# M2D4: Prepare for Expression

3/22/13

1. Turn in M1D3 FNT up front in folder: I'll check off your name and you can come get it if/when you need it

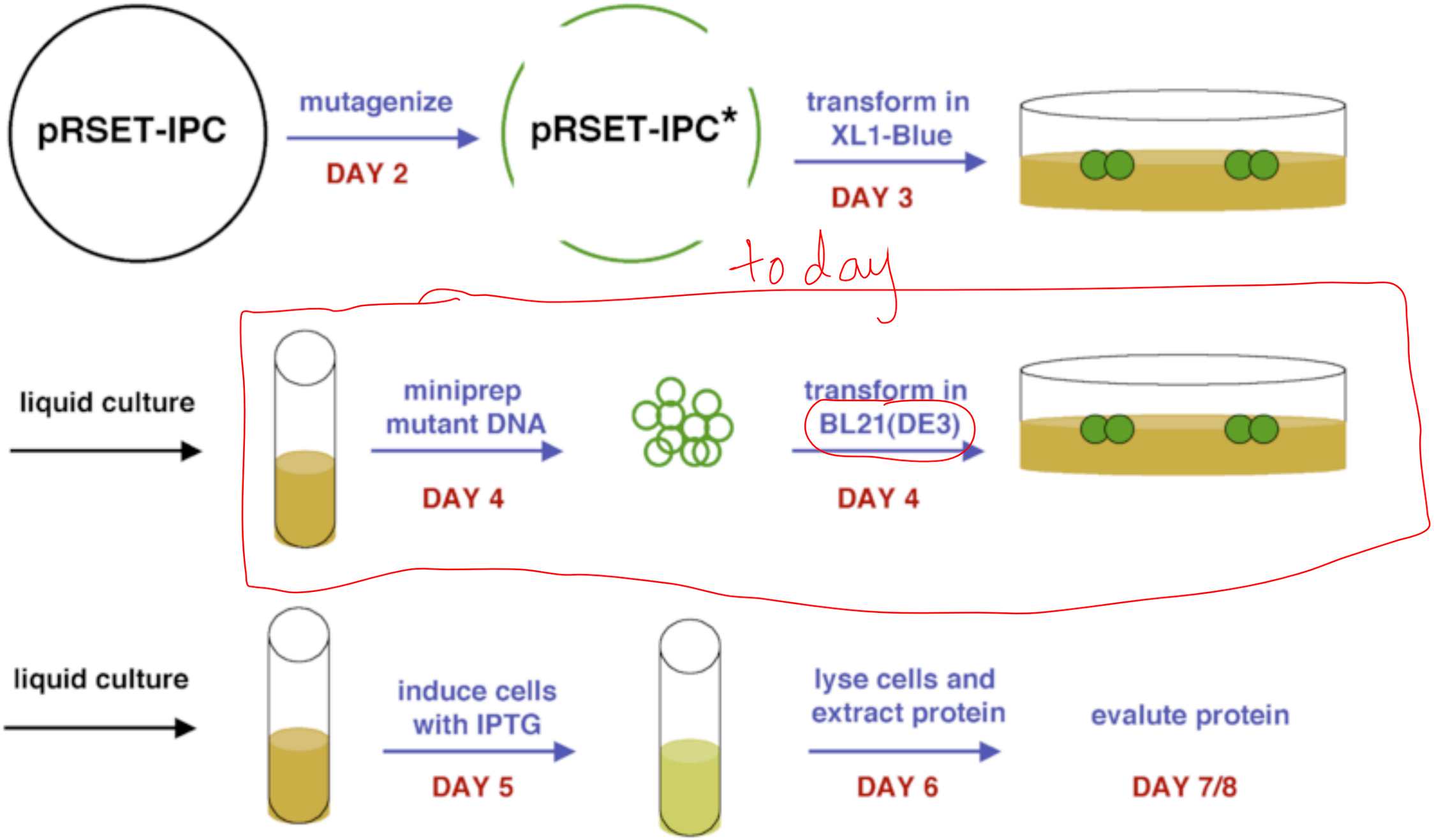
2. Preview of graduate school. Today we will:

   - ☆ Make competent cells
   - ☆ Miniprep DNA
   - ☆ Transform into our homemade competent cells
   - ☆ Set-up diagnostic digests
   - ☆ Set-up sequencing reactions

3. And, hey!, today is not as long as we originally thought!

4. Actual lab treats are out in the break room.

# Protein Engineering -- Experimental Overview

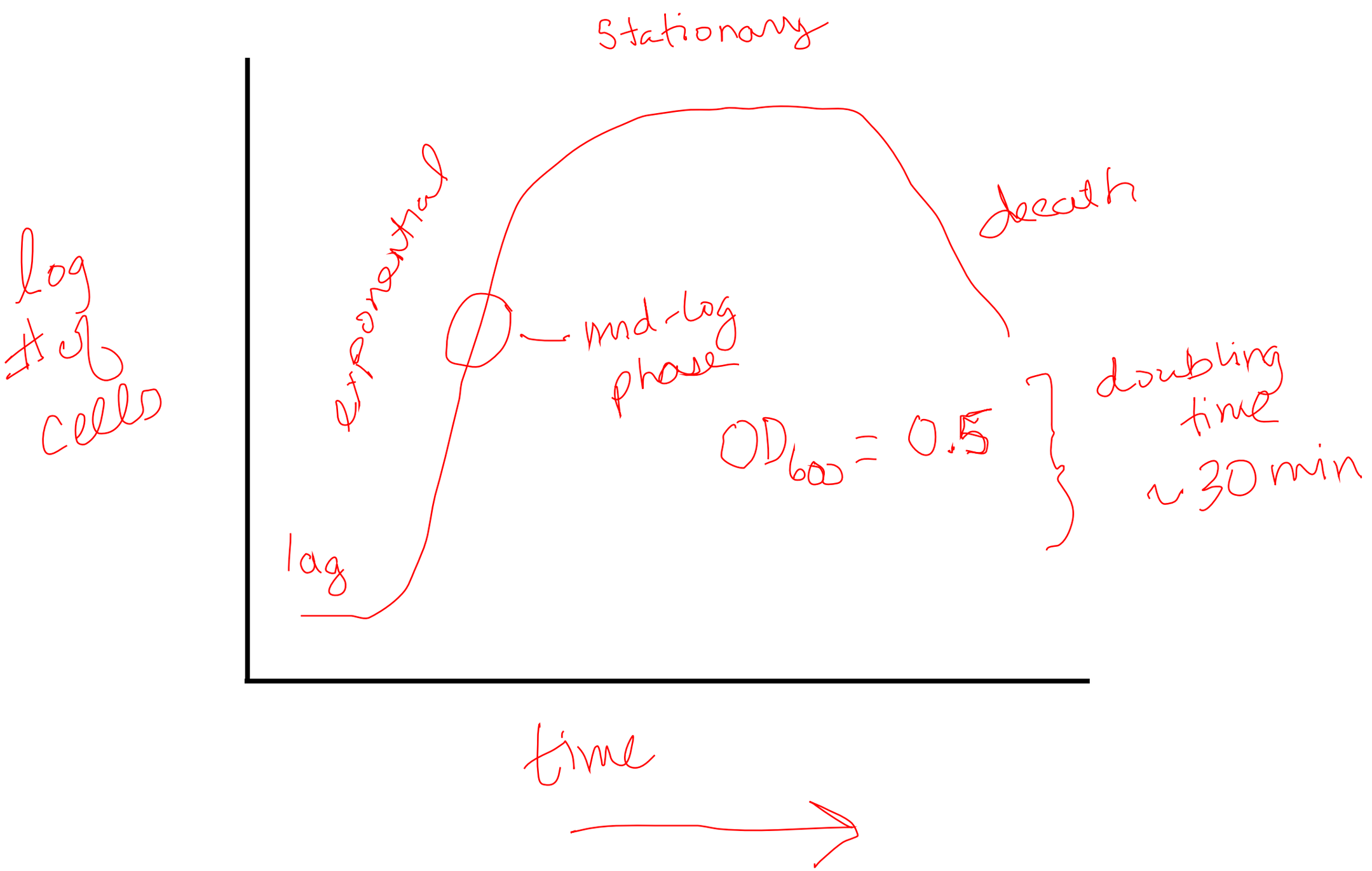


# Let's think about our transformations...

- cells - LB
- DNA - Ab
- Incubator
- You
- other past process

| Sample | Expectation (...what if?) | Role |
|---|---|---|
| no DNA | • Nothing<br>• bad Ab<br>• contamination of media<br>• labeled plate - You<br>• bad Streak technique | Negative Control |
| E67K | • >100<br>• bad cells ⇒ not very competent cells<br>• degraded DNA | positive control |
| Y.M. (X#Z) | ~10-20 colonies<br>PCR-based reasons<br>(Need to optimize!) | experiment |

# E.coli density matters. Why?



# Today in lab:

1. Obtain BL21(DE3) in mid-log phase, make competent -- 1 hr incubation
2. Extract DNA from two mutants

3. Transform BL21 with extracted DNA -- 30 min incubation

4. During incubation(s): set-up diagnostic digests, sequencing reactions, and count colonies -- we will stop digests if they go past 5pm