

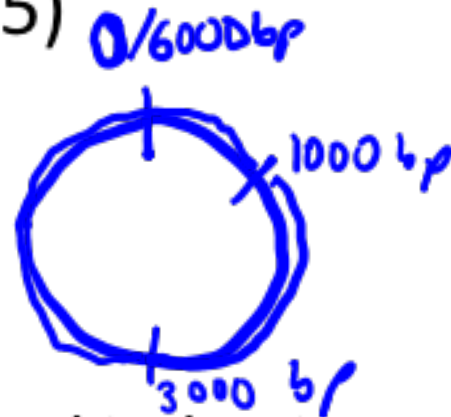
- Announcements
- Pre-lab Lecture
 - ❖ Review approach
 - ❖ Colony data
 - ❖ Genetic control elements
 - ❖ Today in Lab (M2D5)

Announcements: revision

- Report revision and reflection due **Fri 11 AM**
- Resources
 - Agi + Shannon (by appt)
 - Jon (by appt)
 - Writing Fellows (by appt)
 - Writing Lab (Sun-Thu, 7-9 pm, 56-205)
- Jon's comments and guiding questions are *excellent*; take those Q seriously
 - addressing half the comments in depth is better than addressing all of them shallowly!

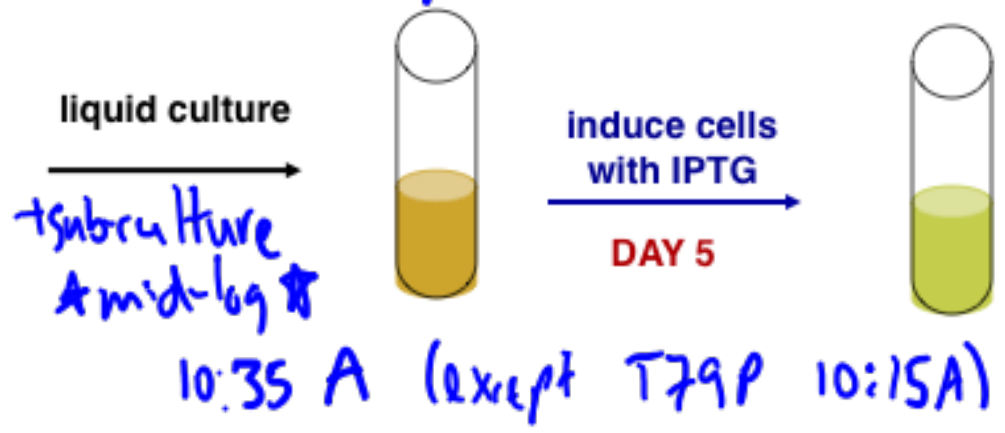
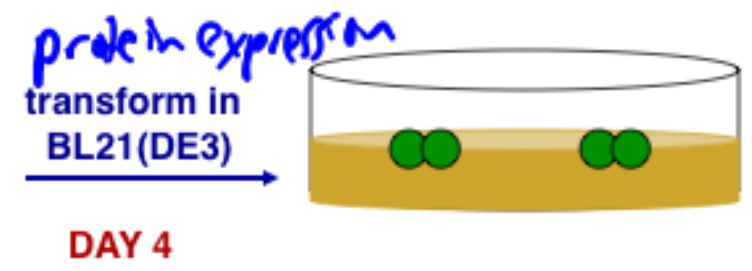
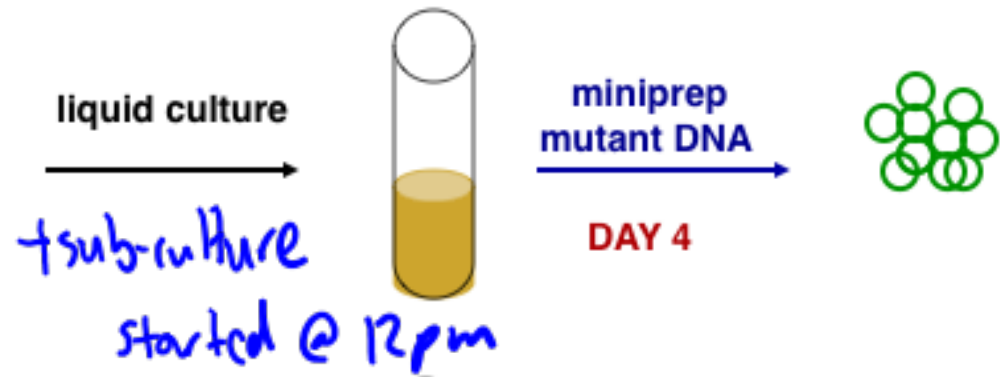
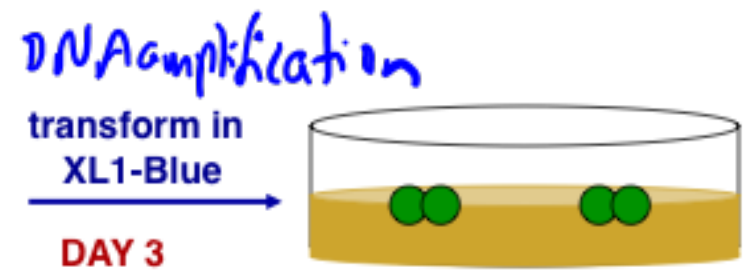
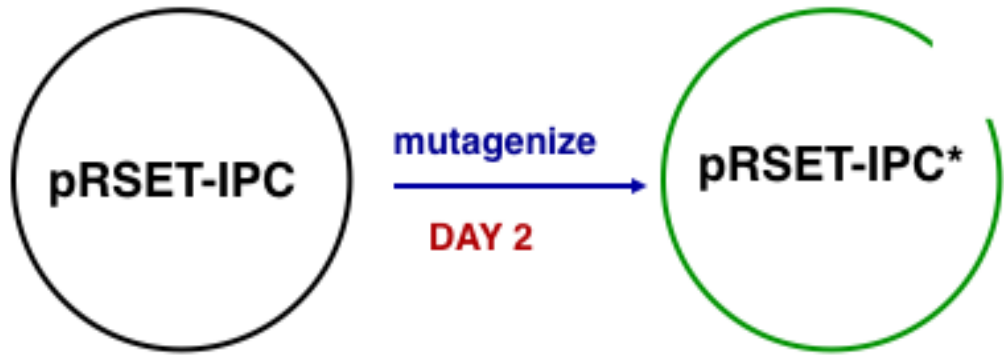
Announcements: review

- Conceptual odds and ends
 - why OD-600 (not A-600) for cell density?
- Quiz next time (M2D3-M2D5)
 - core lecture material (Alan's)
 - NO stats
 - gels and digests
 - cell strains, transformation, and induction



enzyme doesn't
"know" where ϕ is

2000 bp } upon digest
4000 bp }



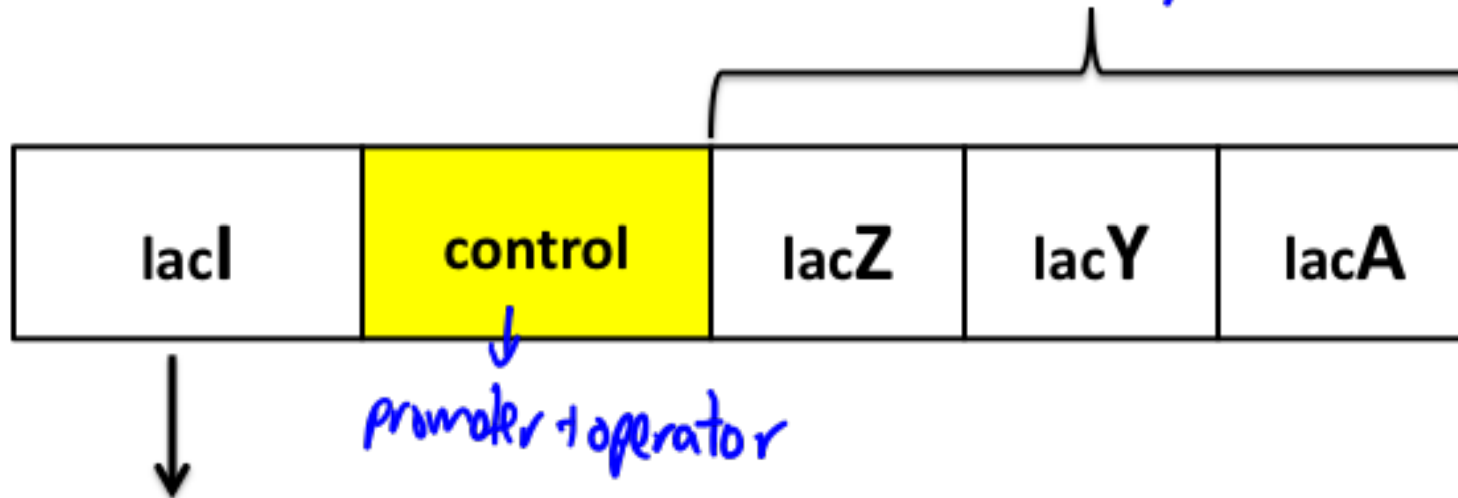
Interpreting class colony counts

Group Colour	no DNA	reference mutant SDM	experiment	no DNA	reference mutant SDM	experiment
Hypothetical Data	0	100	50	0	100	50
Red	0	229	5			
Orange				0	526	1
Yellow				0	658	21
Green	0	433	391			
Blue						
Pink						
Purple	0	150	2	0	1	0
Platinum	1	352	436			

Please post: these counts are interesting data!

lac operon

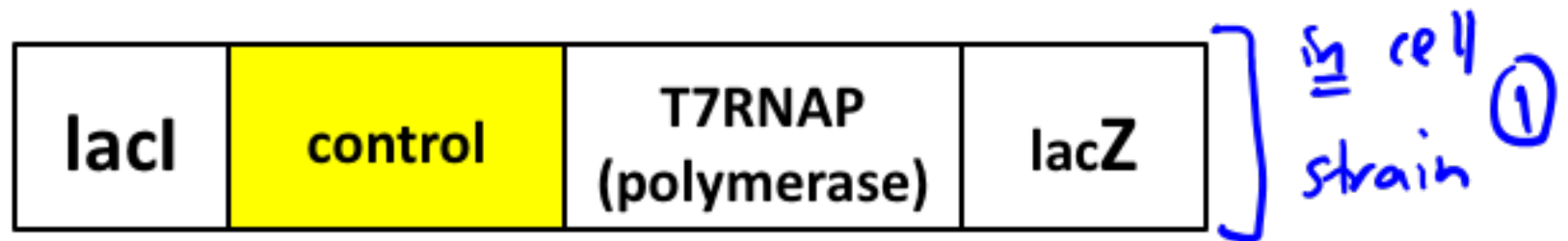
These three genes encode metabolic enzymes



Encodes a repressor
that binds to control area
turning it OFF.

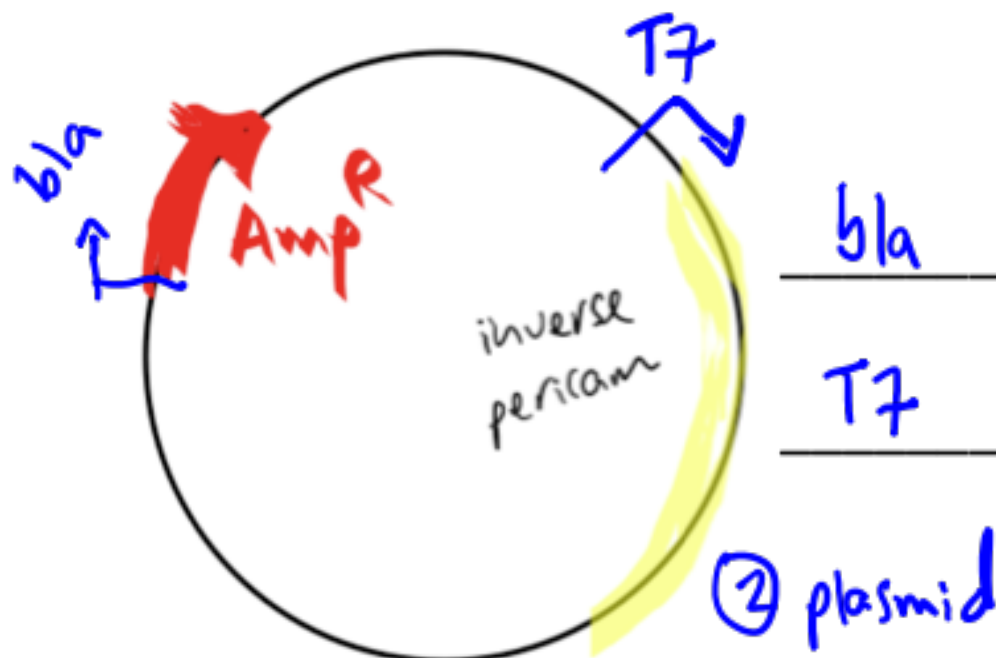
In turn, if lactose binds to the
repressor, it is made
inactive, turning ON expression
of Z, Y, A.

Induction of a chosen protein



T7RNAP gene is expressed in presence of lactose or analogue.

[IPTG] = constant,
not used up



bla promoter is constitutively on.

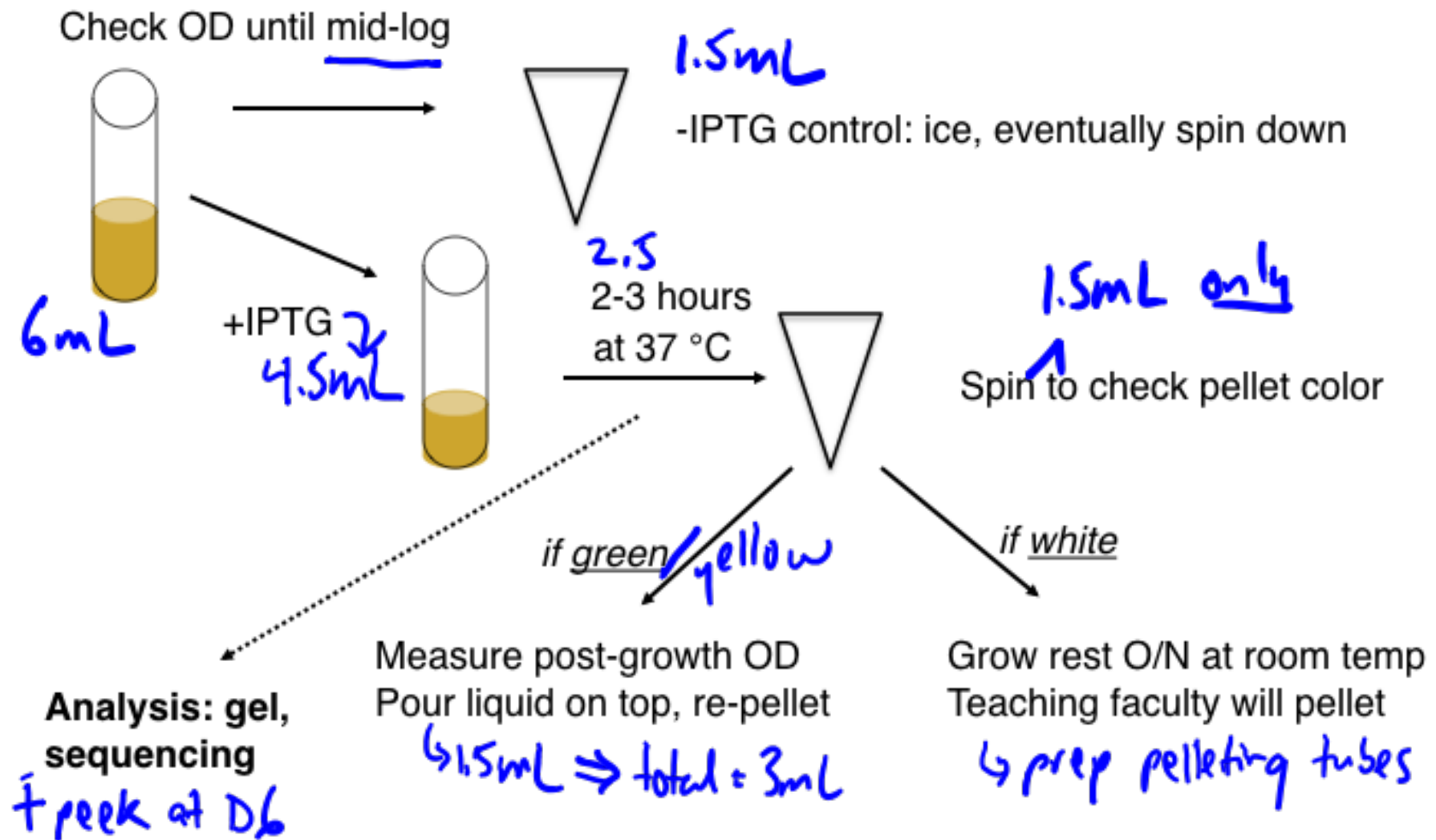
T7 is turned on in presence of T7RNAP.

BL21(DE3) bacterial strain

DE3: bacteriophage (virus) used to integrate
lac/77RNAP construct into E. coli

pLysS: protein that produces lysozyme, which
binds to T7RNAP, reducing basal/"leaky" expression.
Retained by chloramphenicol selection.

Today in Lab (M2D5): Workflow



Today in Lab (M2D5): Samples

- Start with four BL21 cultures:
 - WT
 - Reference mutant
 - X#Z candidates 1 and 2
- After gel and sequencing analysis, pick just *one* X#Z to continue working with
- End of day, “hand in” 6 pellets OR 3 pellets, 3 cultures, and 3 eppendorfs to teaching faculty

- Gnd + IPTG
↑

→ - IPTG only