Gene Expression

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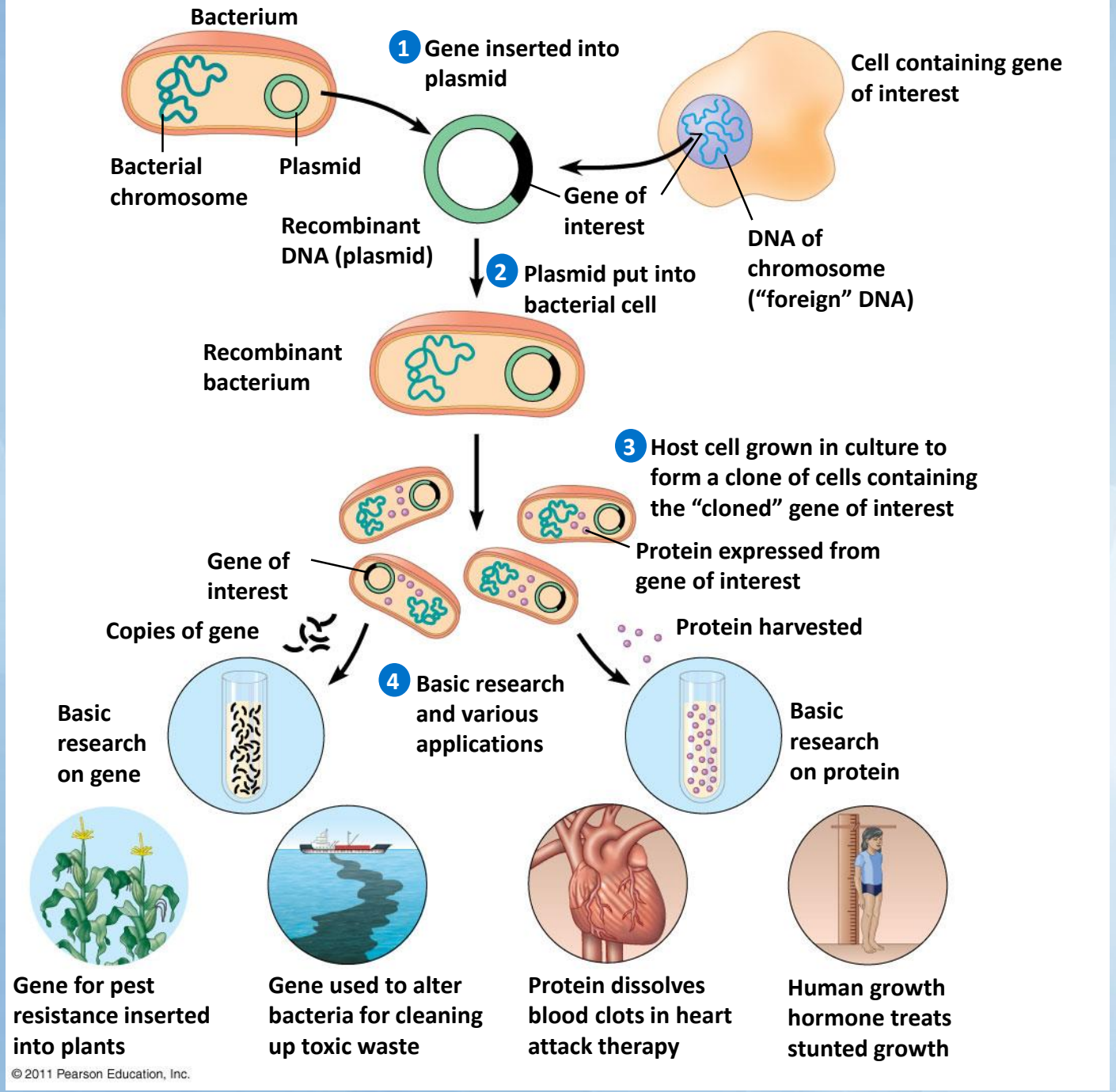
Bacterium
1 Gene inserted into plasmid
Cell containing gene of interest
Bacterial chromosome
Plasmid
Gene of interest
Recombinant DNA (plasmid)
DNA of chromosome ("foreign" DNA)
2 Plasmid put into bacterial cell
Recombinant bacterium
3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest
Protein expressed from gene of interest
Gene of interest
Copies of gene
Protein harvested
4 Basic research and various applications
Basic research on gene
Basic research on protein
Gene for pest resistance inserted into plants
Gene used to alter bacteria for cleaning up toxic waste
Protein dissolves blood clots in heart attack therapy
Human growth hormone treats stunted growth
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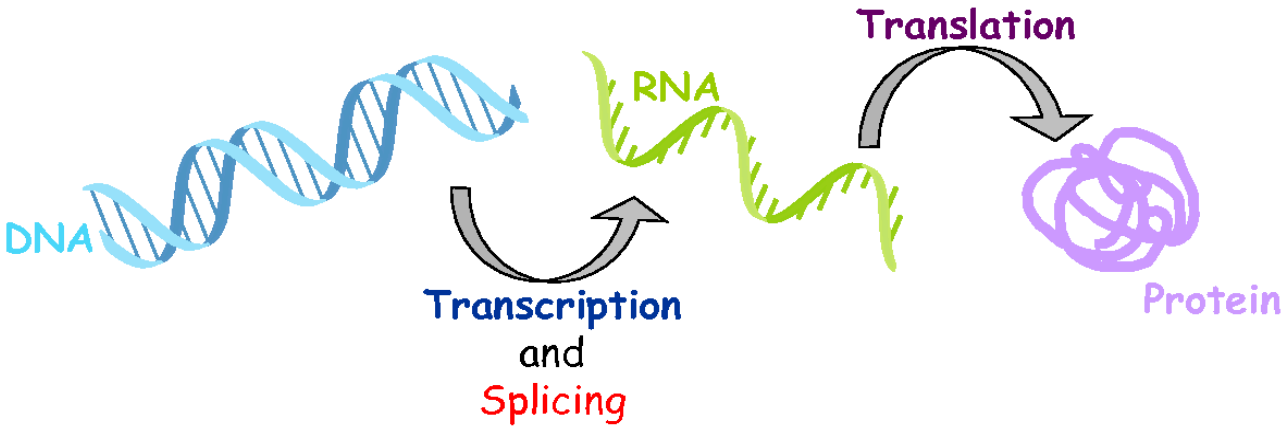
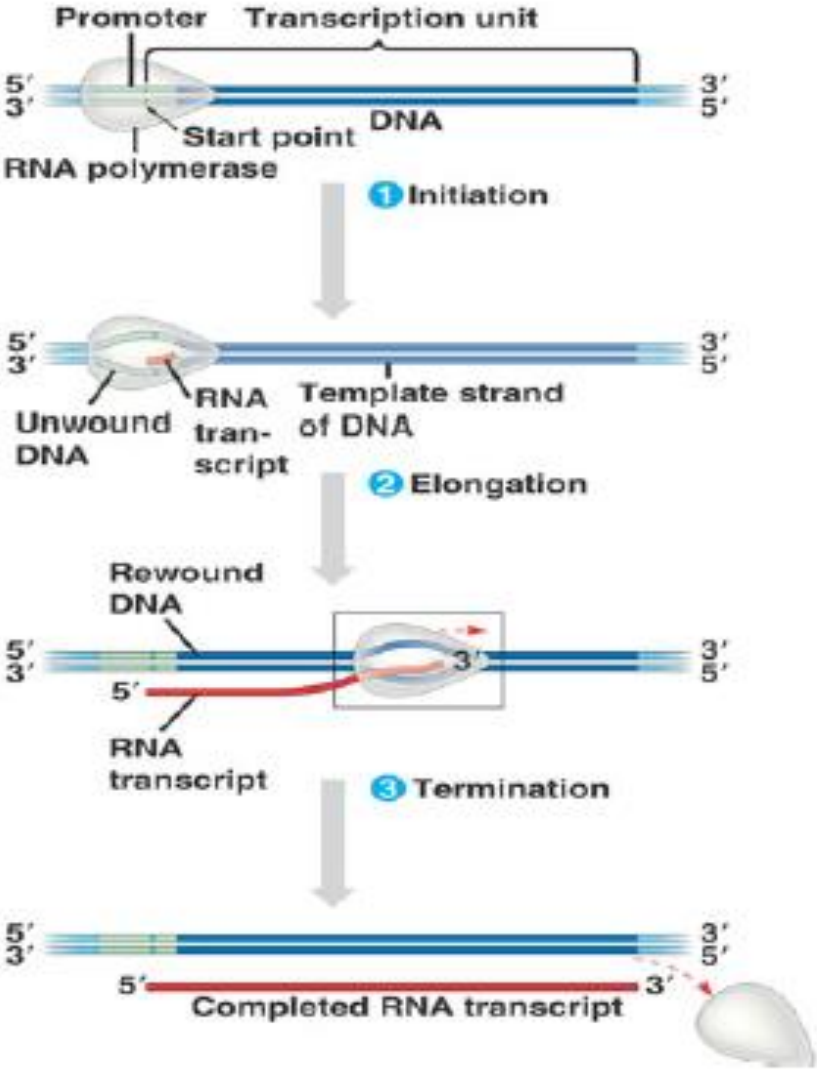
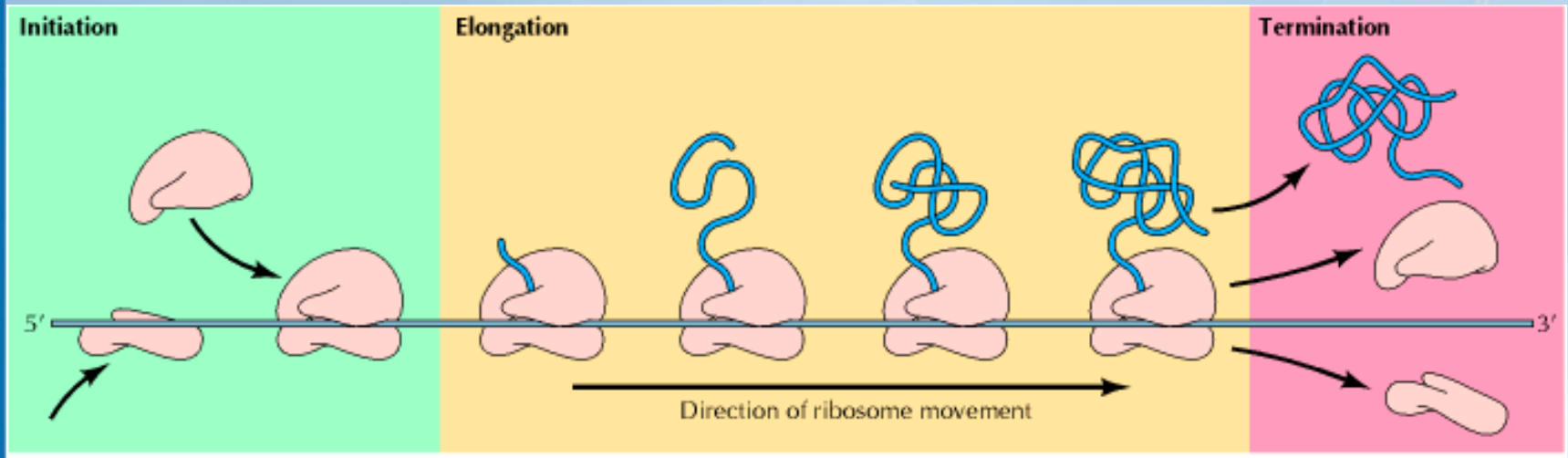
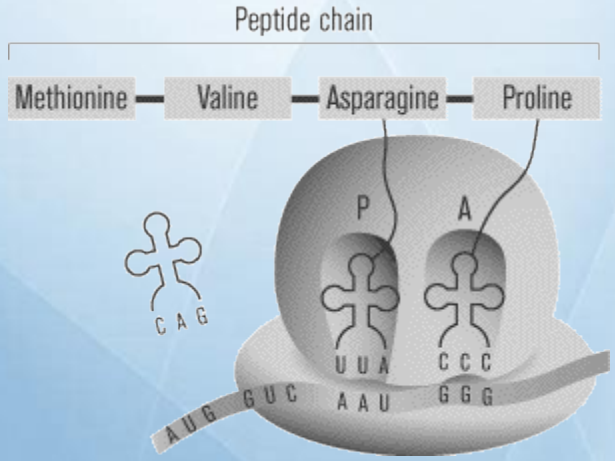

Translation
RNA
DNA
Transcription
and
Splicing
Protein

Transcription



Translation



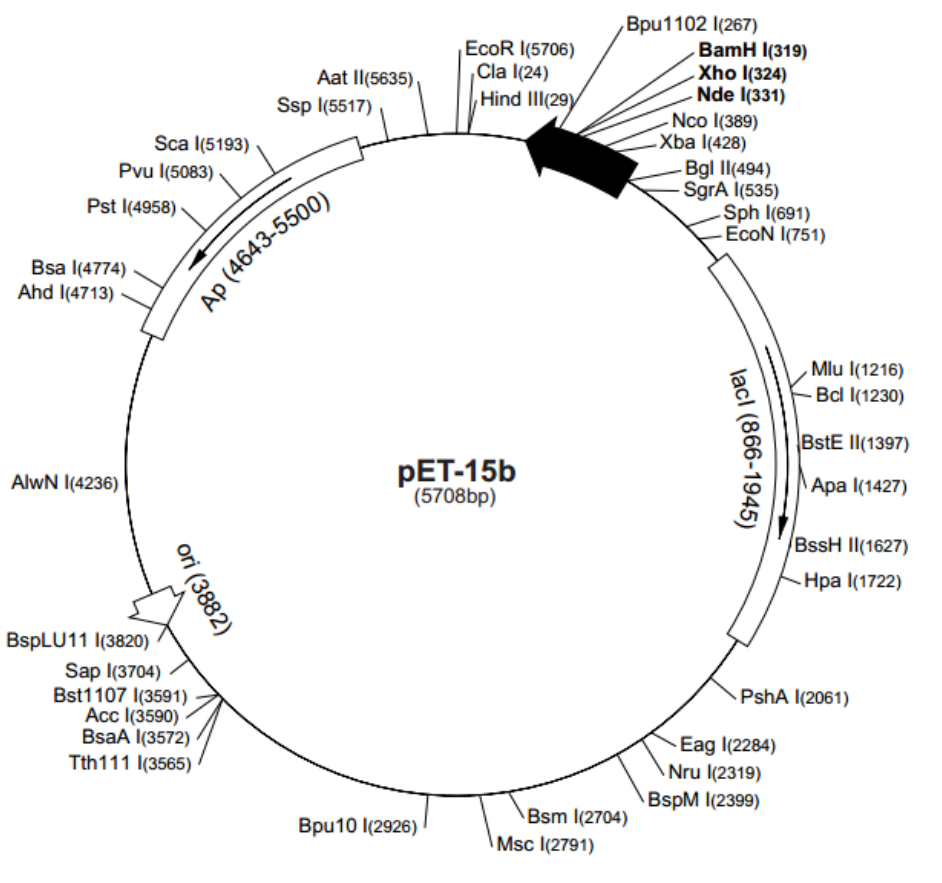


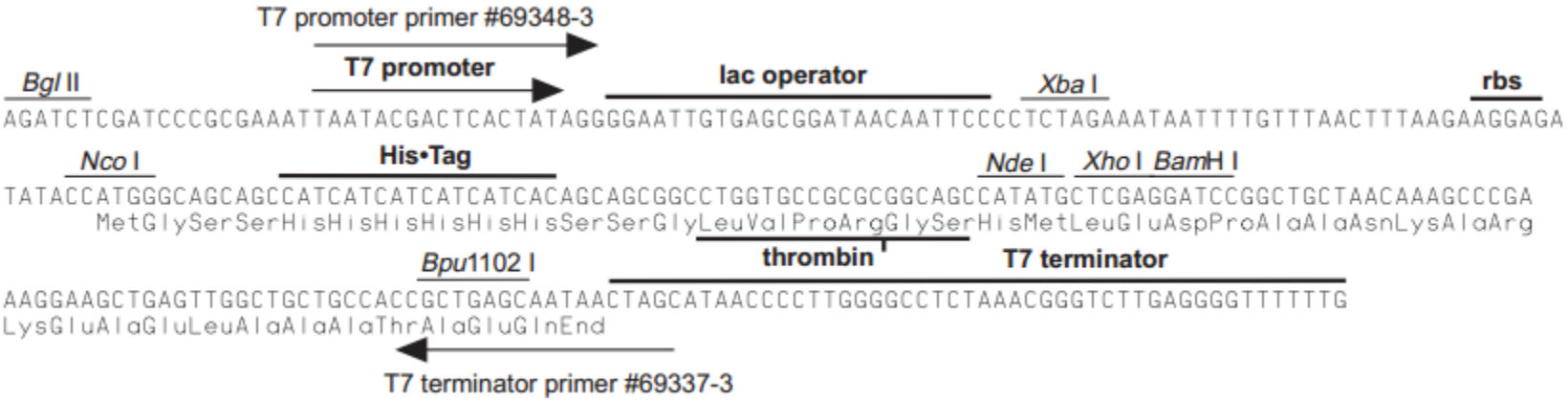
The Regulation of Gene Expression

The Regulation of Gene Expression

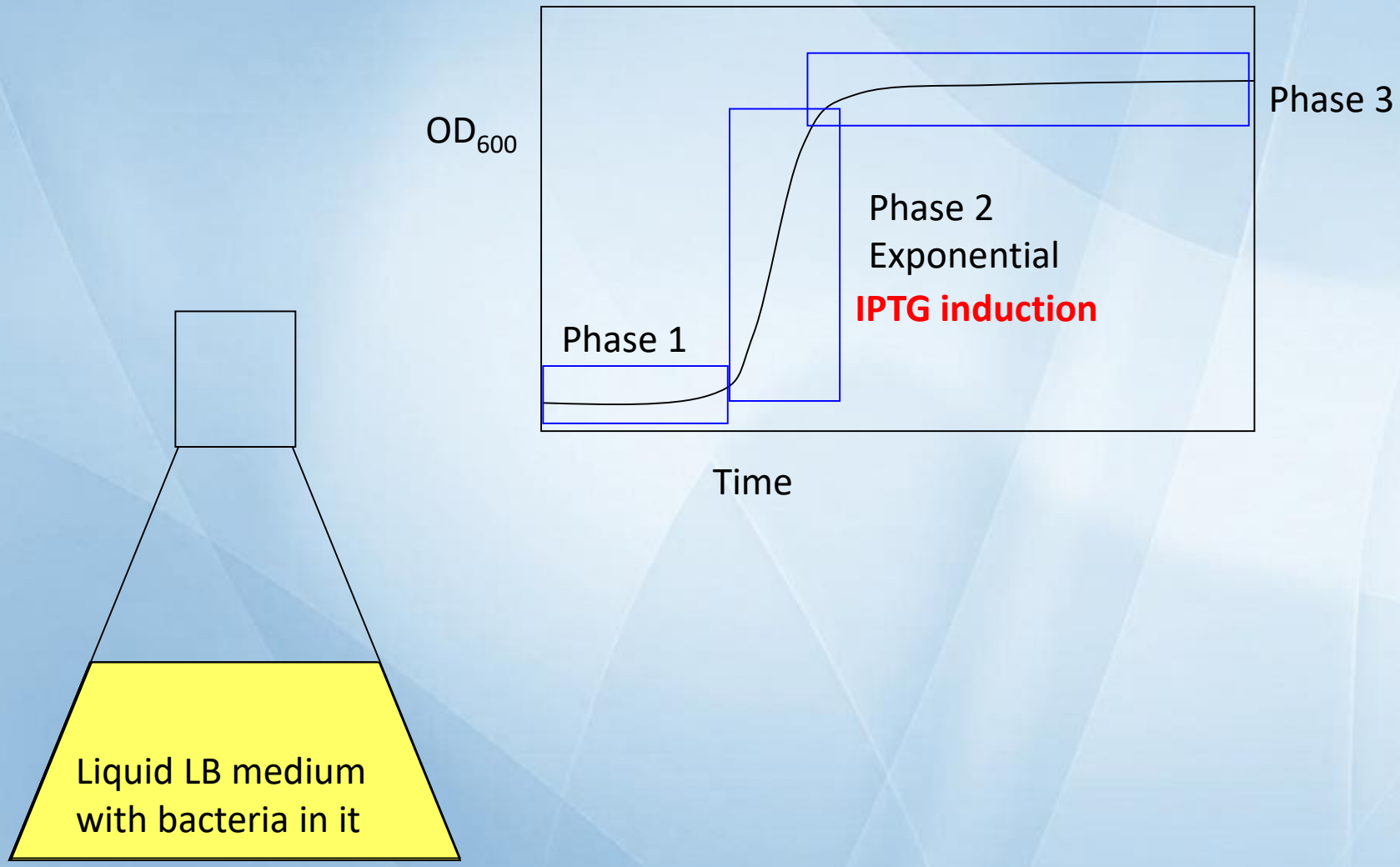
Lac Operon

Animation

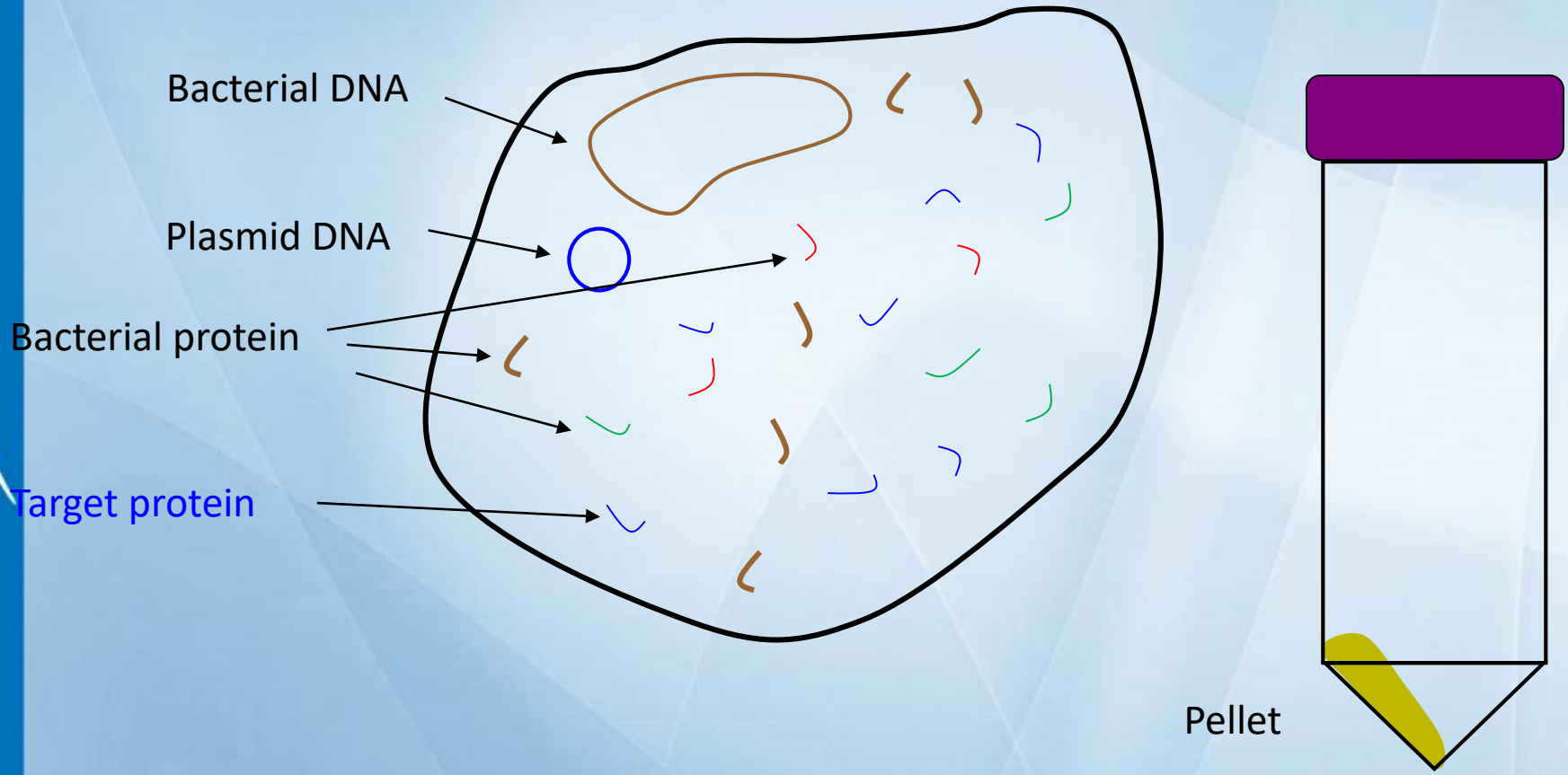

pET-15b
(5708bp)
Bpu1102 I(267)
BamH I(319)
Xho I(324)
Nde I(331)
Nco I(389)
Xba I(428)
Bgl II(494)
SgrA I(535)
Sph I(691)
EcoN I(751)
Mlu I(1216)
Bcl I(1230)
BstE II(1397)
Apa I(1427)
BssH II(1627)
Hpa I(1722)
PshA I(2061)
Eag I(2284)
Nru I(2319)
BspM I(2399)
Bsm I(2704)
Msc I(2791)
Bpu10 I(2926)
BspLU11 I(3820)
Sap I(3704)
Bst1107 I(3591)
Acc I(3590)
BsaA I(3572)
Tth111 I(3565)
AlwN I(4236)
Bsa I(4774)
Ahd I(4713)
Pst I(4958)
Pvu I(5083)
Sca I(5193)
Ssp I(5517)
Aat II(5635)
EcoR I(5706)
Cla I(24)
Hind III(29)
Ap (4643-5500)
lacI (866-1945)
ori (3882)


T7 promoter primer #69348-3
T7 promoter
lac operator
Bgl II
Xba I
rbs
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nco I
His•Tag
Nde I
Xho I
BamH I
TATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGCTCGAGGATCCGGCTGCTAACAAAGCCCGA
MetGlySerSerHisHisHisHisHisHisSerSerGlyLeuValProArgGlySerHisMetLeuGluAspProAlaAlaAsnLysAlaArg
thrombin
Bpu1102 I
T7 terminator
AAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
LysGluAlaGluLeuAlaAlaAlaThrAlaGluGlnEnd
T7 terminator primer #69337-3

Protein Expression



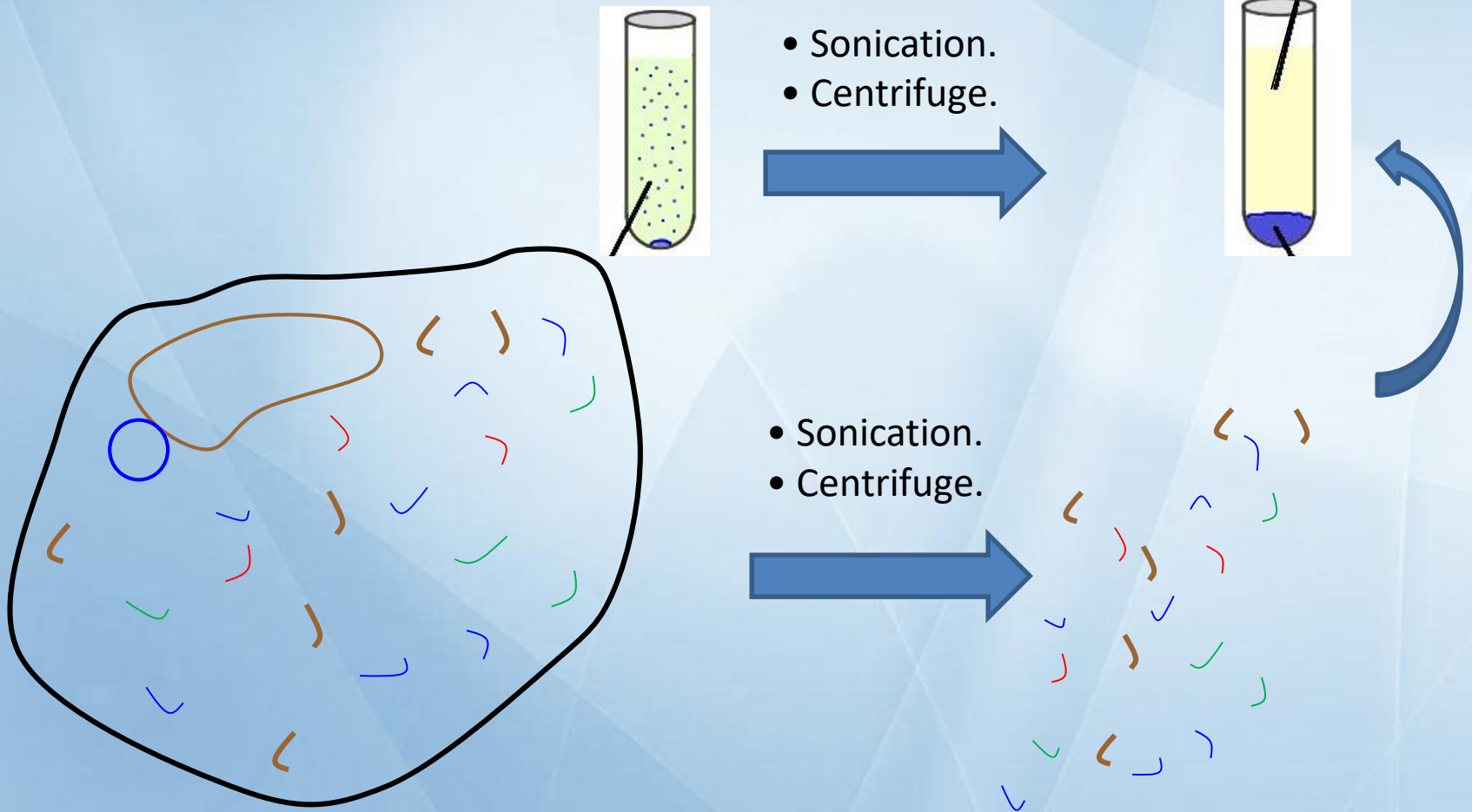
Bacterial Growth



Lysis

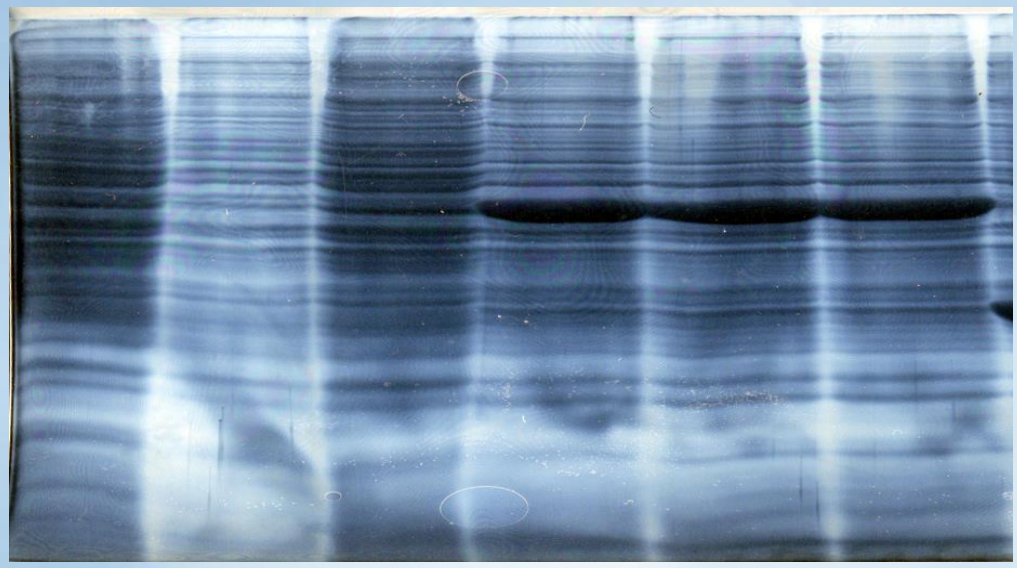
•Pellet is resuspended in the lysis buffer containing, and sonicated to further liberate the protein

• Spin down the denaturing lysis buffer, cell wall and debris will pellet at the bottom and our protein is in the soluble supernatant.



Expression of protein in *E. coli*

Uninduced Induced Samples



We want to work with pure proteins. How do we purify it from all the other *E. coli* proteins?

Why purify a protein?

- To study its function, Activity
- For industrial or therapeutic applications
- Study protein regulation and protein interactions
- Produce Antibodies
- Perform structural analysis by X-Ray and Crystallography

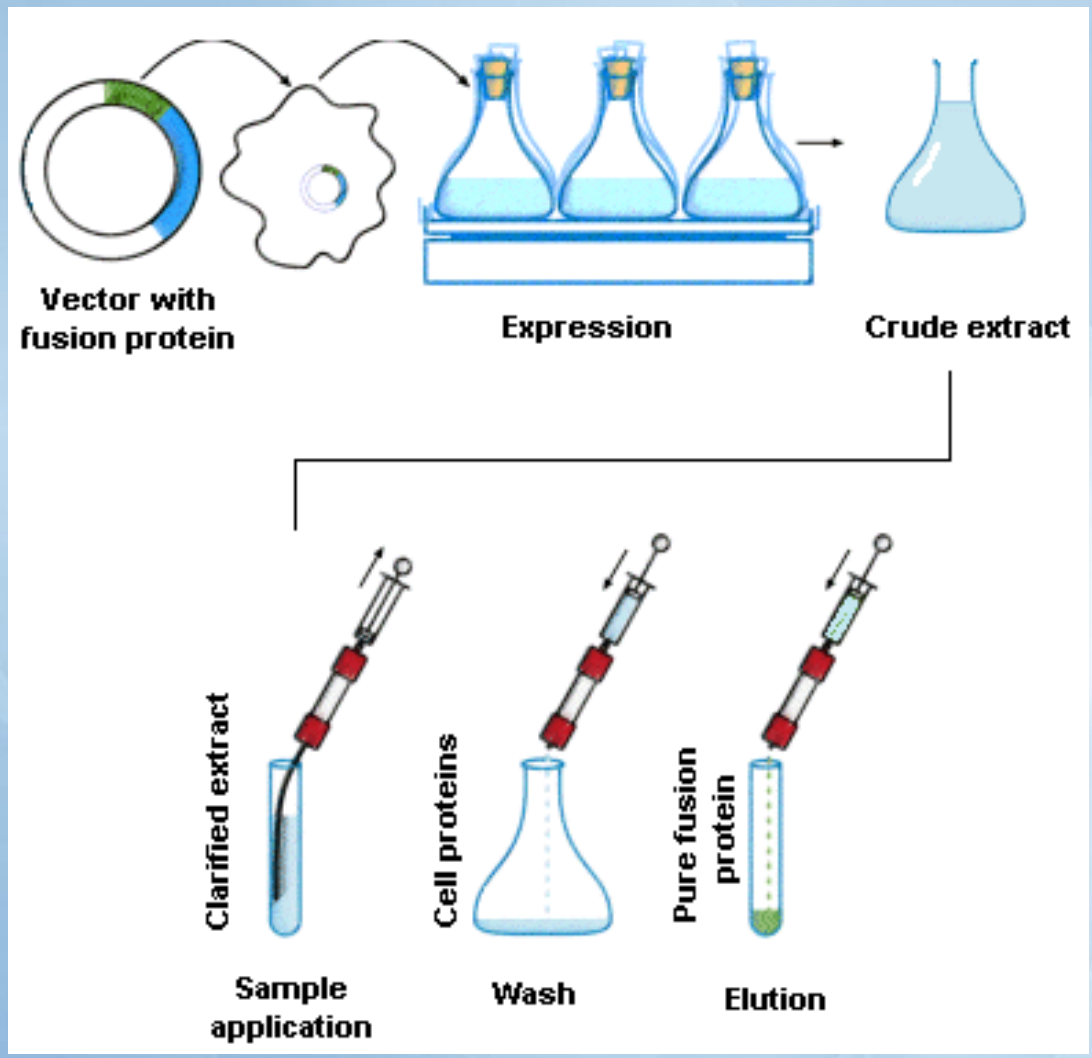
HOW to purify a protein?

Affinity chromatography (AC)

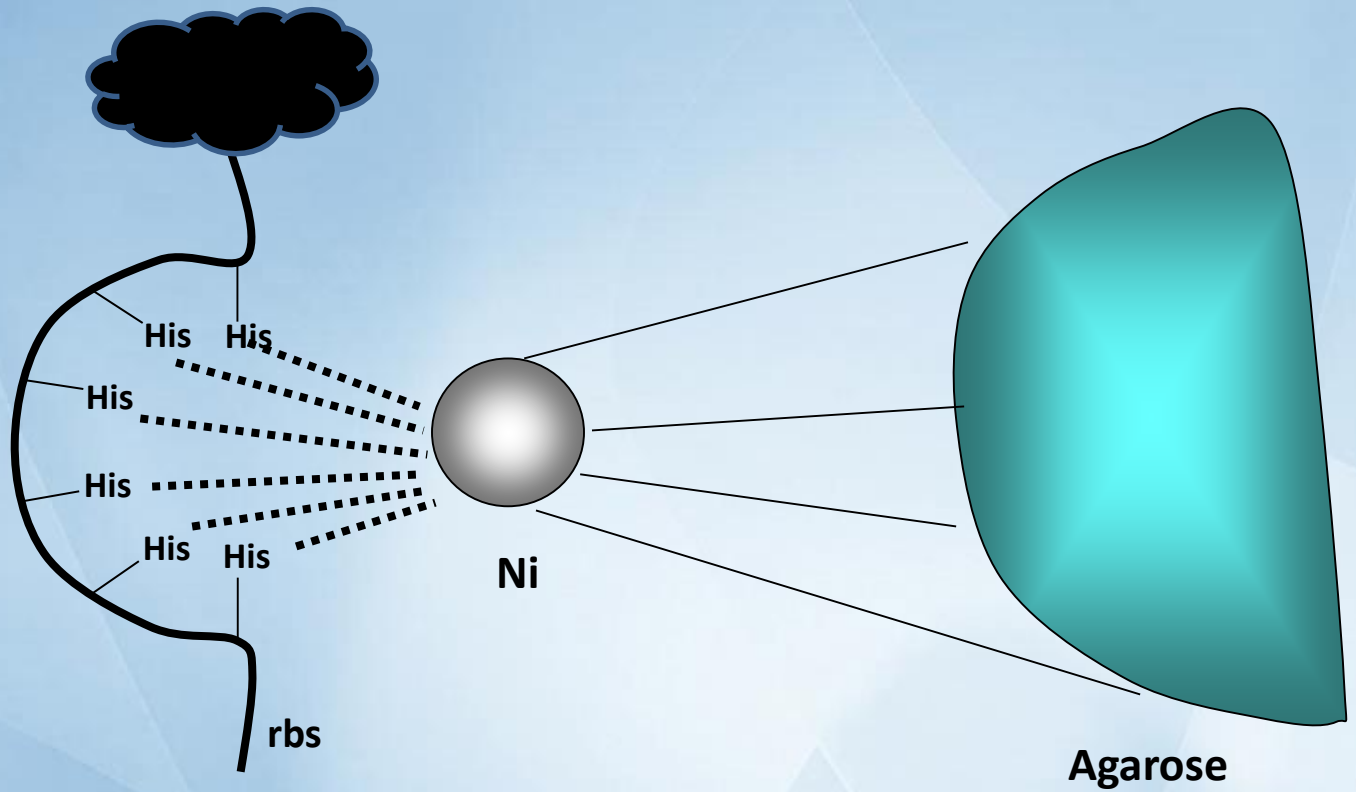
What is AC?

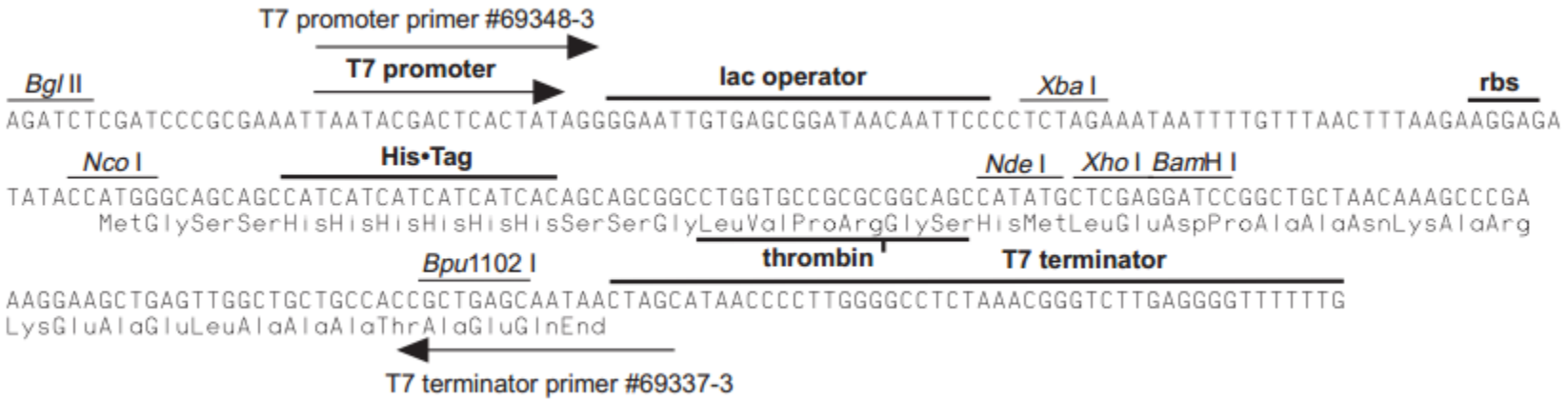
- **AC** is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure.
- AC is designed to purify a particular molecule from a mixed sample.

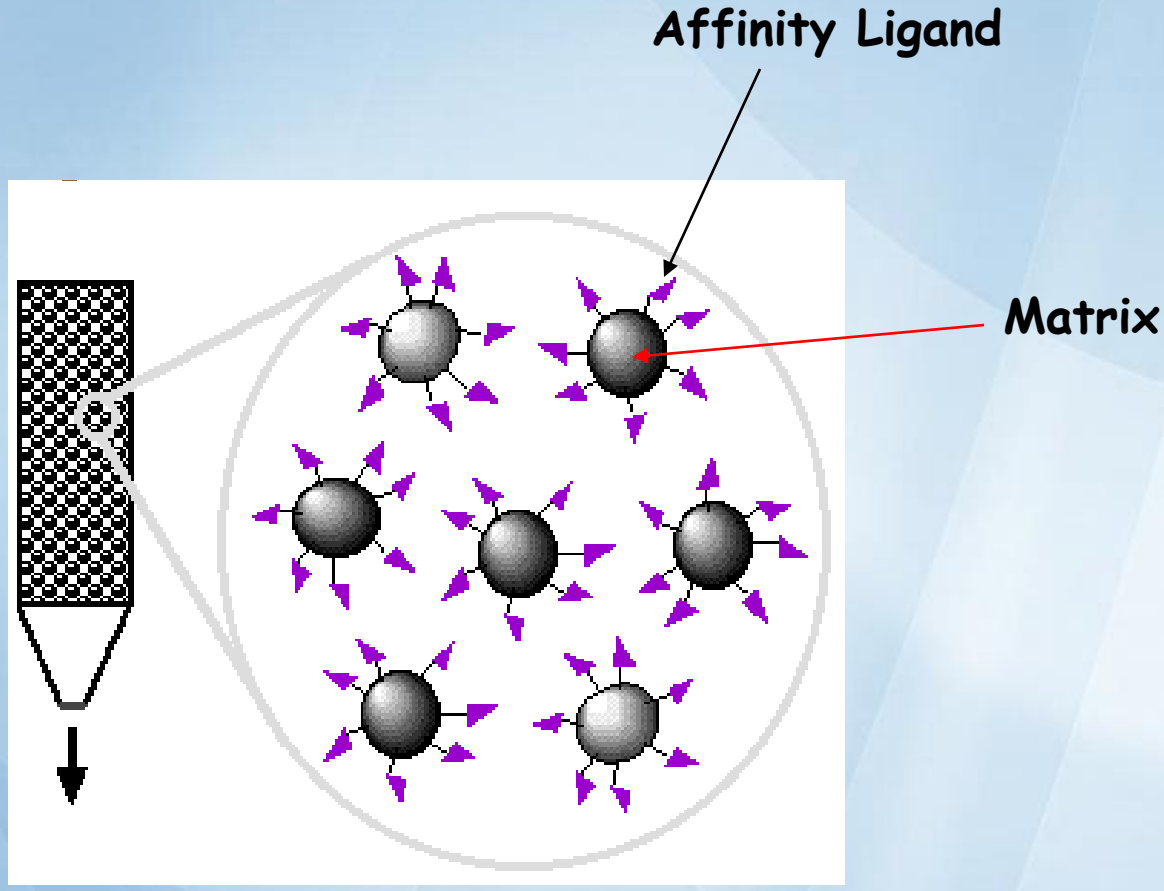
Affinity chromatography applied to recombinant proteins



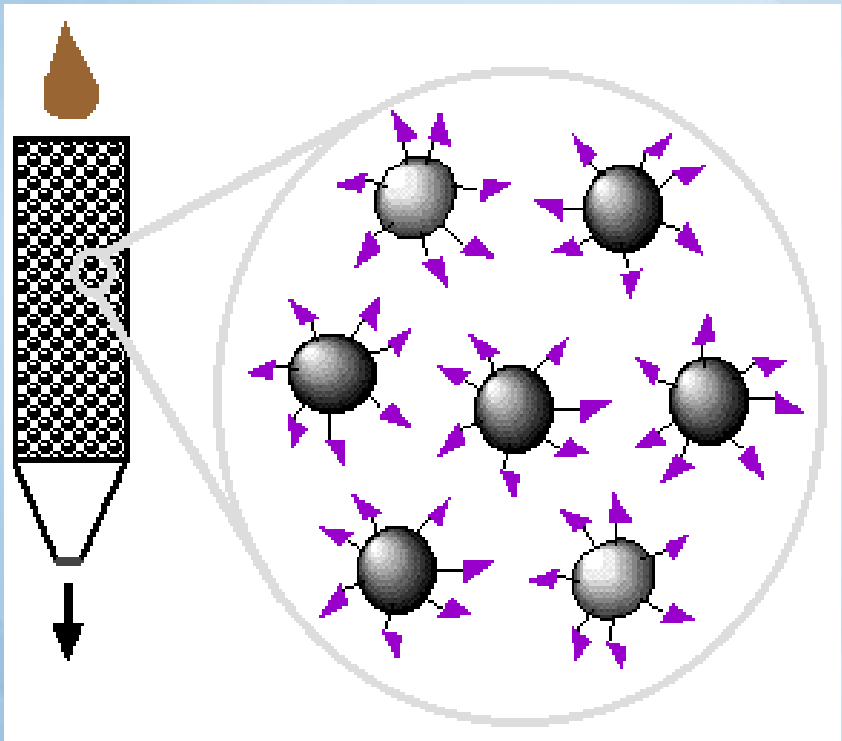
Affinity Chromatography



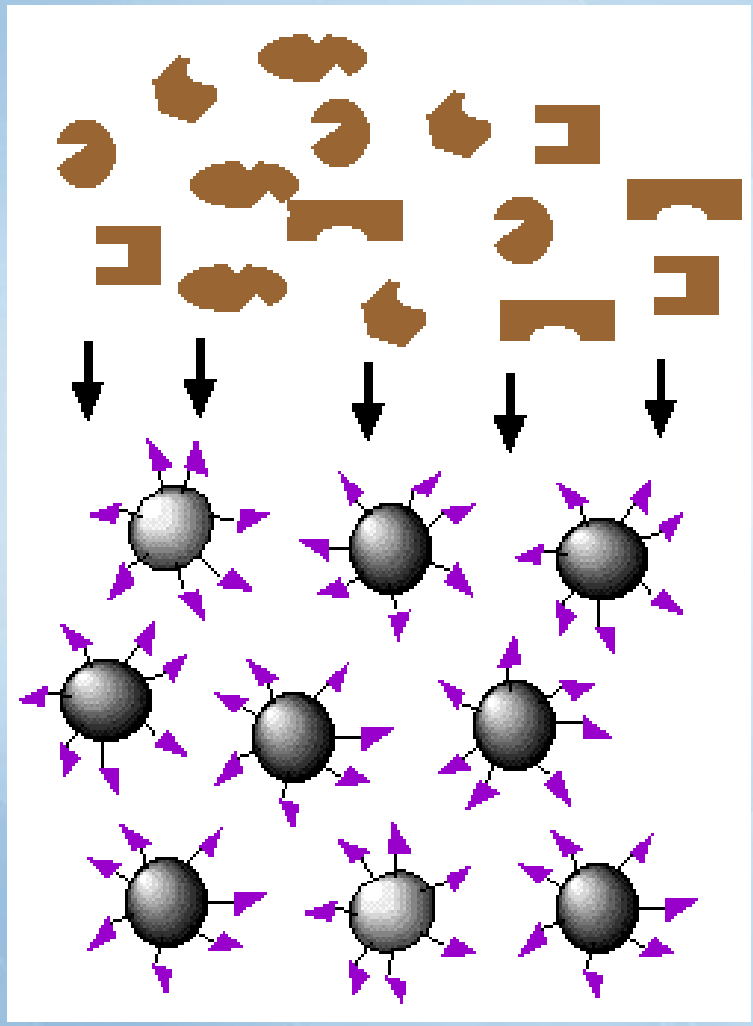



Affinity Ligand
Matrix

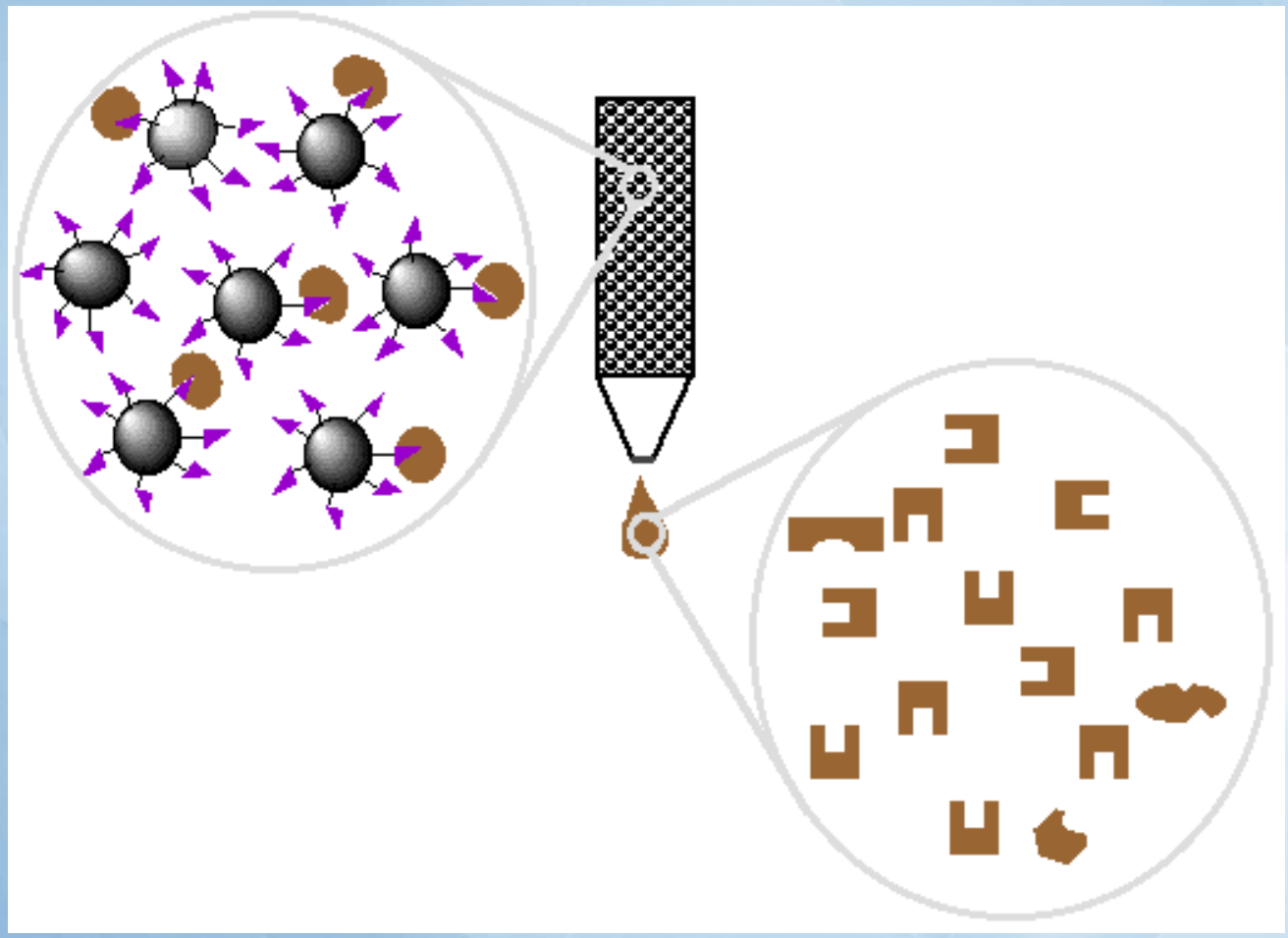
Step 1. Loading affinity column.



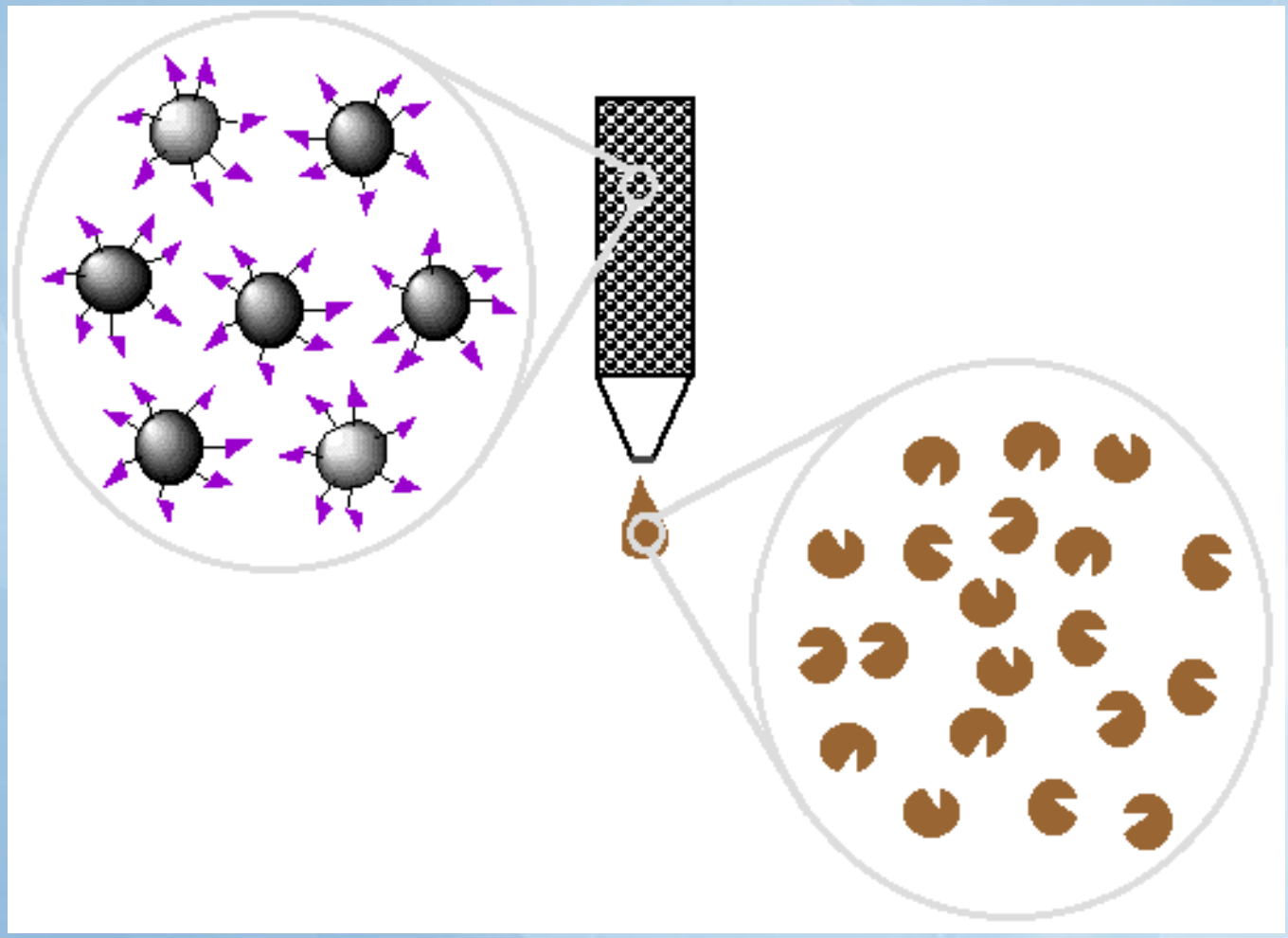
Step 2. Proteins sieve through matrix of affinity beads.



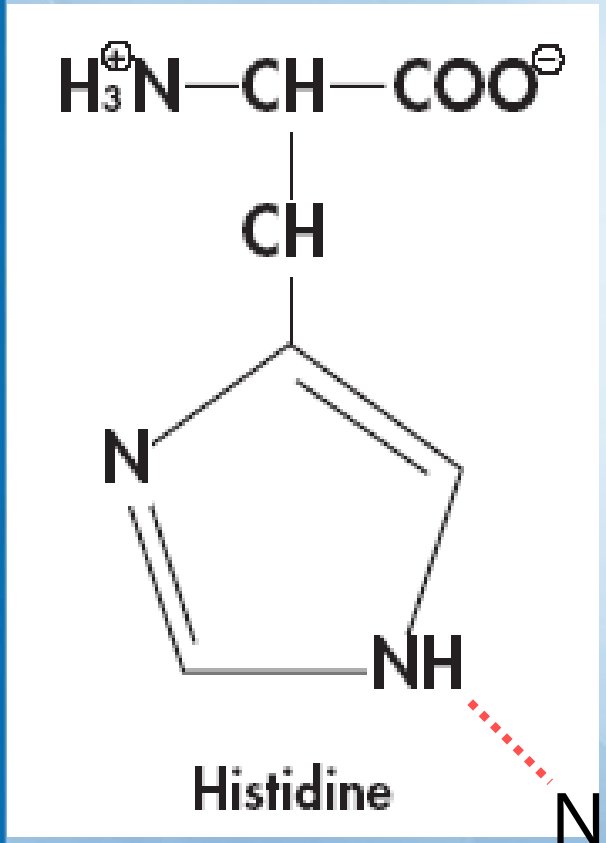
Step 5. Wash off proteins that bind loosely.



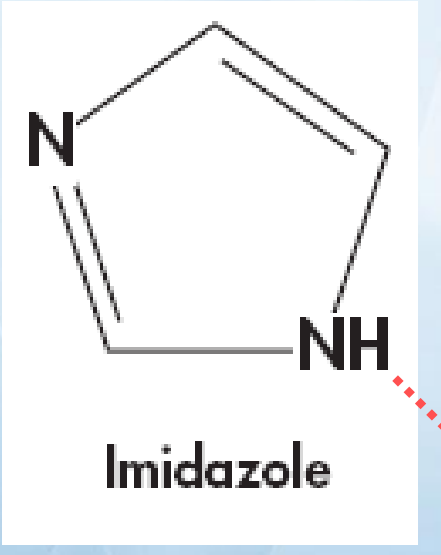
Step 6. Elute proteins that bind tightly to ligand and collect purified protein of interest.



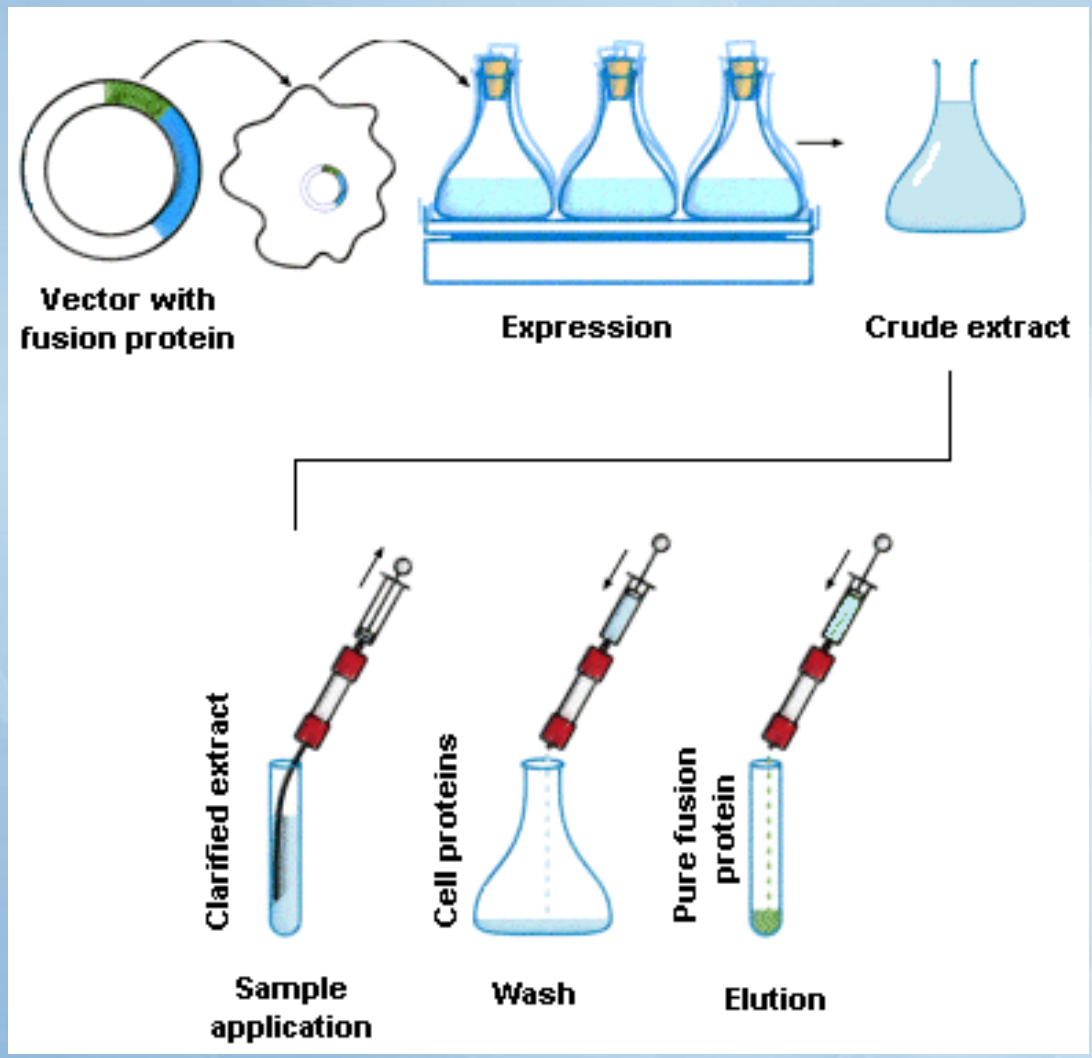
Elution with imidazole
Why imidazole?



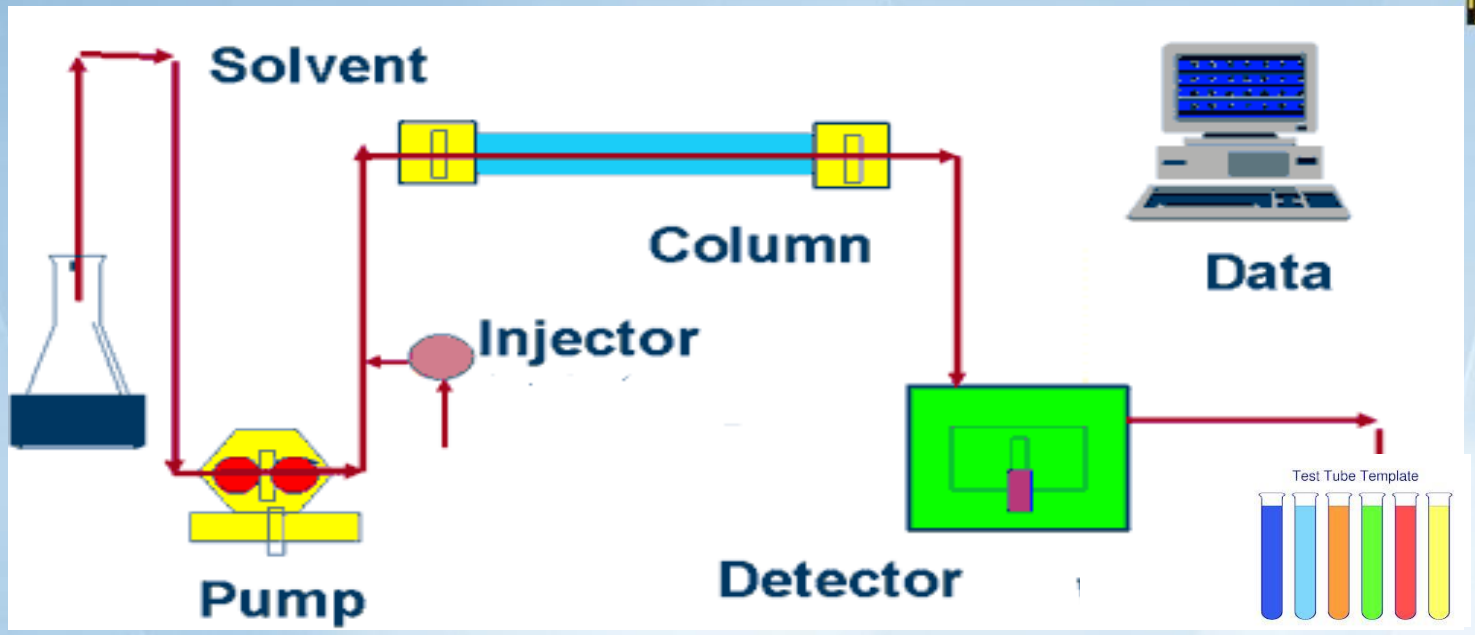
The imidazole ring is part of the structure of histidine

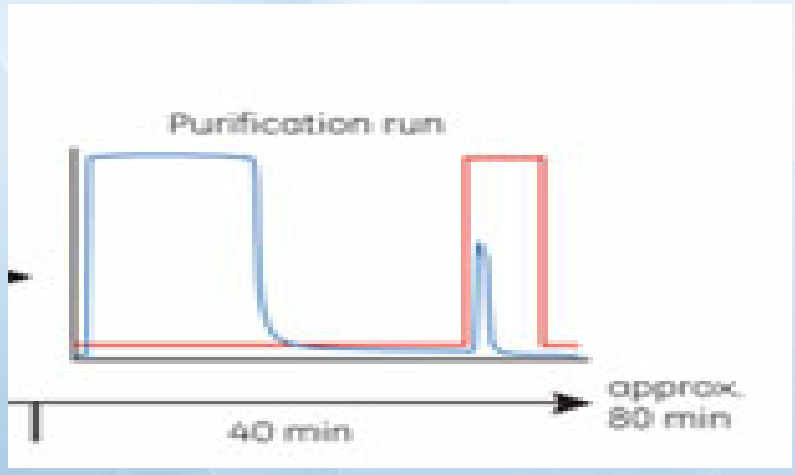


Affinity chromatography applied to recombinant proteins

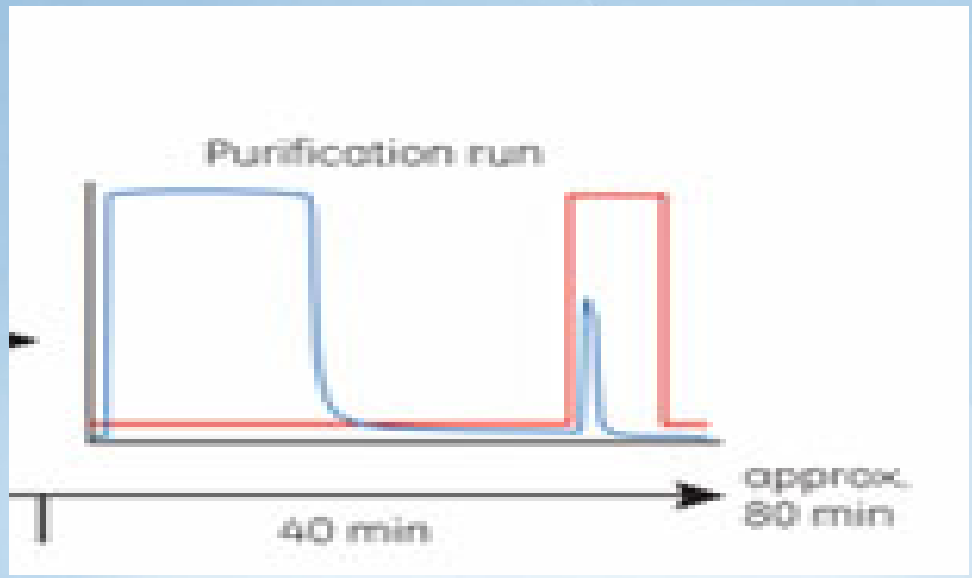


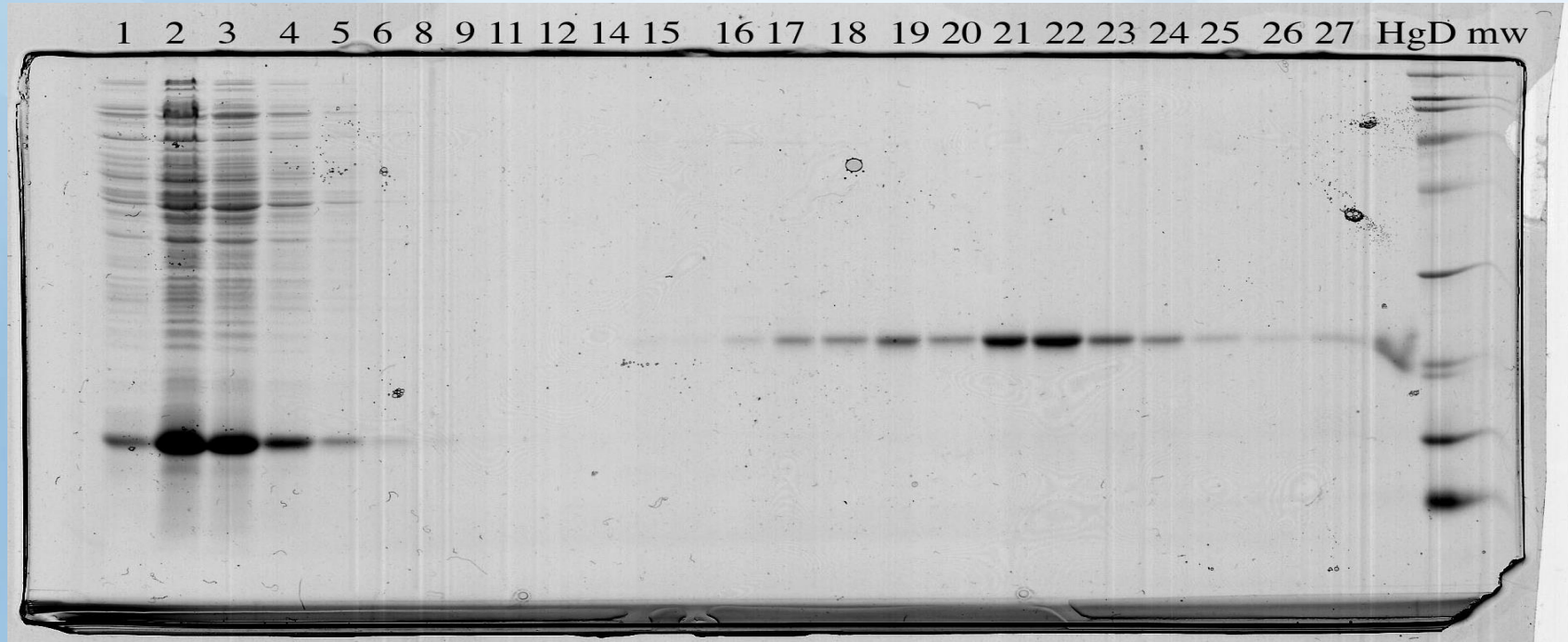
IMAC System


Solvent
Column
Data
Injector
Pump
Detector
Test Tube Template


Purification run
approx.
80 min
40 min

Purity test


Purification run
approx.
80 min
40 min


1 2 3 4 5 6 8 9 11 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 HgD mw

Protein dialysis

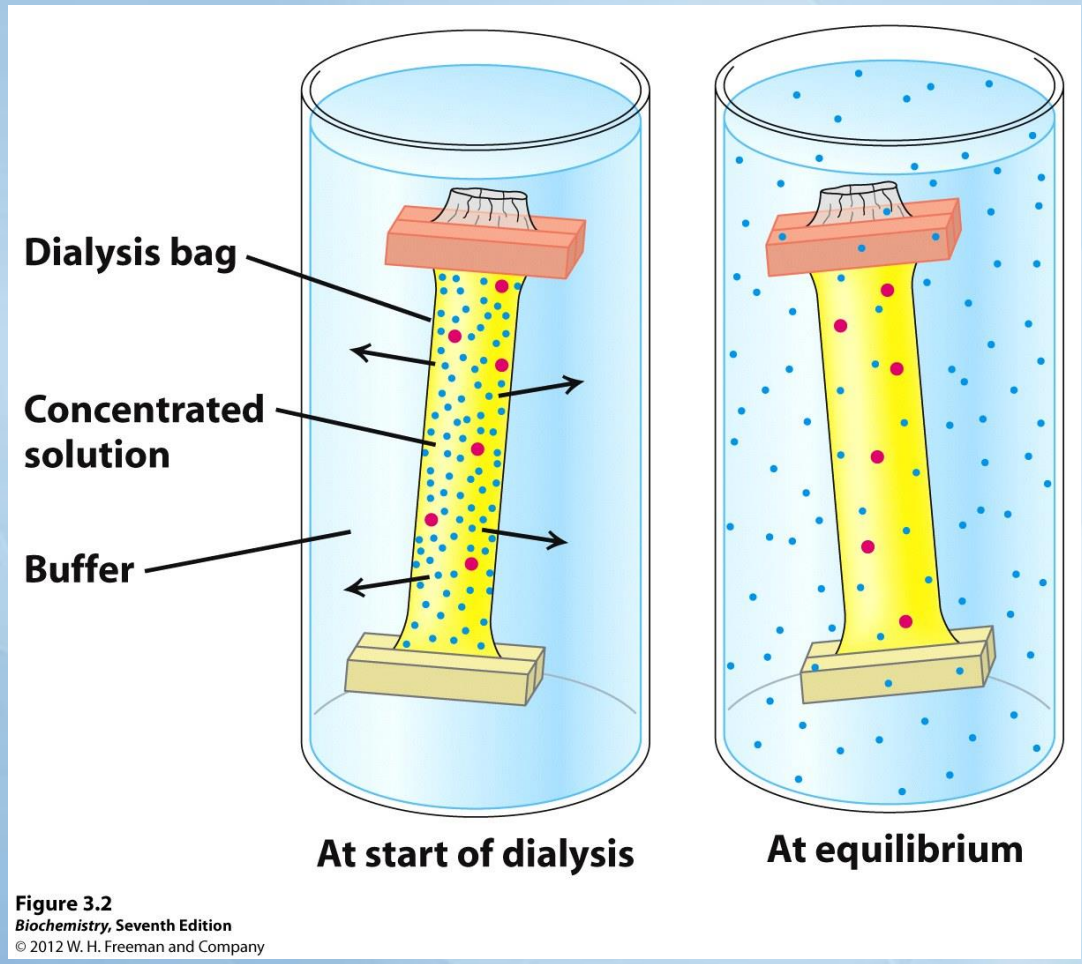


Figure 3.2
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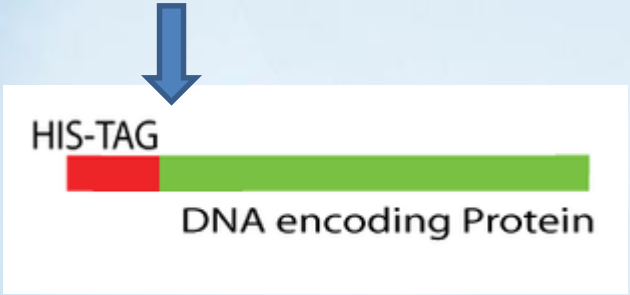
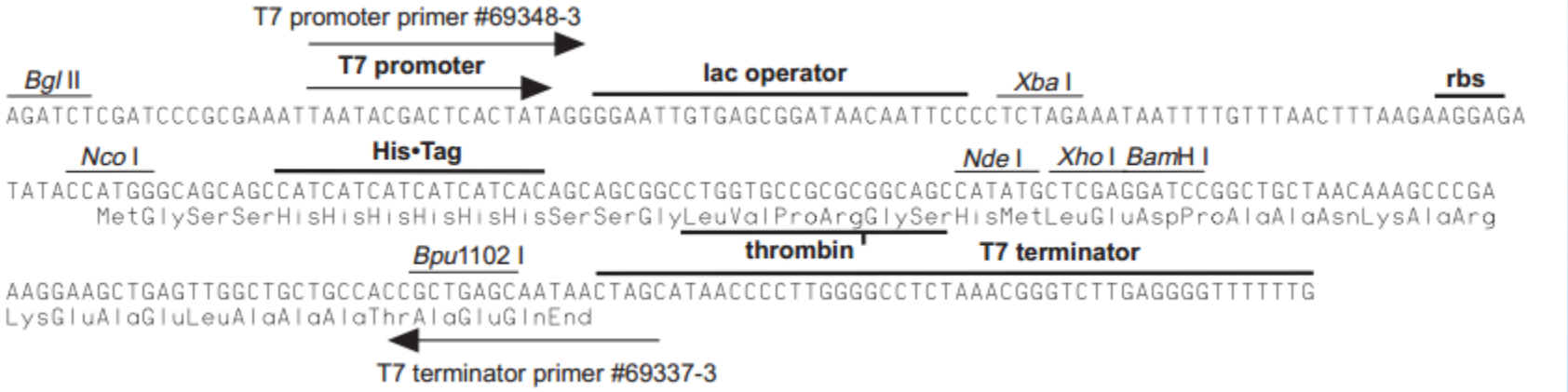
Protein Concentrators

Cleavage of His tag

His tag is not part of the protein. It needs to be removed in order to perform structural and biophysical studies on the protein.

- Thrombin is used to remove the His tag.





Examples of tags and ligands

- His-tag
- FLAG™ peptide
- *Strep*-tag
- GST tag
- Maltose binding protein fusion
- Calmodulin binding protein fusion
- Transition metal ion
- Monoclonal antibody
- Biotin
- Glutathione
- Amylose
- Ca^{2+}

Vector	amp^R	kan^R	T7	T7*lac*	f1 ori	His•Tag	T7•Tag^{11}	T7•Tag^{260}	S•Tag	Trx•Tag	CBD•Tag™	KSI	HSV•Tag	PKA	Dsb•Tag	GST•Tag	protease	signal seq.	LIC available
pET-3a-c	●		●				N												
pET-5a-c	●		●				N												
pET-9a-d		●	●				N												
pET-11a-d	●			●			N												
pET-12a-c	●		●															●	
pET-14b	●		●			N											T		
pET-15b	●			●		N											T		
pET-16b	●			●		N											X		
pET-17b	●		●				N												
pET-17xb	●		●					N											
pET-19b	●			●		N											E		
pET-20b(+)	●		●		●	C												●	
pET-21a-d(+)	●			●	●	C	N												
pET-22b(+)	●			●	●	C												●	
pET-23a-d(+)	●		●		●	C	N												
pET-24a-d(+)		●		●	●	C	N												
pET-25b(+)	●			●	●	C							C					●	
pET-26b(+)		●		●	●	C												●	
pET-27b(+)		●		●	●	C							C					●	
pET-28a-c(+)		●		●	●	N,C	I										T		
pET-29a-c(+)		●		●	●	C			N								T		
pET-30a-c(+)		●		●	●	N,C			I								T,E		
pET-30 Ek/LIC		●		●	●	N,C			I								T,E		●
pET-30 Xa/LIC		●		●	●	N,C			I								T,X		●
pET-31b(+)	●			●	●	C						N							
pET-32a-c(+)	●			●	●	I,C			I	N							T,E		
pET-32 Ek/LIC	●			●	●	I,C			I	N							T,E		●
pET-32 Xa/LIC	●			●	●	I,C			I	N							T,X		●
pET-33b(+)		●		●	●	N,C	I							N			T		
pET-34b(+)		●		●	●	C			I		N						T,E		●
pET-35b(+)		●		●	●	C			I		N						T,X		●
pET-36b(+)		●		●	●	C			I		N						T,E	●	●
pET-37b(+)		●		●	●	C			I		N						T,X	●	●
pET-38b(+)		●		●	●	C			I		C						T	●	
pET-39b(+)		●		●	●	I,C			I						N		T,E	●	
pET-40b(+)		●		●	●	I,C			I						N		T,E	●	
pET-41a-c(+)		●		●	●	I,C			I							N	T,E		
pET-42a-c(+)		●		●	●	I,C			I							N	T,X		
pSCREEN-1b(+)	●		●		●	I		N	I								T,E		

Notes:
T7•Tag^{11} = 11 aa fusion tag T7•Tag^{260} = 260 aa fusion tag signal seq. = signal sequence for potential periplasmic localization
I = internal tag N = N-terminal tag C = optional C-terminal tag
protease cleavage sites: T = thrombin E = enterokinase X = Factor Xa
LIC = ligation independent cloning, vectors available separately as linearized DNA
pSCREEN-1b(+) carries the pUC origin of replication; all other pET vectors carry the pBR322 origin