- Announcements
- Quiz
- Pre-lab Lecture
  - Interpreting transformations
  - *E. coli* growth, ~~bacterial strains~~
  - Today in Lab (Mod 2 Day 4)

Announcements

- Report return today, revision due in 2 wks
- Some general comments
- Module 2 vs. Module 1 expectations
- Previous FNT

#1 careful look @ protocol

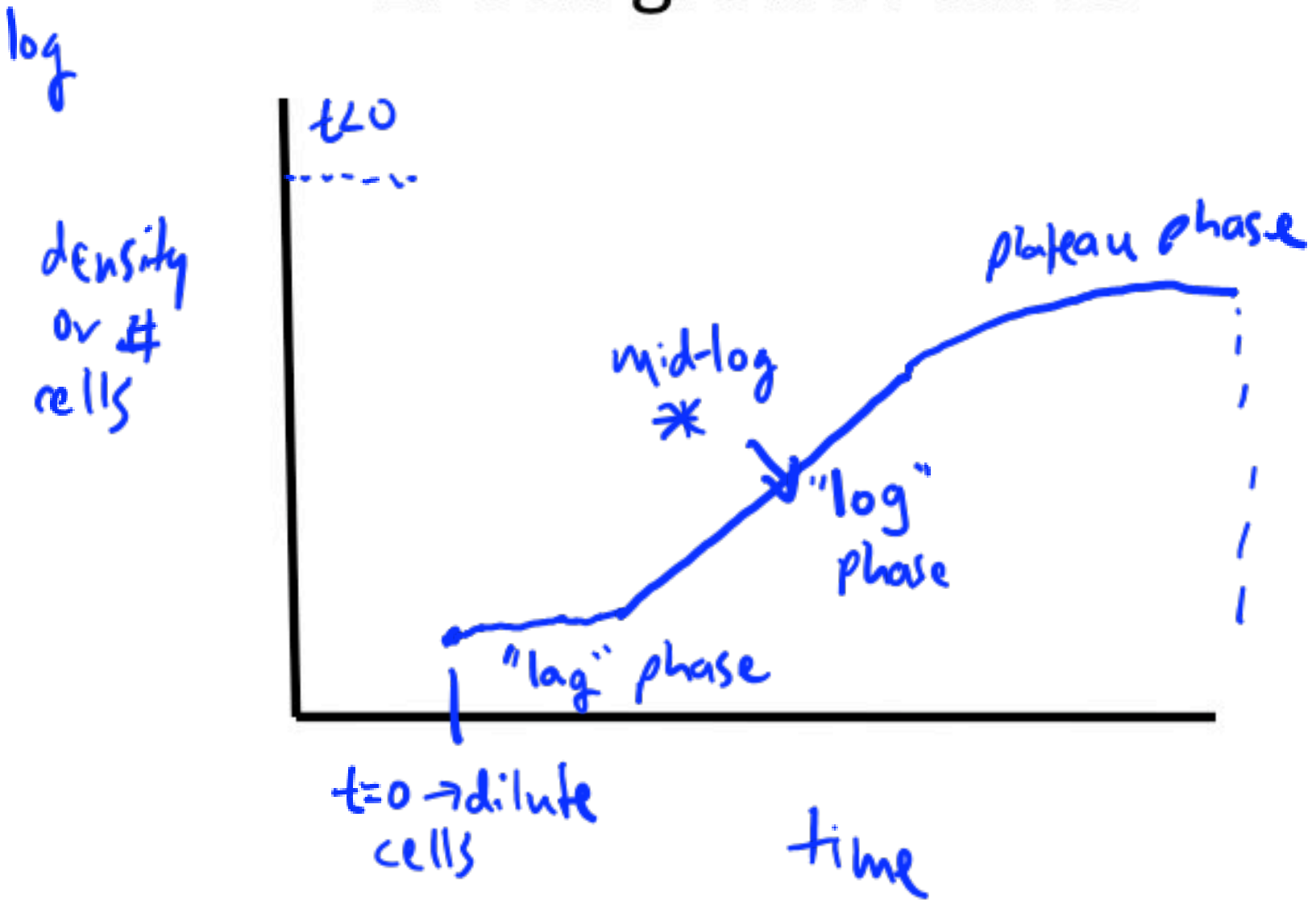
#2 micro and macro

#3 show explicitly

Transformation Controls + Outcomes

Sample	Expectation… What if?	Role
no DNA	Ø Will many? contamination	(—) control w/ cells and/or DNA; wrong plates
Pre-tested sample (M124S or pWhitescript)	Many Will none? killed cells; too little or no DNA; wrong antibiotic	(+) control ↳ for transformation
X#Z	Some-many Will < control? lower [DNA]	and/or lower mutation efficiency

E. Coli growth curve



Extracting DNA from XL1-Blue

Step	Contains	Purpose
Soln. I	EDTA Buffer, glucose	→ weakens cell envelope → otherwise stable
Soln. II	SDS NaOH	→ solubilize proteins, lipids ⇒ disrupt membrane → dsDNA → ssDNA
Soln. III	Acetic acid/KAc	→ neutralize pH ↙ genomic DNA "crashes" ↘ plasmid re-nature
Transfer supernatant	N/A	isolate plasmid
Final steps	EtOH, H_2O, drying	EtOH precipitates DNA, inhibits enzymatic reactions ↳ wash salts

Today in Lab

- Obtain DE3 in mid-log phase, make competent
  - 1 hour incubation 0.4-0.6 OD (keep in mind 1:10)
- Extract DNA from two mutant candidates
- Transform DE3 with the extracted DNA
  - ½ hour incubation
- During incubation(s): count mutant colonies, set up diagnostic digests and sequencing rxns
  - digest 1⁺ hour, we will stop digests if end past 5 pm

tell me if T>37°C ; BseRI ↑✓