- Announcements
- Quiz
- Pre-lab Lecture
  - Interpreting transformations
  - *E. coli* growth
  - Today in Lab (M2D4)

Announcements

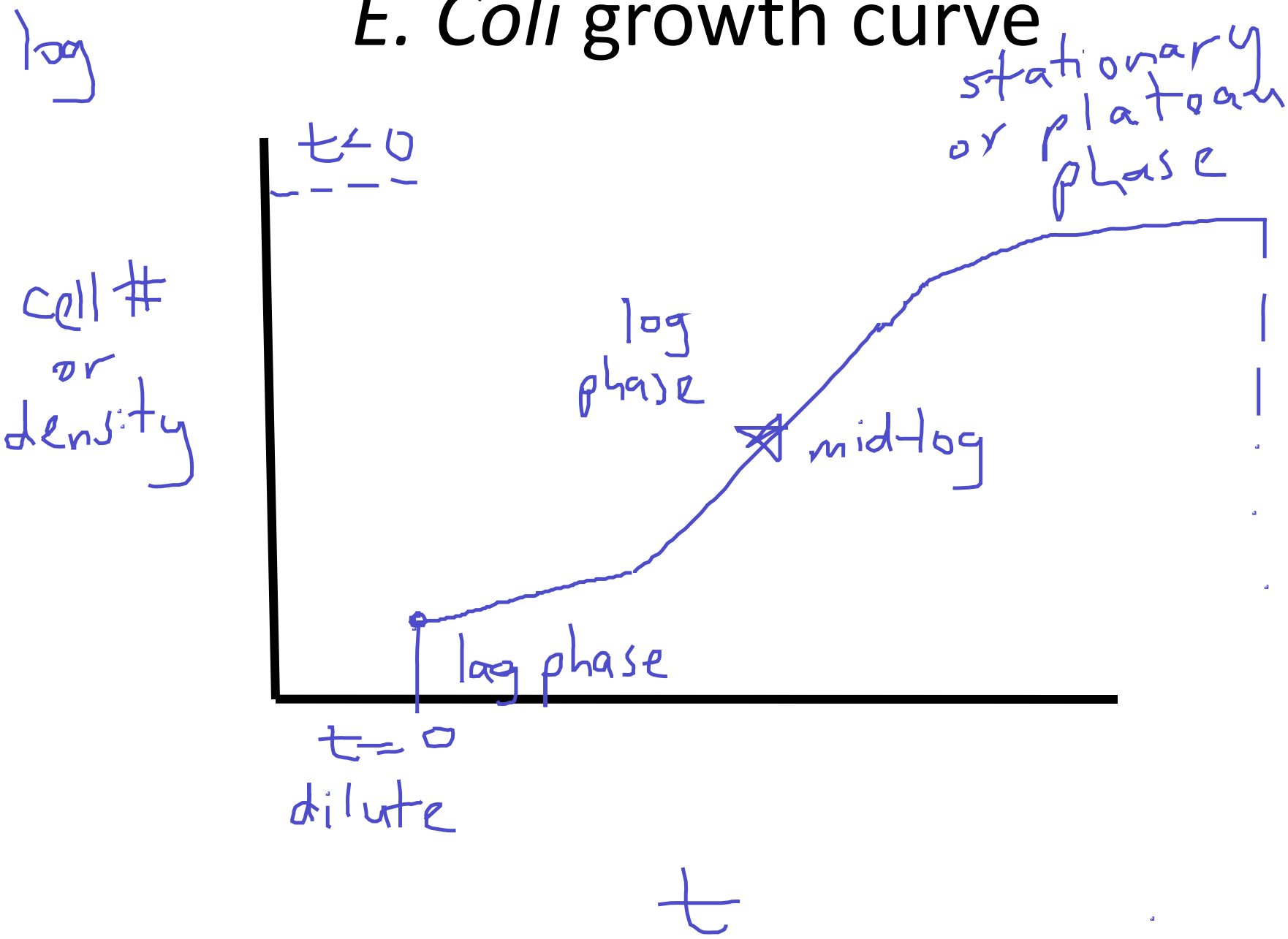
- Revised report due next time
  - to 20109.submit address
  - revisions somehow highlighted/tracked
  - name file as requested

☆ save leftover mini reps ☆

Transformation controls + outcomes

Sample	Expectation… What if? (w/)	Role
no DNA	none w/ many? wrong plates; contam. w/ other cells or w/ DNA	(–) control ↳ for contamination
Pre-tested sample (M124S)	many w/ none? (+) protocol mistake; no/low [DNA], killed cells; wrong antibio	(+) control ↳ for transformation
X#Z	some–many w/ << control? lower [DNA], maybe via lower transformation efficiency	exp.

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 (membrane) dsDNA → ssDNA
Soln. III	Acetic acid/KAc	neutralize pH genomic DNA tangled plasmid renatures
Transfer	N/A	supernatant isolate plasmid
Final steps	EtOH, H_2O, drying	EtOH precipitates DNA, but with aq salts interferes with enzymatic rxns.

Today in Lab (M2D4)

- Obtain BL21(DE3) in mid-log phase, make competent ~0.4-0.8OD for stock
  - 1 hour incubation
- Extract DNA from two mutant candidates ☆
- Transform BL21 with the extracted DNA
  - ½ hour incubation
- During incubation(s): set up diagnostic digests and sequencing rxns, count mutant colonies
  - digest 1⁺ hour, we will stop digests if end past 5 pm

if T≠37°C, tell me