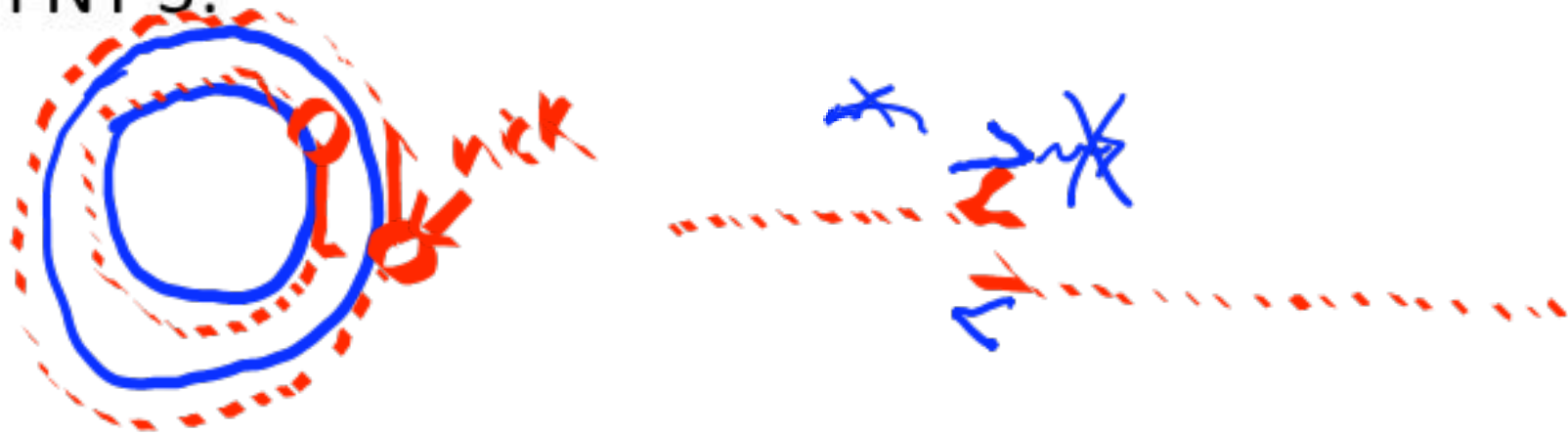


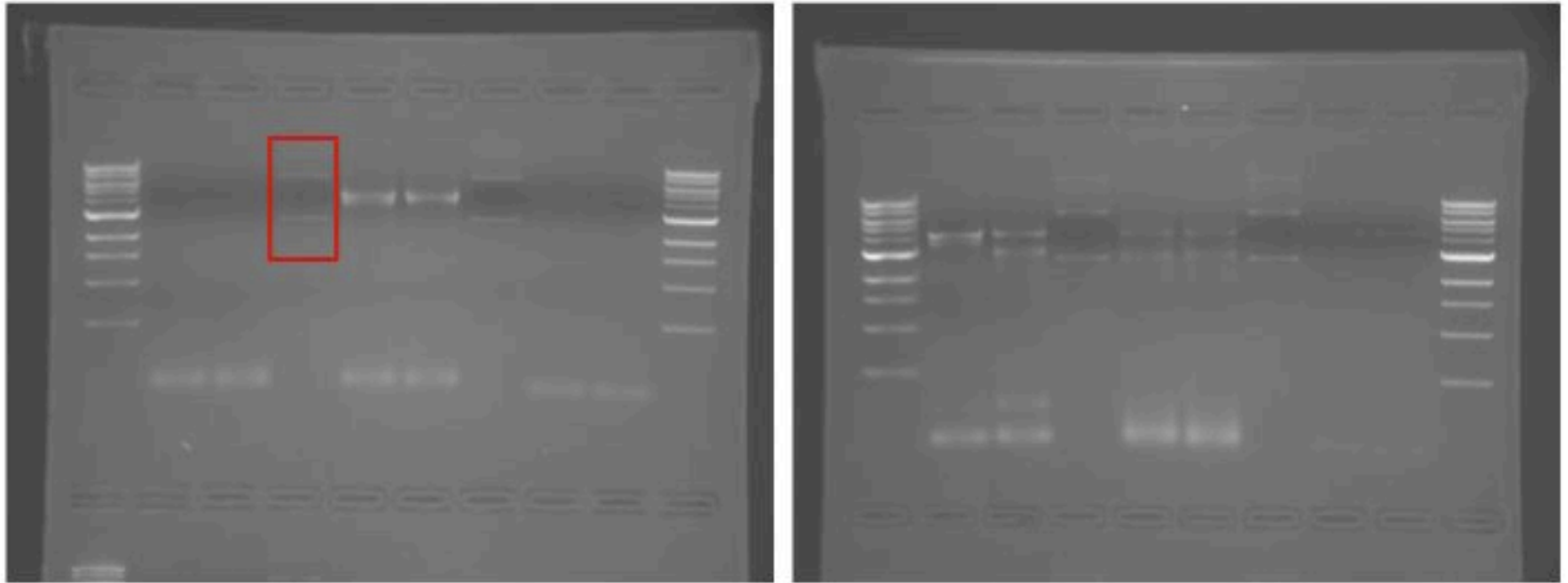
- Announcements
- Quiz
- Pre-lab Lecture
 - ❖ Gel results from last time
 - ❖ Interpreting transformations
 - ❖ *E. coli* growth, bacterial strains
 - ❖ Today in Lab (Mod 1 Day 4)

Announcements

- FNT 1: for methods calculations (or write-ups), look carefully at the protocol
- FNT 2: mostly good, try to answer specifically
- FNT 3:



Day 3 Gel Results



How can we interpret lanes 4+7 (parental control)?

What is the predominant DNA type in the experimental samples?

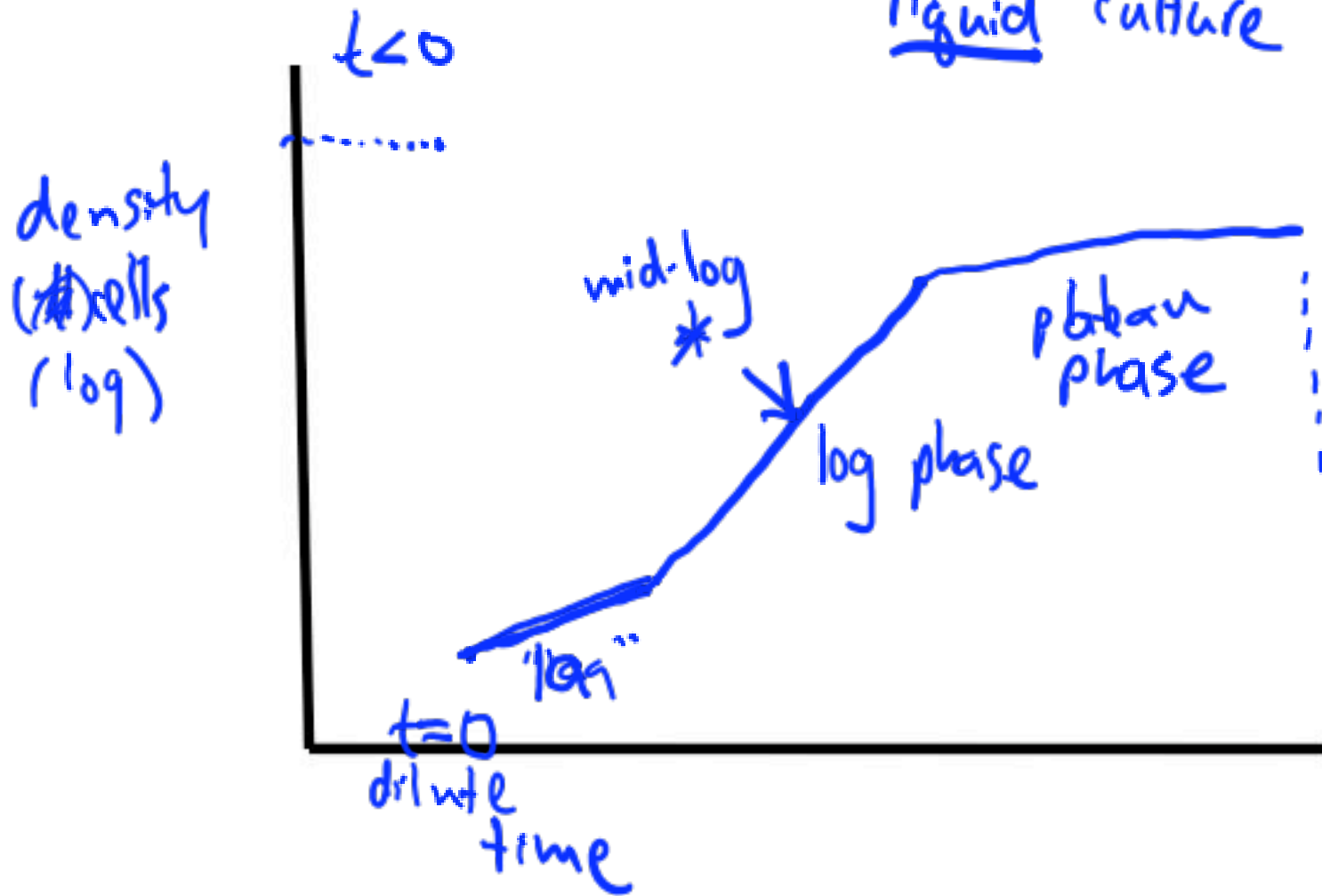
Why might the undigested and digested samples look the same?

Transformation Controls + Outcomes

Sample	Expectation... What if?	Role
no DNA	0 w/ many? contamination w/ cells and/or DNA; plates went bad	(-) control
Known plasmid (pWhitescript)	some-many w/ no? killed cells; wrong antibiotic	+ control
X#Z	few-some w/ < + control? low conc.,	experiment low efficiency for mutation

E. Coli growth curve




liquid culture



Bacterial Strains

Strain:	XL1-Blue	DE3
Function	amplify DNA	make protein
Relevant genotype	recA - no genetic recombination → stable plasmid endA → lack of endonucleases → good quality DNA extracted	lac construct → make protein in response to IPTG. more next time!

Extracting DNA from XL1-Blue

Step	Contains	Purpose
Soln. I	EDTA Buffer, glucose	→ weakens cell envelope → otherwise stable
Soln. II	SDS <i>Na⁺</i> NaOH	→ solubilize lipids, proteins ∴ disrupt membranes ds DNA → ss DNA 
Soln. III	Acetic acid/KAc	→ neutralize pH, precipitate SDS genome "crash"  → plasmid renature 
Transfer <i>Sup.</i>	N/A	isolate plasmid
Final steps	EtOH, H ₂ O, drying <i>wash salts</i>	→ EtOH precipitates DNA, but interferes w/ enzymatic rxns.

Today in Lab

- Obtain DE3 in mid-log phase, make competent
 - 1 hour incubation *Keep in mid log: 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
- FNTs: intro this time, figure/text next time