M2D3:
Induce protein expression

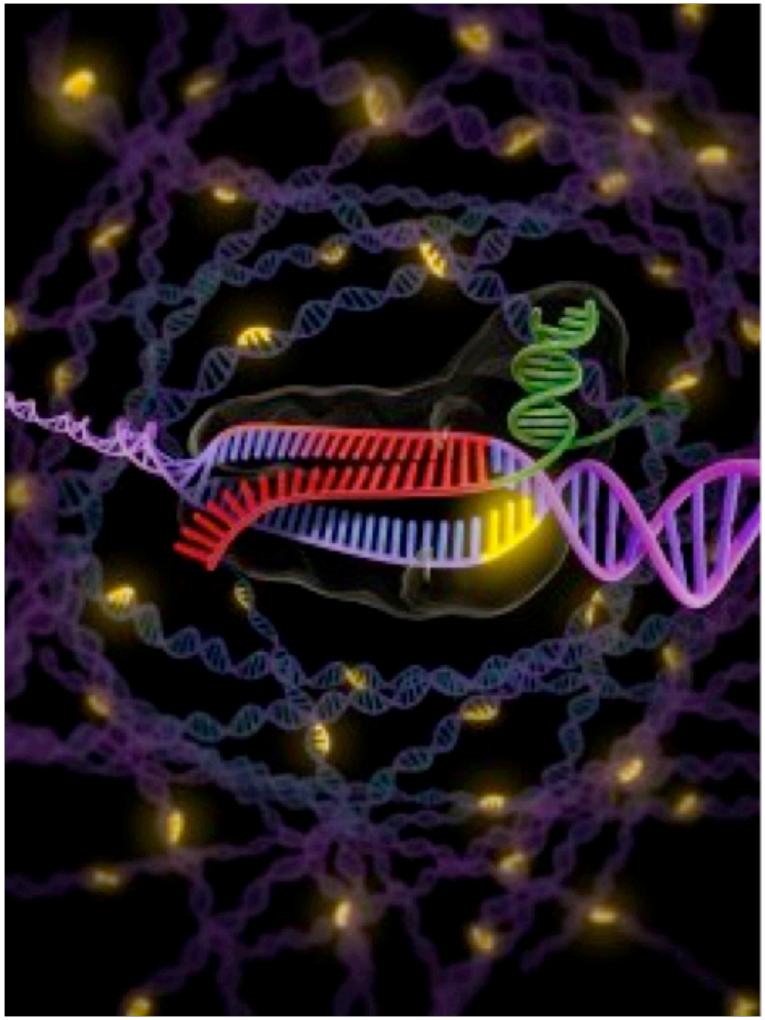
10/20/2015

Many activities today

start right now with PART 1 of the lab

- IPTG induction of inverse pericam protein expression
  - we'll explain the rationale in prelab lecture
- BE Communication Lab presentation on journal club
- Quiz 3
- Prelab lecture
- More lab exercises…

Seminar of great interest



Prof. Feng Zhang

Development and applications of CRISPR-Cas for genome editing

Wednesday, October 21

10:00 am

in 46-3002

Sign up for journal club

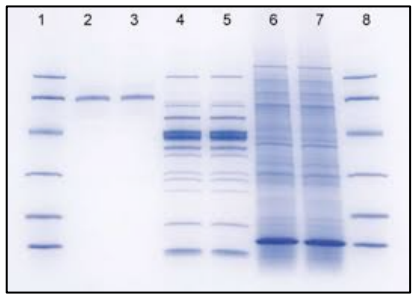
Slot	Day 5 (T/R)	Day 8 (T/R)	Day 5 (W/F)	Day 8 (W/F)
1		Lucy	Paola	Charlotte
2		Cathy	Julia	Emily
3		Trinh	Andrew	Ashley
4		Rozanne	Wangui	Tammy
5		Cristie	Jiapei	Raymond
6				

- Pick 1 of 25 papers, or suggest your own
- Present M2D5 (Oct. 27) or M2D8 (Nov. 5)
- Sign up by adding your name next to listing **[MJ/TR/Rainbow]**
  - first come first serve!
  - one T/R and one W/F per article

Developing and examining calcium sensors [edit]

1. Robin, J. et al. *Differential nanosecond protein dynamics in homologous calcium sensors.* (2015) ACS Chem Biol epub ahead of print. PMID:26204433
2. Cai, B. et al. *A cell-based functional assay using a green fluorescent protein-based calcium indicator dCys-GCaMP.* (2014) Assay Drug Dev Tech 12:342-351. PMID:25105973 **[MJ/TR/Rainbow]**
3. Wu, J. et al. *Red fluorescent genetically encoded Ca2+ indicators for use in mitochondria and endoplasmic reticulum.* (2014) Biochem J 464:13-22. PMID:25164254

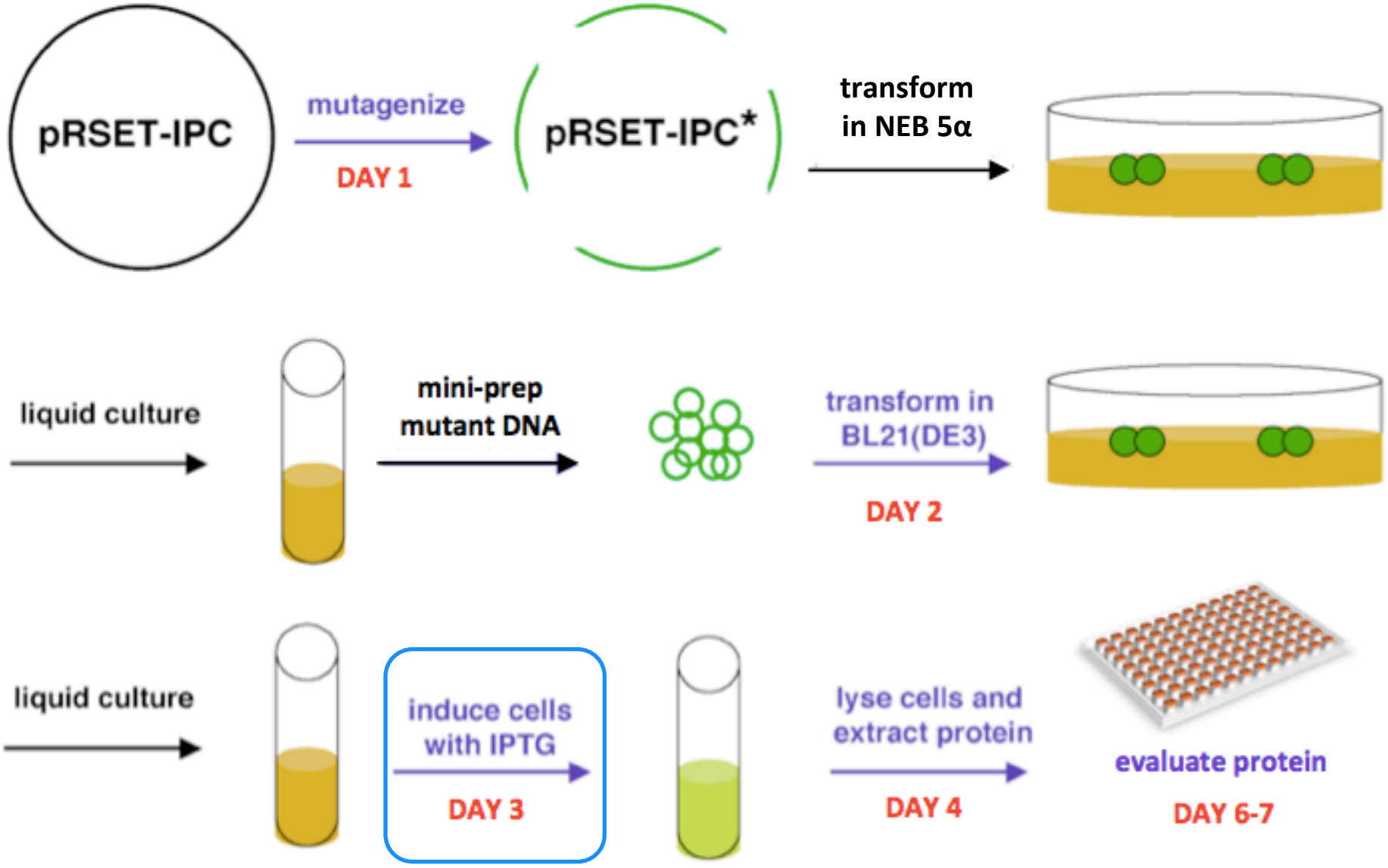
Assignments on the horizon



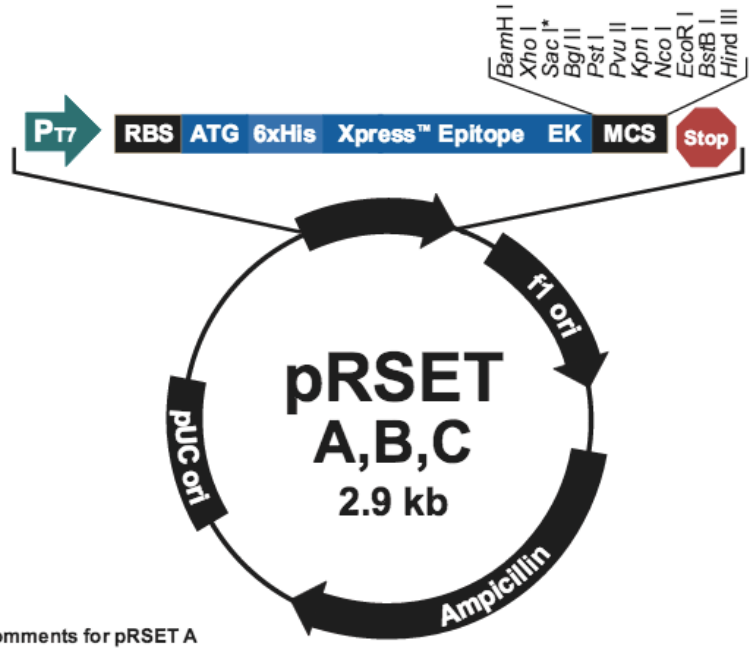
- DNA engineering summary revision
  - due by 5pm on Saturday, Oct. 24
- Blog post for M1
  - due by 5pm on Sunday, Oct. 25

- For M2D4:
  - one slide, “journal club style”, to illustrate *your* Nagai paper data from M2D2
  - prepare SDS-PAGE calculations
- For M2D5:
  - journal club readings

We're making progress… and proteins today!



BL21(DE3)pLysS competent cells



Comments for pRSET A
2897 nucleotides

*Version C does not contain *Sac* I

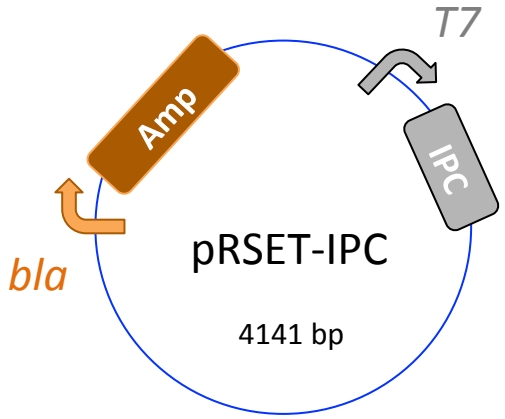
T7 promoter: bases 20-39
6xHis tag: bases 112-129
T7 gene 10 leader: bases 133-162
Xpress™ epitope: bases 169-192
Multiple cloning site: bases 202-248
T7 reverse priming site: bases 295-314
T7 transcription terminator: bases 256-385
f1 origin: bases 456-911
bla promoter: bases 943-1047
Ampicillin (*bla*) resistance gene (ORF): bases 1042-1902
pUC origin: bases 2047-2720 (C)

- BL21: E. coli bacterial strain
- can express IPC protein
  - induction by lactose or analog: isopropyl β-D-thiogalactoside
  - under T7 promoter control in pRSET vector
- DE3: bacteriophage (virus)
  - used to integrate the *lac*/T7RNAP construct into *E. coli*
- pLysS: protein that produces
  - lysosyme, which binds to T7RNAP, reducing basal "leaky" expression
  - retained by chloramphenicol (Cam) selection

Let's piece together this "protein induction" story

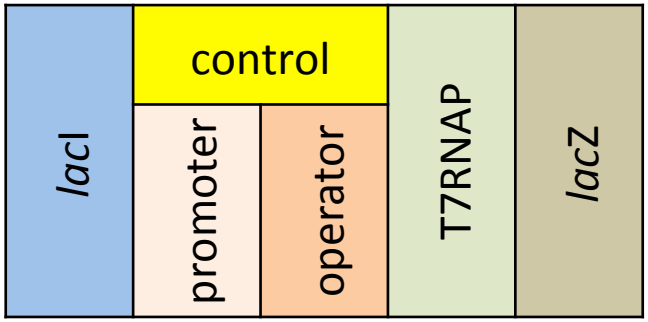
① in the pRSET plasmid

- bla promoter is constitutively *on*
- T7 promoter is turned *on* in the presence of T7 RNA polymerase



② in BL21(DE3)pLysS

- T7RNAP gene engineered in DE3 cells under a modified *lac* operon control
- *lac*I encodes a ___repressor___ that binds to ___control area___ , thereby turning it *off*
- in addition, T7 lysosyme inactivates T7 promoter



genes of the lac operon

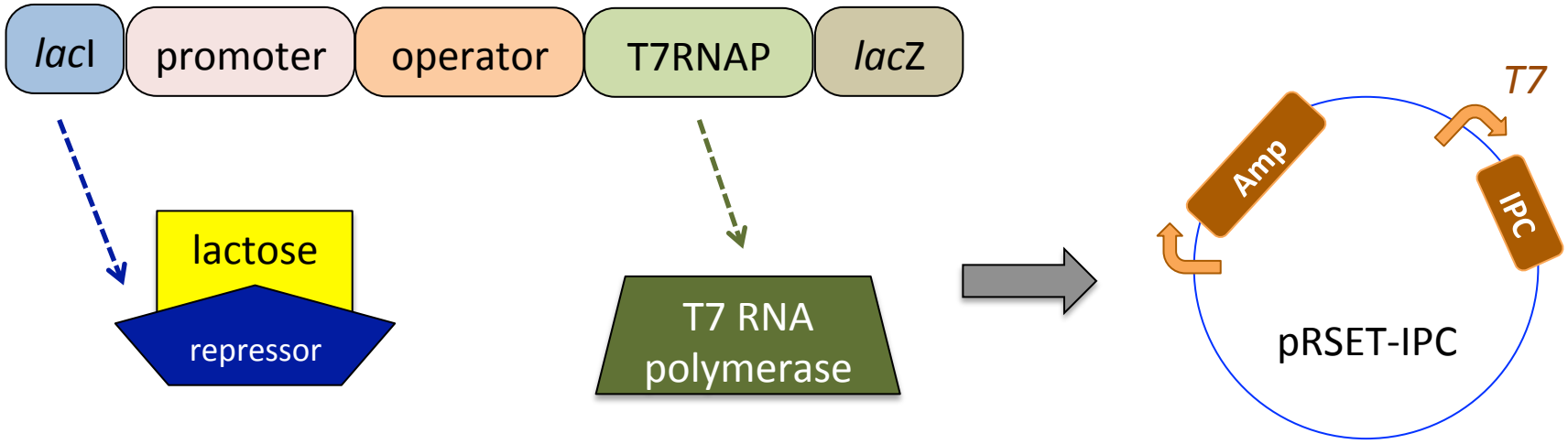
Let's piece together this "protein induction" story

① in the pRSET plasmid, T7 promoter *on* only if T7RNAP present

② in BL21(DE3)pLysS, *lac*I => repressor binds control area => T7RNAP turned *off*

③ if lactose is present

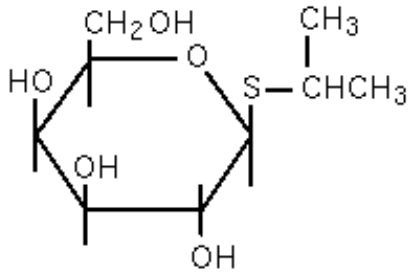
- lactose binds to repressor and makes it ___inactive___ , thus turning ___on___ expression of T7RNAP
- with T7RNAP present, the T7 promoter is ___on___, and IPC expressed



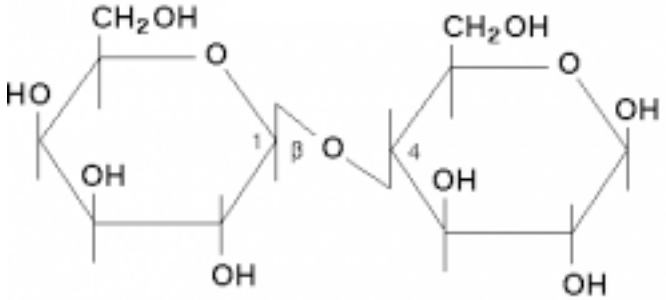
IPTG is a lactose analogue

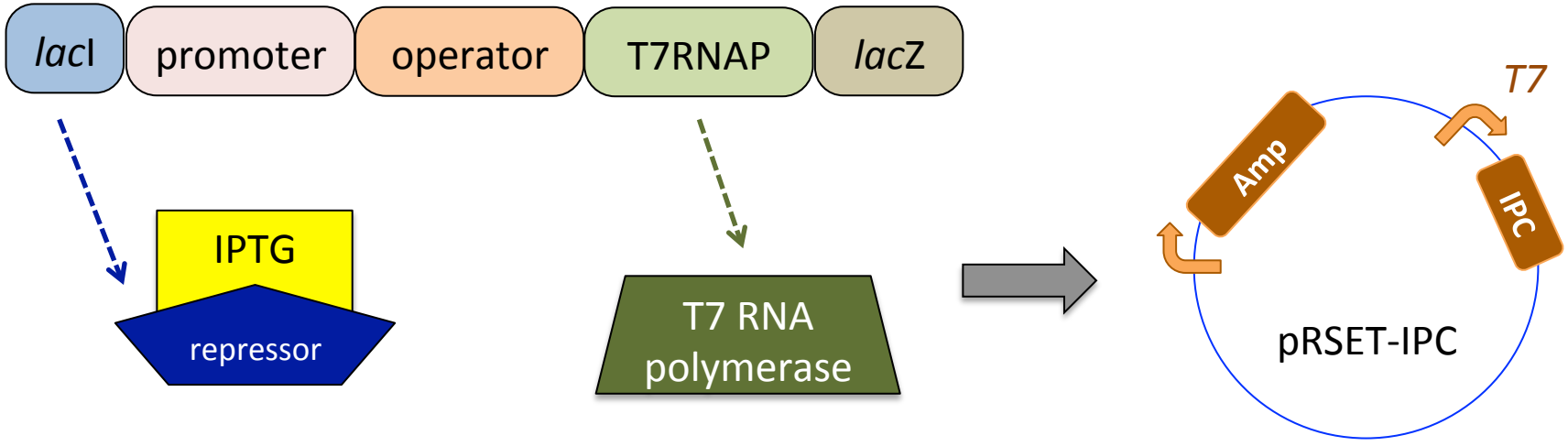
- isopropyl β-D-1-thiogalactoside
- structural mimic of lactose
- unlike lactose, IPTG is not part of any metabolic pathways and so will not be broken down or used by the cell ➔ [IPTG] constant

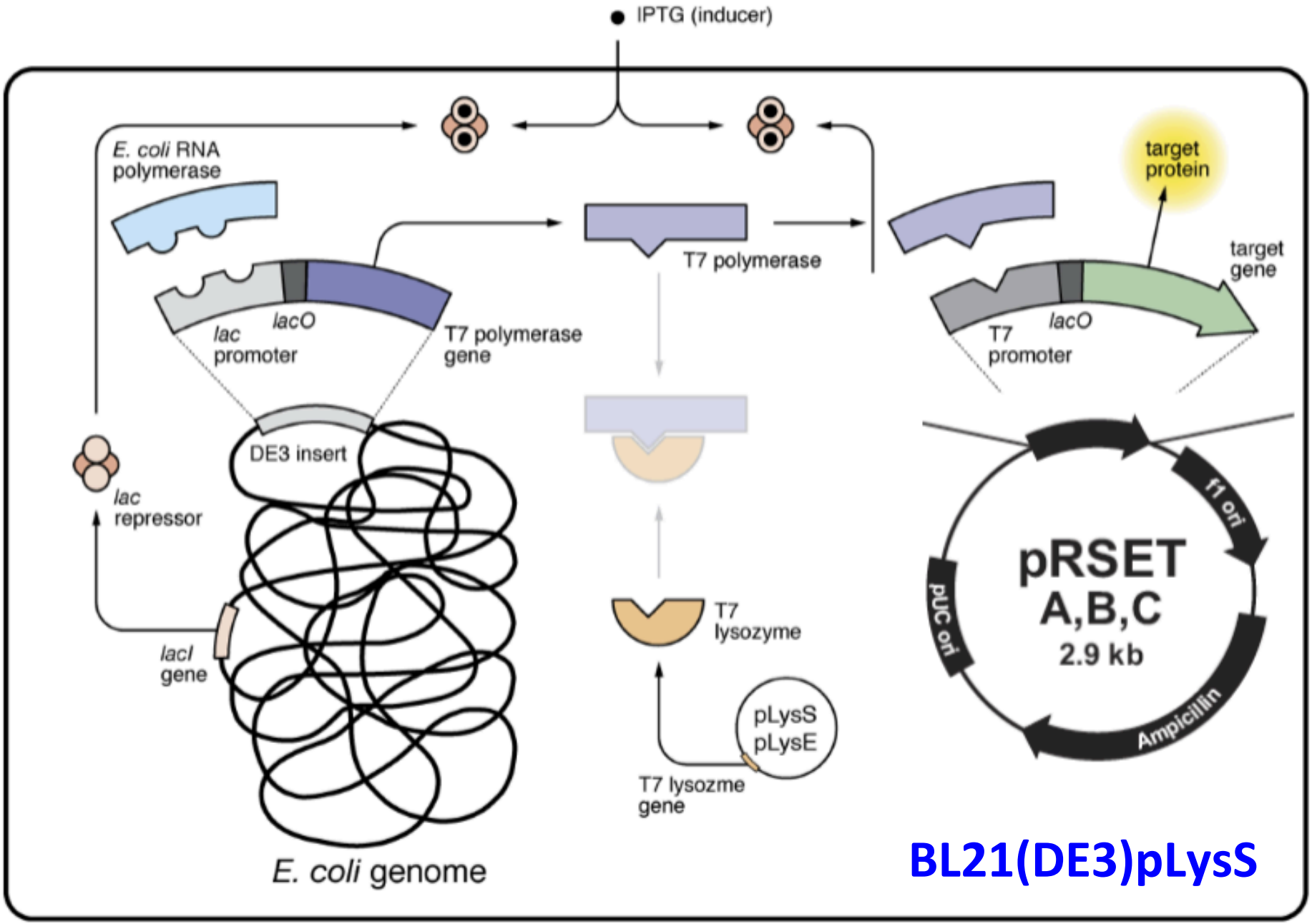
IPTG



lactose



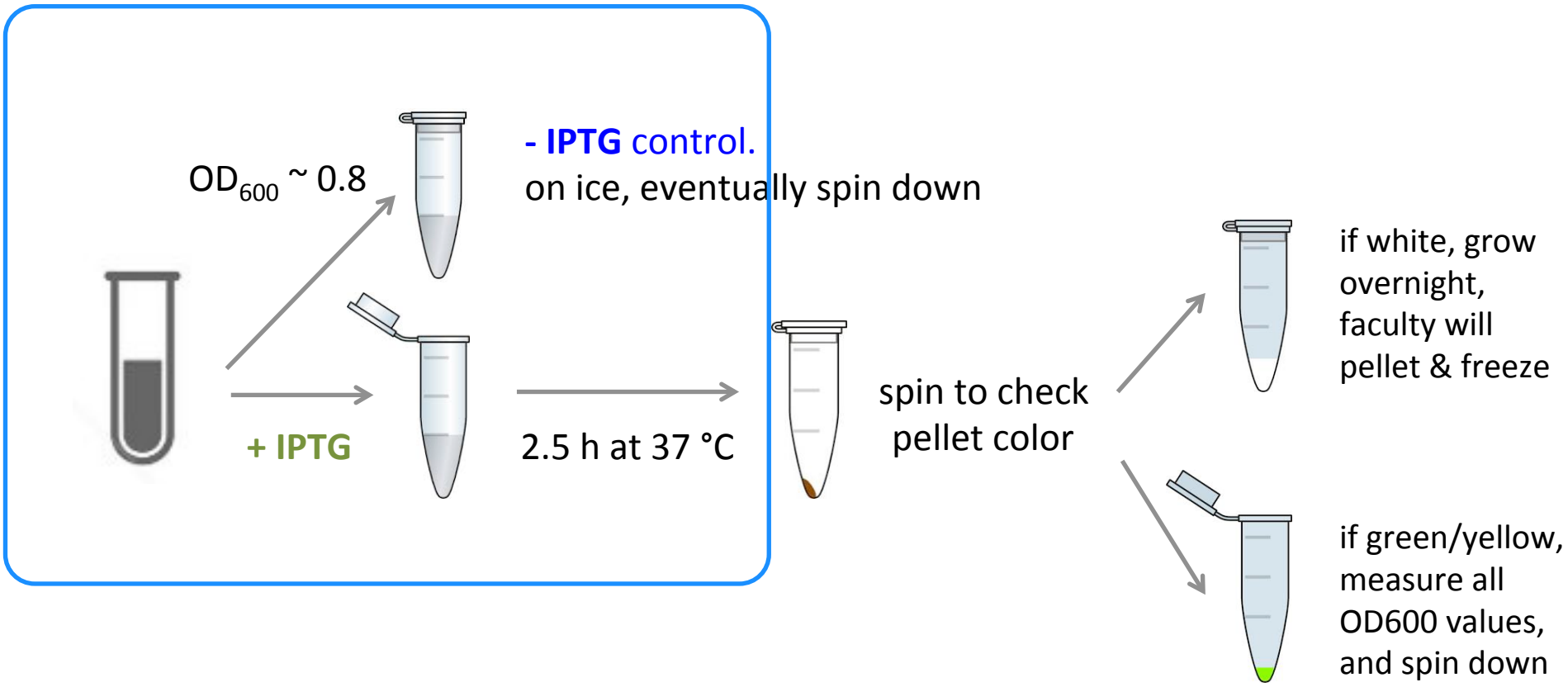



IPTG (inducer)
E. coli RNA polymerase
lac promoter
lacO
T7 polymerase gene
T7 polymerase
target protein
target gene
T7 promoter
lacO
DE3 insert
lac repressor
lacI gene
E. coli genome
T7 lysozyme
pLysS pLysE
T7 lysozme gene
pRSET A,B,C
2.9 kb
f1 ori
pUC ori
Ampicillin
BL21(DE3)pLysS

start right now with Part 1 of the M2D3 lab:

Induce IPC protein expression

- for three samples: X#Z #1, X#Z #2, and wt inverse pericam



In lab today

- IPTG induction of IPC protein expression
- BE Communication Lab presentation on journal club
- Quiz 3
- Prelab lecture
- Analysis of DNA sequences
- Count mutant colonies (in BL21)
- Measure OD_{600} of, and spin down six samples
  - wt IPC 1.5 mL – IPTG 3 mL + IPTG
  - X#Z #1 1.5 mL – IPTG 3 mL + IPTG
  - X#Z #2 1.5 mL – IPTG 3 mL + IPTG