- Announcements
- Lab Quiz
- Pre-lab Lecture
  - Where are we/going?
  - Cells and transformation
  - Plasmid miniprep
  - Today in Lab: M2D5

Announcements

- Module 1 ~~drafts~~ revisions due next time (11 AM)
- Vacuum aspirators contain bleach for biohazardous waste (i.e., cells)
  - after bleach treatment, these go down the sink
- Chemical waste and sink-safe chemicals should not be aspirated
  - the former is a safety risk, the latter just a hassle

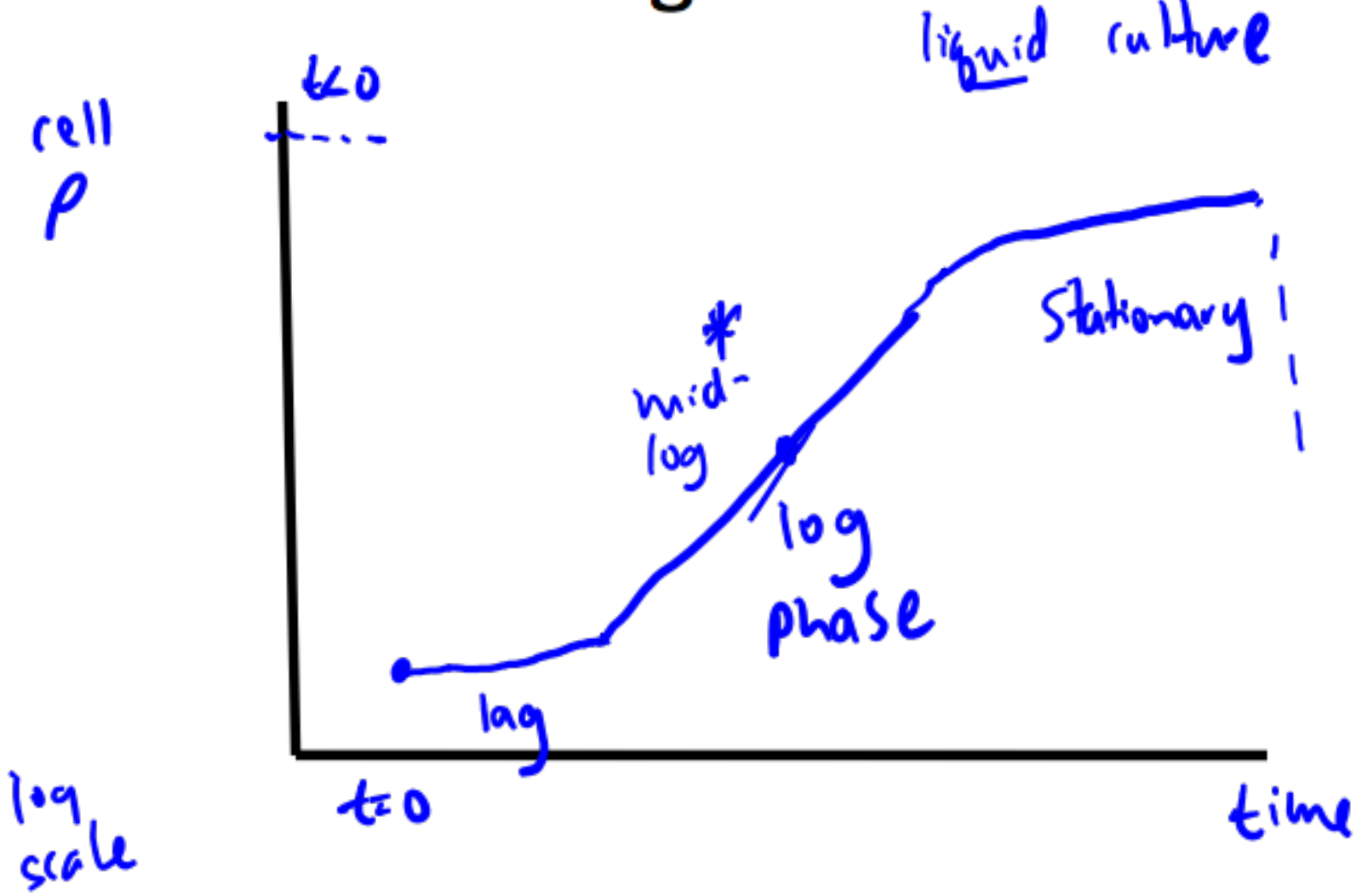
Module 2 Overview

- So far: designed new Plwy-λ
  Cloned into IPTG-sensitive system
- Next: test clones for correctness
  transform into edge detector base strain
  get new truth table (β-gal assay)
- Why: genetic reduce Plwy-λ leakiness
  physical improved contrast b/w edge + background
- Enrich understanding w/ modeling + transfer functions

Transformation Controls + Outcomes

Sample	Expectation… What if?	Role
No DNA	∅ Wl many?: contamination w/other cells, no antibiotic, plates went bad; (mutation);	(−) control DNA
+ pED-IPTG-INS	many Wl none?: killed cells; too little DNA; wrong antibiotic	(+) control
+ Bkb Ligation	few-some Wl many?: uncut and/or singly cut plasmid	digestion control
+ Bkb/Ins Ligation	some-many Wl way<< (+)?: low conc. DNA	expt'l

E. Coli growth curve



Bacterial Strains

Strain:	**XL1-Blue**	**JW3367c**
Function	amplify DNA	work w/ edge detector Strain
Relevant genotype	recA – no genetic recombination → stable plasmid endA. endonuclease (–) → intact plasmid	ΔlacZ no background → black pigment ΔenvZ no cross-talk w/ CpxR → fusion EnvZ/CpxA

Extracting DNA from XL1-Blue

Miniprep

Step	Contains	Purpose
Prepare	EDTA Buffer, glucose	→ weaken cell envelope → otherwise stable
Lyse	SDS ~~~ Na^+ NaOH	solubilize lipids (membrane), proteins ds → ss DNA
Neutralize	Acetic acid/KAc	pH to neutral, precipitate SDS genomic DNA crashes out, plasmid renatures
Transfer	N/A	* supernatant * isolate plasmid
Wash, collect	A) Column B) EtOH, dry	- better purity - precipitate DNA, resuspend

Today in Lab (M2D5)

- Obtain JW3367c in mid-log phase, make competent
  - 1 hour incubation
- Extract DNA from three candidate clones
- Transform JW3367c with the extracted DNA
  - ½ hour incubation
- During incubation(s): count colonies and set up sequencing rxns