

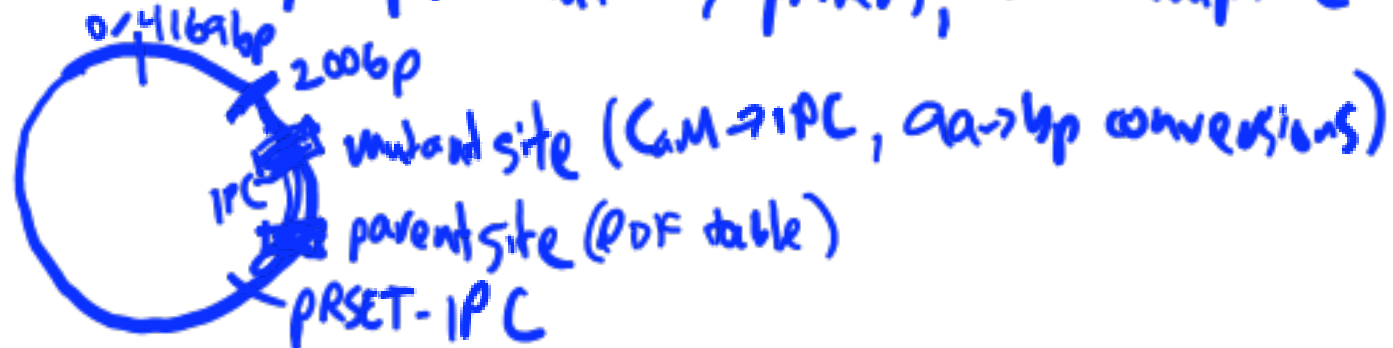
- Announcements
- Quiz
- Pre-lab Lecture
  - ❖ Review so far, colony data
  - ❖ Genetic control elements
  - ❖ Sequencing recap
  - ❖ Today in Lab (Mod 2 Day 5)

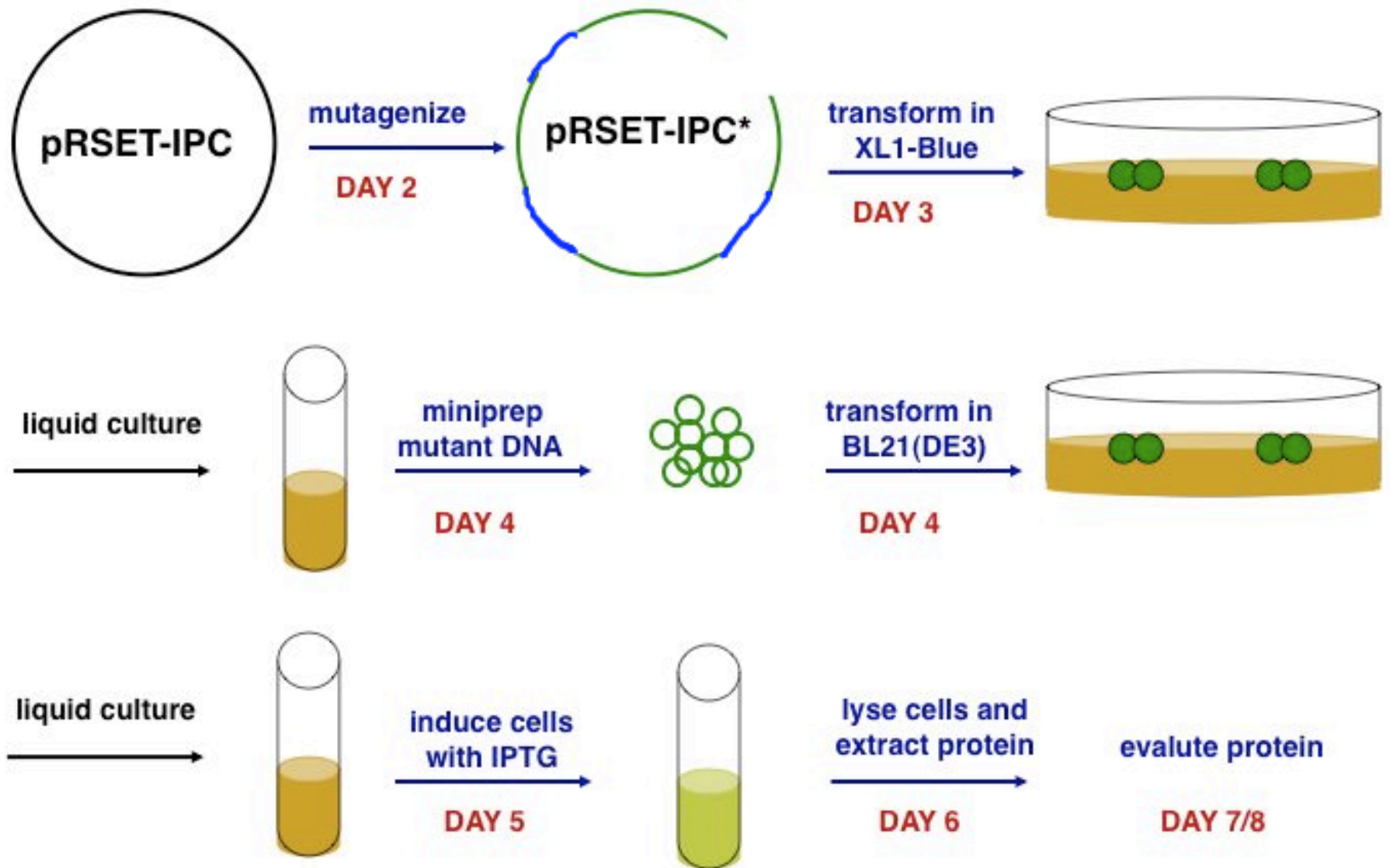
# Announcements

- No quiz next time (full day)!
  - Responsible for Day 5 + 6 material for Day 7 quiz
- Module 1 revision due by 11 am next time
- Previous quiz and FNT:

ctrl + exp dont work → Master Mix, cycle ✓

ctrl worked, exp. didn't → primers, DNA template





# Colony counts

Group Colour	(-) control	+ control plasmid	experiment
Hypothetical Data	0	100	50
Red			
Orange	0	274	323
Yellow	0	156	285
Green	0	285	341
Blue	0	625	351
Pink	0	140	12
Purple	0	233	195

Group Colour	(-) control	+ control plasmid	experiment
Hypothetical Data	0	100	50
Red	0	145	2
Orange			
Yellow	0	123	299
Green	0	400	41
Blue			
Pink	0	338	621
Purple	0	108	0

## Teaching faculty plates

pWhitescript: ~600

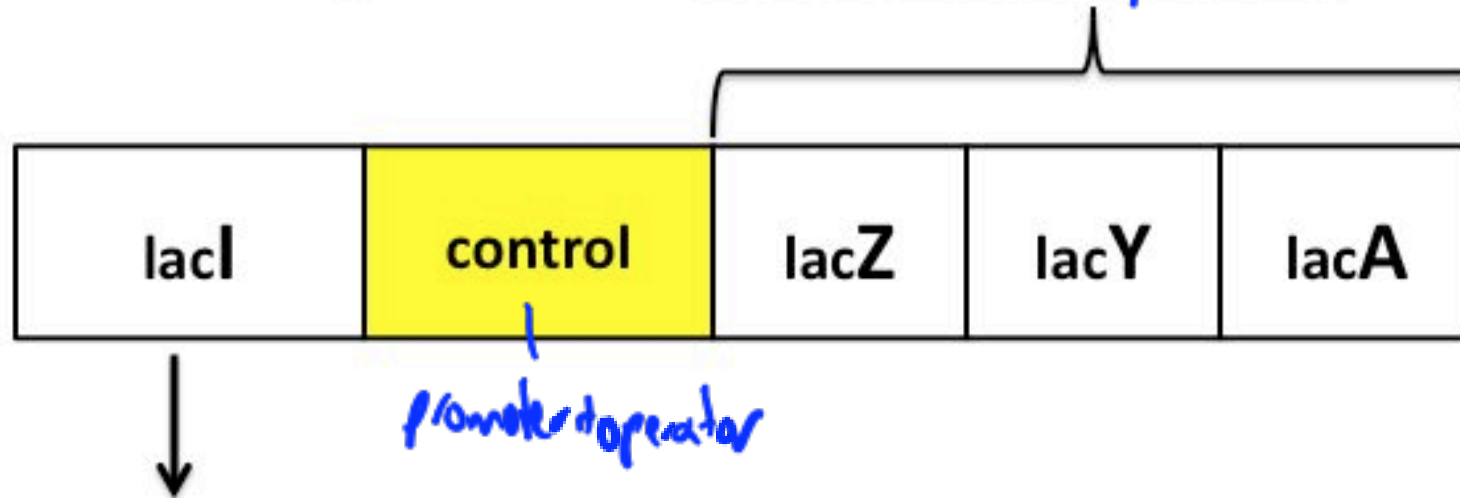
M124S (1:300): ~225

M124S (1:400): 52



# lac operon

These three genes encode metabolic enzymes



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

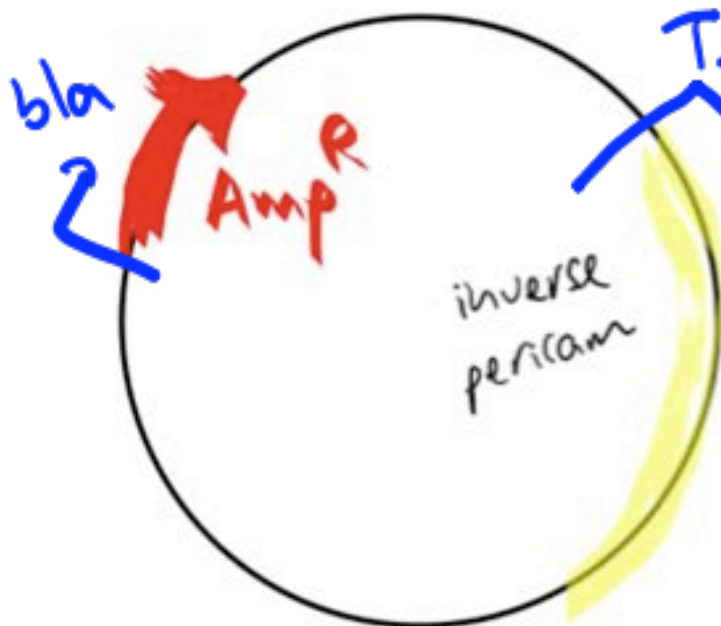
In turn, if lactose binds to the repressor, it is made inactive, turning ON expression of z, y, A.

# Induction of a chosen protein

lacI	control	T7RNAP (polymerase)	lacZ
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T7RNAP gene is expressed in presence of lactose or analogue.

$$[IPTG]_0 = [IPTG]_t$$



bla promoter is constitutively on.

T7 promoter is turned on in presence of T7RNAP.

# BL21(DE3) bacterial strain

genome

**DE3:** bacteriophage (virus) used to integrate lac / T7RNAP into *E. coli*






plasmid

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

# Sequencing reactions

Dideoxy method: no 3' OH → can't elongate  
 Run 4 rxns: (d)dT, dA, dG, dC and 3 others

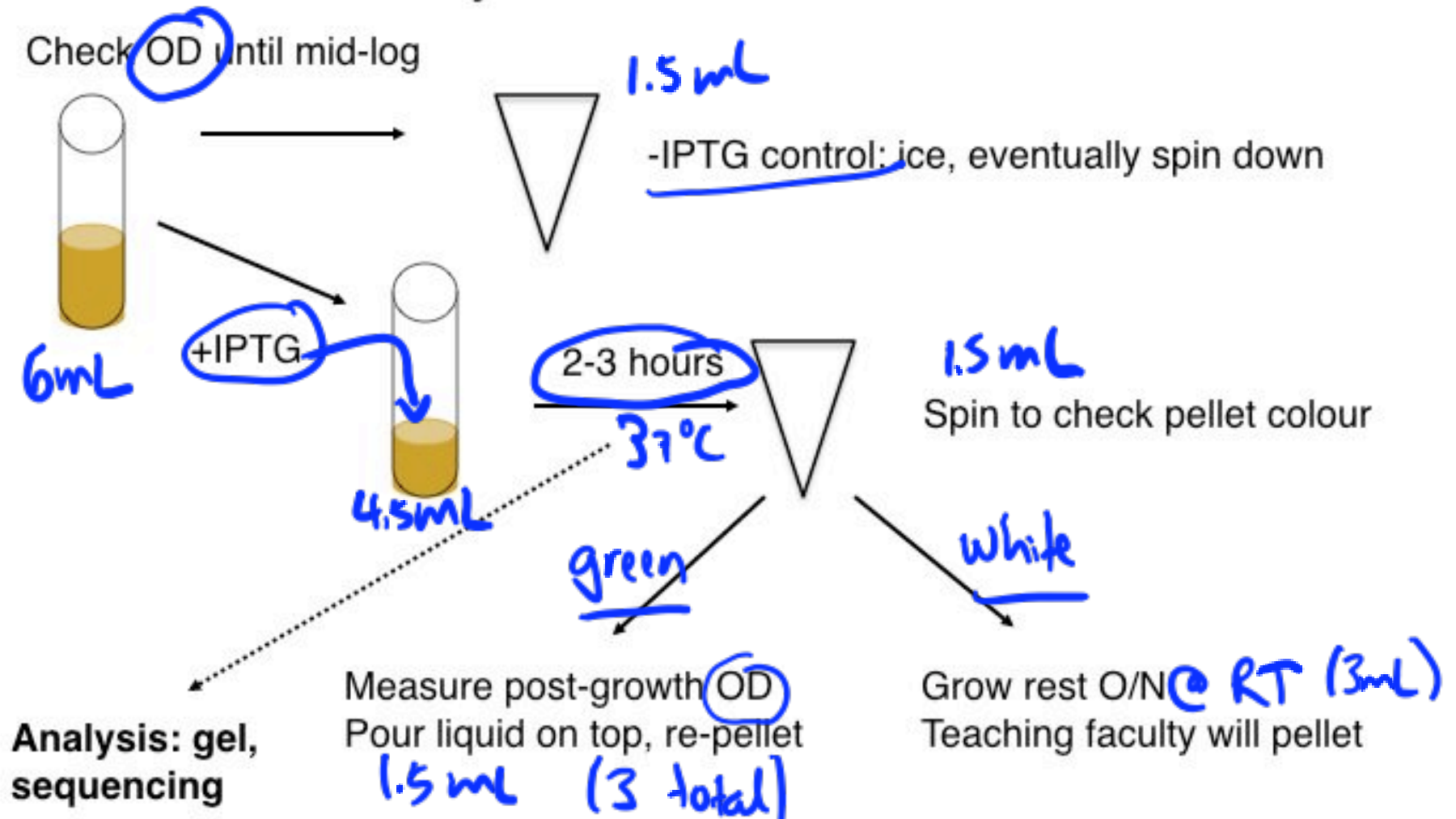
*different fluorophore*  
 ddA<sup>I</sup>, G<sup>II</sup>, C<sup>III</sup>, T<sup>IV</sup>

<i>ddT</i> Reactions		<i>ddA</i>		Gel	
<i>←</i>	TAAATT	<i>←</i>	TAAATT	ddT	ddA
	AT*		A*		 7bp
	ATT*		ATTTA*		
	ATTT*		ATTTAA*		
			ATTTAAA*		 1bp

\* = radioactive or fluorescent label



# Today in Lab: Workflow



# Today in Lab: Samples

- Start with four DE3 samples carrying plasmid
  - WT
  - M124S
  - 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

→ IPTG