- 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 don't work → Master Mix, cycler
ctrl worked, exp. didn't → primers, DNA template

pRSET-IPC —— mutagenize —— pRSET-IPC* —— transform in XL1-Blue

DAY 2

DAY 3

liquid culture —— miniprep mutant DNA —— transform in BL21(DE3)

DAY 4

DAY 4

liquid culture —— induce cells with IPTG —— lyse cells and extract protein —— evalute protein

DAY 5

DAY 6

DAY 7/8
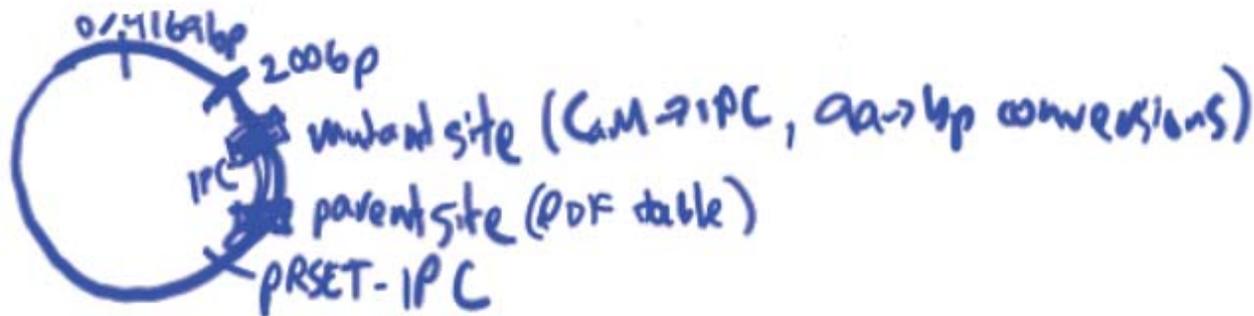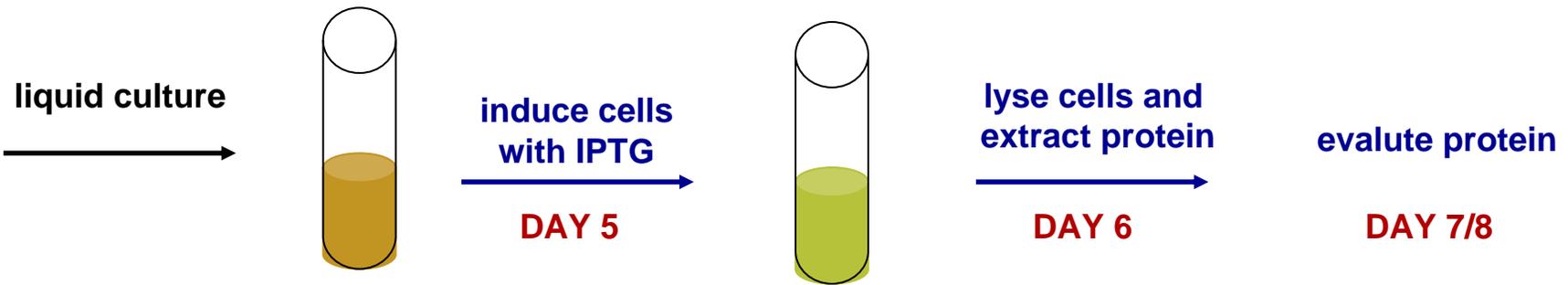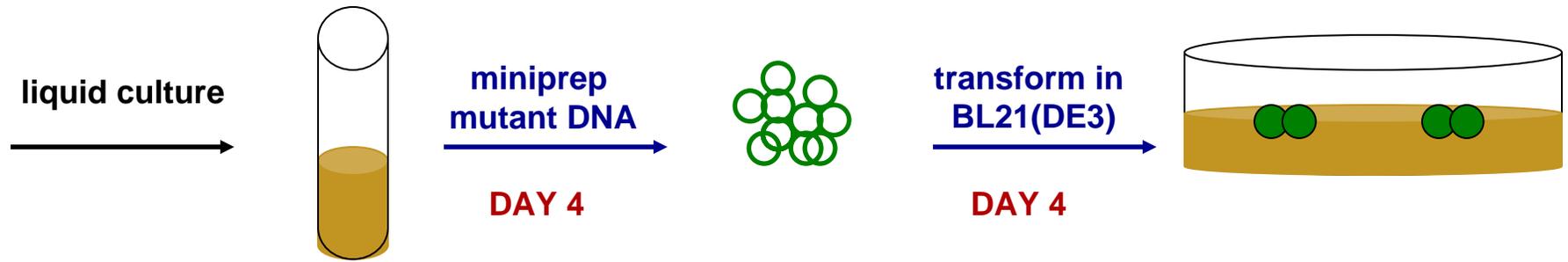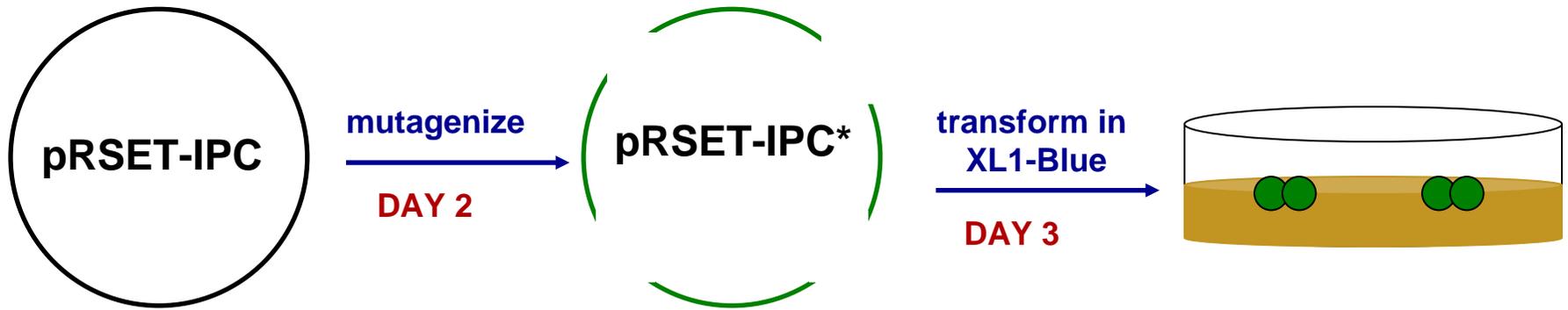
Colony counts

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
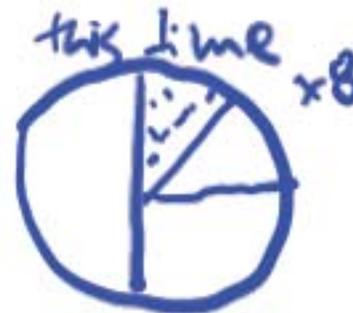
lac operon

These three genes encode <u>metabolic enzymes</u>

lac**I**	**control**	lac**Z**	lac**Y**	lac**A**

promoter + operator
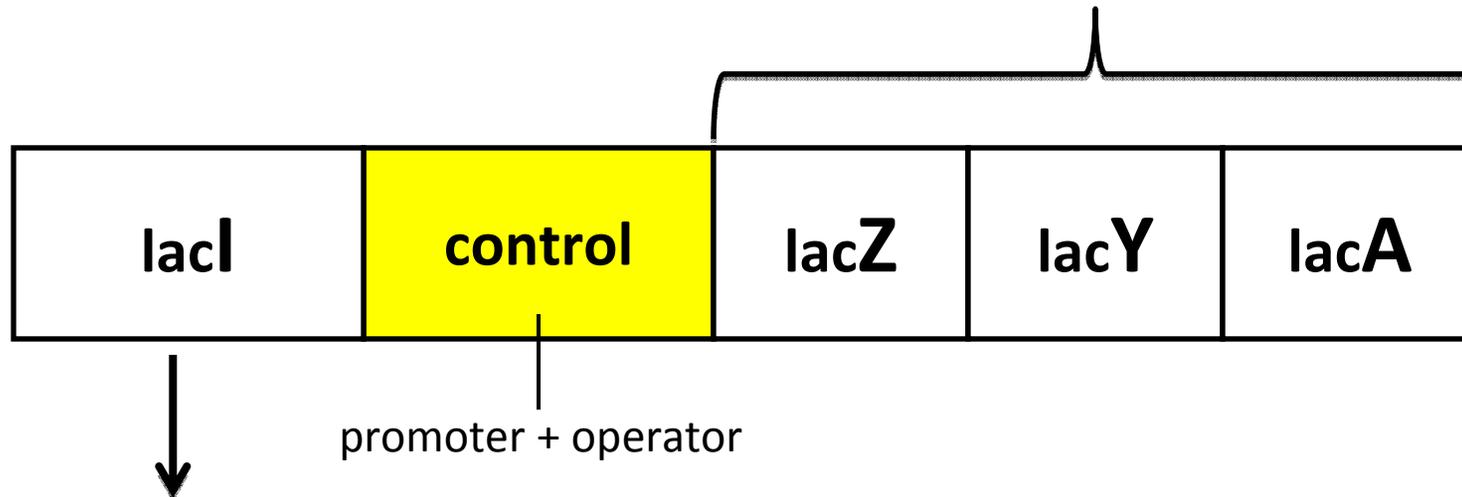
Encodes a <u>repressor protein</u> that binds <u>to control area</u> turning it <u>OFF</u>.

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

Induction of a chosen protein

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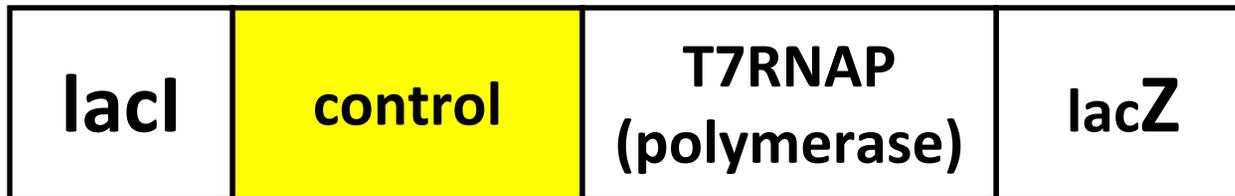
T7RNAP gene is expressed in presence of lactose or <u>analogue</u>.

$$[IPTG]_0 = [IPTG]_t$$
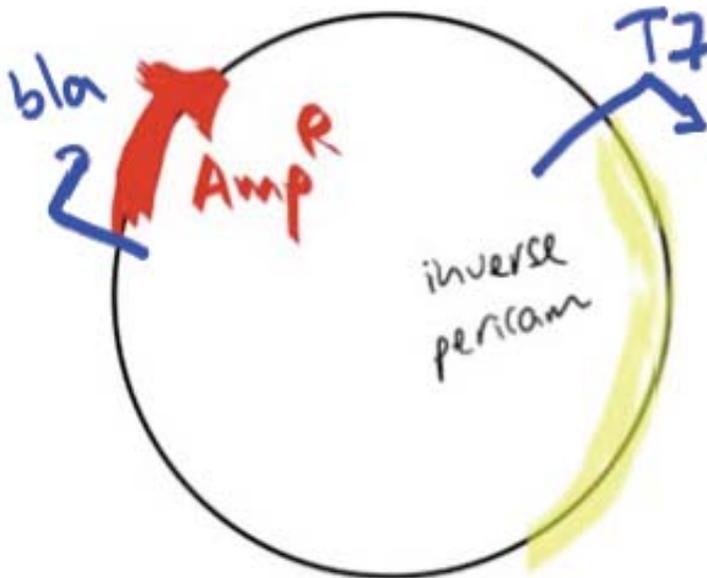
<u>bla</u> promoter is constitutively on.

<u>T7 promoter</u> 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', A'', C''', T''''

Reactions ddT

~~ TAAATT

AT*

ATT*

ATTT*

ddA

TAAATT

A*

ATTTA*

ATTTAA*

ATTTAAA*

Gel

ddT   ddA

7bp

1bp
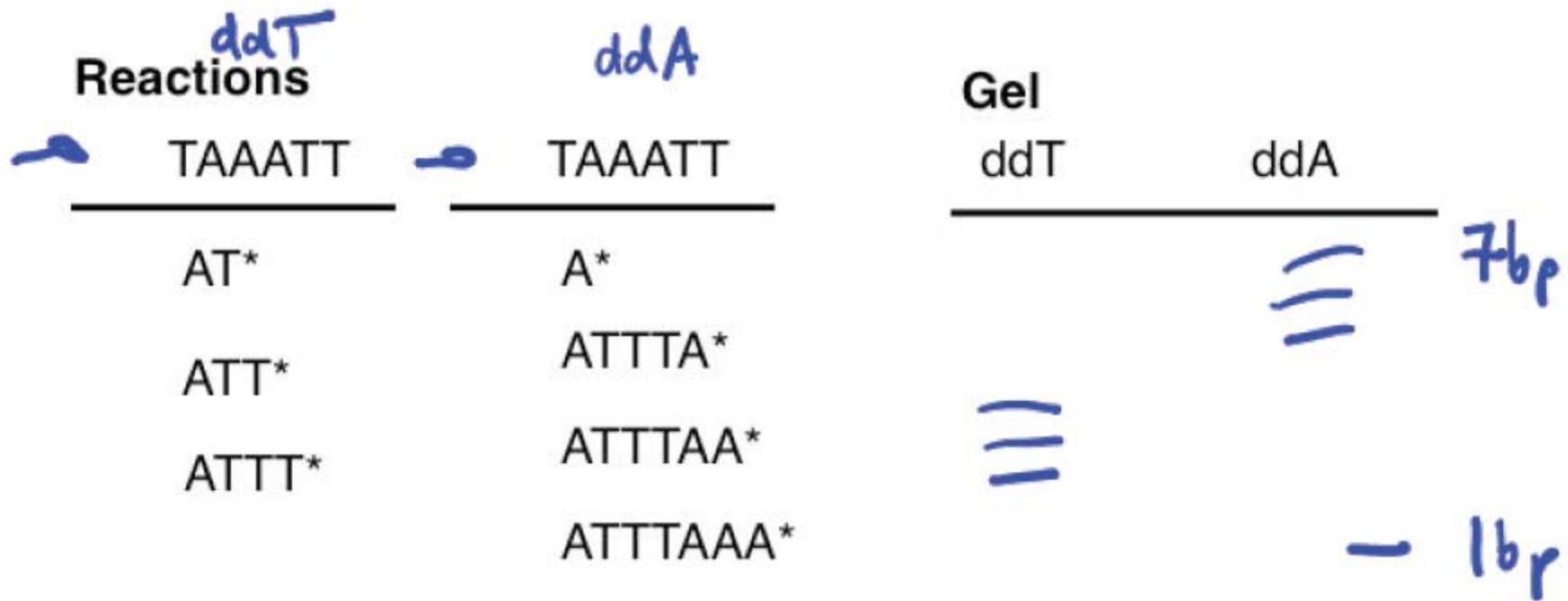
* = radioactive or fluorescent label

Today in Lab: Workflow

Check OD until mid-log

1.5 mL

-IPTG control: ice, eventually spin down

6mL

+IPTG

4.5mL

2-3 hours

37°C

1.5 mL

Spin to check pellet colour

green

white

Analysis: gel, sequencing

Measure post-growth OD
Pour liquid on top, re-pellet
1.5 mL   (3 total)

Grow rest O/N @ RT (3mL)
Teaching faculty will pellet
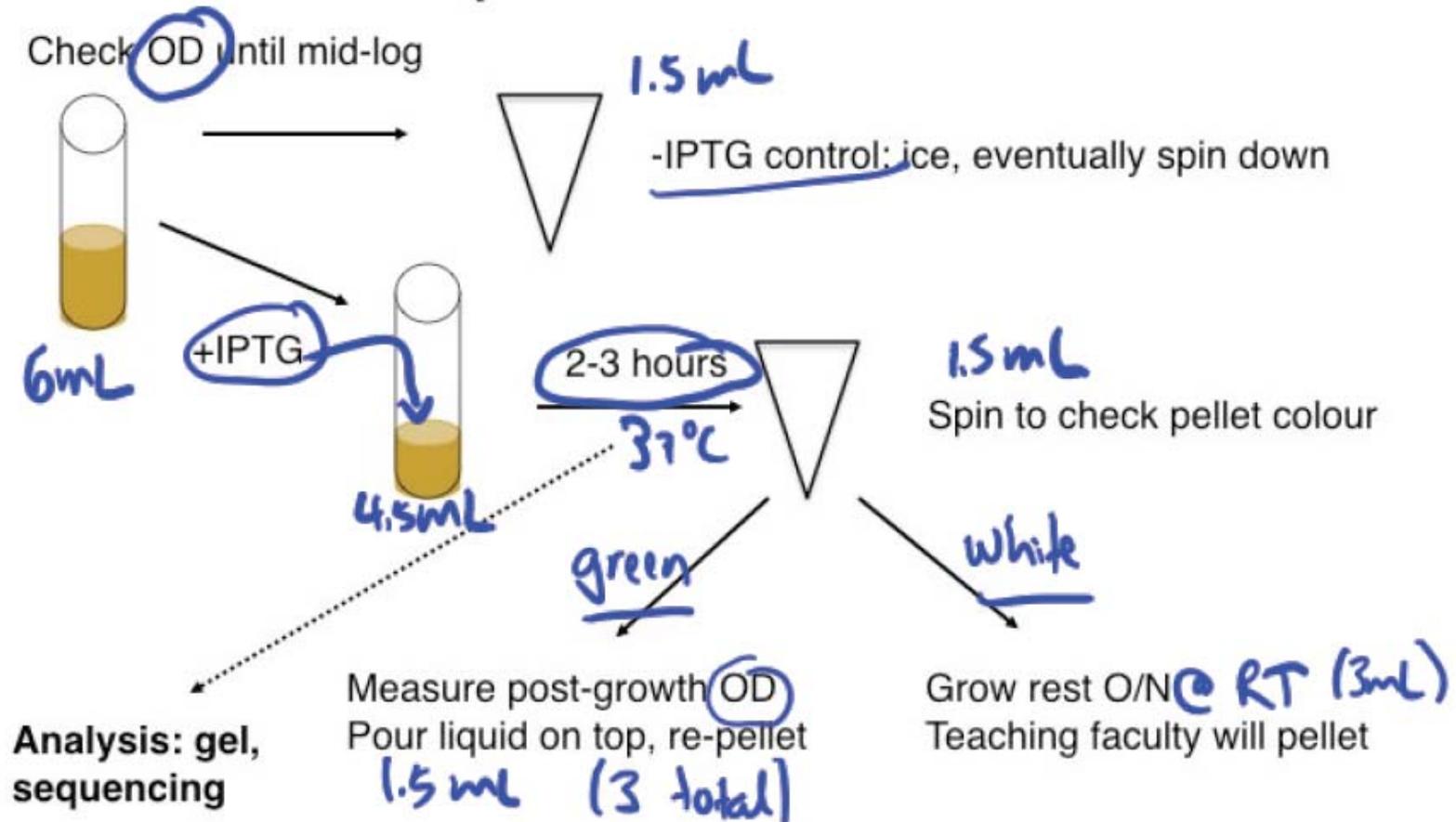
Today in Lab: Samples

- 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

20.109 Laboratory Fundamentals in Biological Engineering

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