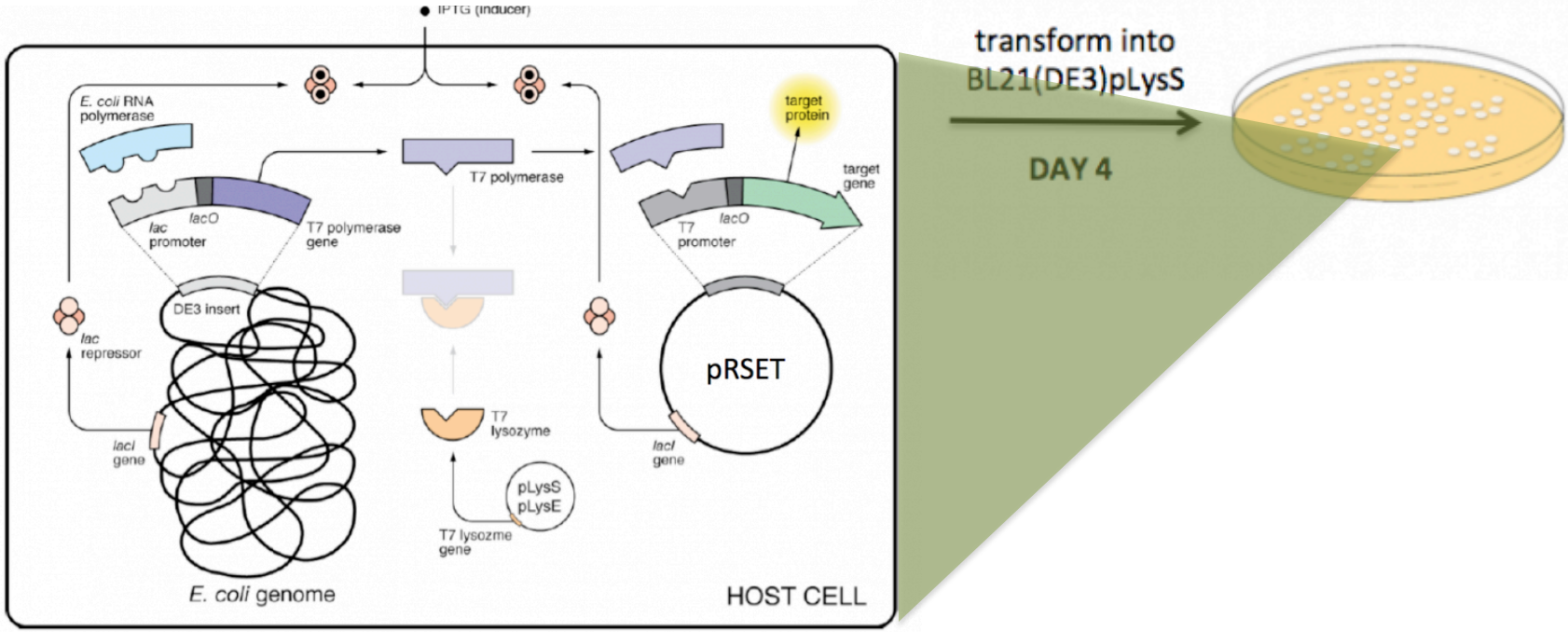


steps	contents	purpose
prepare P1	Tris/EDTA buffer RNase	resuspend NEB 5alpha cells weaken the cell walls
lyse P2	SDS surfactant NaOH alkaline lysis	solubilize proteins, denature DNA
neutralize N3	acetic acid, chaotropic salt, potassium acetate	short (plasmid) renature long DNA (rest of E. Coli) precipitate
		clear lysate
concentrate	spin: bind to silica column	pellet “garbage”
wash PB, PE	ethanol use 'Qiagen waste'	** get rid of *all* ethanol
elute	water, pH 8.0	high-purity DNA

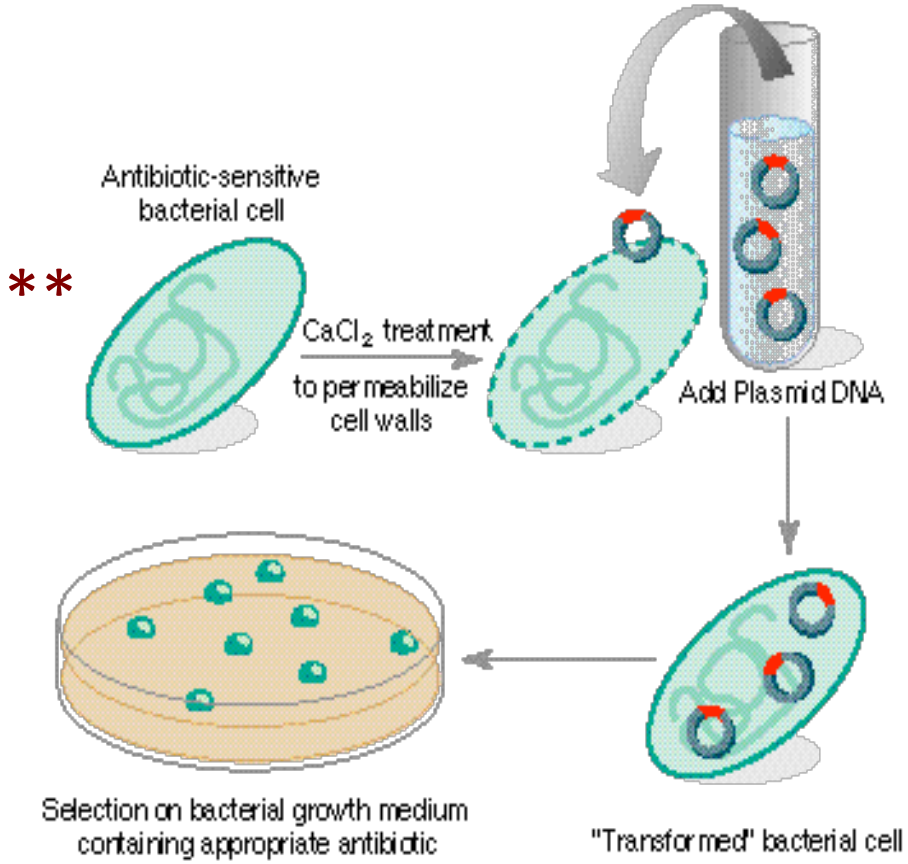
DNA vs. protein amplification in NEB 5α vs. BL21(DE3)pLysS *E. Coli*

- "BL21" can express IPC protein
  - when induced by lactose analog…
  - …review details on M1D5!





Transform BL21(DE3)pLysS competent cells



- can express IPC protein
- made **competent** by $CaCl_2$
  - Ca^{2+} ions attract both DNA and liposaccharides
  - 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 **

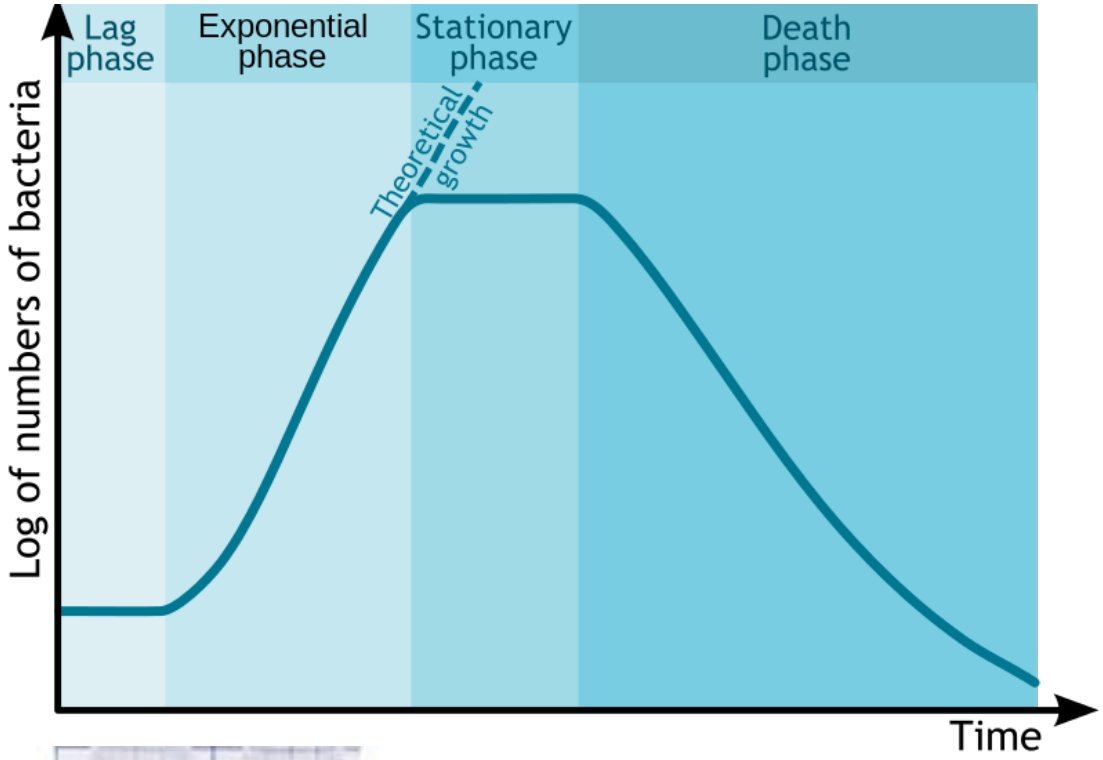
fragile

- Cam (chloramphenicol) resistant *E. coli* strain
  - also Amp (ampicillin) resistant if IPC insert uptaken

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

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

- OD ≠ absorbance
  - **OD: optical density**
  - **scattering, turbidity**
  - **yellow cells don't absorb 600 nm light!**
  - **UV light not safe**



Today in lab

- ~~Quiz~~
- ~~Prelab~~
- Isolate mutants DNA by Qiagen mini-prep
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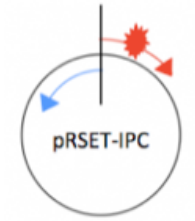
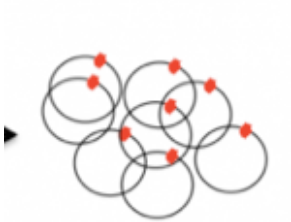
OD600 = 0.75

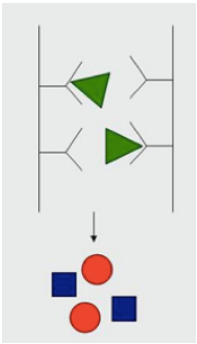
You have an entire week for M1D6 homework: get started now!

Tips to write Methods section

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







Homework due M1**D6**

- Methods from M1D3-M1D5:
  - 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