- Announcements
- Quiz
- Pre-lab Lecture
  - ❖ Review
  - ❖ 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
- FNT updated by noon tomorrow
- Report revision reflection due next time
- Can still hand in evals today
- Conceptual odds and ends
  - why OD-600 (not A-600) for cell density?
  - heat shock mechanism

Interpreting class colony counts

Spring 2010

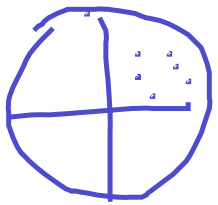
Group Colour	(-) control	+ control plasmid	experiment
Hypothetical Data	0	100	50
Red			
Orange	0	274	323
Yellow	0	156	285
Green	0	285	341
Blue	0	625	351
Pink	0	140	12
Purple	0	233	195

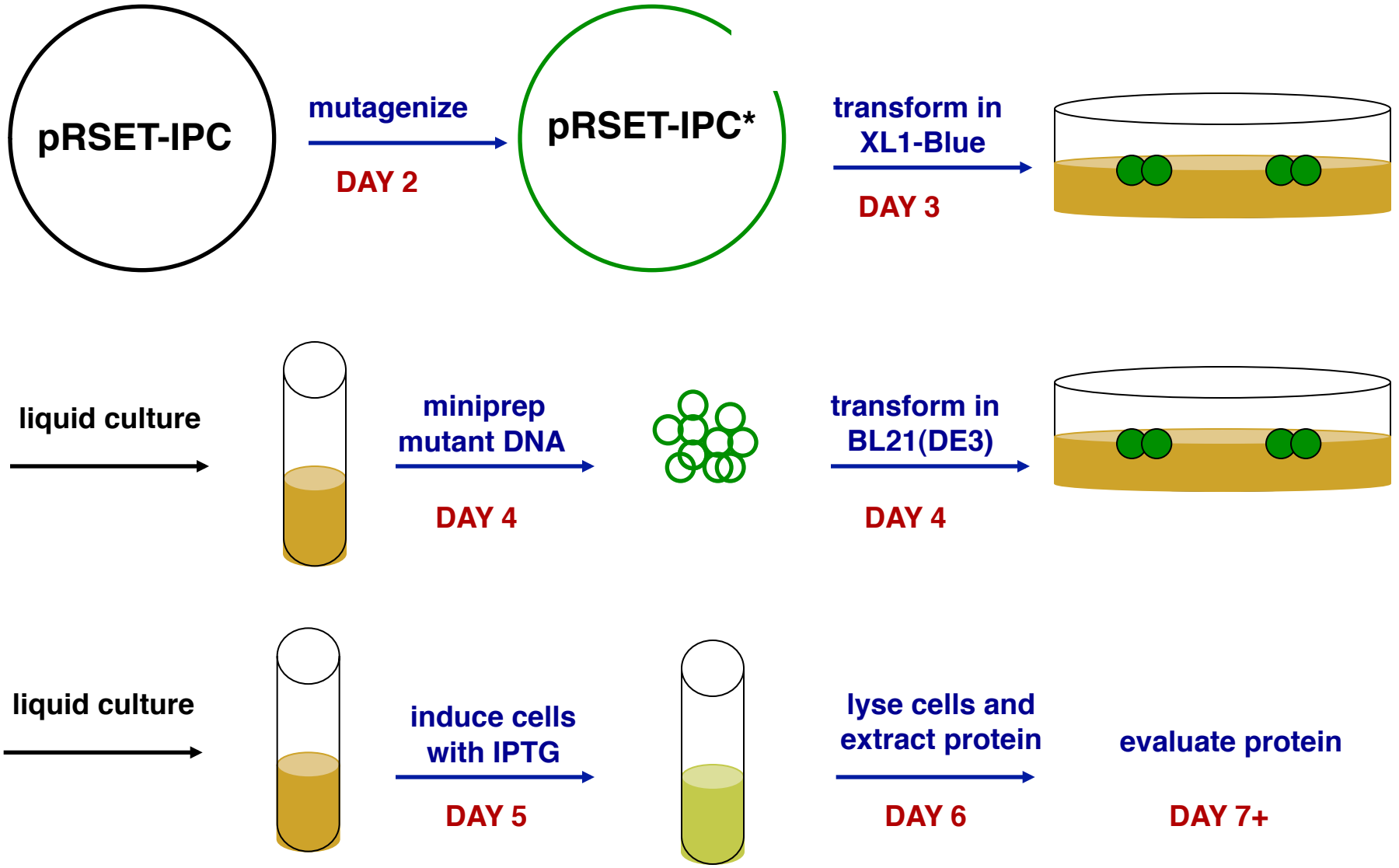
Spring 2012

Group Colour	(-) control	+ control plasmid	experiment
Hypothetical Data	0	100	50
Red	0	entire plate	~200
Orange	0	entire plate	2
Yellow	0	entire plate	35
Green	0	entire plate	186
Blue			
Pink			
Purple	0	Entire plate	51

What positive control do you think you were given (in error)?

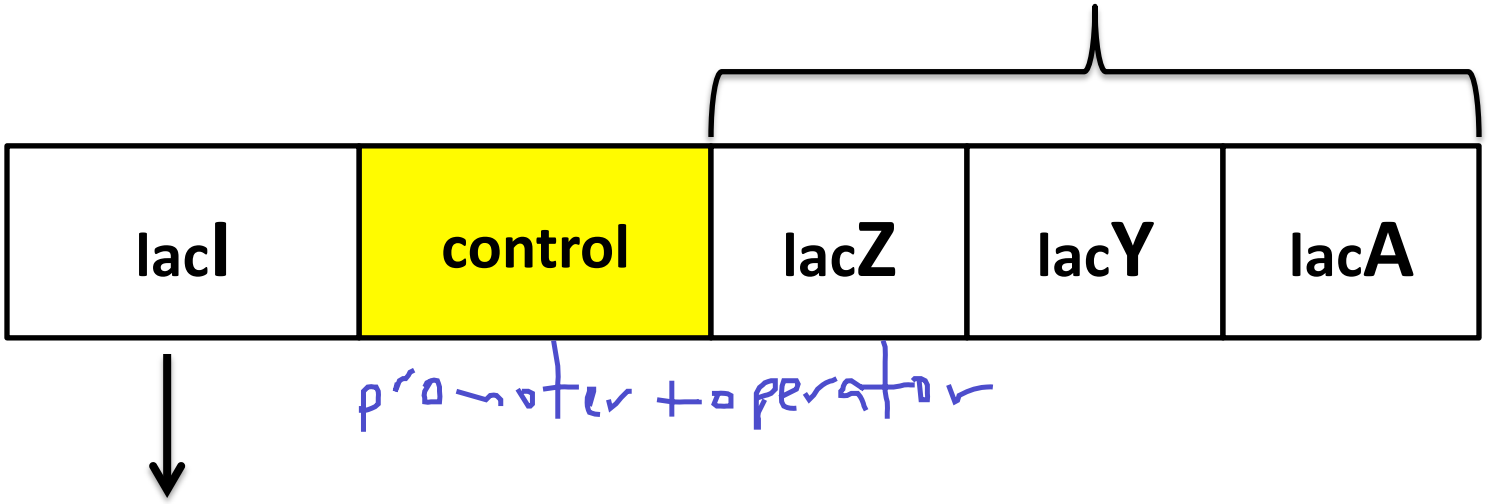
MIZ45 plasmid instead of SDM (or undigested SDM)




pRSET-IPC
mutagenize
DAY 2
pRSET-IPC*
transform in
XL1-Blue
DAY 3
liquid culture
miniprep
mutant DNA
DAY 4
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+

lac operon

These three genes encode metabolic enzymes



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

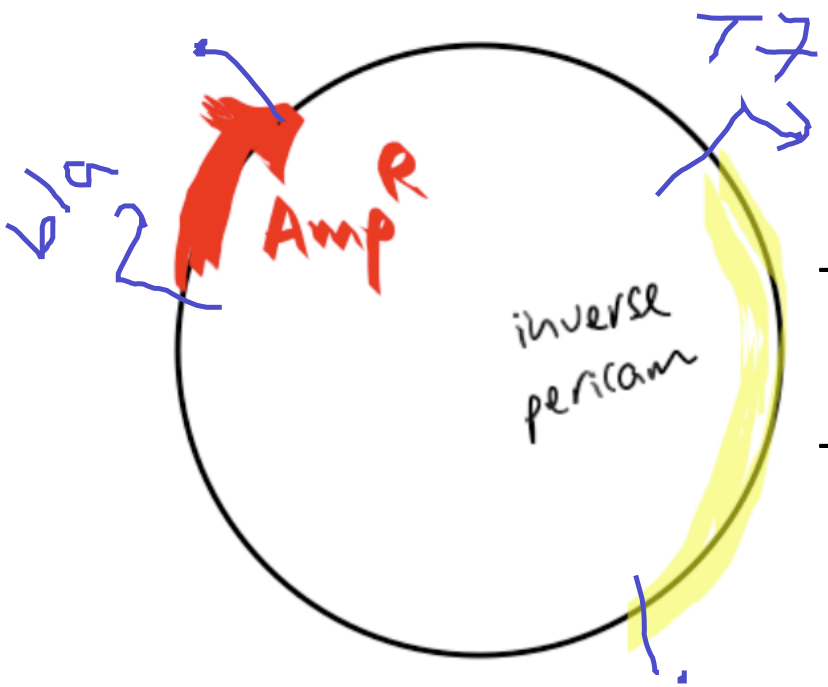
In turn, if lactose binds to the repressor, it is made inactive, turning ON expression of Z, Y, A.

Induction of a chosen protein

lacI	control	T7RNAP (polymerase)	lacZ

T7RNAP _____ gene is expressed in presence of lactose or analogue.

[IPTG] = constant



bla _____ promoter is constitutively on.

T7 _____ is turned on in presence of T7RNAP.

BL21(DE3) bacterial strain

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

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

Sequencing reactions

Dideoxy method: no 3' OH → can't elongate
Run 4 rxns: (d)dT, dA, dG, dC together and 3 others
* Radioactive or fluorescent label. Different color for each.

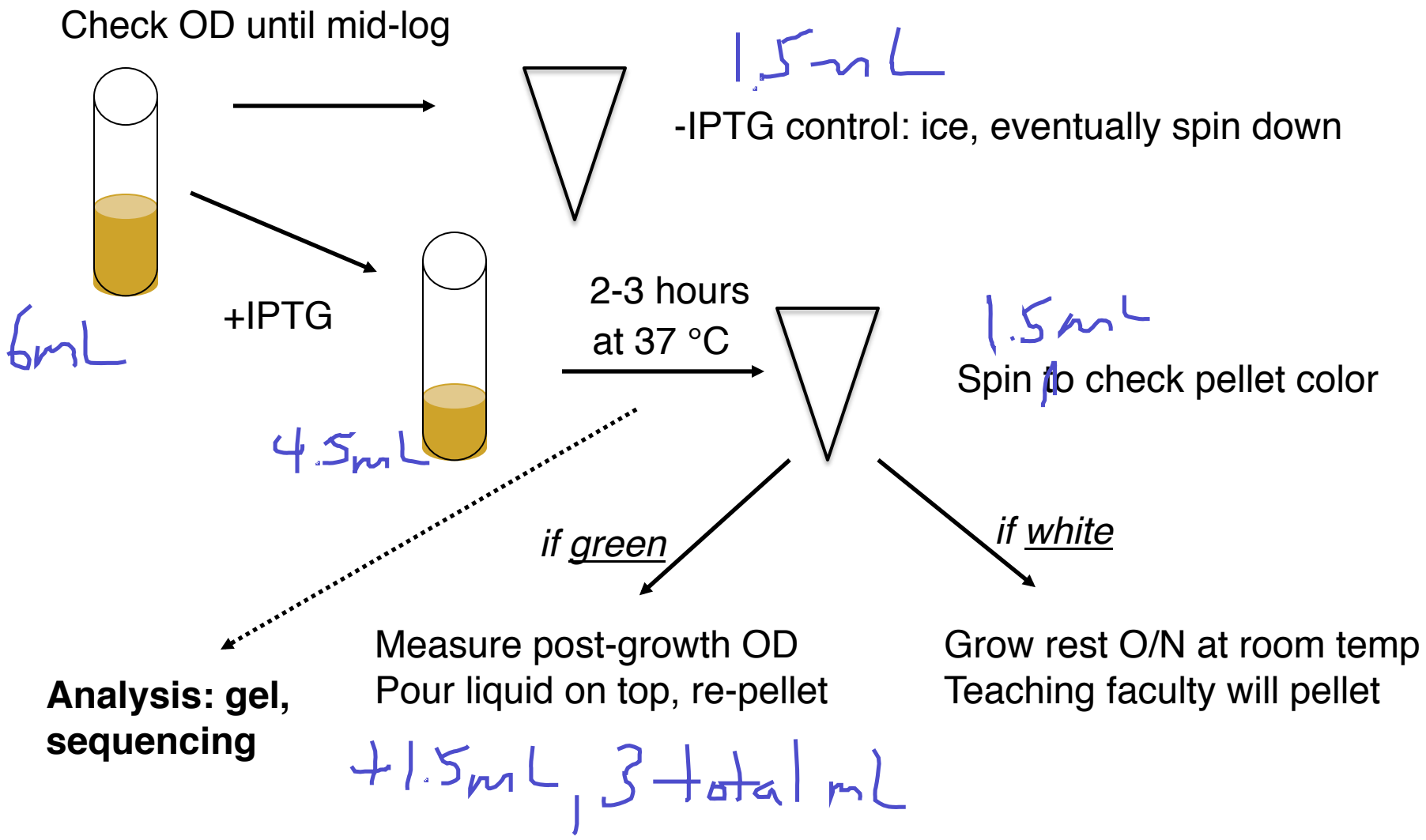
Reactions

TAAATT	TAAATT
AT*	A*
ATT*	ATTTA*
ATTT*	ATTTAA*

Gel

ddT	ddA	(bp)
		6
		1

Today in Lab (M2D5): Workflow



Today in Lab (M2D5): Samples

- Start with four BL21 samples carrying plasmid
  - WT
  - E67K or T79P or 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