M2D5: Induce Protein and Evaluate DNA

4/3/13

1. Turn in M1D4 FNT up front in folder and mid-semester eval in the other folder.

Welcome Back!

2. Module 1 report revisions: **Due 11am on Friday**
   - Resources:
     - Shannon + Agi (by appt)
     - Jon (by appt)
     - Writing fellows (by appt)
     - Writing lab (Sun-Thu, 7-9pm, 56-205)

3. Pay close attention to Jon's comments and guiding questions -- take them seriously, in many cases they are specific (and leading)!
   - Better to address 50% of comments in depth, than 100% comments shallowly

M2D5: Induce Protein and Evaluate DNA

4/3/13

1. Welcome Back -- let's review a bit

2. Mod2 Quiz 2 next time!
   - Covers the following:
     - Lecture material (Alan's lectures)
     - Diagnostic gels and digests
     - Bacterial strains, transformations, inductions
     - NO stats :-(

3. Pay close attention to Jon's comments and guiding questions -- take them seriously, in many cases they are specific (and leading)!
   - Better to address 50% of comments in depth, than 100% comments shallowly

Protein Engineering -- Experimental Overview

modified DNA

pRSET-IPC

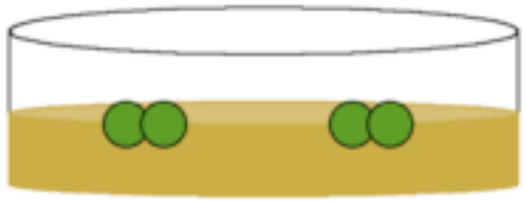
mutagenize

DAY 2

pRSET-IPC*

transform in XL1-Blue

DAY 3



liquid culture

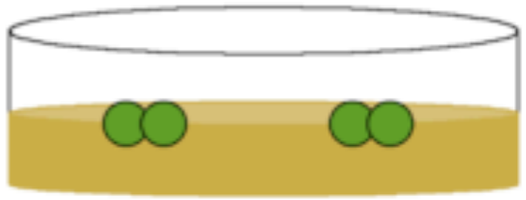
liquid culture - diluted @ noon ~1:30 OD ~0

miniprep mutant DNA

DAY 4

transform in BL21(DE3)

DAY 4



Switched ~9:45-10am

liquid culture

OD ⇒ 0.4 - 0.8

induce cells with IPTG

DAY 5

lyse cells and extract protein

DAY 6

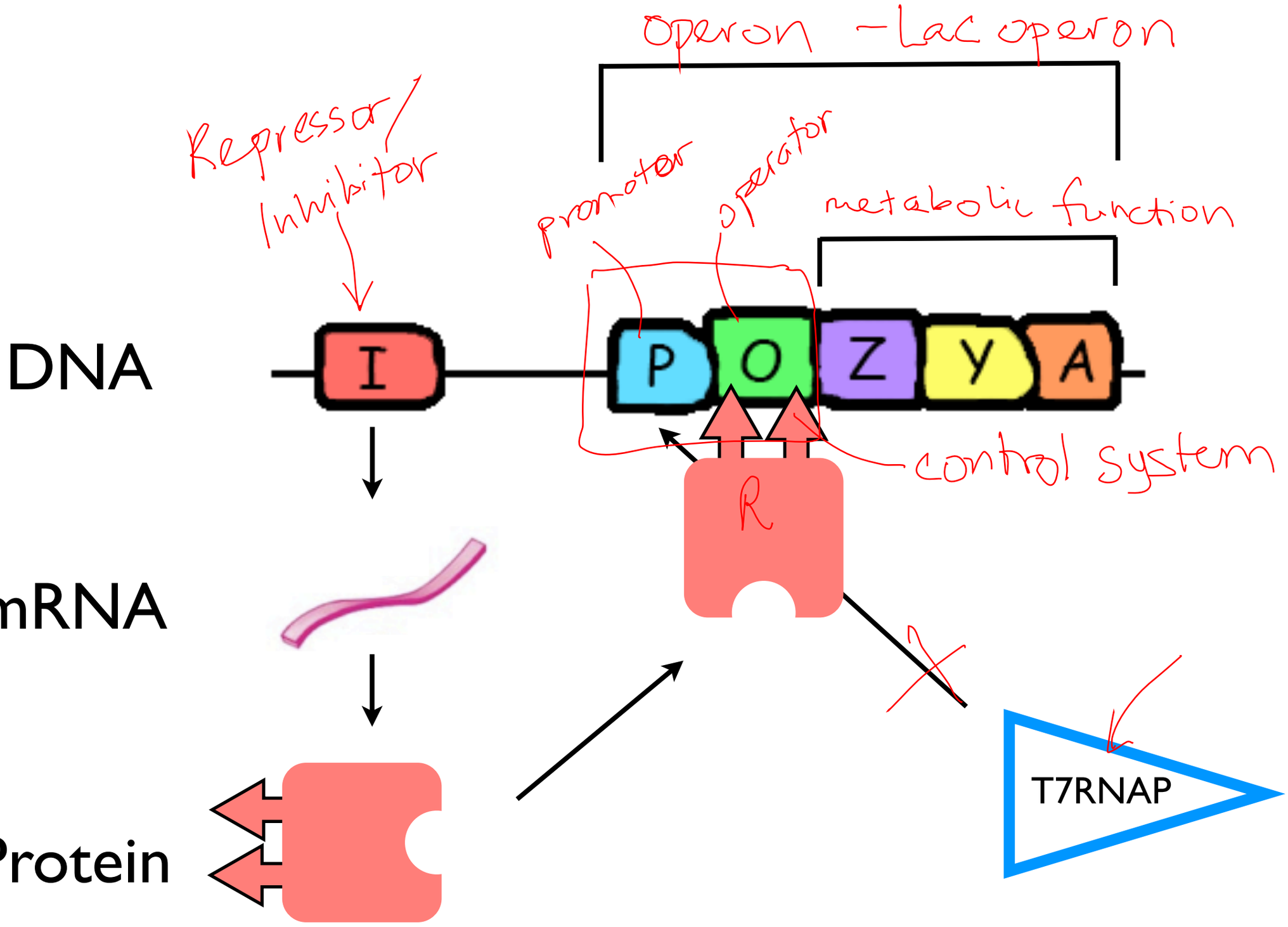
evalute protein

DAY 7/8

produce protein

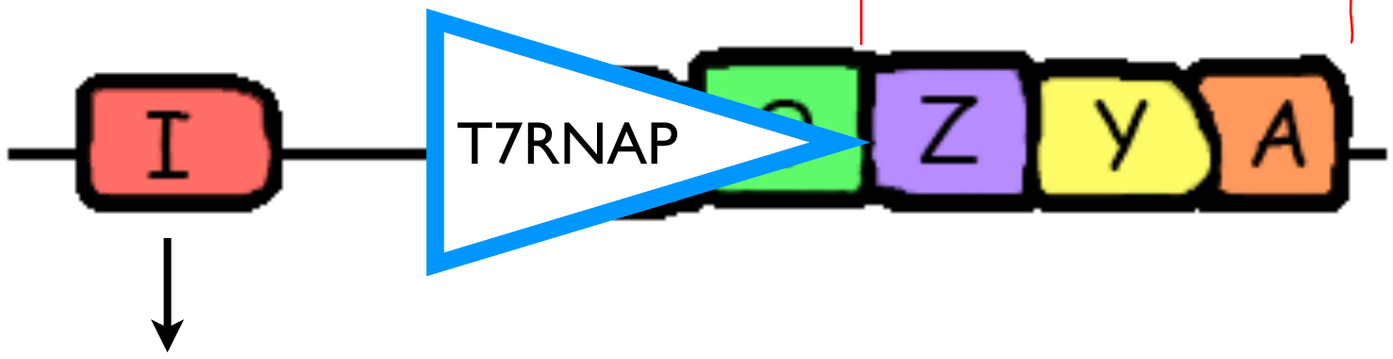
quantify mutation

Protein Engineering -- Controlling Expression



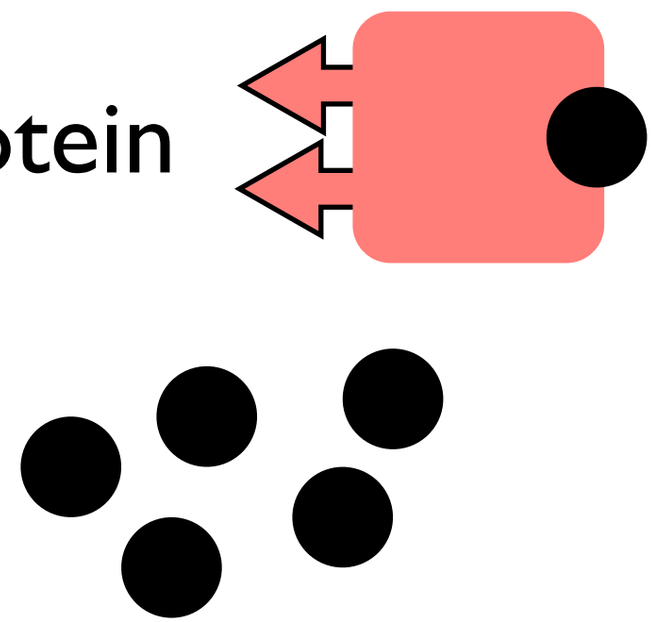
Protein Engineering -- Controlling Expression

DNA

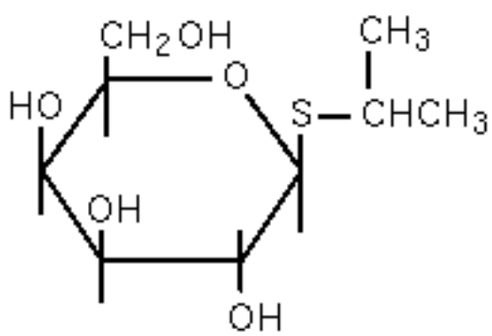


mRNA

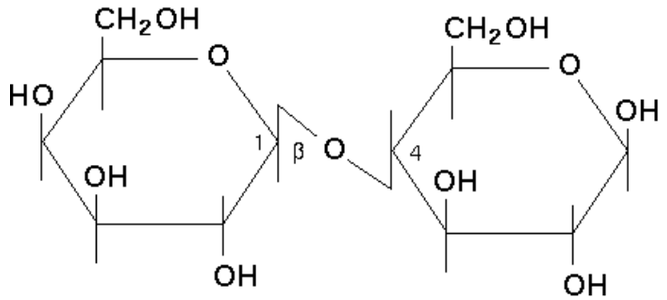
Protein



IPTG



Lactose



Protein Engineering -- Our Expression System

In the cell:

BL21(DE3) ↔ bacteriophage virus encoded

repressor

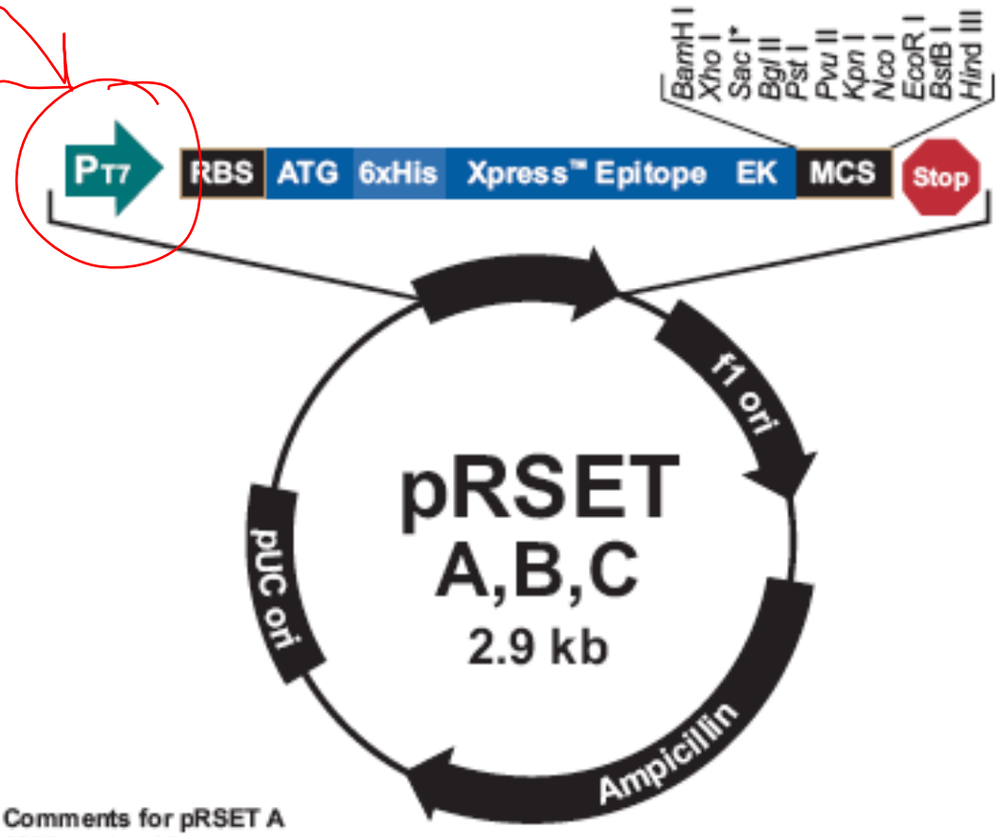
T

lactose

ON: T7RNAP (IPG lactose)

IPC

In the plasmid:

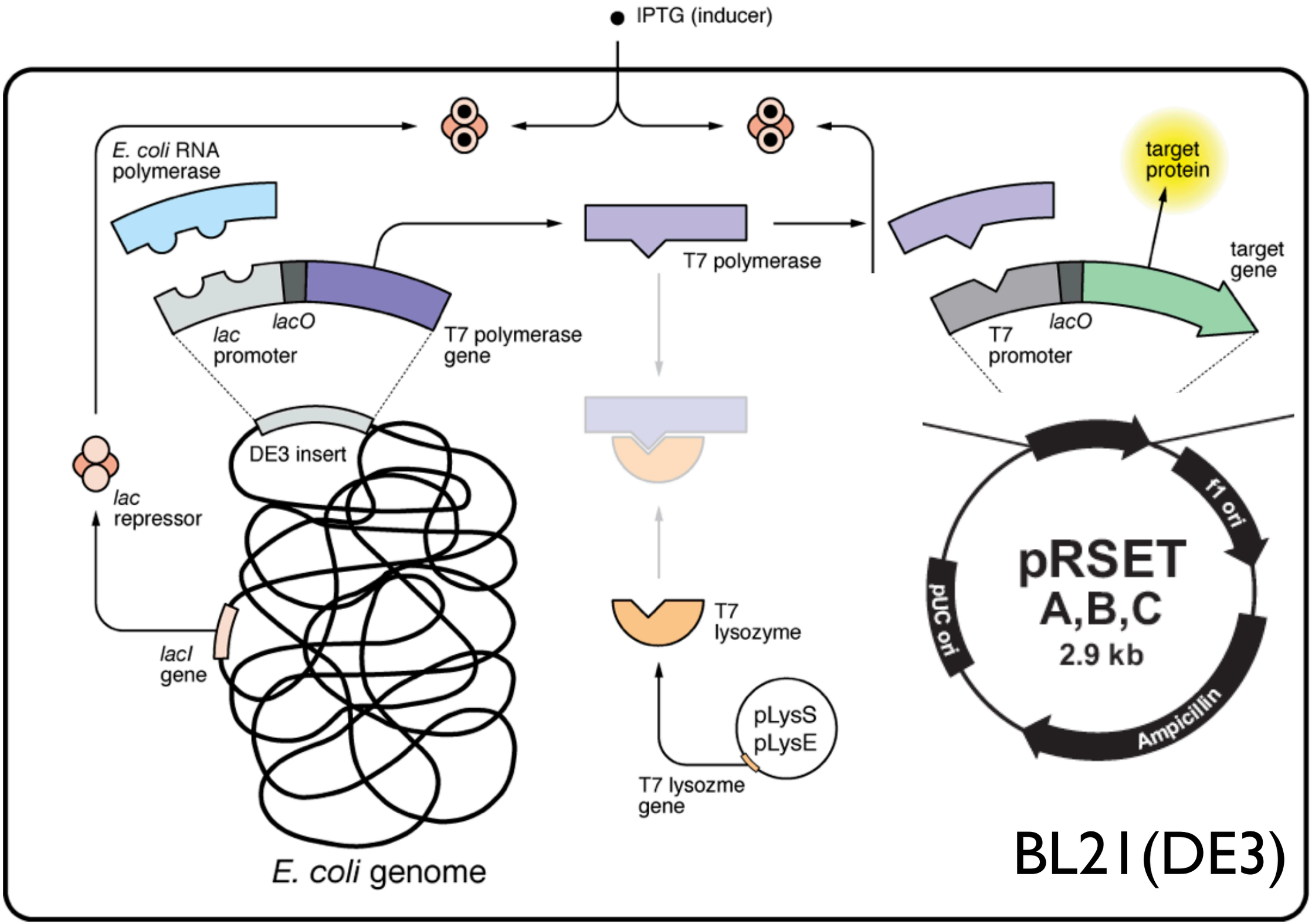


Comments for pRSET A
2897 nucleotides

*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)

Protein Engineering -- Our Expression System

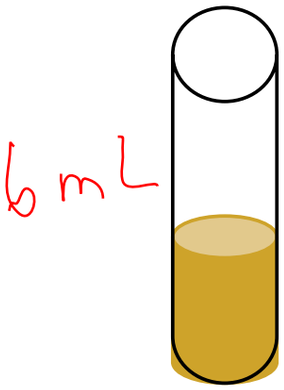


Protein Engineering -- Workflow M2D5

700µL 1:10 dilution

OD_{600} = 0.4-0.8

Check OD until mid-log



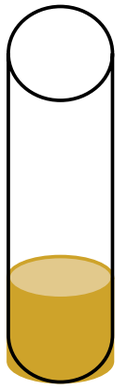
6 mL

1.5 mL

-IPTG control: ice, eventually spin down

+IPTG

4.5mL

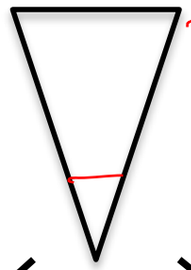


– 2.5hrs

2-3 hours

at 37 °C

1.5mL



Spin to check pellet color

if green

if white

Analysis: gel, sequencing

Measure post-growth OD

Pour liquid on top, re-pellet

+1.5mL

Grow rest O/N at room temp

Teaching faculty will pellet

Today in lab:

1. Start with **FOUR** BL21 cultures:
   - ⭐ WT
   - ⭐ Reference
   - ⭐ X#Z candidates 1 & 2
2. After gel and sequencing analysis, pick **ONE** X#Z candidate
3. End of day -- two possibilities:
   - ⭐ Hand in 6 pellets
   - ⭐ Hand in 3 pellets + 3 cultures + 3 eppendorfs