Module 2 overview

lecture	*lab*
1. Introduction to the module	1. Start-up protein eng.
2. Rational protein design	2. Site-directed mutagenesis
3. Fluorescence and sensors	3. DNA amplification
4. Protein expression	4. Prepare expression system
SPRING BREAK	
5. Review & gene analysis	5. Gene analysis & induction
6. Purification and protein analysis	6. Characterize expression
7. Binding & affinity measurements	7. Assay protein behavior
8. High throughput engineering	8. Data analysis

Lecture 4: Protein expression & purification

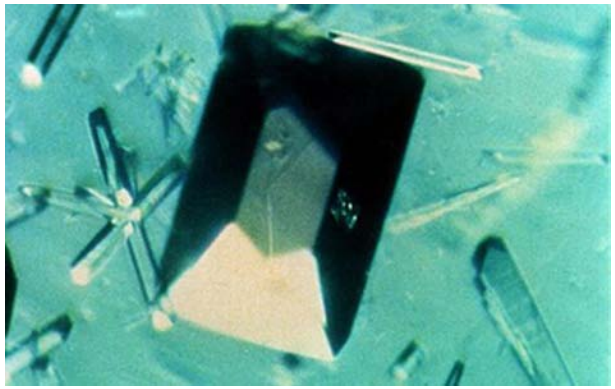
I. Why express & purify proteins?
   A. Scientific applications
   B. Applications in industry, *etc.*

II. Protein expression systems
   A. Alternatives to protein expression
   B. Prokaryotic and eukaryotic systems

Laboratory uses of purified proteins

Biochemistry analysis

Structural biology



Research biochemicals

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Photo of New England BioLabs biochemical vials.

From top, left: biochemistry lab, public domain (c. 1920), McGill University; protein crystal, public domain, NASA; NMR tubes photo, courtesy of Agilent Technologies, Inc, used with permission.

Protein therapeutics

Table 1 Top ten recombinant therapeutic proteins and their global sales between 2001 and 2003

Product (generic)/ marketing company	2001 ($million)	2002 ($million)	2003 ($million)	Growth (decline) 2002–2003 (%)
Procrit (epoetin alfa)/ Johnson & Johnson	3,430	4,269	3,986	(6.6)
Epogen (epoetin alfa)/ Amgen	2,108	2,261	2,435	7.7
Neupogen (filgrastim)/ Amgen	1,346	1,380	1,268	(8.1)
Neulasta (pegfilgrastim)/ Amgen	0	464	1,255	170.5
Novolin (insulin systemic)/ Novo Nordisk	2,244	2,255	2,235	(0.9)
Avonex (interferon beta-1a)/ Biogen IDEC	971	1,034	1,170	13.2
PEG-Intron A franchise (pegylated interferon alpha)/ Schering Plough	1,447	2,736	1,851	(32.3)
Enbrel (etanercept)/ Amgen	856	521	1,300	149.5
Aranesp (darbepoetin alfa)/ Amgen	42	416	1,544	271.2
NeoRecormon (epoetin-beta)/ Roche	479	766	1,318	72.1
Top ten product sales	*12,923*	*16,102*	*18,362*	*14.0*
Others	*8,547*	*10,833*	*13,703*	*26.5*
Total market value	*21,470*	*26,935*	*32,065*	*19.0*

Source: Datamonitor and company-reported information.

PEGylated

PEGylated

TNF ligand binding domain + Fc antibody domain

epo engineered to have additional glycoslyation sites

Pavlou & Reichert (2004) *Nat. Biotechnol.*

Product images removed due to copyright restrictions.
Box of laundry detergent; packet of dry beer enzyme;
book cover of "What's in your Milk?"; bottle of whey protein
isolate nutritional supplement; box of cosmetic Botox.

Photos removed due to copyright restrictions. Bulgarian dissident Georgi Markov, assasinated with ricin in 1978.
Replica of umbrella gun used to kill Georgi Markov:
see http://www.washingtontimes.com/news/2008/sep/04/london-umbrella-killing-likely-to-remain-unsolved/
Castor beans, above right, used to manufacture the toxin ricin. (Public domain image, USDA)

How can proteins be produced?

1. Purify from natural source
   advantages: no chemistry or DNA manipulation required, proteins likely to fold properly, assemble with native cofactors, *etc.*
   disadvantages: usually only practical for high abundance proteins, source-specific purification method required

2. Synthesize *de novo*
   advantages: no DNA manipulation required, synthesis methods well established, proteins produced are relatively pure
   disadvantages: relatively expensive, becomes extremely difficult for polypeptides > 50 amino acids

3. Express and purify from a dedicated expression system
   advantages: cheap and frequently high-yield, versatile expression systems already established
   disadvantages: cloning required, troubleshooting often needed to obtain high expression and proper folding

Solid phase peptide synthesis is a reliable technique for generating short polypeptides

Images removed due to copyright restrictions.
See Chan, W. C., and P. D., White. *Fmoc Solid Phase Peptide Synthesis*.
New York, NY: Oxford University Press, 2000. ISBN: 9780199637249.

www.pitt.edu

Chan & White (2000) *Fmoc Solid Phase Peptide Synthesis*

E. coli are the most common host for recombinant gene expression

inserted genes may be homologous or heterologous proteins, fusion proteins, or entirely novel constructs

Diagrams removed due to copyright restrictions.

Once a foreign gene has been introduced, how does protein expression take place?

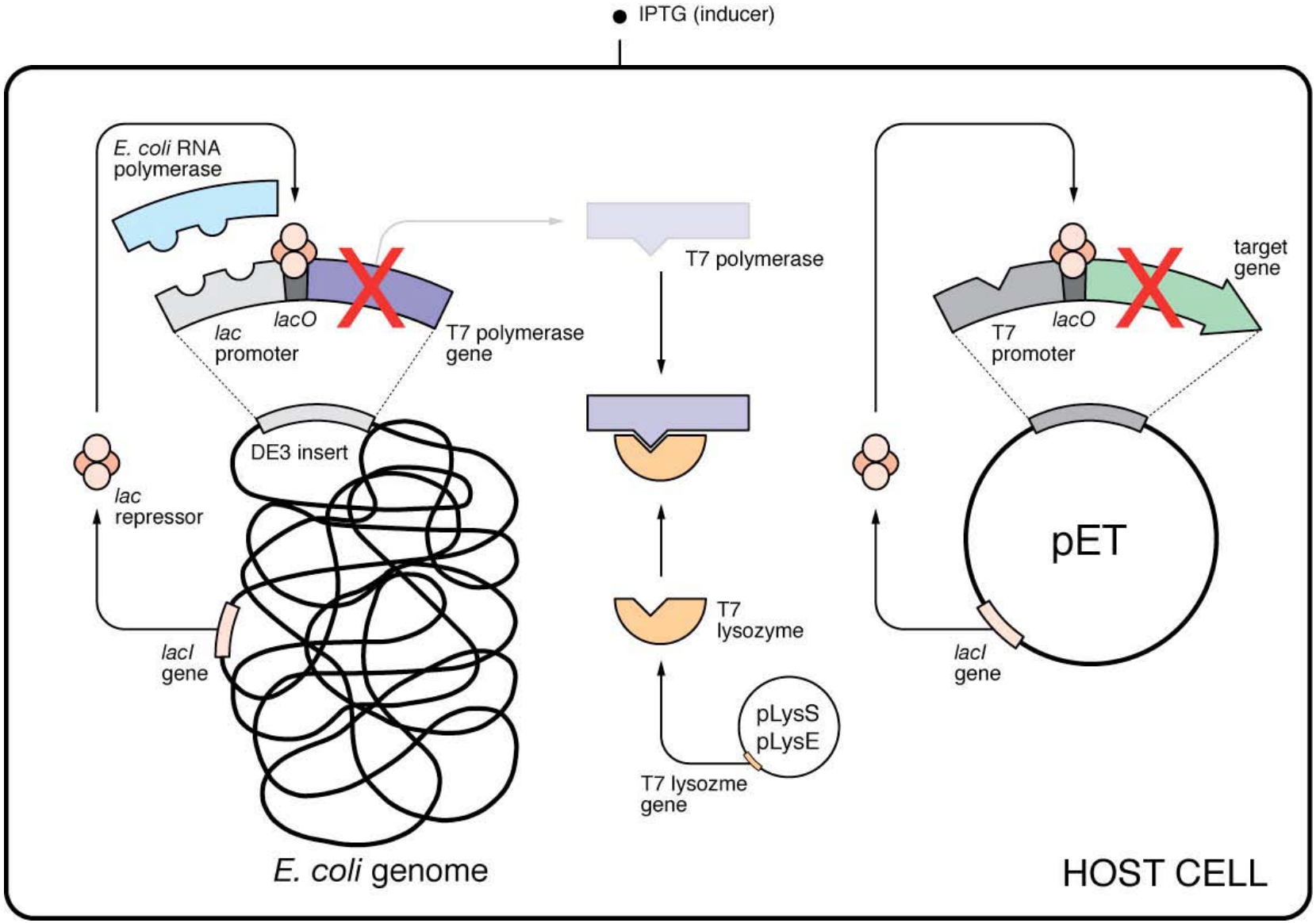
Stryer (1988) *Biochemistry, 3rd ed.*

The *lac* operon is the basis for the most common bacterial protein expression systems

Two diagrams removed due to copyright restrictions.
Lactose Hydrolyzed into Galactose and Glucose; structures of 1,6 allolactose and IPTG
Fig 31.8, *Induction of the LAC Operon.* In Berg, Tymoczko, and Stryer. *Biochemistry.* 5th ed. W. H., Freeman, 2002.

Stryer (1988) *Biochemistry, 3rd ed.*

T7 expression system



Other induction systems can also be used for protein expression in *E. coli:* arabinose system is considered to be more tightly controlled than the *lac* operon

Diagrams removed due to copyright restrictions.

ara system is also compatible with T7-based vectors

Stryer (1988) *Biochemistry, 3rd ed*.

Differences between prokaryotic and eukaryotic proteins sometimes require eukaryotic expression systems.

These two proteins exemplify characteristics that frequently call for eukaryotic expression:

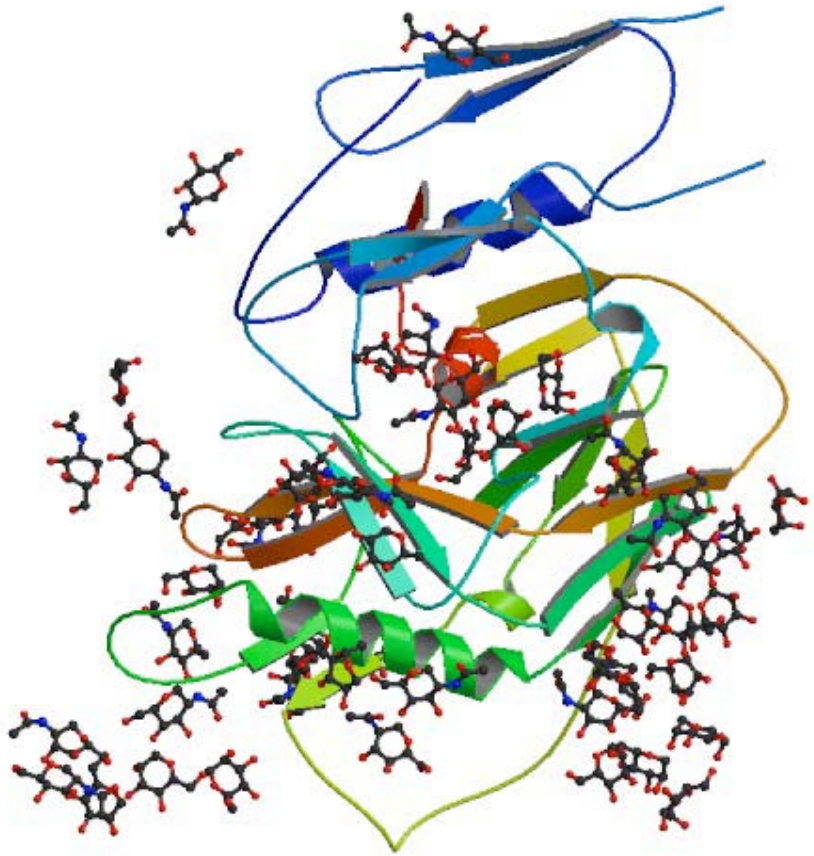
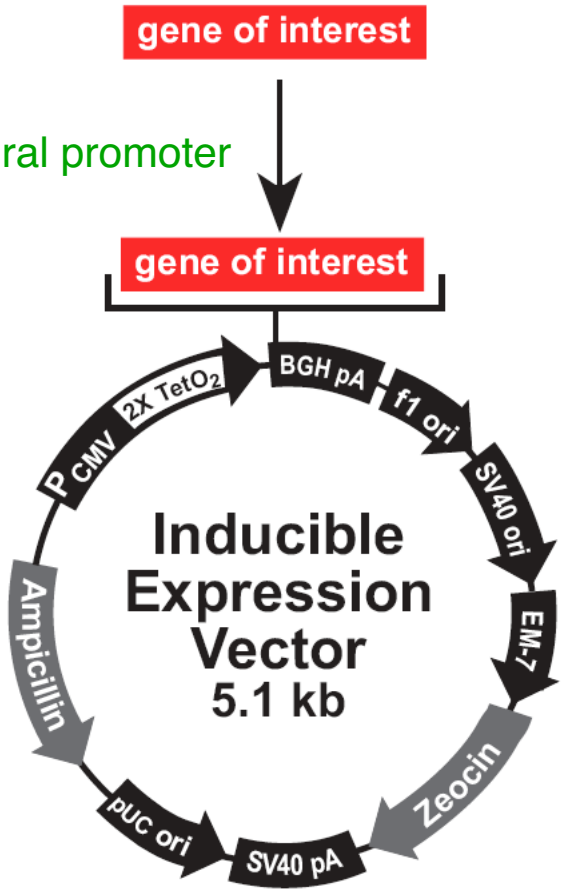


Image removed due to copyright restrictions.
Three dimensional structure of bovine rhodopsin.

Eukaryotic expression vectors share features with bacterial systems



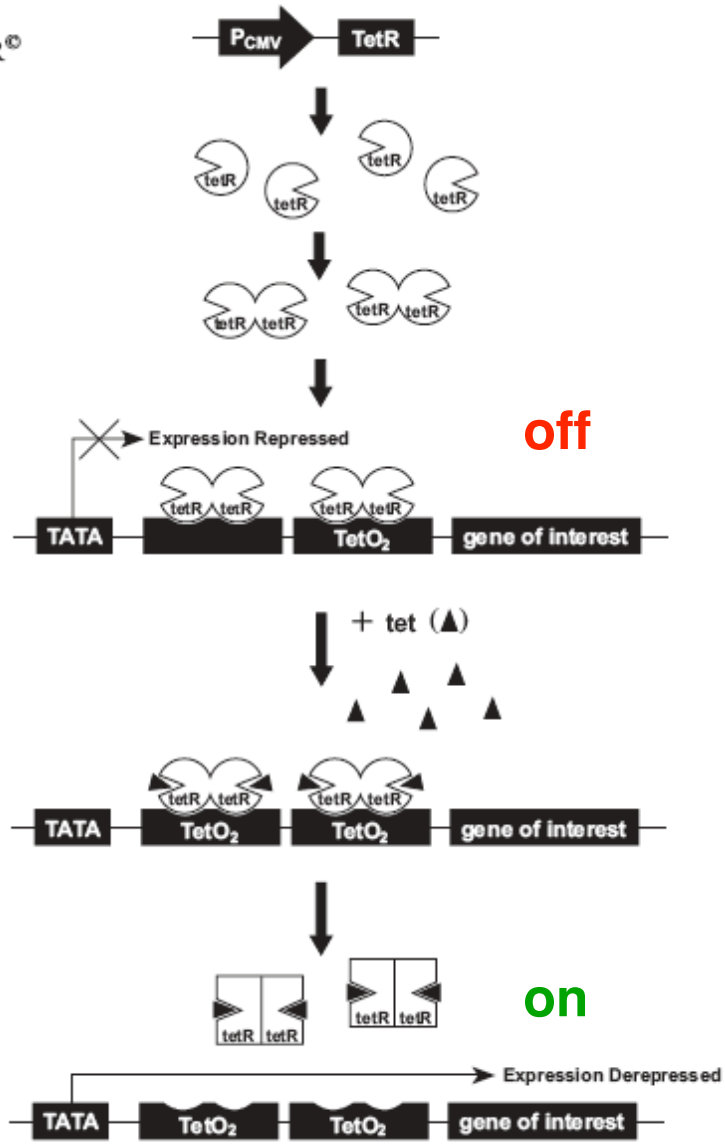
selection markers for bacterial and eukaryotic cell growth

1. Tet repressor (tetR) protein is expressed from pcDNA6/TR© in cultured cells.

2. TetR homodimers bind to Tet operator 2 ($TetO_2$) sequences in the inducible expression vector, repressing transcription of the gene of interest.

3. Upon addition, tetracycline (tet) binds to tetR homodimers.

4. Binding of tet to tetR homodimers causes a conformational change in tetR, release from the Tet operator sequences, and induction of transcription from the gene of interest.



Invitrogen (2006) *T-REx System*

Prokaryotic *vs.* eukaryotic protein expression

property	*prokaryotic*	*higher eukaryotic*
yield/(L culture)	1-100 mg	widely variable
cost/(L medium)	~$5	~$50
introduction of DNA	transformation of competent cells	viral or nonviral transfection
handling	sterile needles, *etc*.	tissue culture hood
cell incubation	shaking incubator	usu. w/CO_2-control
induction	usually IPTG	none, tetracycline
glycosylation, *etc.*	no	yes
notes	best for small, globular proteins	best for complex, eukaryotic proteins

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