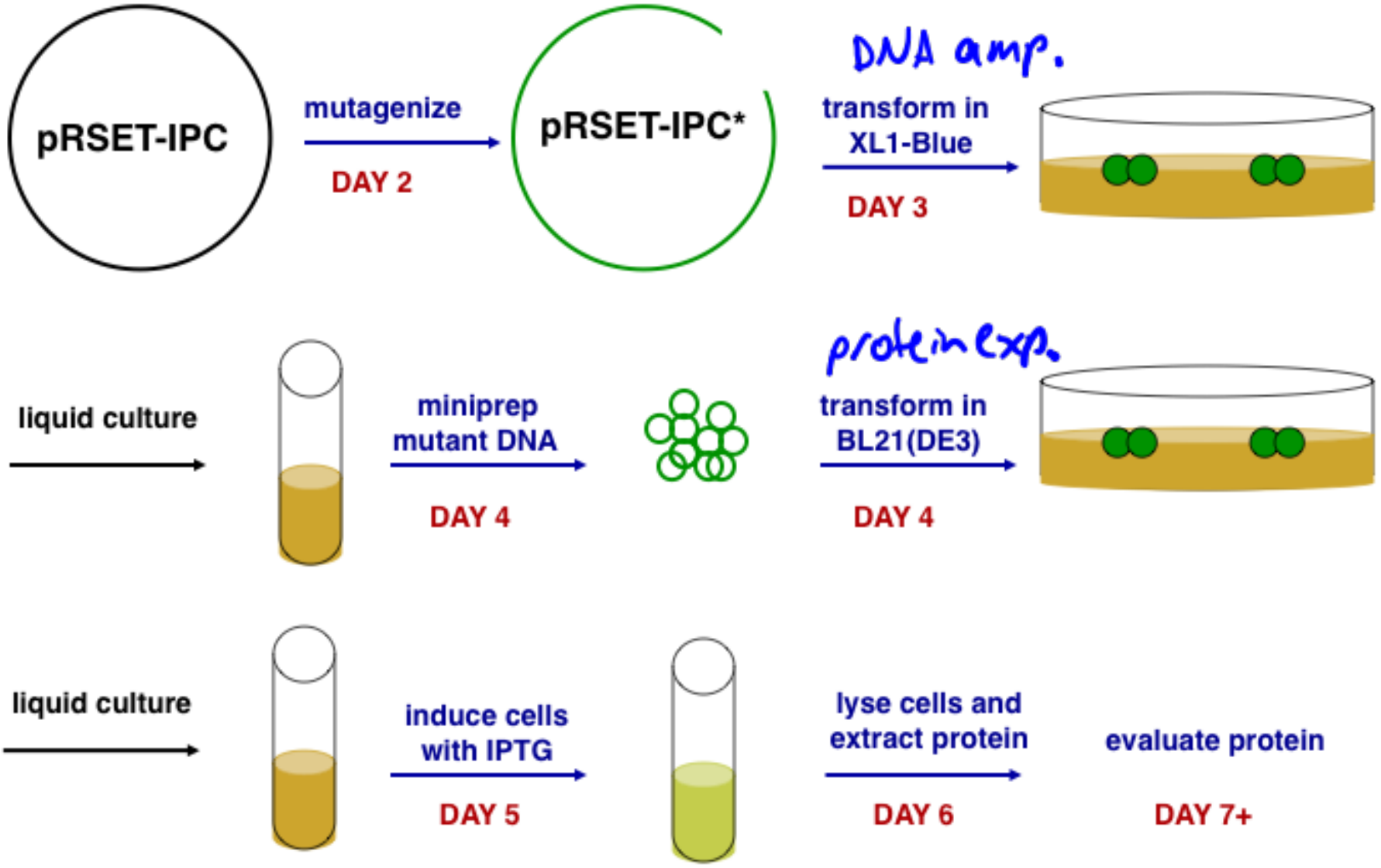
- Announcements
- Pre-lab Lecture
  - Interpreting transformations
  - *E. coli* growth
  - Today in Lab (M2D4)

Announcements

- Next FNT will be updated by tomorrow morning
- Module 1 lab report
  - Leslie's comments returned today
  - Jon's comments tomorrow electronically
  - revision due 2 weeks from tomorrow → individual office hours work best
- Next pre-lab will hit
  - more about XL1-Blue vs BL21(DE3)
  - review protein expression in BL21

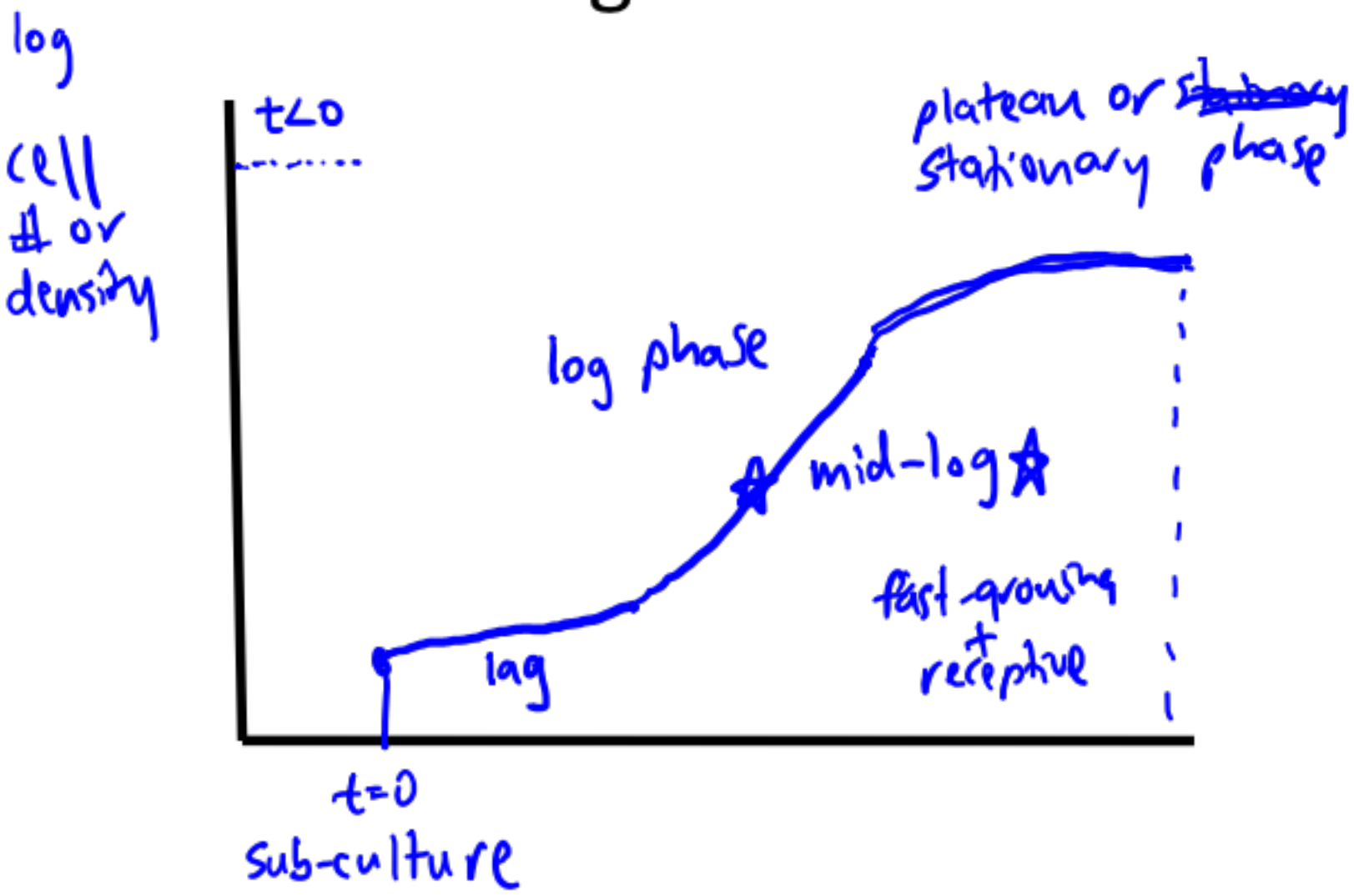
• today's FNT → in for now, borrow as needed


pRSET-IPC
mutagenize
DAY 2
pRSET-IPC*
DNA amp.
transform in
XL1-Blue
DAY 3
liquid culture
miniprep
mutant DNA
DAY 4
protein exp.
transform in
BL21(DE3)
DAY 4
liquid culture
induce cells
with IPTG
DAY 5
lyse cells and
extract protein
DAY 6
evaluate protein
DAY 7+

Transformation controls + outcomes

Sample	Expectation… What if? (WI)	Role
no DNA	none WI many? -contamination by cells or DNA - wrong or ineffective plates	(–) control for contamination (Amp-resistant)
Pre-tested sample (E67K)	many WI none? or few? - Killed some cells (vortex etc.) - wrong antibiotic - low [DNA]	(+) control for transformation (reagents, technique)
X#Z	some-many WI << control? low [DNA], maybe via lower mutation efficiency	experiment

E. Coli growth curve



Today in Lab (M2D4)

- Obtain BL21(DE3) in mid-log phase, make competent
  - 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

goal: ~0.4–0.8 for stock

* save minipreps

* check day step w/us

+ label tubes

if T ≠ 37°C, tell me.

Have a great spring break!