

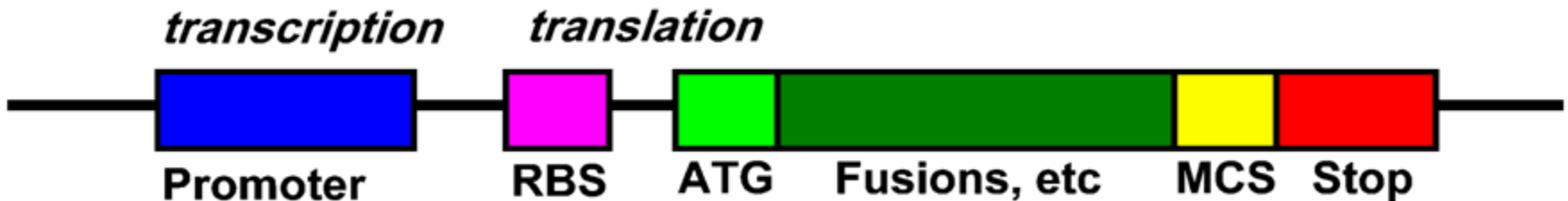
Expression of Recombinant Proteins

Uses of Cloned Genes

- sequencing
- reagents (eg, probes)
- protein production
 - insufficient natural quantities
 - modify/mutagenesis
 - library screening

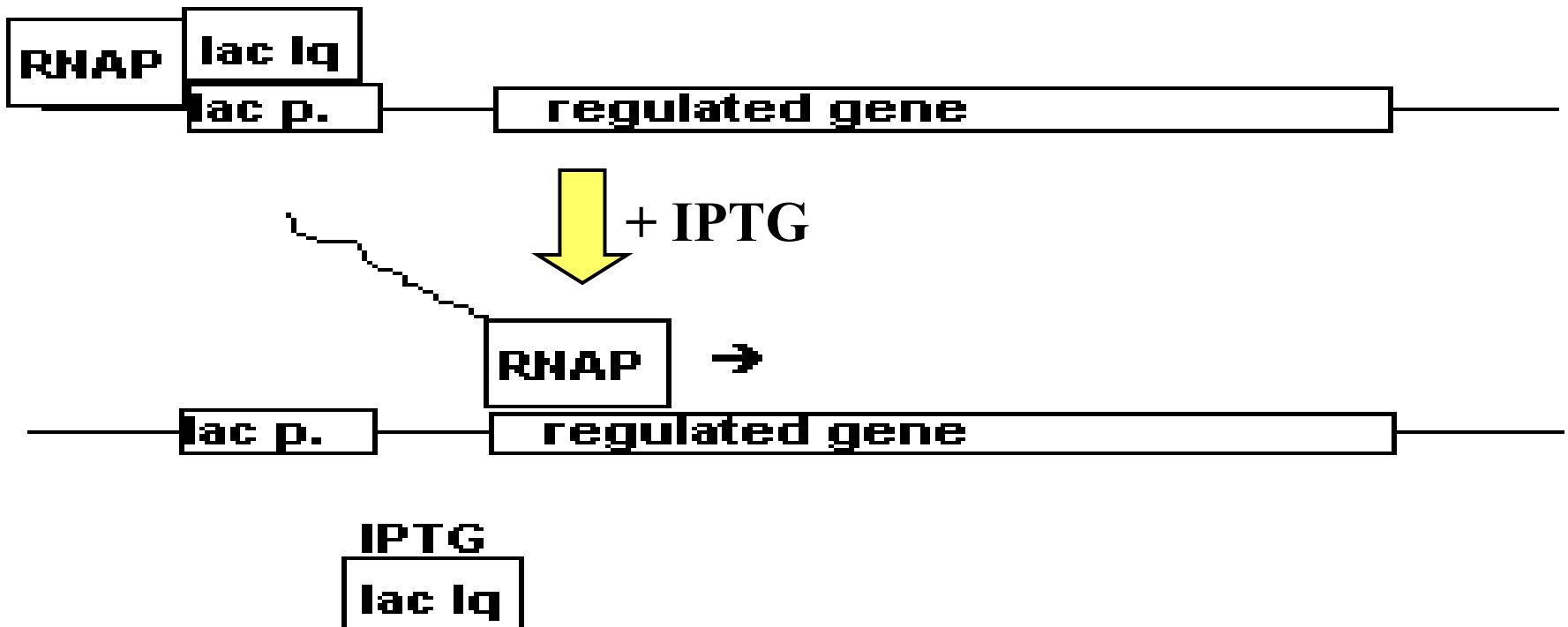
Expression Vector Features

- gene dosage
- **strong promoter**
- **regulatable promoter**
- translation signals, etc
- **± fusion proteins**
- protease defective hosts



Bacterial Promoters

Promoter	Repressor	Induction
λP_L	cl857 (ts)	32° → 42°
lac	lacI ^q	IPTG
tac	lacI ^q	IPTG



Fusion Proteins

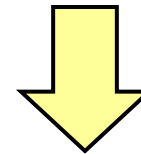
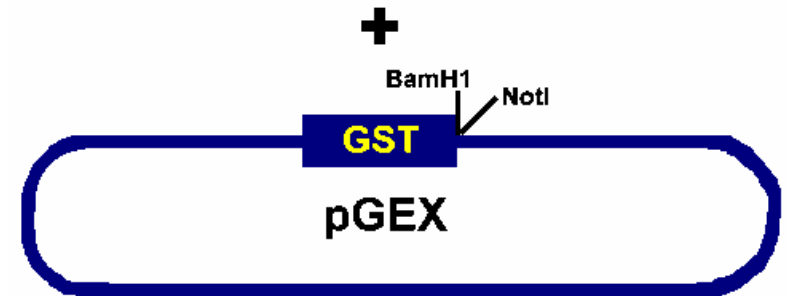
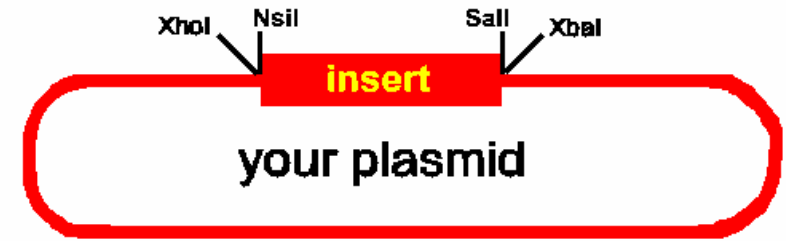
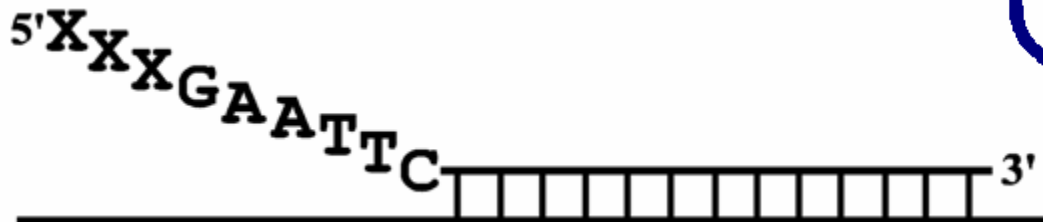
- increase stability
- affinity purification
- detection/assay
 - spectrophotometric
 - binding assays
 - antibodies
- export signals



Fusion Partner	Affinity Ligand
Glutathione-S-Transferase	glutathione
Thioredoxin	phenylarsine oxide
Maltose Binding Protein	amylose
Six Histidine Residues (His₆)	nickel

Preparation of Expression Vector

- subclone insert from current vector into expression vector
- design PCR primers to amplify region of interest
- expressed protein must be:
 - correct orientation
 - 'in-frame'



Reading Frames

Insert

|EcoRI|
NNN GAA TTC TTG ACT AGG NNN ...
... Glu Phe Leu Thr Arg Xaa ...

Vectors

|EcoRI|
P_{tac} ... ATG AAT TCN NNN
Met Asn Ser Xaa

⇒

|EcoRI|
P_{tac} ... ATG AAT TCT TGA CTA GGN
Met Asn Ser ***

|EcoRI|
P_{tac} ... ATG GAA TTC NNN
Met Glu Phe Xaa

⇒

|EcoRI|
P_{tac} ... ATG GAA TTC TTG ACT AGG NNN ...
Met Glu Phe Leu Thr Arg Xaa ...

|EcoRI|
P_{tac} ... ATG GGA ATT CNN
Met Gly Ile Xaa

⇒

|EcoRI|
P_{tac} ... ATG GGA ATT CTT GAC TAG GNN
Met Gly Ile Leu Asp ***

Generation of a -1 Frameshift

 | SalI | | ClaI || HIII |
GGT CGA CGG TAT CGA TAA GCT TGA
CCA GCT GCC ATA GCT ATT CGA ACT
Gly Arg Arg Tyr Arg *** Ala ***

⇓ Sal I

GG T CGA CGG TAT CGA TAA GCT TGA
CCA GCT GCC ATA GCT ATT CGA ACT

⇓ + Klenow + dNTPs

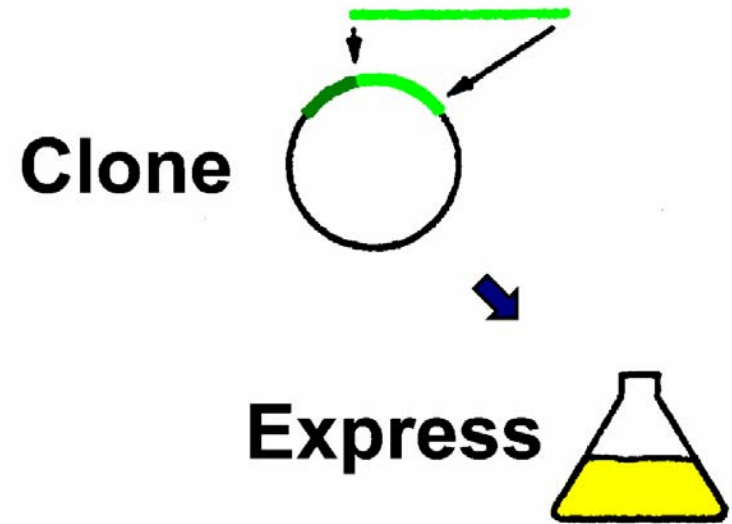
GGT CGA T CGA CGG TAT CGA TAA GCT TGA
CCA GCT A GCT GCC ATA GCT ATT CGA ACT

⇓ + DNA ligase

GGT CGA TCG ACG GTA TCG ATA AGC TTG A
CCA GCT AGC TGC CAT AGC TAT TCG AAC T
Gly Arg Ser Thr Val Ser Ile Ser Leu

Expression of Recombinant Protein

- transform bacteria with engineered plasmid
- grow to appropriate density
- induce expression (eg., IPTG)



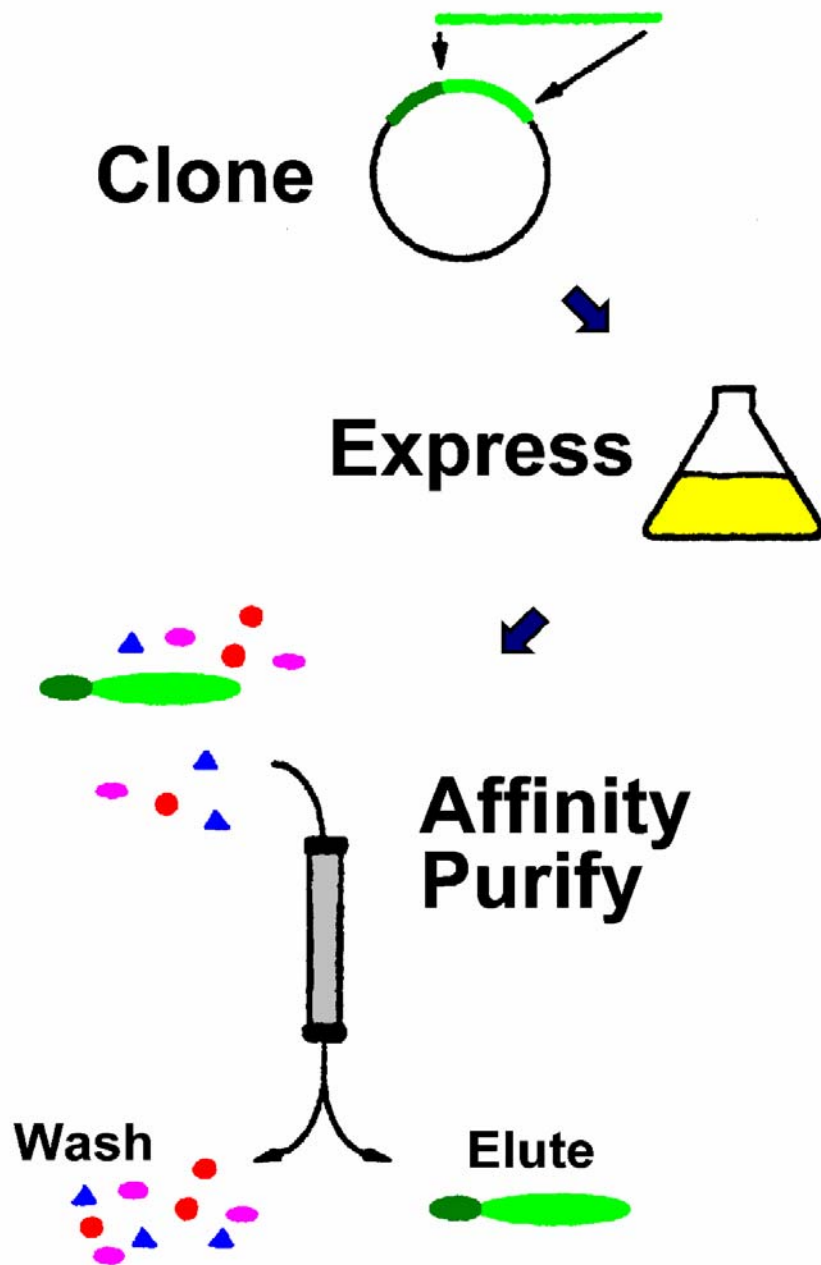
Potential problems

- **toxicity**
- **proteolysis**
- **inclusion bodies**

Possible IB Cure

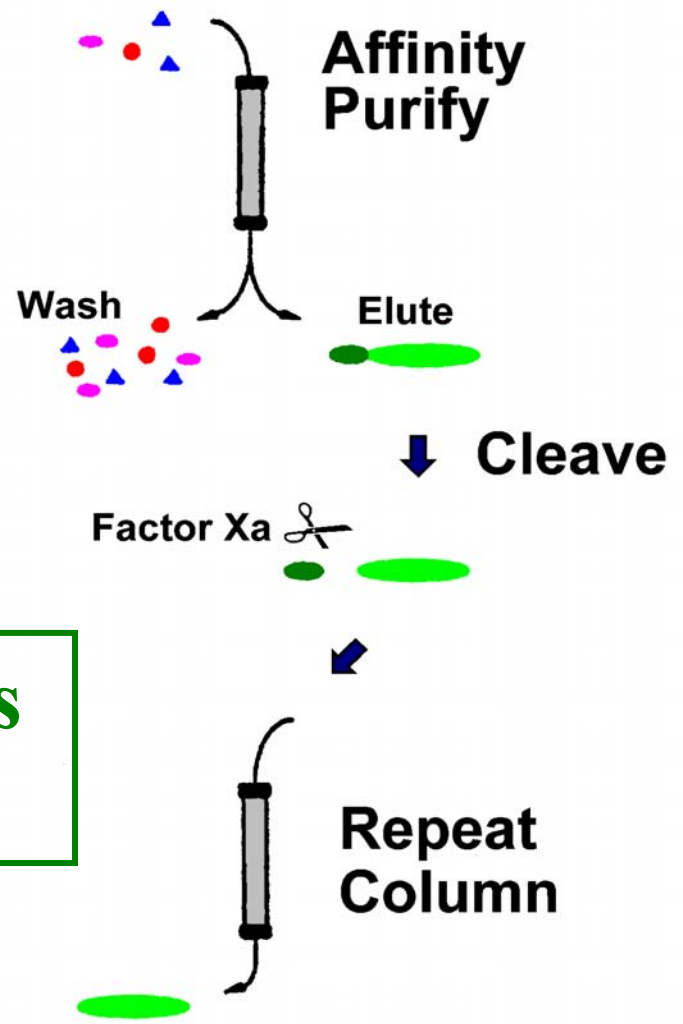
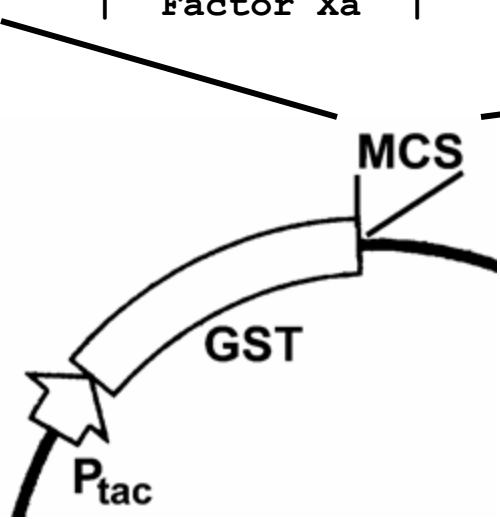
- **isolate by differential centrifugation**
- **solubilize in urea**
- **re-nature protein (?)**

Protein Isolation



- conventional
- affinity based on fusion partner

... ATC GAA GGT CGT GGG ATC CCC AGG AAT TCC CGG GTC GAC TCG AGC GGC CGC ...
 ... TAG CTT CCA GCA CCC TAG GGG TCC TTA AGG GCC CAG CTG AGC TCG CCG GCG ...
 ... Ile Glu Gly Arg Gly Ile Pro Arg Asn Ser Arg Val Asp Ser Ser Gly Arg ...
 | Factor Xa |

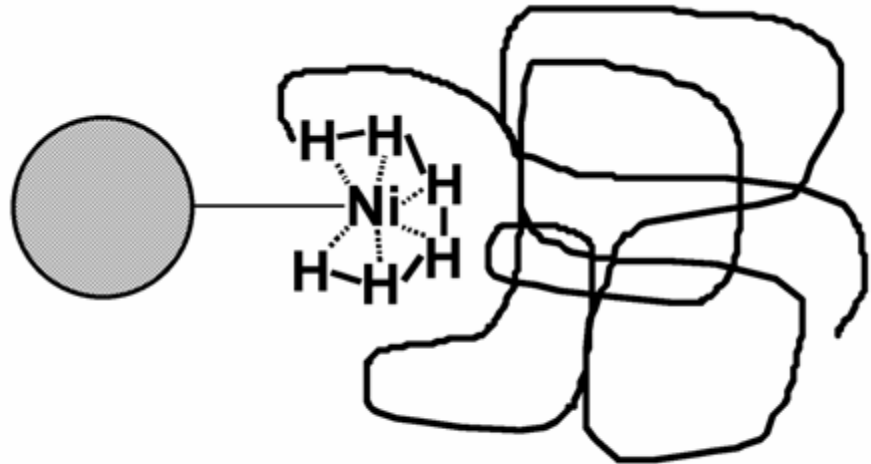


Engineered protease site allows removal of fusion partner.

Addition of a few residues should have minimal effect on recombinant protein.

His₆ Tag

- add 6 consecutive His to either end
- binds metals



Metal Chelate Chromatography

Epitope Tag

- 6-12 amino acids
- mAb for detection or purification

Problems with Expression of Eukaryotic Proteins in Prokaryotes

- **stability (protein and gene)**
- **proper folding and disulfide formation**
- **post-translational modifications**
- **asking species specific questions**

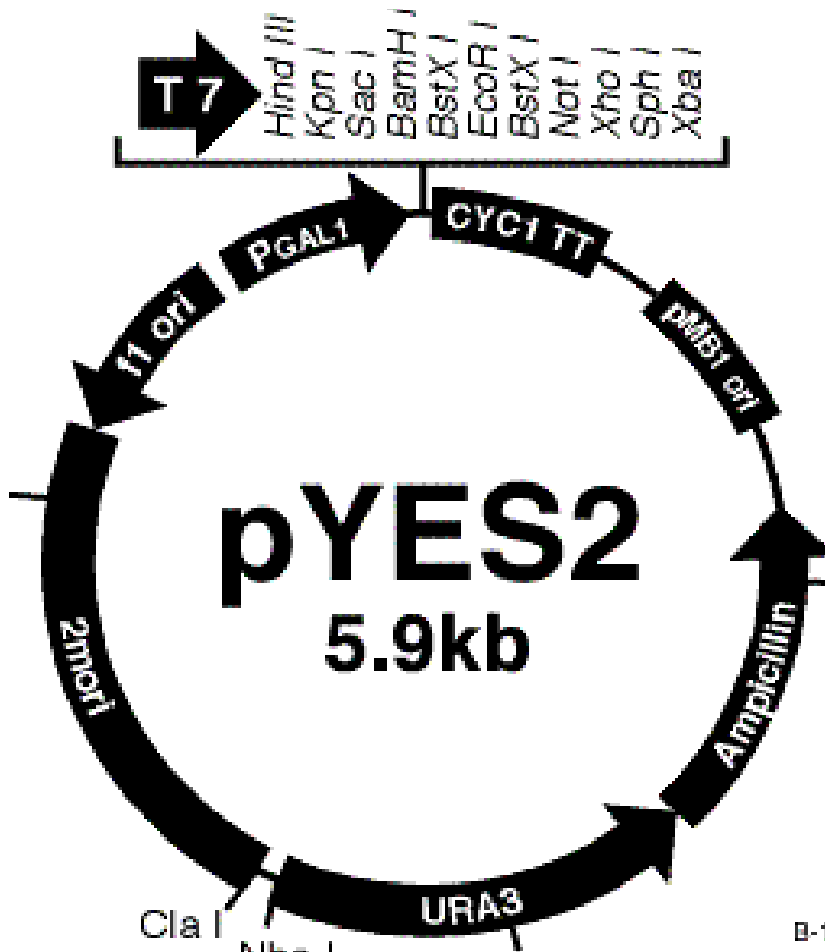
Eukaryotic Expression Systems

- in theory, plasmids can be introduced into any host
- yeast are easy to maintain in lab
 - *Saccharomyces cerevisiae*
 - *Pichia pastoris*
- viruses
 - several mammalian
 - baculovirus (insect)

vaccinia (lytic)
adenovirus (lytic)
papilloma (episomal)
retrovirus (integrated)

Shuttle Vectors

- *E. coli* replication origin and selectable marker
- eukaryotic replication origin, selectable marker, promoters/enhancers, polyA signals



PGal1	galactose-inducible promoter (yeast)
cyc1 TT	transcription terminator
pMB1 ori	<i>E. coli</i> origin of replication (pUC)
Amp^r	<i>E. coli</i> selectable marker
URA3	yeast selectable marker (ura3 host)
2μ ori	yeast origin of replication
f1 ori	ssDNA origin of replication
T7	phage promoter (in vitro transcription)

Expression in Baculovirus

- high level recombinant protein expression
- post-translational modifications
- polyhedrin gene
 - strong promoter
 - not needed for replication
 - replace with target gene
- co-transfection with baculovirus and transfer plasmid
- recombination replaces polyhedrin gene with target
- efficiency enhanced by disrupting an essential gene

