UNDERGRAD & GRAD STUDENT UROP MIXER

Course 20 | Course 7 | Course 5 | Course 10B | Course 6-7

2/25 4:30-5:30

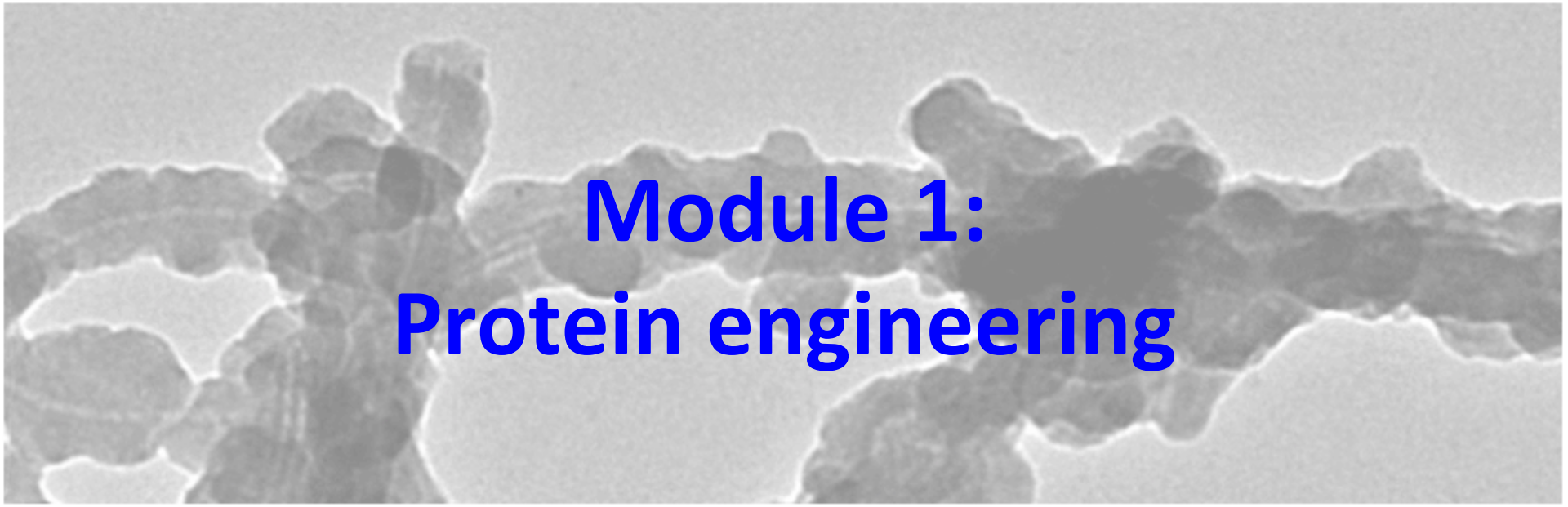
56-614

Present your research to a variety of undergrads and get a UROP!

Refreshments will be served!

RSVP at: https://goo.gl/A69WLQ

Email questions to biotech-undergrad-officers@mit.edu



Module 1:
Protein engineering

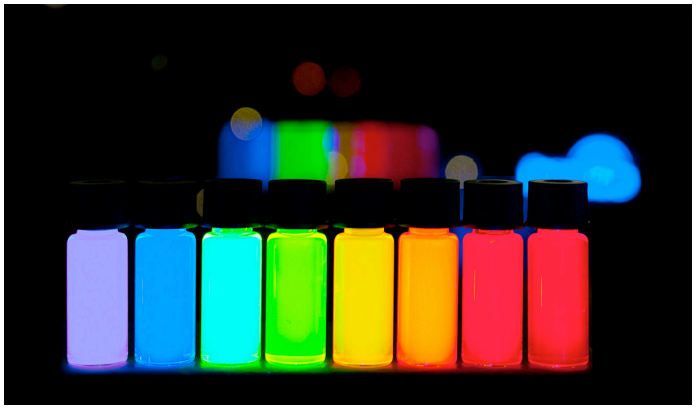
I Protein expression

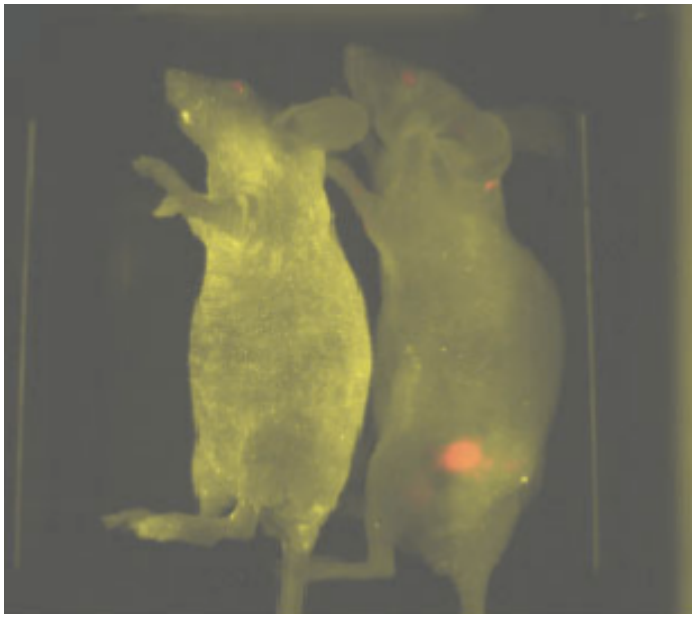
II Building an expression system

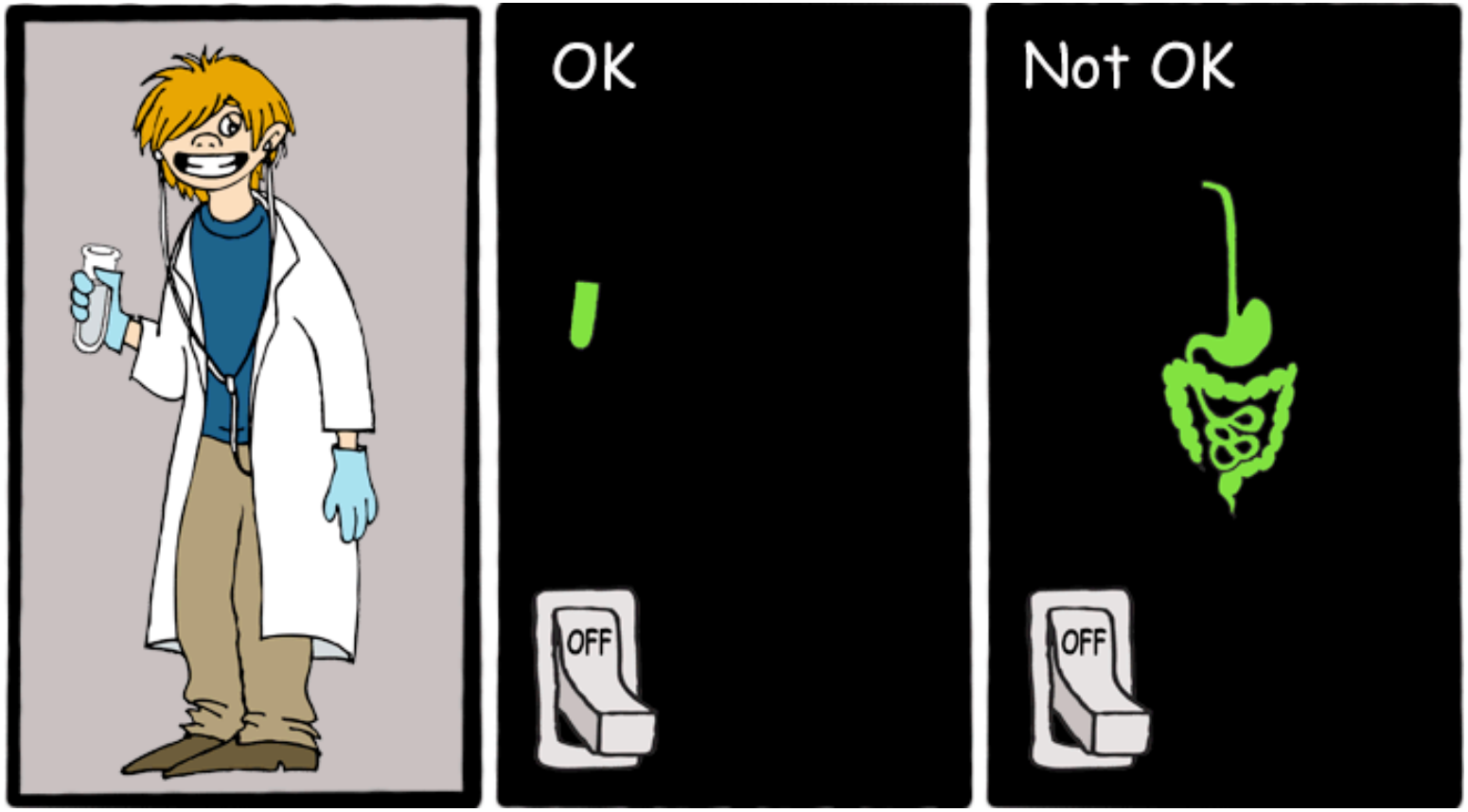
2/18/16

Biology vs. Technology

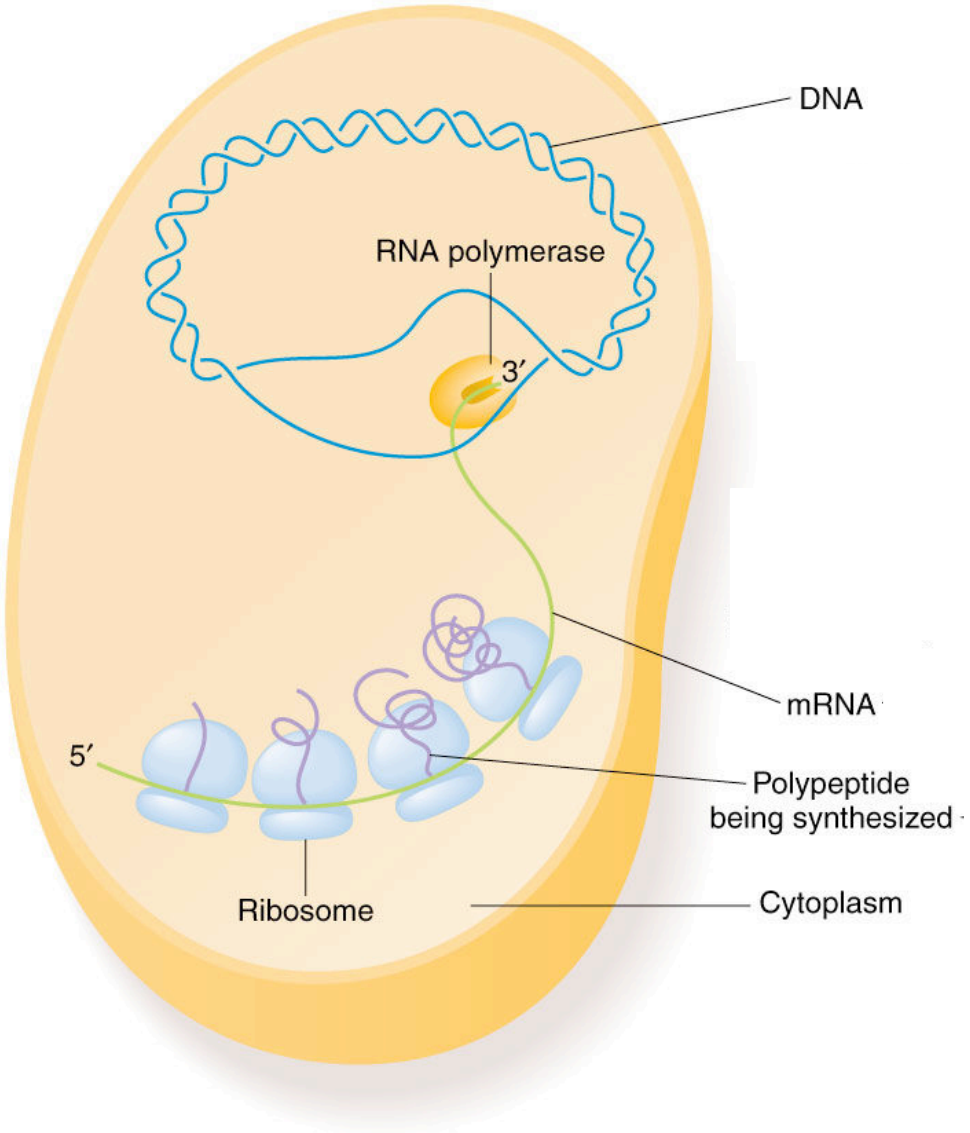
- Quantum dots (QDs) are small semiconductors that are able to convert light into nearly any color
  - Color is determined by size
- QDs can be targeted to cancer cells



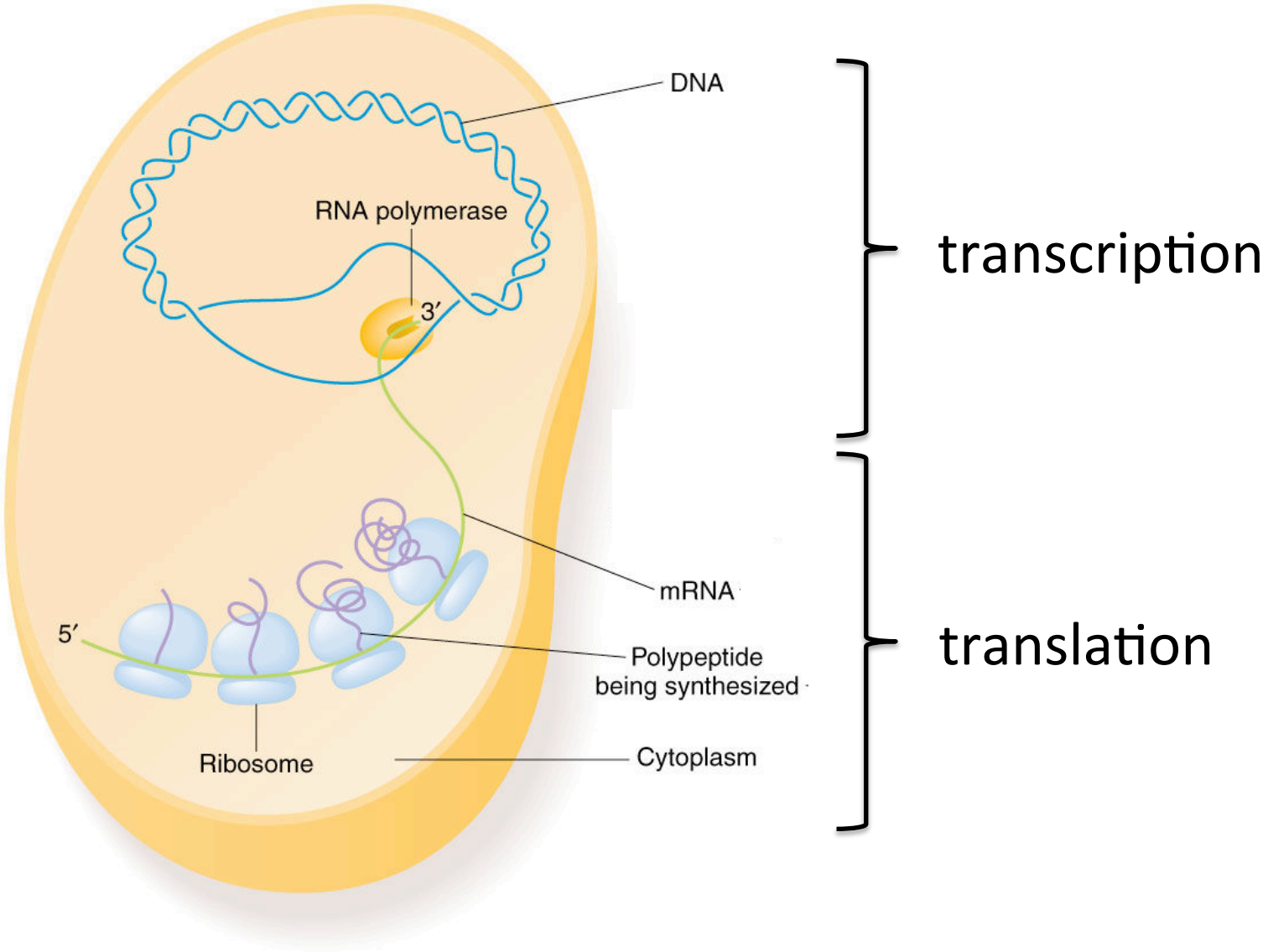



OK
OFF
Not OK
OFF

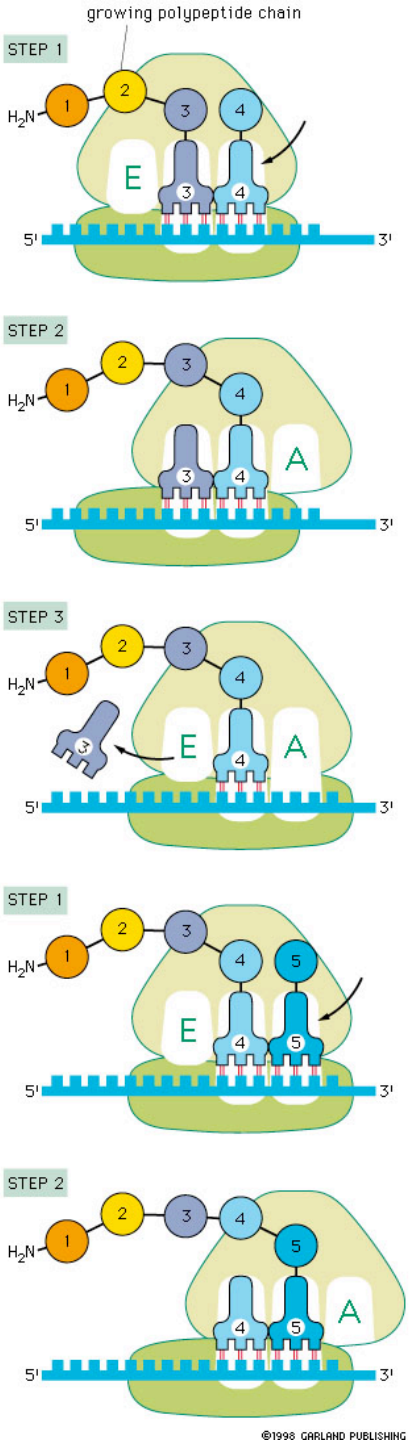
How are proteins generated?



How are proteins generated?



How *specifically* are proteins generated?



Protein synthesis

- Ribosome has three sites for tRNA binding
  - Aminoacyl (A) site binds incoming tRNA
  - Peptidyl (P) site holds tRNA with forming peptide chain
  - Exit (E) site

What controls protein expression?

What controls protein expression?

- At the level of transcription
  - Environmental regulation of promoters
  - Repressor binding to operator sequences
  - Sigma factor binding to promoters
- At the level of translation
  - Proteasome degradation of proteins
  - Modifications

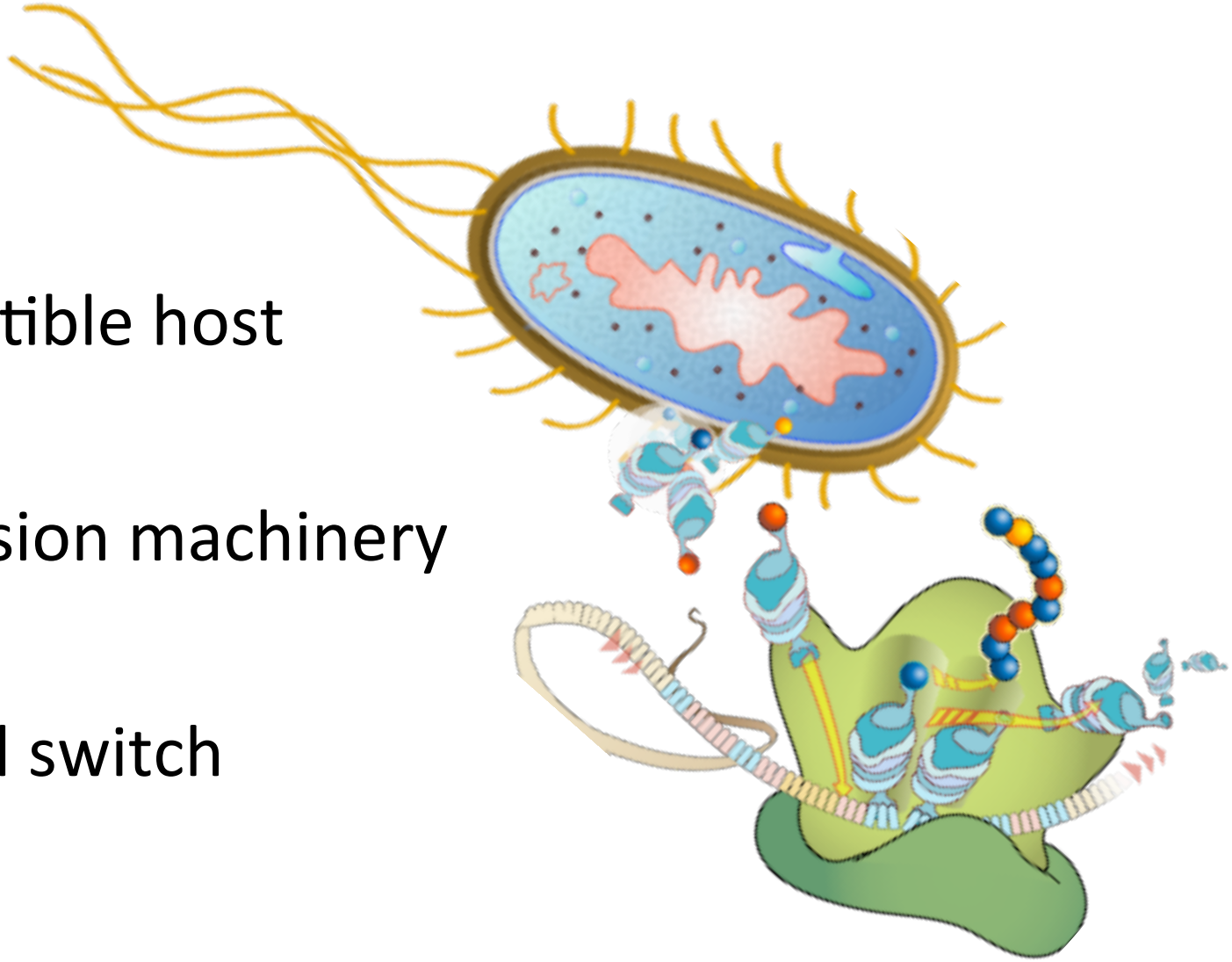
Generating proteins for research

- Purify from native source
  (+) no DNA manipulation, correct folding likely
  (–) need high abundance, only native proteins
- Synthesize de novo
  (+) no DNA manipulation, pure product
  (–) expensive, difficult for >50 amino acids
- Produce using expression system
  (+) inexpensive, generally high-yield
  (–) cloning required, troubleshooting

What do we need to build a protein expression system?

Building a protein expression system

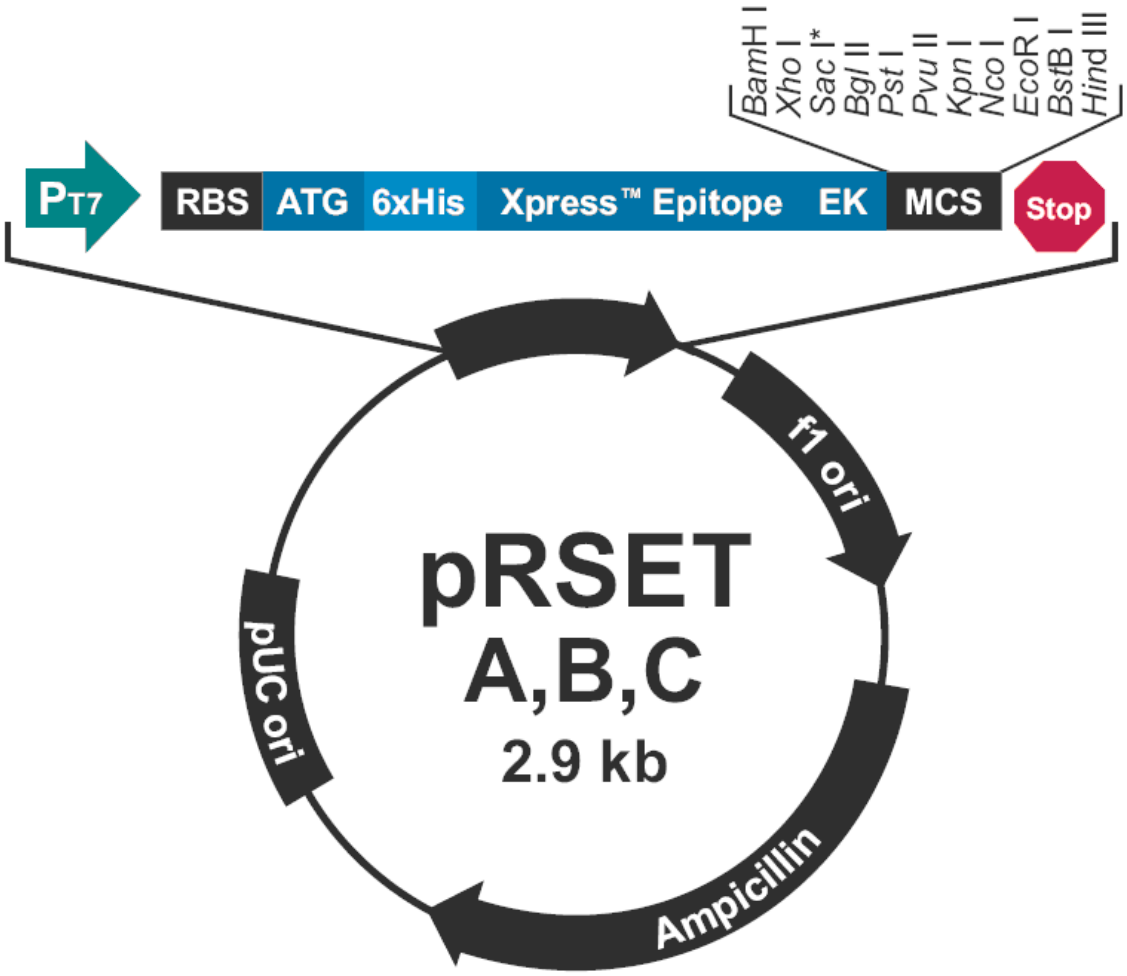
- Vector
- Compatible host
- Expression machinery
- Control switch



Considerations for the vector

- Multiple cloning site (MCS)
- Resistance cassette, typically antibiotic marker
- Origin of replication
- Ribosome binding site (RBS)
- Copy number
  - High copy number gives greater yield
  - Lower copy number alleviates stress, toxicity

pRSET engineered for protein expression



*Version C does not contain *Sac* I

Considerations for the host

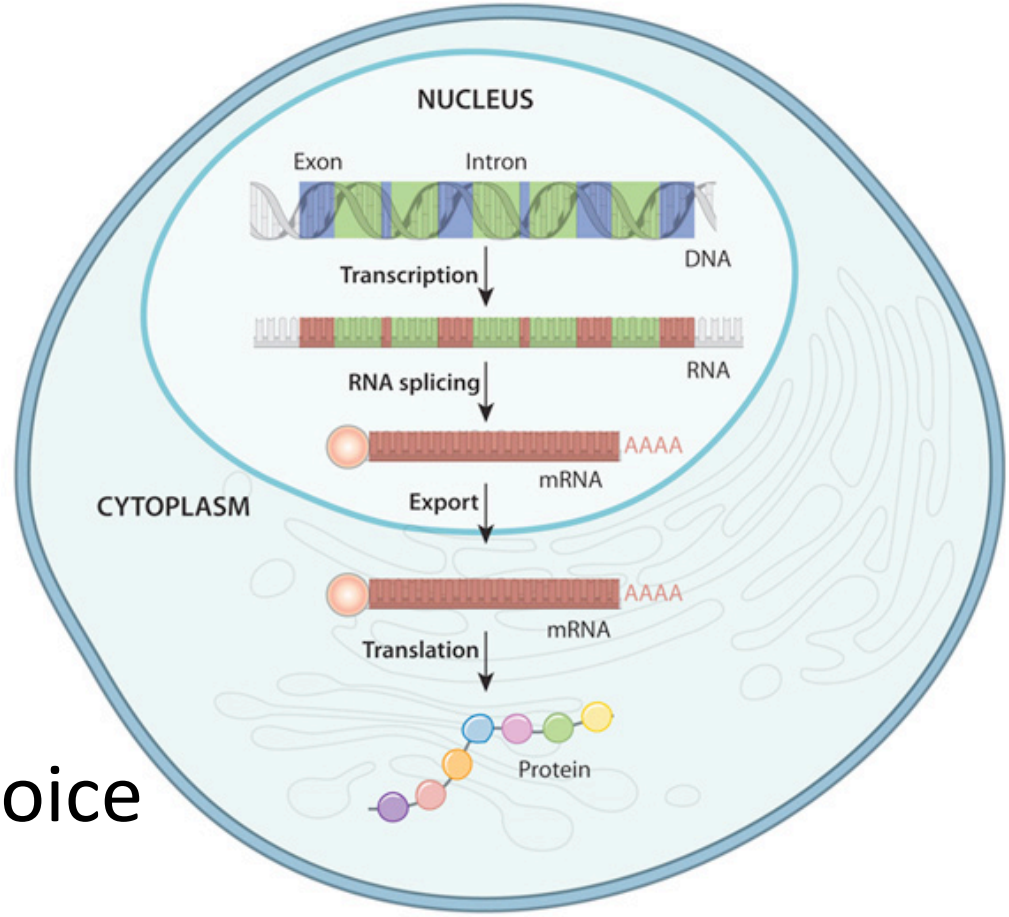
- Growth rate
- Growth conditions
- Genotype
  - Presence of transcriptional, translational machinery
  - Absence of enzymes that decrease yield, efficiency

BL21(DE3)pLysS engineered for protein expression

- Lacking *ompT* and *lon*
  - Encode for proteases
- Carries *gal*
  - Allows for utilization of galactose as carbon source
- Lacking *dcm*
  - Unable to methylate cytosine
- Lacking *hsdS*$_B$(r_B^- m_B^-)
  - Inactive restriction/methylation system

Considerations for the machinery

- Codon usage
- Chassis
  - Prokaryote versus Eukaryote
- Linked with host choice



Considerations for the control switch

- Responsiveness
- Chemical used for ‘on’
- Tight control
  - Weak ‘off’ may allow leaky expression of toxic protein
  - Weak ‘on’ may delay protein expression or give low yield

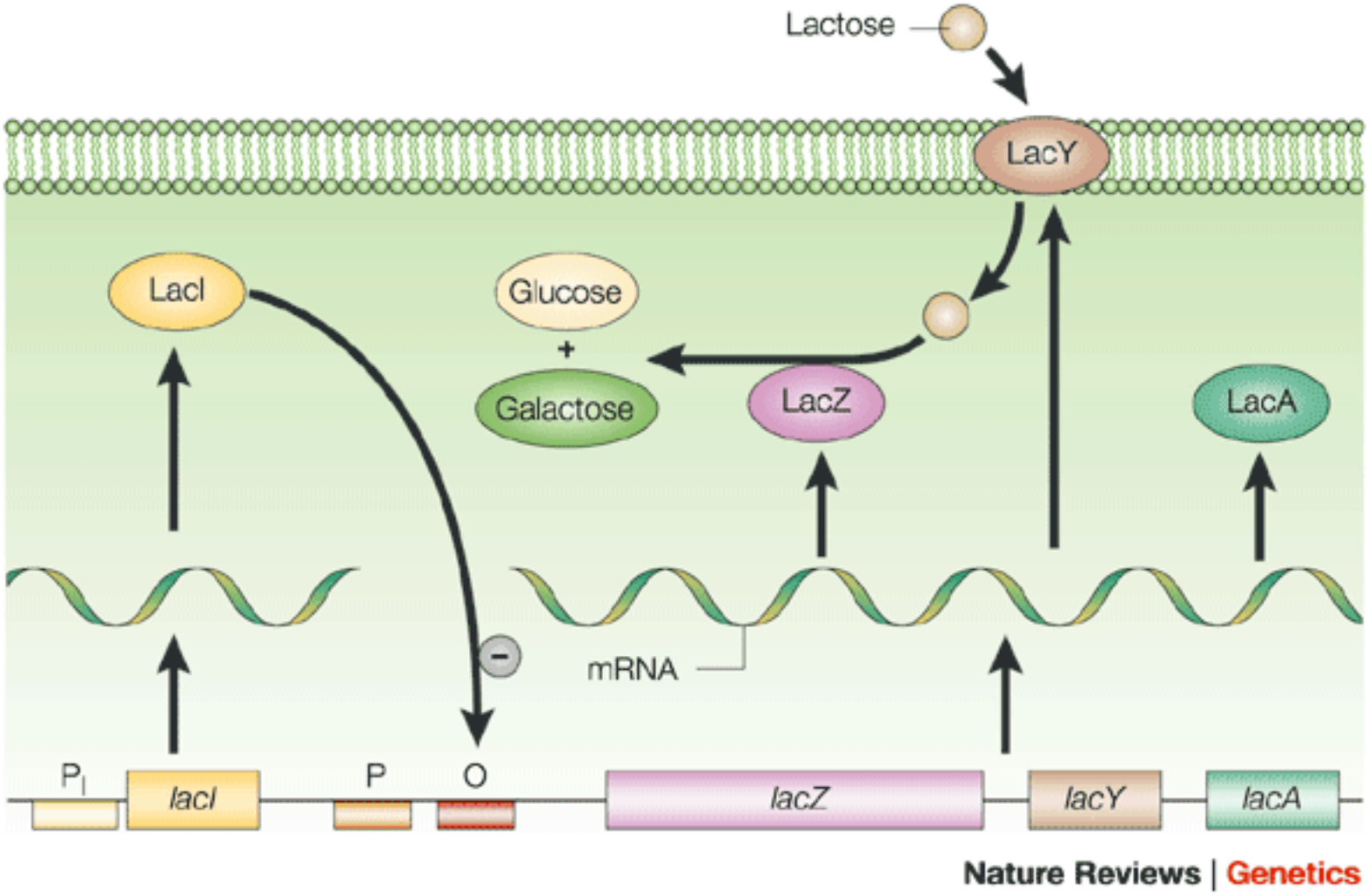
- Linked with vector and host choice

Our system uses two switches

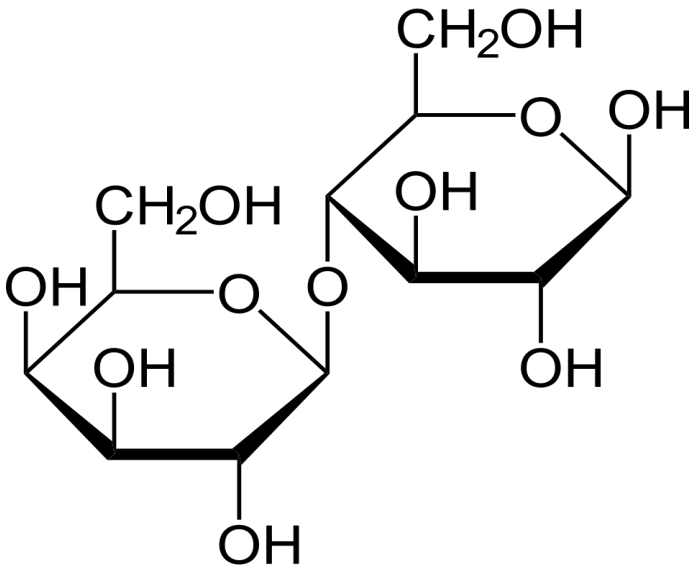
- IPTG induction
  - Used to induce protein expression by activating transcription of T7 polymerase

- T7 control
  - Used to eliminate leaky control of T7 polymerase expression

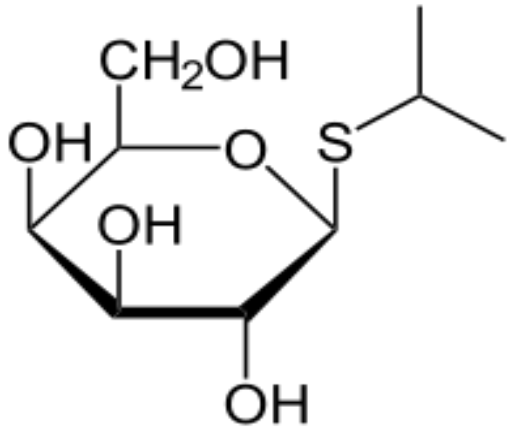
Quick review of the *lac* operon



IPTG induction of protein expression



β-D-galactopyranosyl-(1-4)-D-glucose
(lactose)

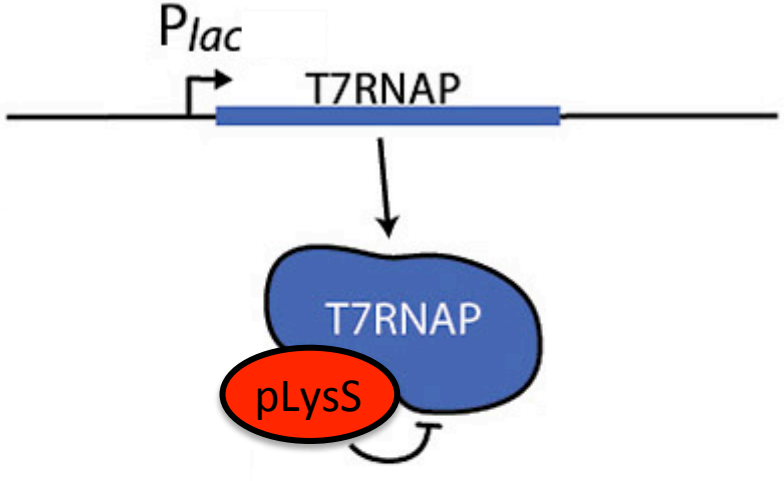


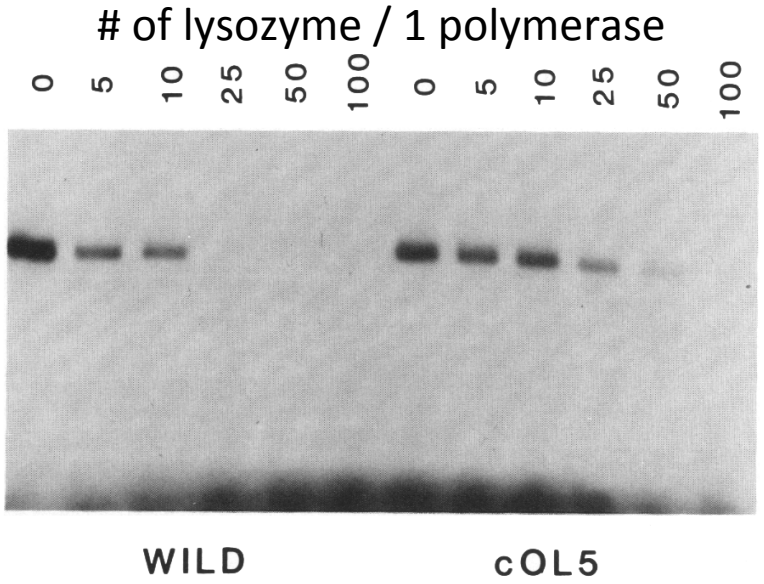
isopropyl β-D-1-thiogalactopyranoside
(IPTG)

- P_{lac} drives expression of T7 polymerase gene
- P_{T7} drives expression of gene of interest
- Both promoters upstream of *lacO* sequence

T7 control of protein expression

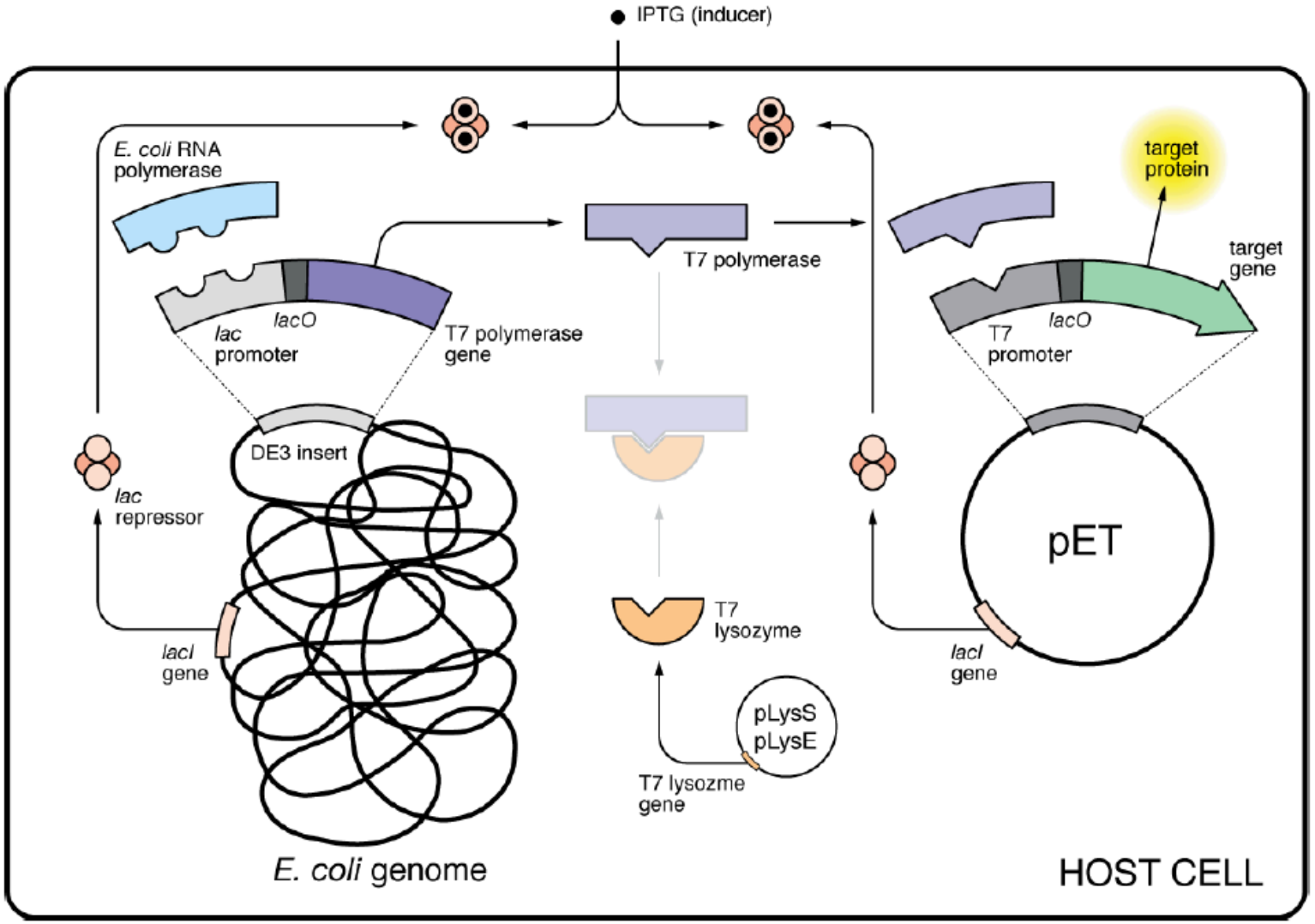
- pLysS encodes T7 lysozyme
  - Forms complex with T7RNAP
  - Prevents elongation of transcripts
- Corrects for leaky T7RNAP expression





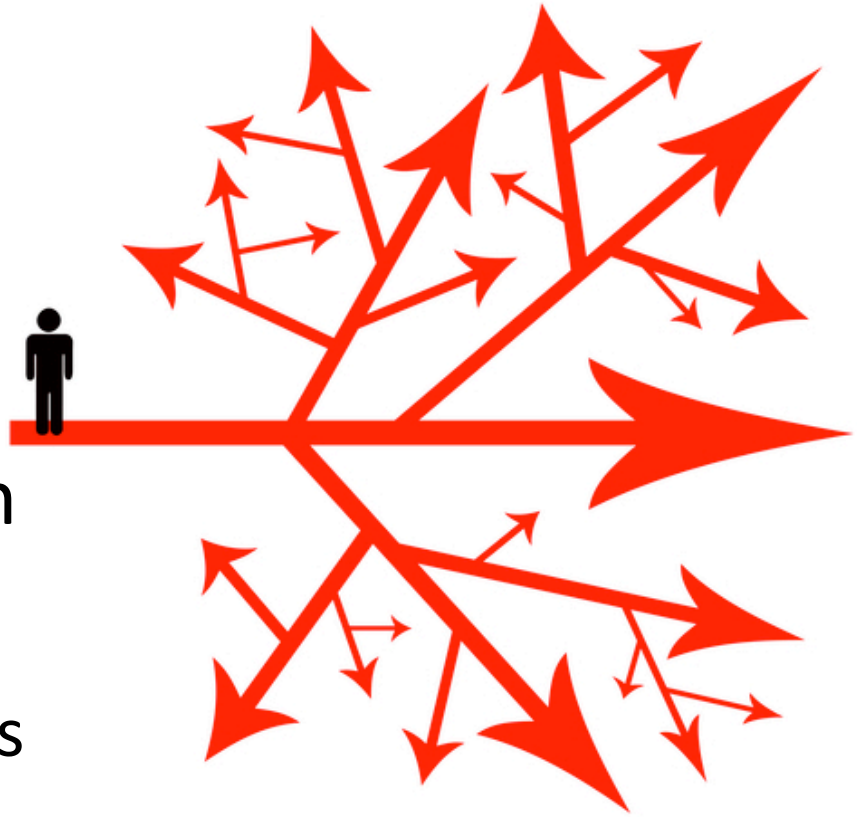
Moffatt and Studier. (1987) *Cell* 49:221-227.

BL21(DE3)pLysS and pRSET system includes both levels of control



Additional considerations…

- Growth phase of expression host
- Concentration of inducing chemical
- Timeframe of induction
- Stability of protein
  - Storage time, conditions
- And…



In the laboratory…

- Isolate X#Z plasmid potentials
  - Two clones inoculated ~18 h prior to laboratory
- Submit X#Z plasmids for sequencing check
- Transform X#Z plasmids into BL21(DE3)pLysS

- Homework due M1D5
  - Write methods draft