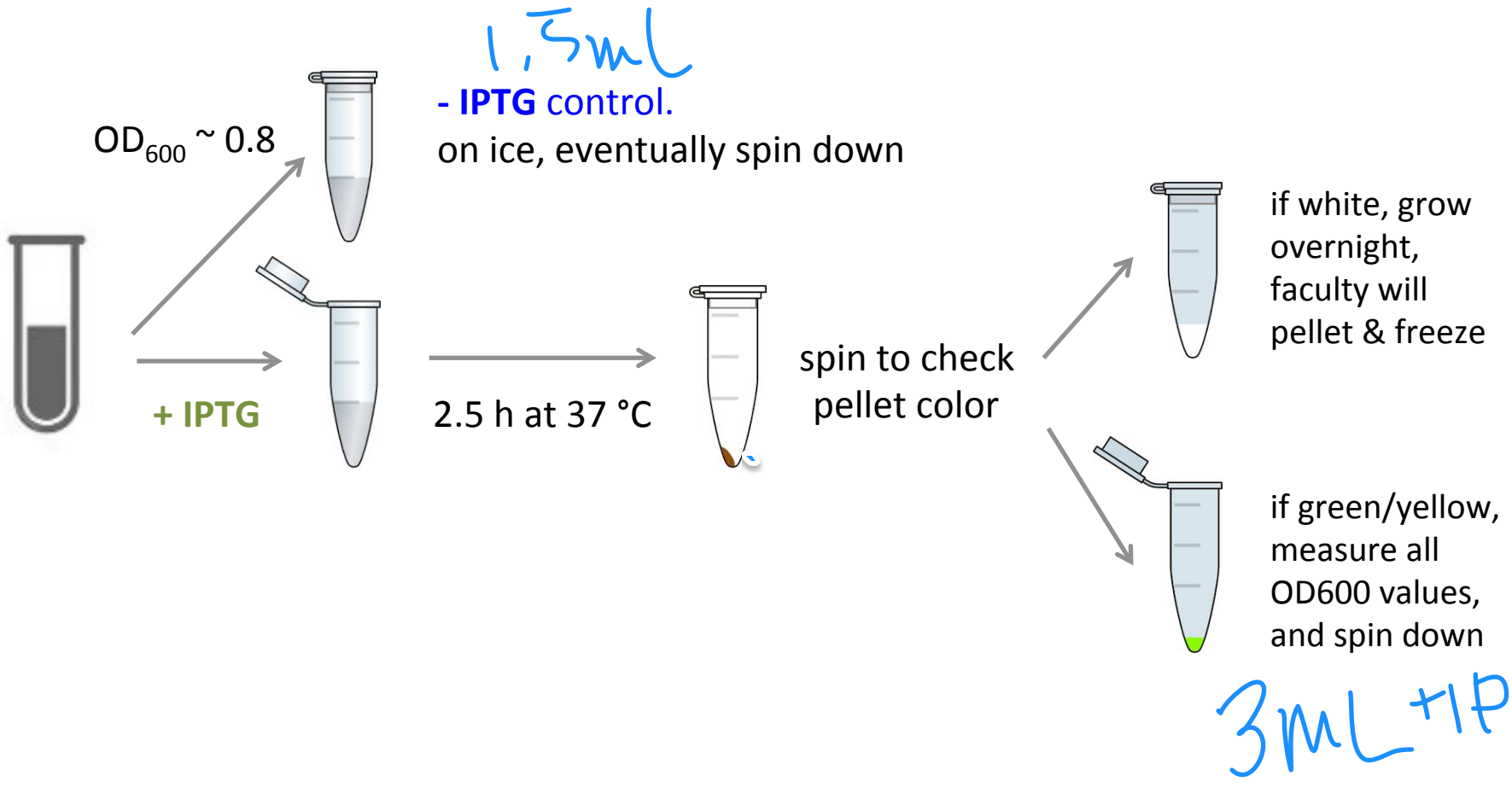
Induce IPC protein expression

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



M2D3: Induce protein expression

10/21/2015

1. IPTG induction of inverse pericam protein expression
2. Journal Club Workshop
3. Lab Treat
4. Prelab lecture
5. Analyze Sequence Data, Count Colonies and Harvest Cells

Sign up for journal club and choose a paper

1. Pick 1 of 25 papers, or suggest your own
2. Sign up by adding your name next to paper[LMM/WF/Rainbow]
   - **first come first serve!**
   - **one T/R and one W/F per article**
3. Sign up for a time slot: M2D5 (Oct. 28) or M2D8 (Nov. 6)

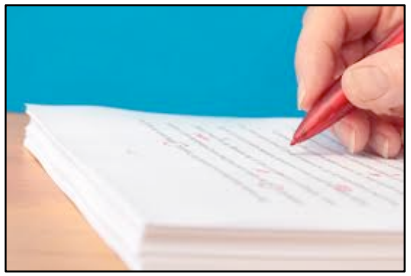
Developing and examining calcium sensors [edit]

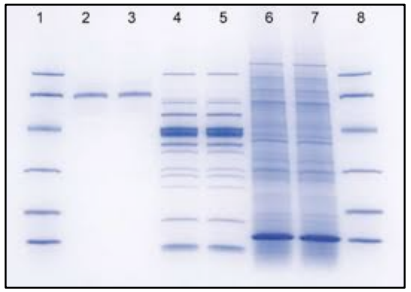
1. Robin, J. et al. *Differential nanosecond protein dynamics in homologous calcium sensors.* (2015) ACS Chem Biol epub ahead of print. PMID:26204433
2. Cai, B. et al. *A cell-based functional assay using a green fluorescent protein-based calcium indicator dCys-GCaMP.* (2014) Assay Drug Dev Tech 12:342-351. PMID:25105973
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

Using calcium sensors in biological systems [edit]

1. Muto, A. et al. *Real-time visualization of neuronal activity during perception.* (2013) Curr Biol 23:307-311. PMID:23375894
2. Luongo, F. et al. *Putative microcircuit-level substrates for attention are disrupted in mouse models of autism.* (2015) Biol Psych epub ahead of print. PMID:26022075
3. Tang, W. et al. *Stimulation-evoked Ca2+ signals in astrocytic processes at hippocampal CA3-CA1 synapses of adult mice are modulated by glutamate at ATP.* (2015) J Neurosci 35:3016-3021. PMID:25698739

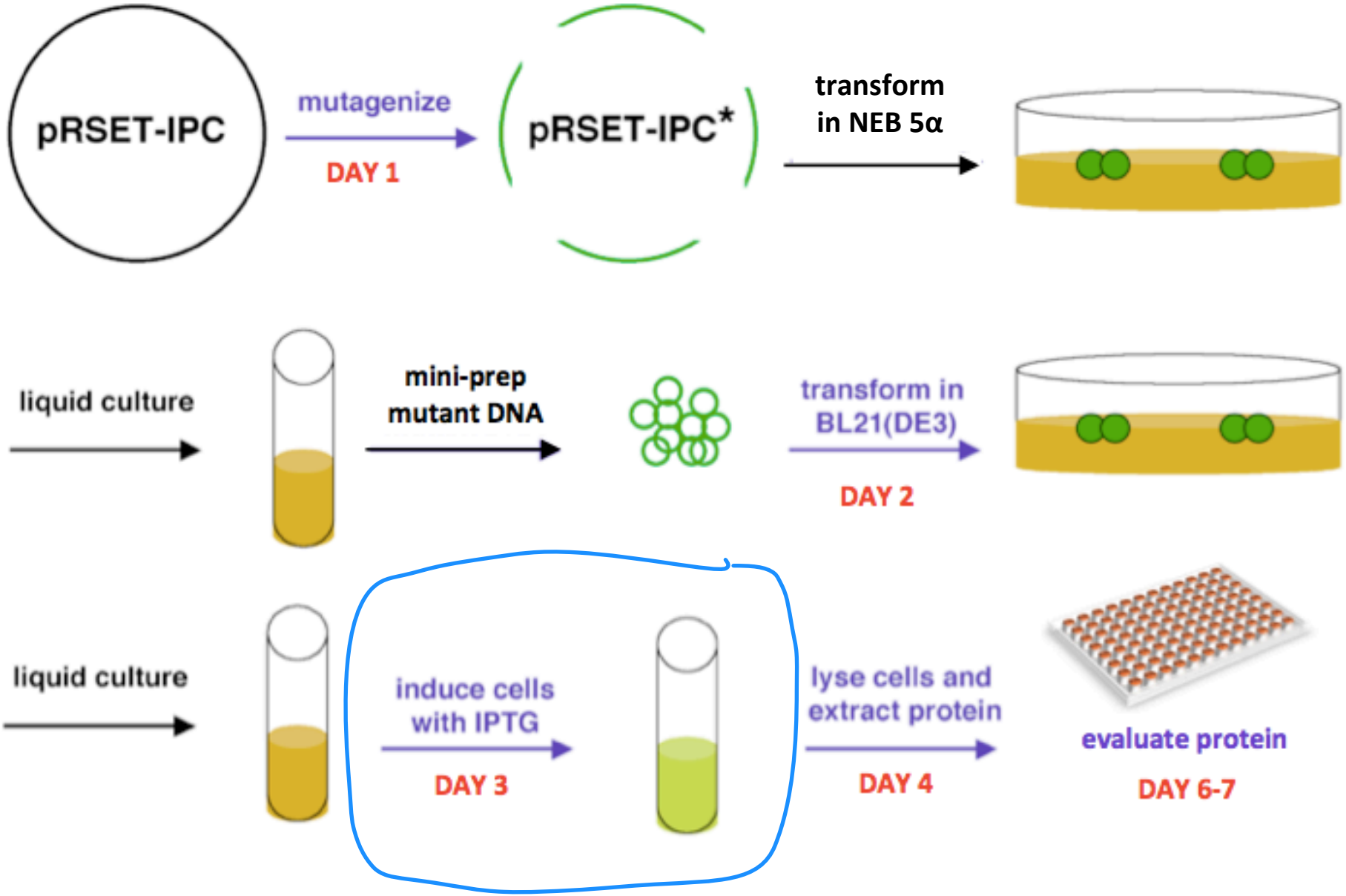
Assignments on the horizon



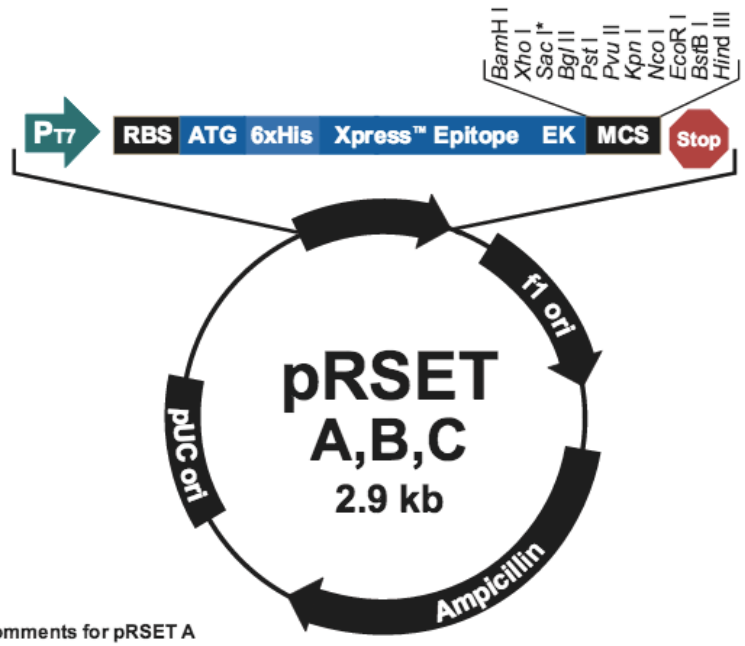


- DNA engineering summary revision
  - due by 5pm on Saturday, Oct. 24
- Blog post for M1
  - due by 5pm on Sunday, Oct. 25
- For M2D4:
  - prepare a (JC style) PP slide that represents your groups contribution to the Nagai et al discussion (ex. Fig. 3+ part of table)
  - prepare SDS-PAGE calculations
- 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

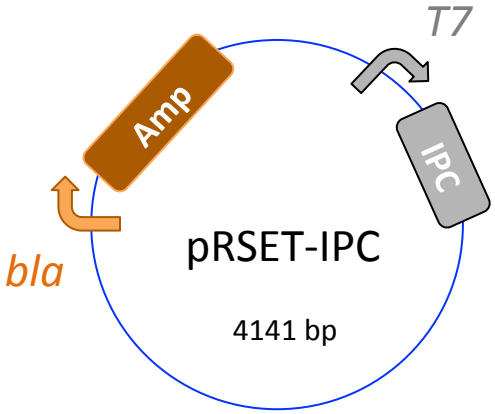
T7 promoter: bases 20-39
6xHis tag: bases 112-129
T7 gene 10 leader: bases 133-162
Xpress™ epitope: bases 169-192
Multiple cloning site: bases 202-248
T7 reverse priming site: bases 295-314
T7 transcription terminator: bases 256-385
f1 origin: bases 456-911
bla promoter: bases 943-1047
Ampicillin (*bla*) 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 =| PTG
  - under T7 promoter control in pRSET vector
- DE3: bacteriophage (bacterial 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

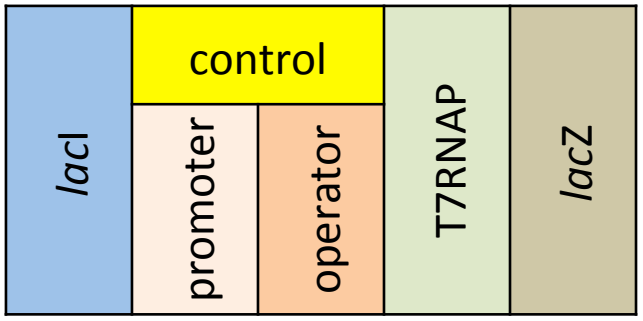
① in the pRSET plasmid

- 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, thereby turning it *off*
- in addition, T7 lysosyme inactivates T7 promoter



genes of the lac operon

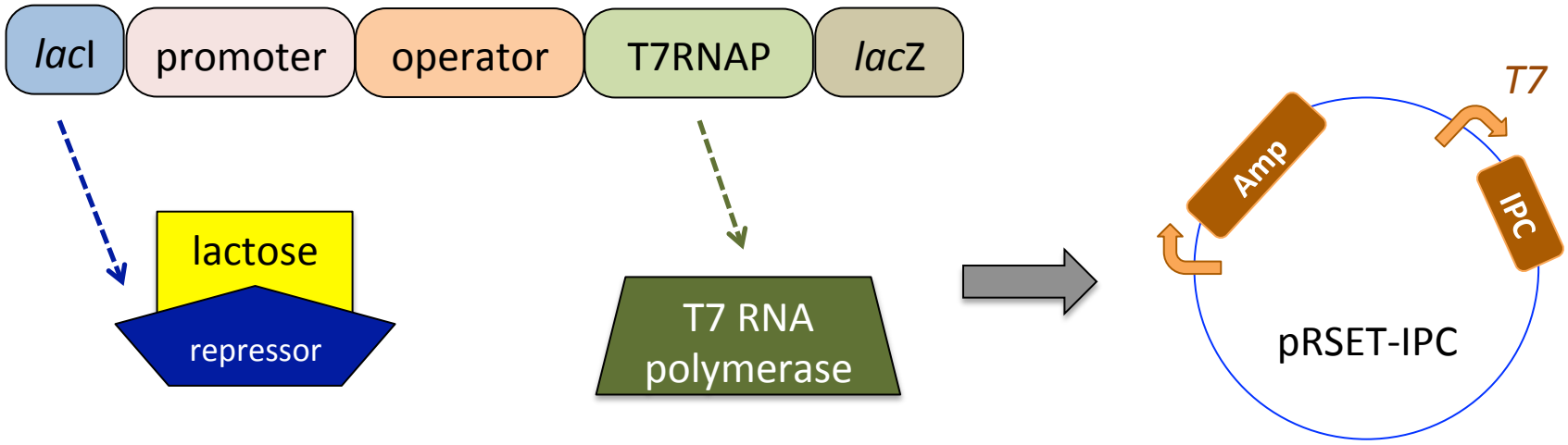
Let's piece together this "protein induction" story

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

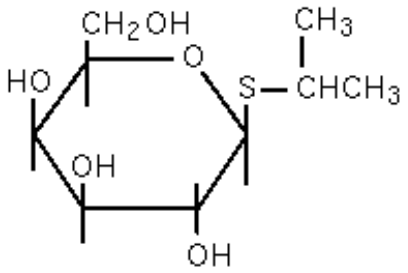
- lactose binds to repressor and makes it inactivate, thus turning ON expression of T7RNAP
- with T7RNAP present, the T7 promoter is ON, and IPC expressed



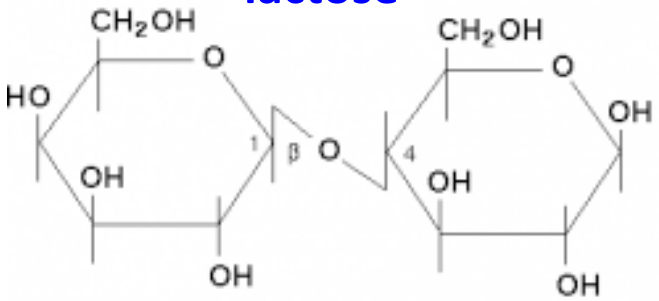
IPTG is a lactose analogue

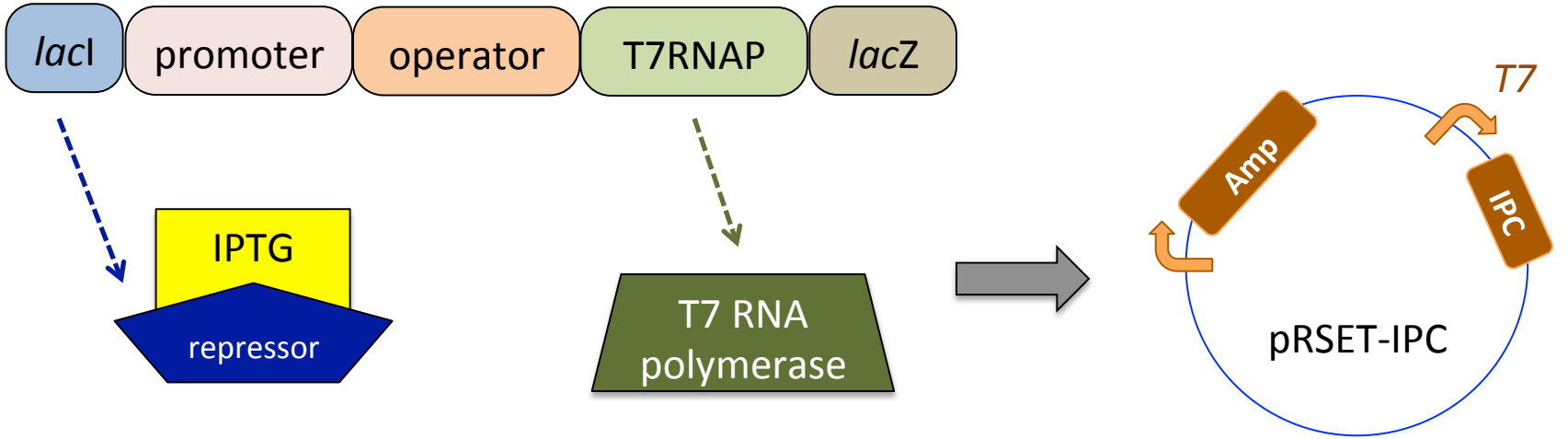
- isopropyl β-D-1-thiogalactoside
- structural mimic of lactose
- Both lactose and IPTG are taken up by *E. coli* but unlike lactose, IPTG is not part of any metabolic pathways and not hydrolyzable by β-galactosidase.

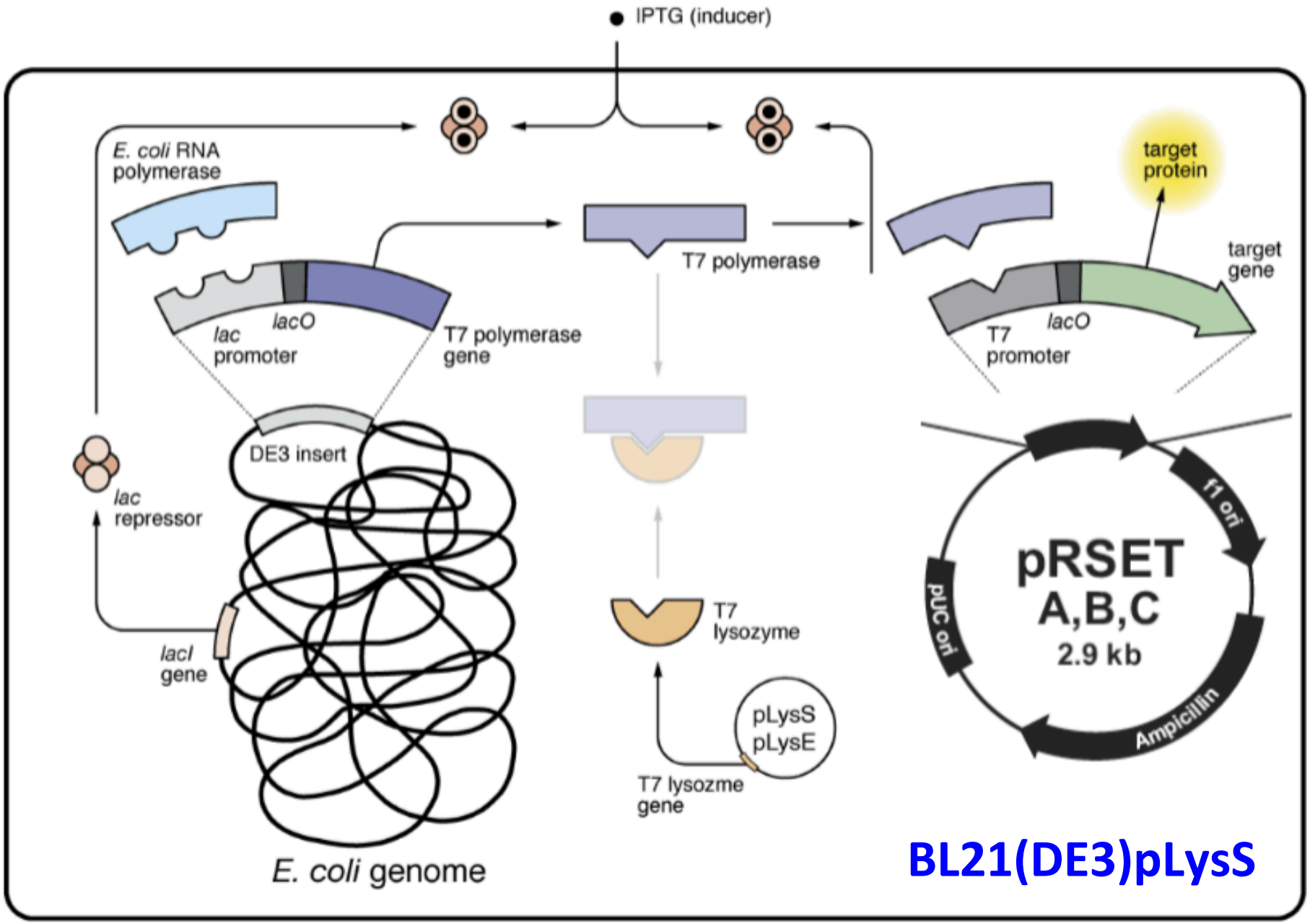
IPTG



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

In lab today…

- Analysis of DNA sequences
- Count colonies in BL21
- 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