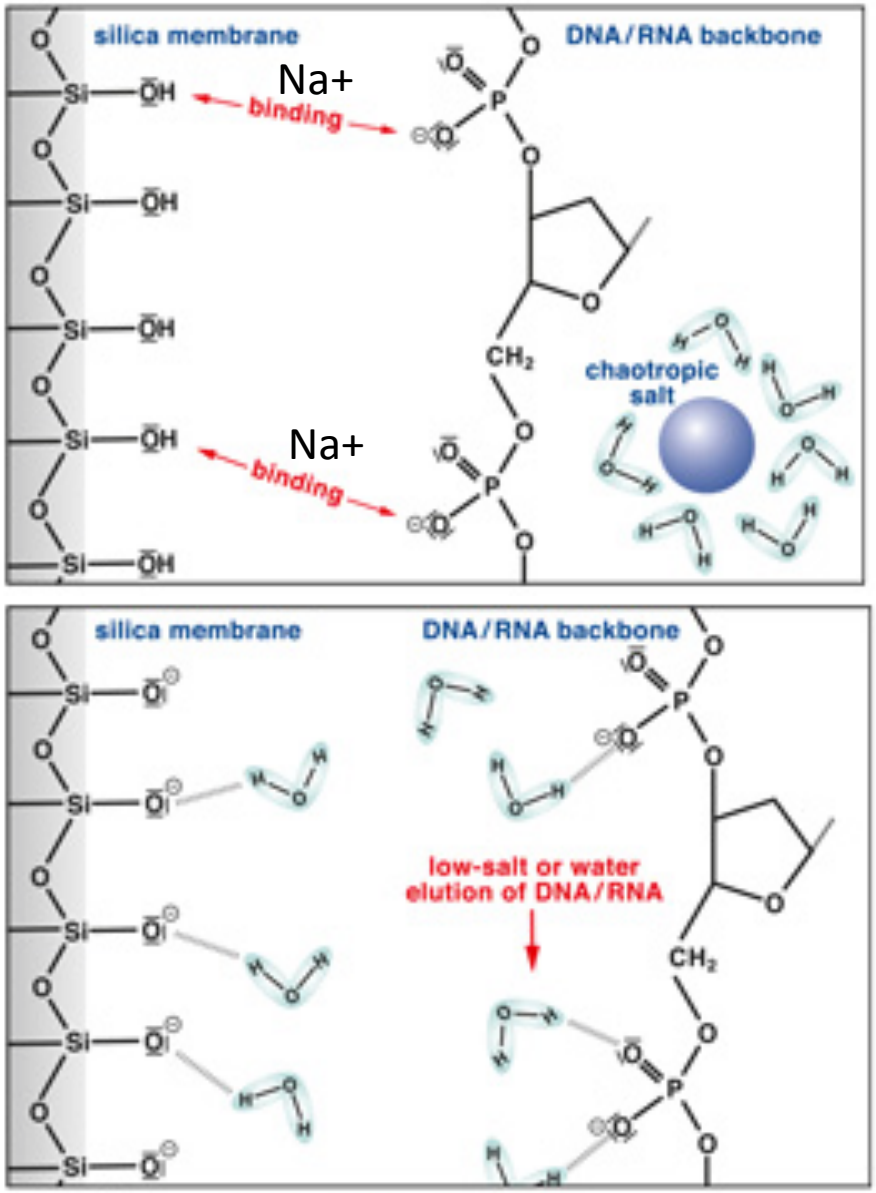
Purifying DNA using silica and chaotrophic salt



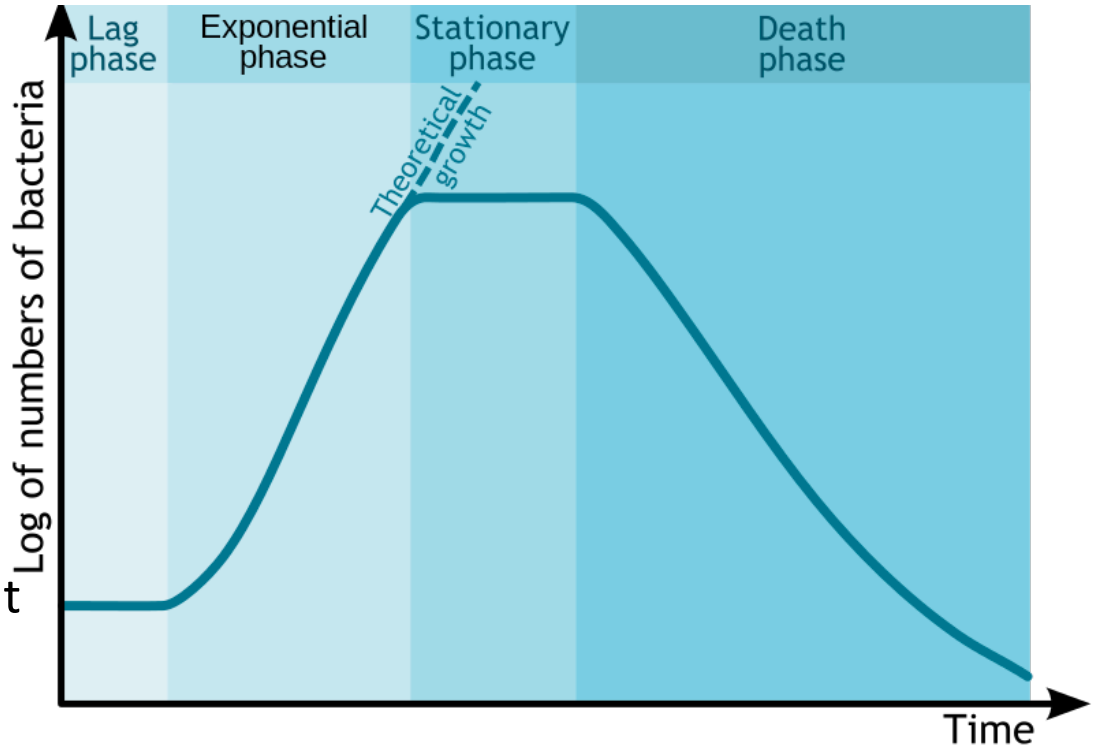
1) At high salt concentrations and low pH, hydrogen bonds are broken between the hydrogens in water and the negatively charged oxygen ions in silica. Sodium (from salt) serves as a cation bridge and attracts the negatively charged oxygen in the phosphate backbone of the DNA molecule.

2) At low salt concentrations and higher pH, the silica and DNA is hydrated and the DNA is eluted from the membrane.

E. coli (NEB5alpha or BL21) growth curve

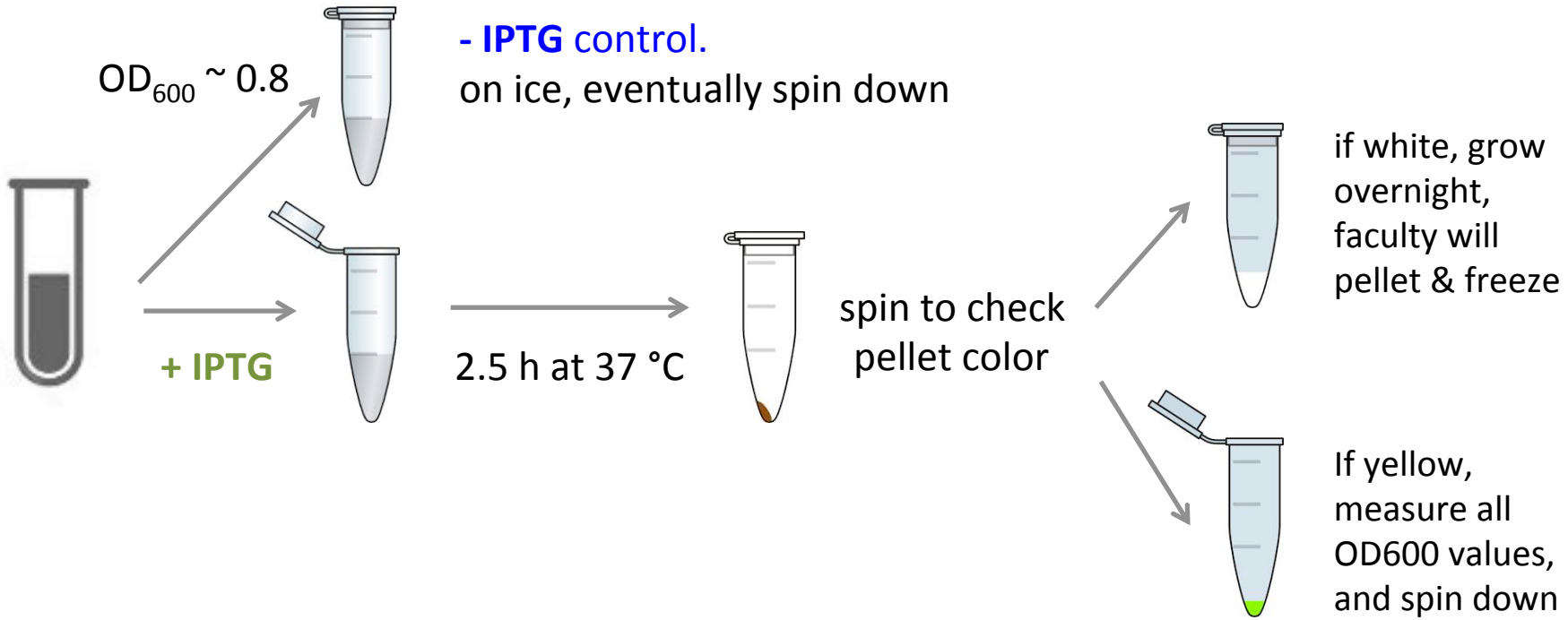
- exponential phase
  - binary fission
  - OD600 ~ 0.4 - 0.8
  - machinery ready

- OD ≠ absorbance
  -OD=Opitcal density
  -measuring turbidity rather than absorption, light scattering predominates
  -*E. coli* yellowish, so they don't absorb 600nm (=orange)
  -600nm is safer than UV (UV~300nm) for DNA in *E. coli*



Induce IPC protein expression

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



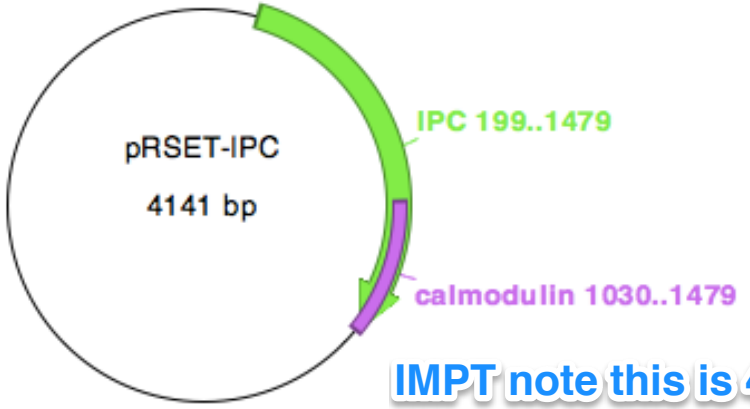
M2D5: Induce protein expression

2/23/2016

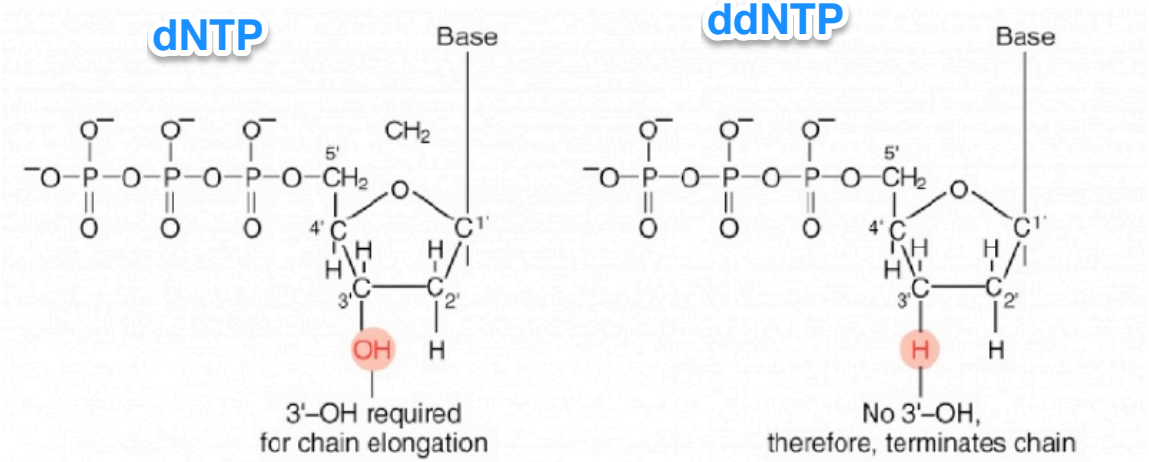
1. IPTG induction of inverse pericam protein expression
2. Prelab lecture
3. Analyze Sequence Data
4. Count Colonies
5. Harvest BL21 for protein purification

Do we have the intended mutant?

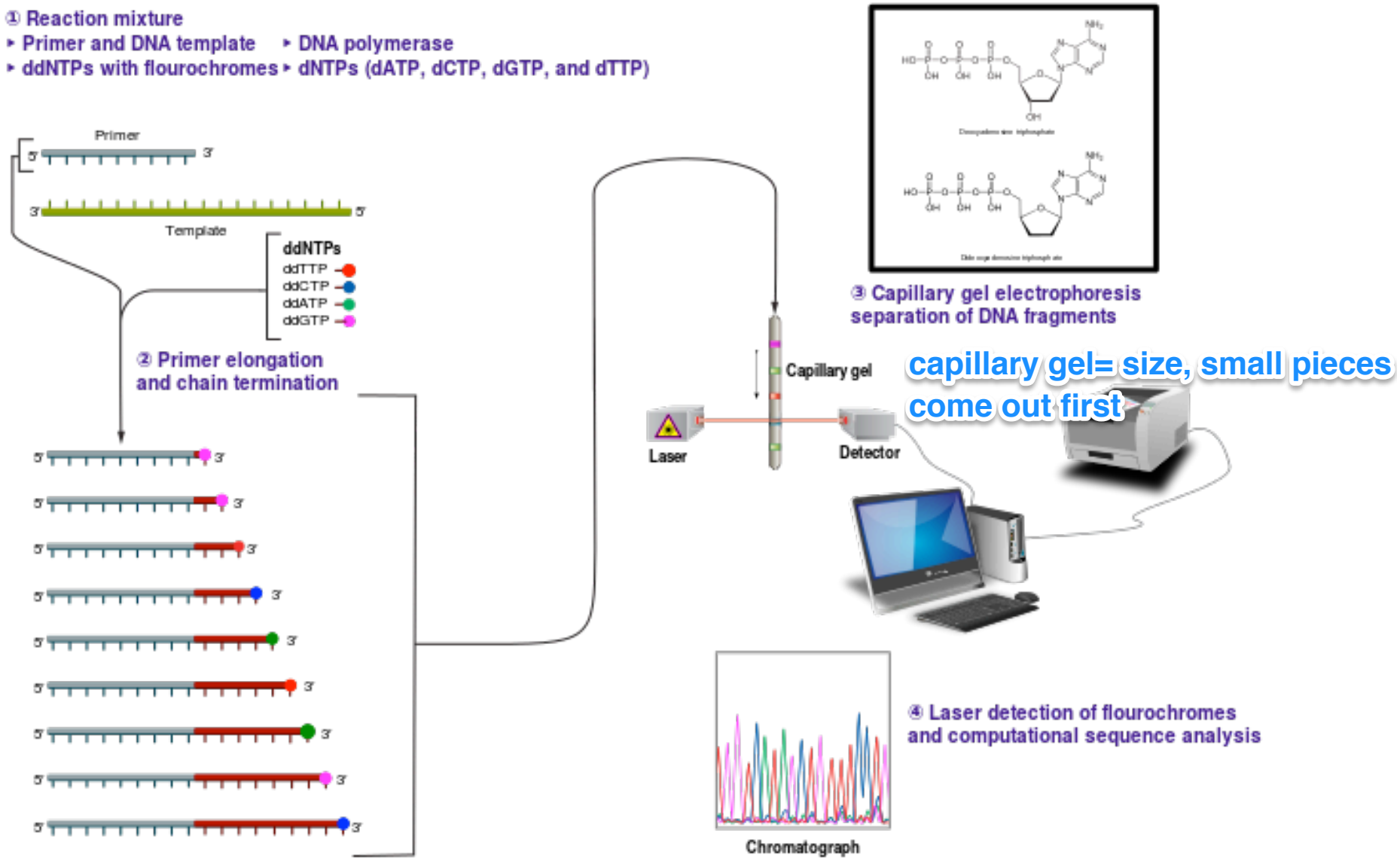
- Diagnostic digests
- Sequencing
  - good to have both F and R primers
    - 2X coverage of seq. of interest
    - good coverage of greater than 500bps
  - di-deoxynucleotides terminate elongation



IMPT note this is 4 separate rxns



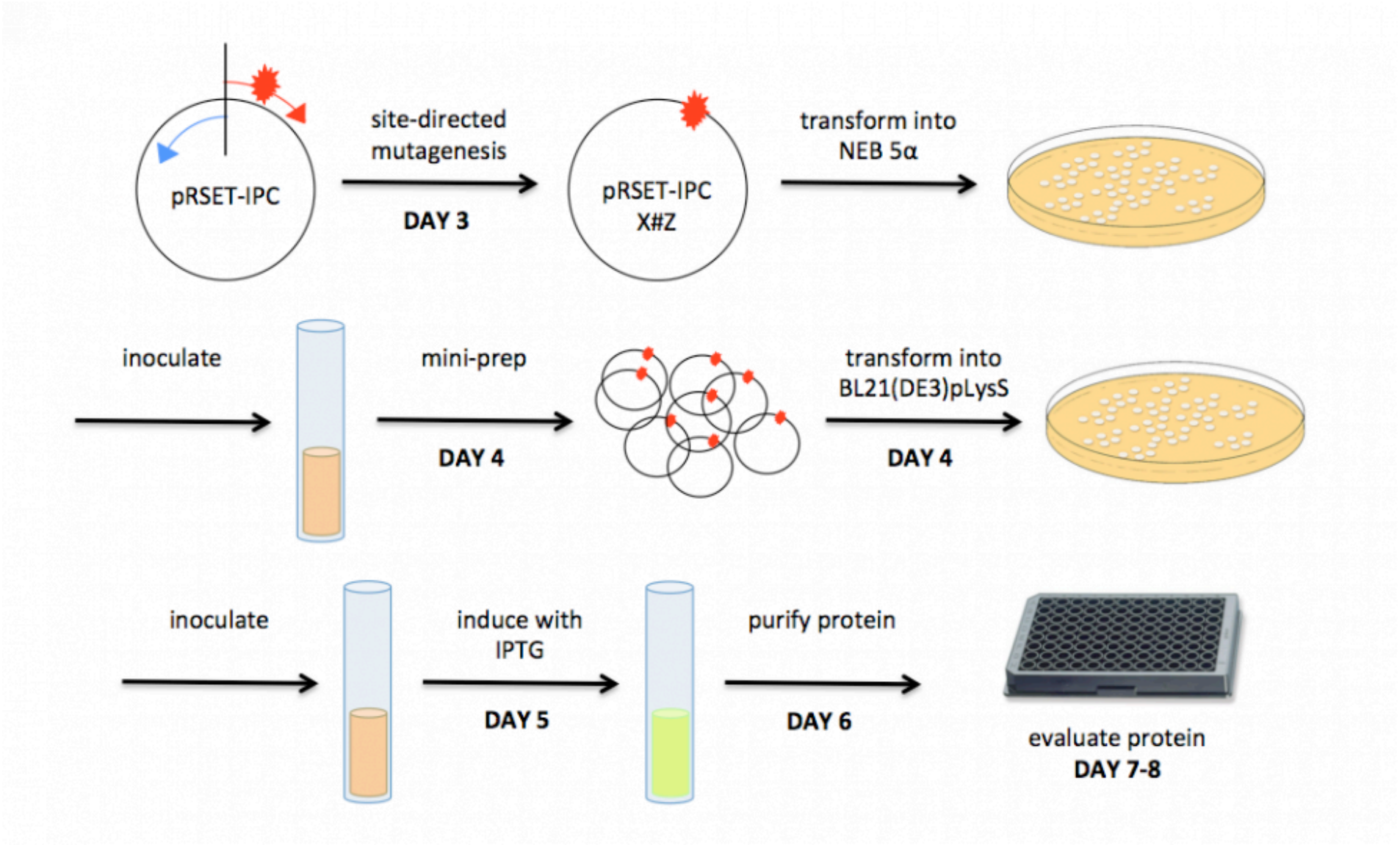
Sanger sequencing by Genewiz



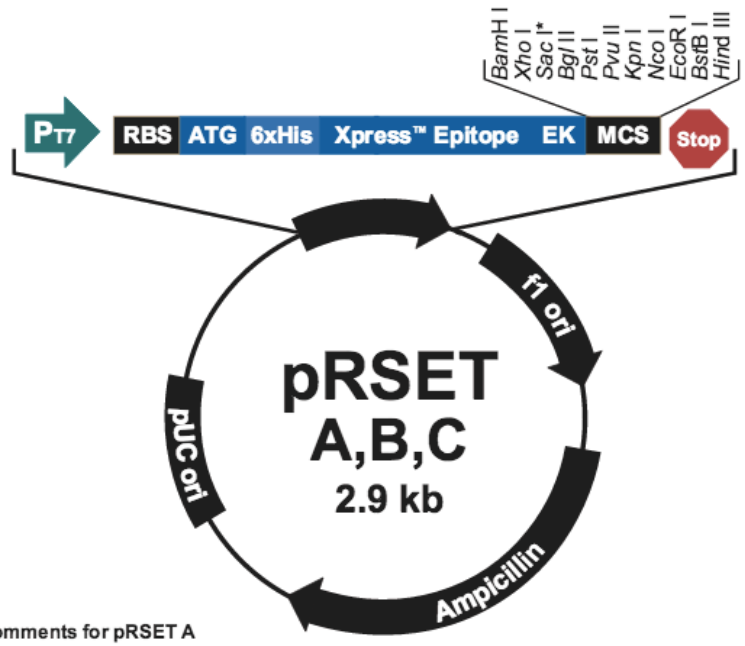
Aligning your sequencing data to pRSET-IPC:

```
  1 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~GACCAACTGACAGAAGAGCAGATTGCAGAGTTCAAAGAAGCCTTCTCATTATTCGACAAGGATGGGG 67
101 ATCCTGGGGCACAAGCTGGAGTACAACGGTACCGACCAACTGACAGAAGAGCAGATTGCAGAGTTCAAAGAAGCCTTCTCATTATTCGACAAGGATGGGG 200

 68 ACGGCACCATCACCACAAAGGAACTTGGCACCGTTATGAGGTCGCTTGGACAAAACCCAACGGAAGCAGAATTGCAGGATATGATCAATGAAGTCGATGC 167
                                                                                                       #
201 ACGGCACCATCACCACAAAGGAACTTGGCACCGTTATGAGGTCGCTTGGACAAAACCCAACGGAAGCAGAATTGCAGGATATGATCA-TGAAGTCGCTGC 299

168 TGATGGCAATGGAACGATTTACTTTCCTGAATTTCTTACTATGATGGCTAGAAAAATGAAGGACACAGACAGCGAAGAGGAAATCCGAGAAGCATTCCGT 267
300 TGATGGCAATGGAACGATTTACTTTCCTGAATTTCTTACTATGATGGCTAGAAAAATGAAGGACACAGACAGCGAAGAGGAAATCCGAGAAGCATTCCGT 399

268 GTTTTTGACAAGGATGGGAACGGCTACATCAGCGCTGCTCAGTTACGTCACGTCATGACAAACCTCGGGGAGAAGTTAACAGATGAAGAAGTTGATGAAA 367
400 GTTTTTGACAAGGATGGGAACGGCTACATCAGCGCTGCTCAGTTACGTCACGTCATGACAAACCTCGGGGAGAAGTTAACAGATGAAGAAGTTGATGAAA 499

368 TGATAAGGGAAGCAGATATCGATGGTGATGGCCAAGTAAACTATGAAGAGTTTGTACAAATGATGACAGCAAAGTAA~~~~~~~~~~~~~~~~~~~~~~~ 444
500 TGATAAGGGAAGCAGATATCGATGGTGATGGCCAAGTAAACTATGAAGAGTTTGTACAAATGATGACAGCAAAGTAAGAATTCGAAGCTTGATCCGGCTG 599
```

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

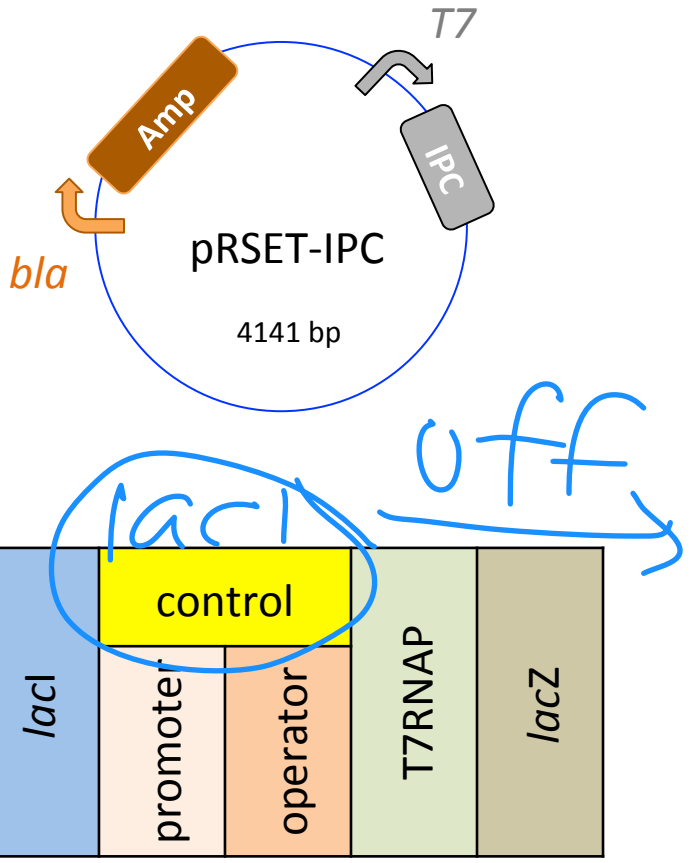
Let's piece together this "protein induction" story

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



genes of the lac operon

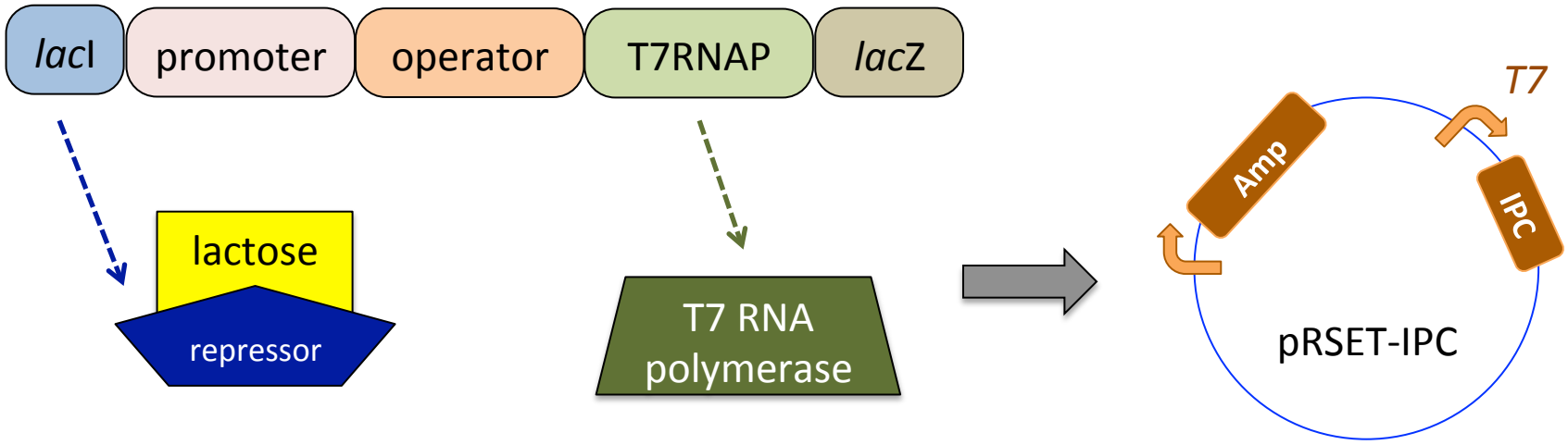
Let's piece together this "protein induction" story

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

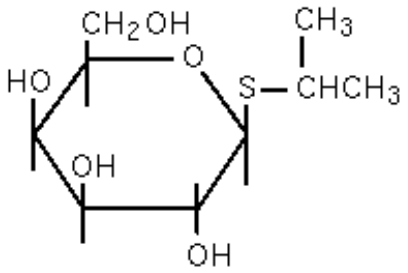
- lactose binds to repressor and makes it _inactive____ , thus turning ___ON___ expression of T7RNAP
- with T7RNAP present, the T7 promoter is _____ON_____, and IPC expressed



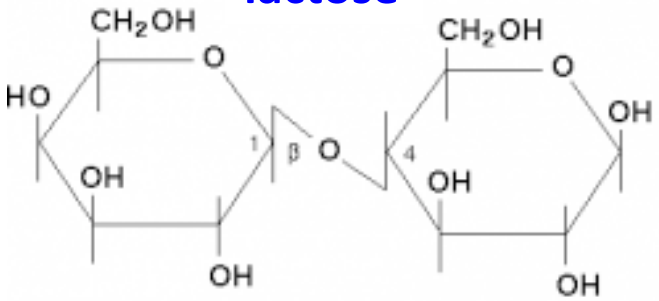
IPTG is a lactose analogue

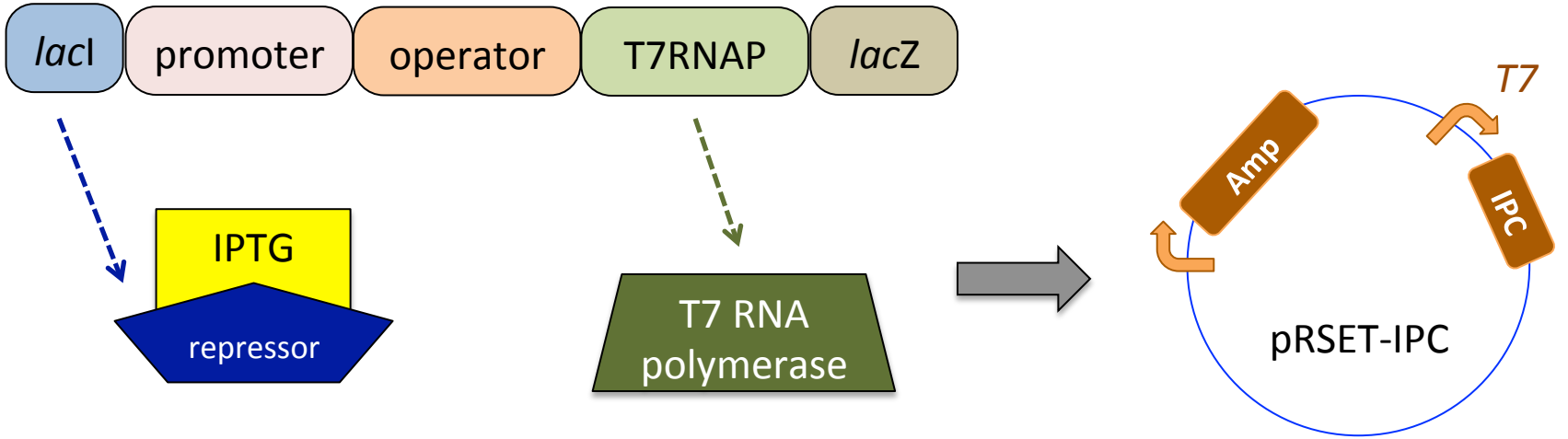
- isopropyl β-D-1-thiogalactoside
- structural mimic of lactose
- Both lactose and IPTG are taken up by *E. coli* but unlike lactose, IPTG is not part of any metabolic pathways and not hydrolyzable by β-galactosidase and utilized by the cell.

IPTG

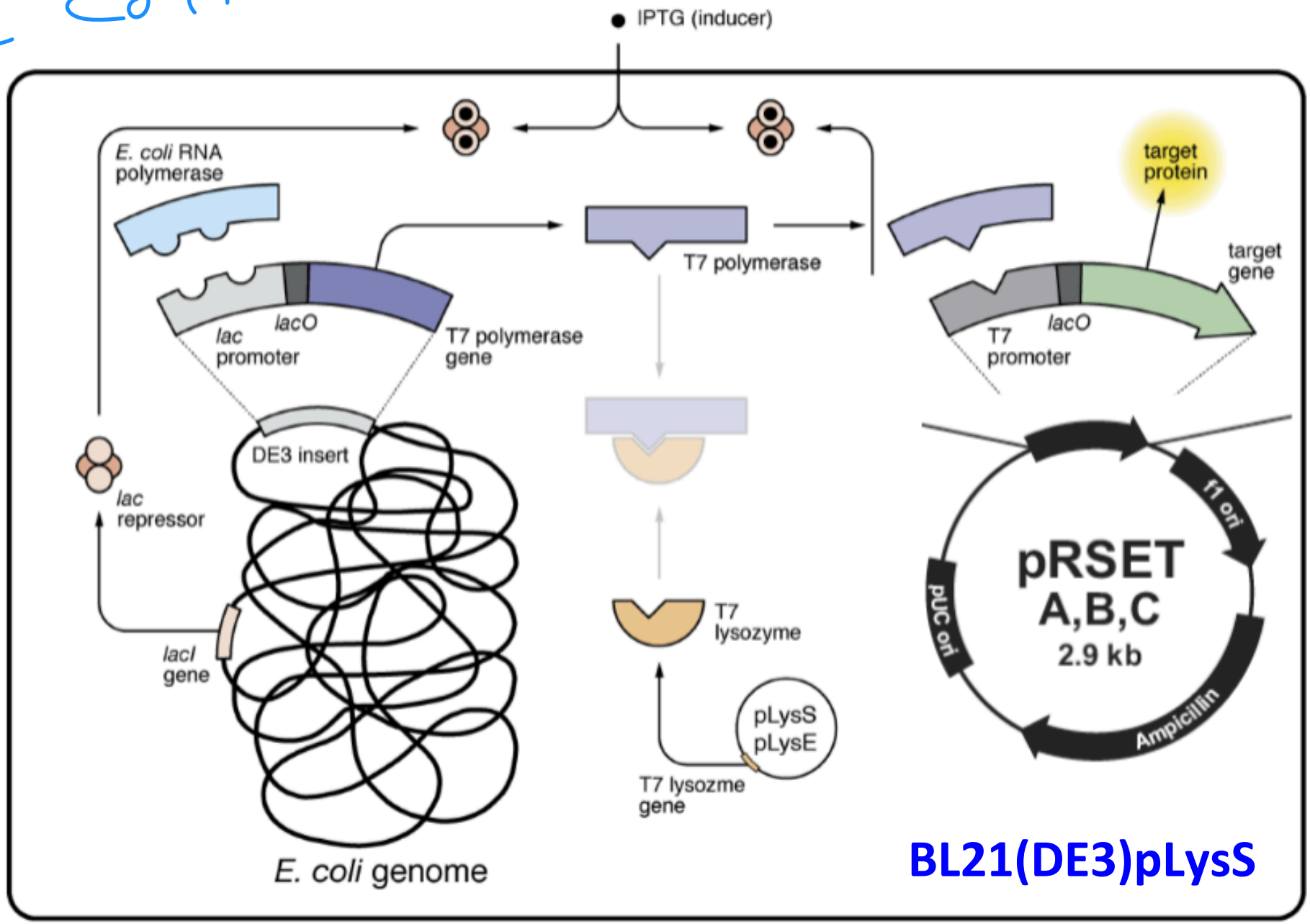


lactose





E coli



BL21(DE3)pLysS

In lab today

- IPTG induction of IPC protein expression
- 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

Methods homework due M1D6

- Methods M1D3-M1D5: SDM, Prep of expression system, protein induction
  - Eliminate 109 specific details
  - Do not include obvious details such as info about tubes and water
  - Avoid repeating information
- Use subsections with descriptive titles
  - Put in logical order
  - Begin with topic sentence
- Use clear and concise full sentences
  - Avoid tables and lists
- Use the most flexible units
  - Write concentration rather than volume
  - Report concentrations (NOT volumes)

-more than 1 way to write a methods section
-assume the reader has some molecular biology experience

Improving your Methods, Example 1:

Inverse Pericam (IPC) was amplified from pcDNA3-IPC → 2pmol/ul IPC forward (CATTAG...)

1ng/ul

Template ~~DNA (5ng)~~ and primers ~~were mixed~~

using 1X → with

~~with 20 uL of 2.5X~~ Master Mix ~~in a PCR tube~~.

1X Master Mix (5Prime, City, St)

~~Water was added to 50 uL. A tube without~~

included.

No template was ~~prepared and labeled control~~.

** thermocycler conditions should be included as a sentence

Improving your Methods, Example 2:

1.5ml NEB5 alpha (insert genotype) overnight culture was collected

A liquid bacteria culture was pelleted and the

pRSETb-IPC-D49H

DNA was purified using a Qiagen kit.

QIAquick ....(Qiagen, City, st)

according to manufacturer's protocl with the final elution in 30ul of dH2O pH8.