# Protein Expression and Purification

**Goals for this unit:**

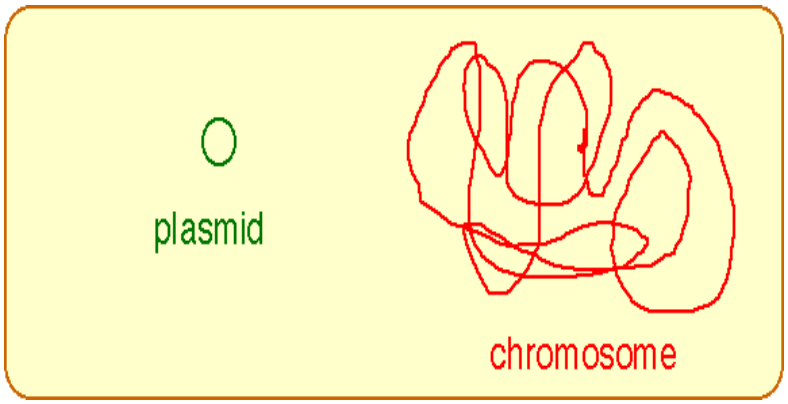
1. **Review steps in cloning and expression**
   - **Vectors / Restriction sites / Induction / Tags**

2. **Protein Purification**
   - **Factors**
   - **Assay / Purification Table**
   - **Basic Methods (Chromatographic Methods)**

     *GF / HIC / IEX / AC*

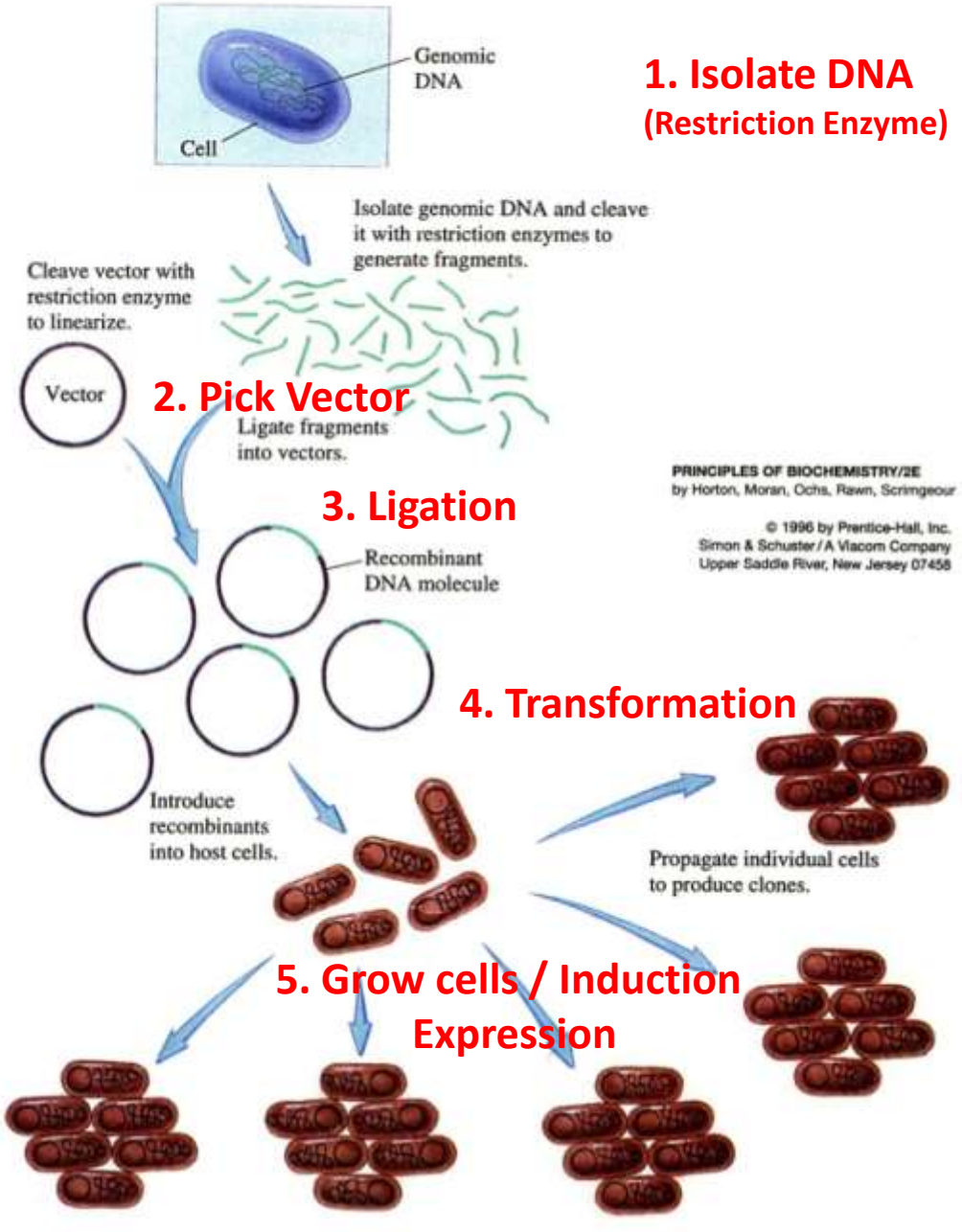
# Why do we do cloning?

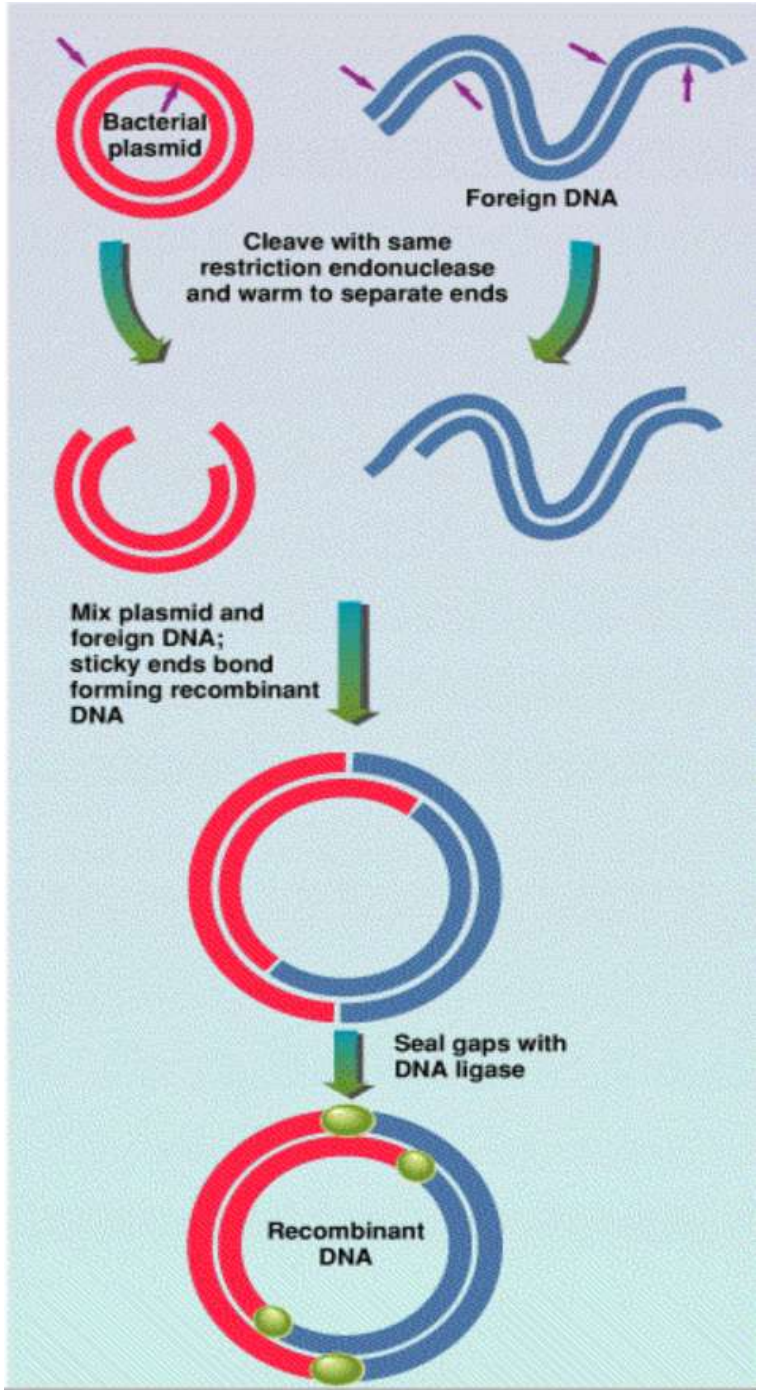
- **A single DNA molecule can be amplified allowing it to be:**
  - Studied - Sequenced
  - Manipulated - Mutagenesis or Engineered
  - Expressed - Generation of Protein

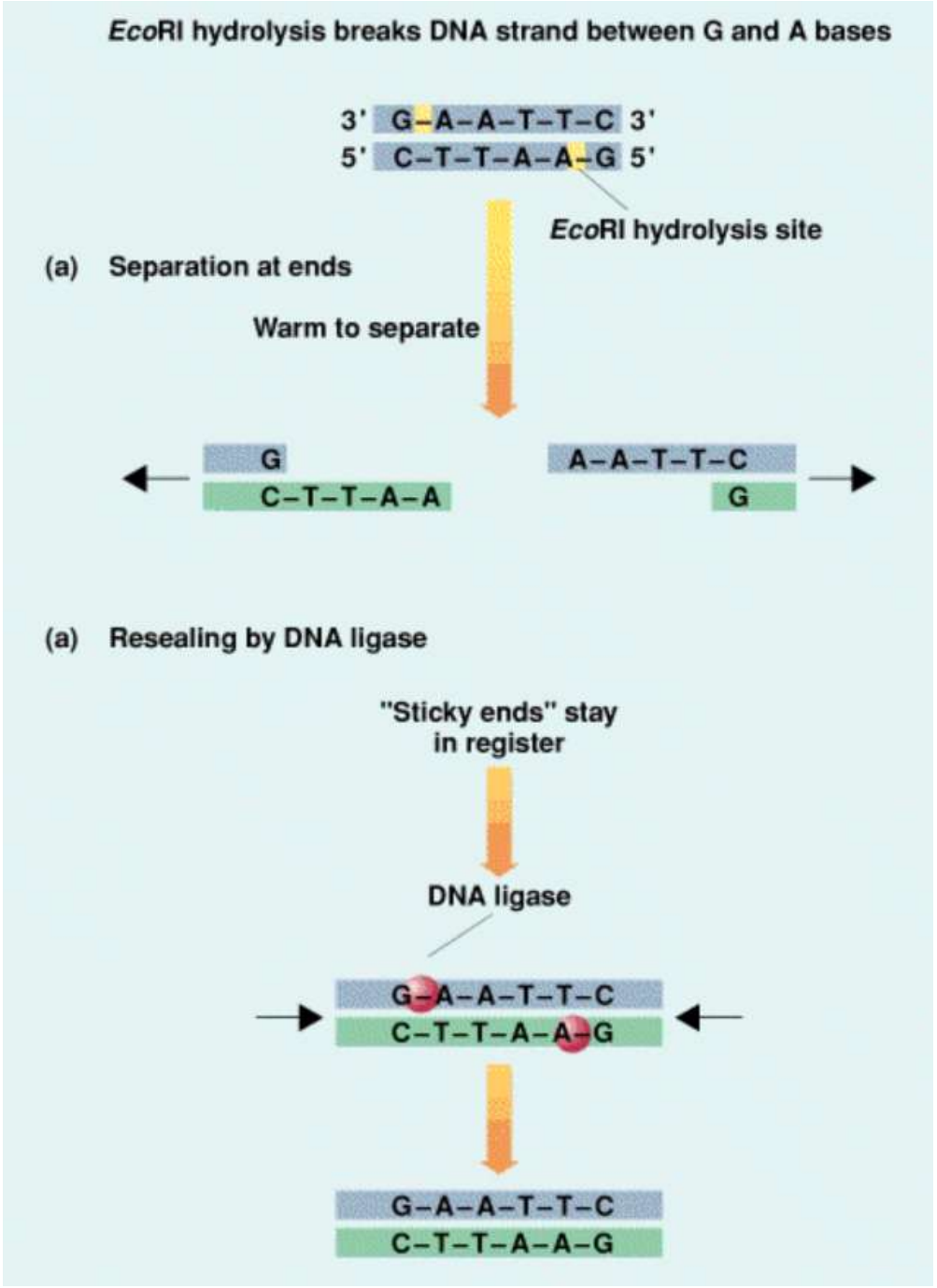



- Bacterial cells may contain extra-chromosomal DNA called plasmids.
- Plasmids are usually represented by small, circular DNA.
- Some plasmids are present in multiple copies in the cell

T–100 Fig. 24.1 General steps in the generation of a recombinant DNA molecule




Bacterial plasmid
Foreign DNA
Cleave with same restriction endonuclease and warm to separate ends
Mix plasmid and foreign DNA; sticky ends bond forming recombinant DNA
Seal gaps with DNA ligase
Recombinant DNA

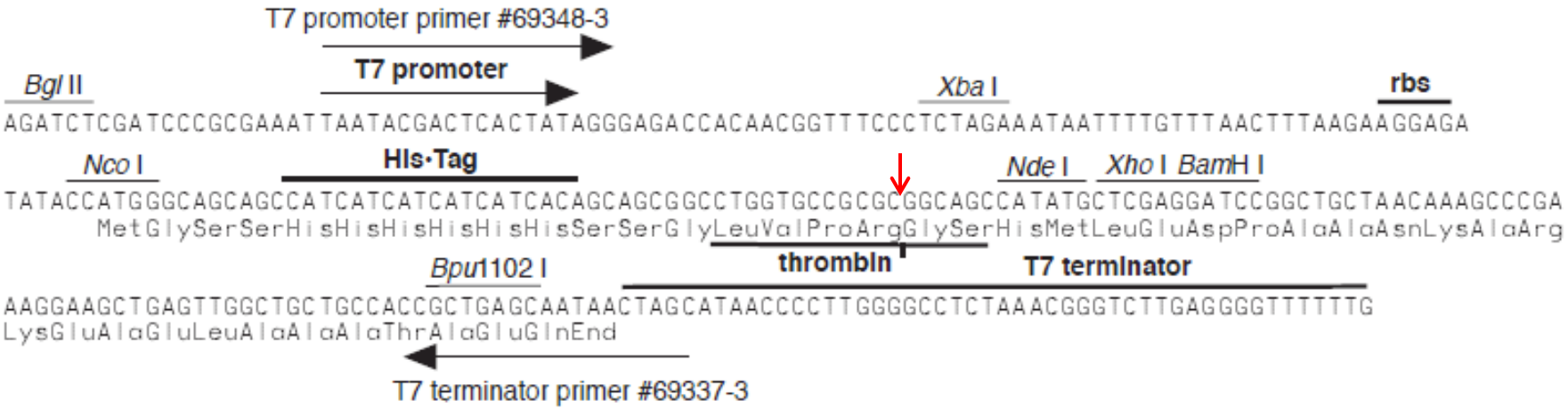

EcoRI hydrolysis breaks DNA strand between G and A bases
3' G–A–A–T–T–C 3'
5' C–T–T–A–A–G 5'
EcoRI hydrolysis site
(a) Separation at ends
Warm to separate
G
C–T–T–A–A
A–A–T–T–C
G
(a) Resealing by DNA ligase
"Sticky ends" stay in register
DNA ligase
G–A–A–T–T–C
C–T–T–A–A–G
G–A–A–T–T–C
C–T–T–A–A–G

# 1. Isolate DNA (Restriction Enzyme)

## What restriction enzyme to choose?

First check the vector you want to insert your gene into.

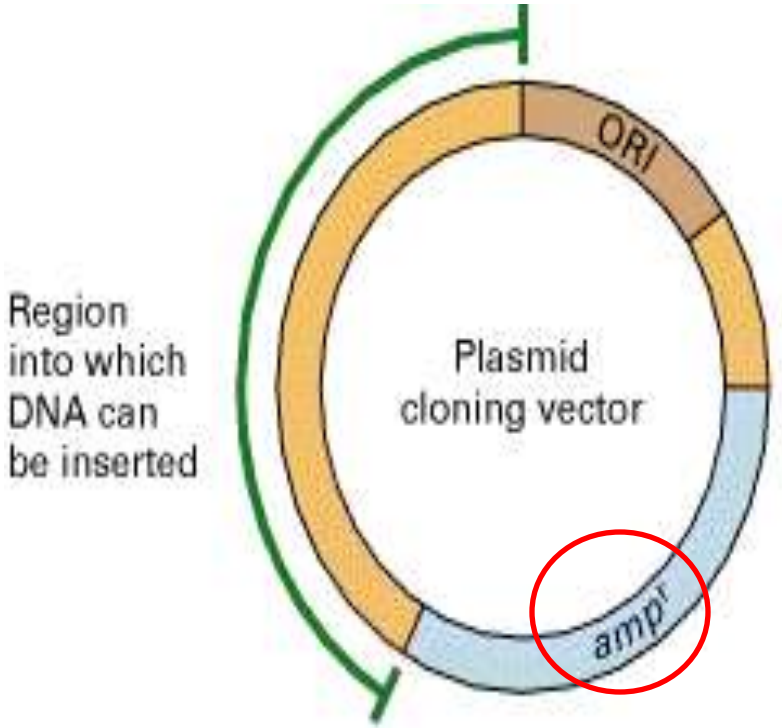
[PDF] **pET14b**
biochem.web.utah.edu/hill/links/**pET14b**.pdf
Novagen • ORDERING 800-526-7319 • TECHNICAL SUPPORT 800-207-0144 ori (2845). A p (3606-4463). Cla I(24). Hind III(29). Nhe I(229). Bpu1102 I(458).



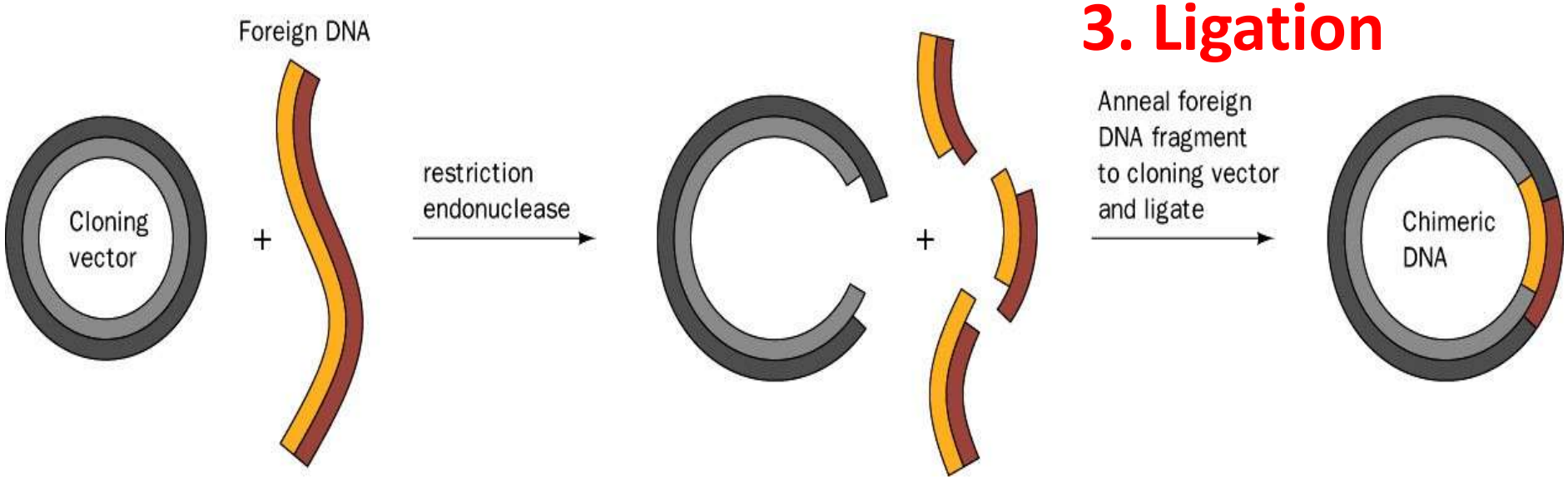

**pET-14b cloning/expression region**

# 2. Pick Vector

- replication origin (ORI) sequence
- a gene that permits selection,
- Here the selective gene is *ampr*; it encodes the enzyme b-lactamase, which inactivates ampicillin.
- Exogenous DNA can be inserted into the bracketed region .
- Under the selective conditions, only cells that contain plasmids with selectable marker can survive



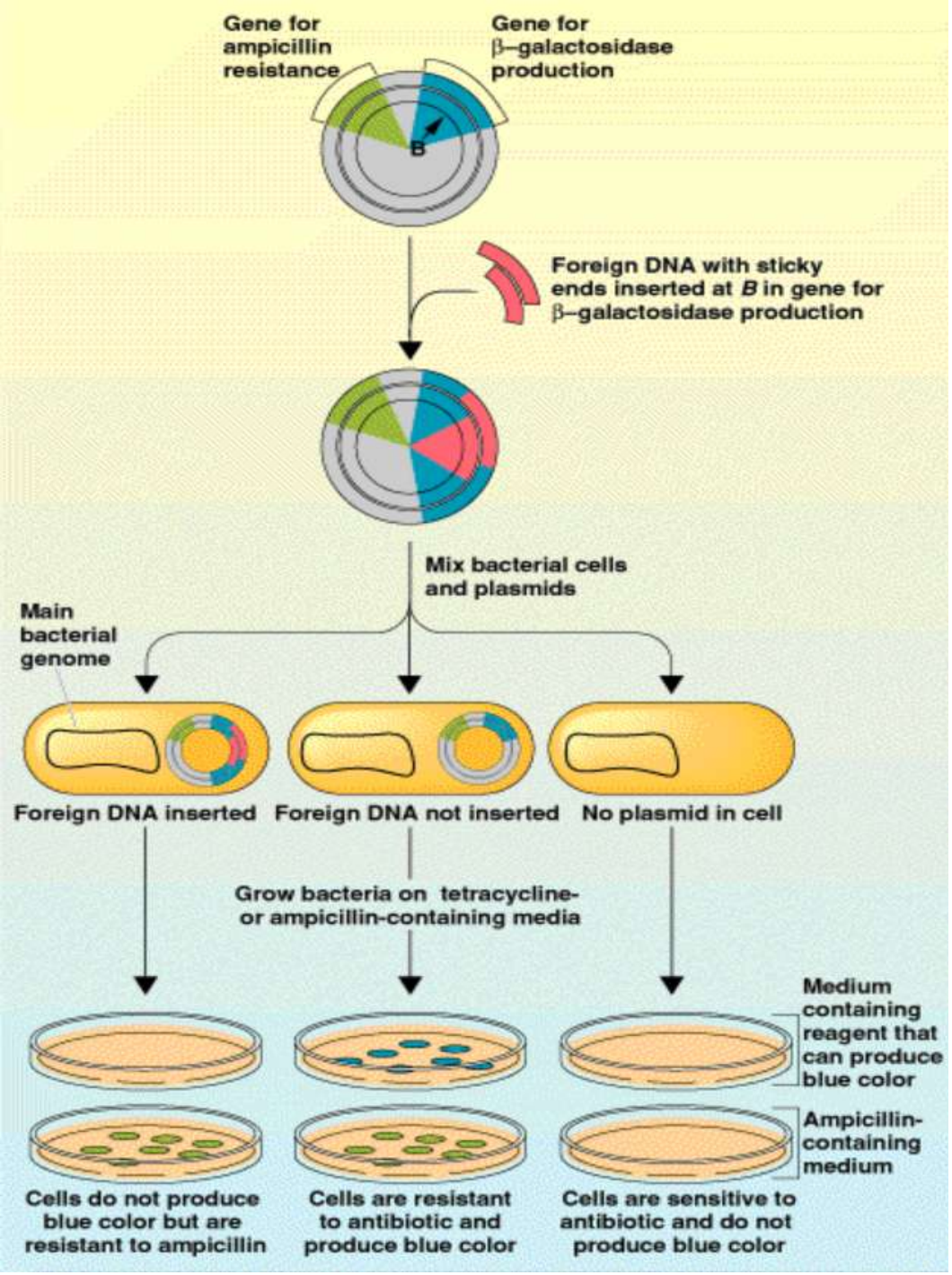

# 3. Ligation



# Transformation

- Upon heat shock or electric pulses, bacterial cell walls can temporarily become permeable and allow DNA to get in.
- The mechanism for such take-up is still unknown.
- Overall take-up efficiency is low. But once the plasmid enters, the ligated ones will be replicated in bacteria along with bacteria growth.
- *Use antibiotics to select those bacterial cells that take-up the foreign DNA.*
- So the specific DNA you want are duplicated many times in bacterial cells.
- When you spread them on agar plate and put it to selection, the ones that have plasmid will grow as colonies.

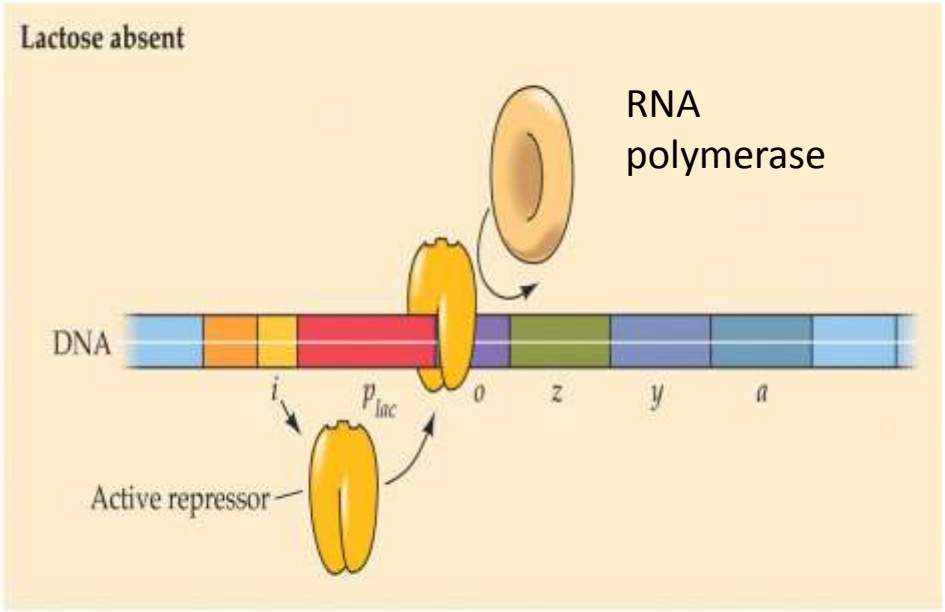
## 4. Transformation

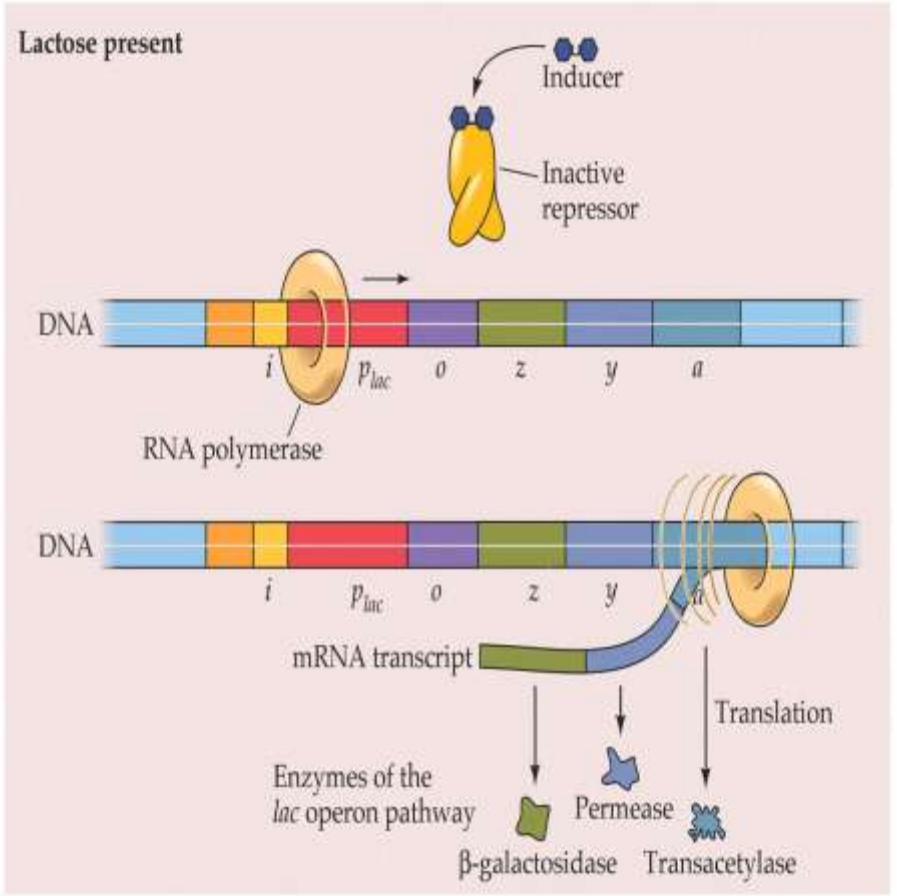


# IPTG-inducible protein expression

Isopropyl β-D-1-thiogalactopyranoside

## 5. Grow cells / Induction Expression




LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 13.17 The lac Operon: An Inducible System (Part 2)
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LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 13.17 The lac Operon: An Inducible System (Part 1)
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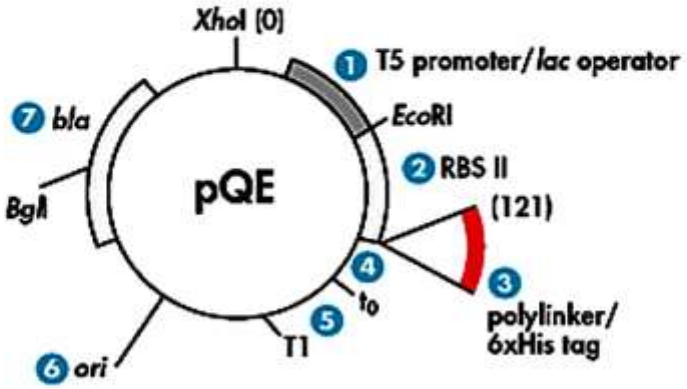
# Elements present in QIAexpress pQE Vectors

**pQE Vectors**




| | Element | Description |
|---|---|---|
| 1 | Optimized promoter/operator element | Consists of the phage T5 promoter and two lac operator sequences, which increase the probability of lac repressor binding and ensure efficient repression of the powerful T5 promoter |
| 2 | Synthetic ribosomal binding site - RBSII | For efficient translation |
| 3 | 6xHis-tag coding sequence | Either 5' or 3' to the polylinker cloning region |
| 4 | Translational stop codons | In all reading frames for convenient preparation of expression constructs |
| 5 | Two strong transcriptional terminators | t0 from phage lambda, and T1 from the rrnB operon of E. coli, to prevent read-through transcription and ensure stability of the expression construct |
| 6 | ColE1 origin of replication | From pBR3227 |
| 7. | beta-lactamase gene (bla) | Confers ampicillin resistance |

# Most used organisms for protein overexpression

- **Bacterial culture**
  - Easy to manipulate.
  - Don’t get contaminated easily.
  - Grow fast. (one day turn-around).
  - Cheap!
- **Eukaryotic systems**:
  - Yeast
    - Cheap and fast turn-around
    - Sometimes good yield.
    - Translational modification still very different from mammalian system.
  - Baculovirus and insect cells.
    - Stable expression.
    - Great yield.
    - Overcome problem like folding or glycosylation.
    - Less non-specific band during purification.
    - But long turn-around.
    - $$$
  - Mammalian
    - Very pricey.
    - Yield low.
    - Expression unstable
    - Only used when small amount is required.

# So, why not use a bacterial expression system every single time?

- Codon usage.
- Toxicity
- Sugar linkage.
- Folding issue and disulfide bond formation.

***Companies have developed vectors and bacterial strains to improve the chances of success!***

# QIAgenes:* Easy access to high-yield expression of human proteins — in *E. coli*, insect, and mammalian cells

Lack of sufficient amounts of protein often slows down or makes structural and functional analyses of proteins impossible. Expression of optimized genes helps to overcome this obstacle by improving codon usage and avoiding mRNA secondary structure or motifs that interfere with the transcription/translation process (internal ribosomal binding sites, sequence repeats etc.).

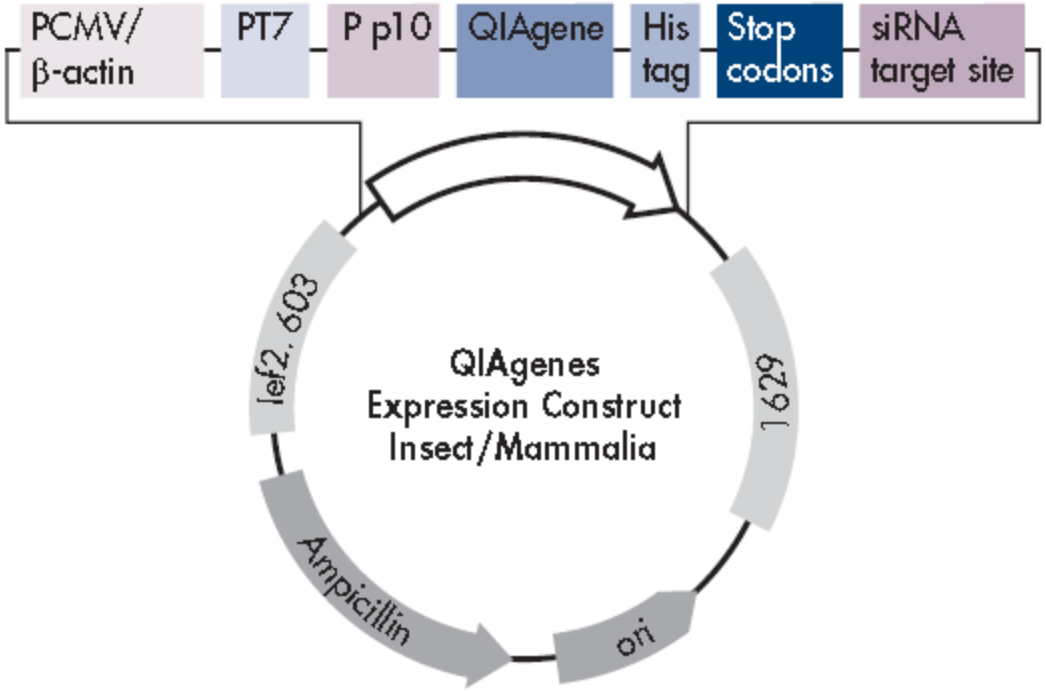



**Figure 1. Elements within QIAgenes Expression Constructs Insect/Mammalia.**

# Strategy to overcome the problems

- If the protein is toxic, use (DE3) strain to prevent expression before induction. Additionally, pLysS or pLysE strain can make sure no protein expression until addition of **IPTG**.

- If the protein is from eukaryotes, use codon plus strain if there are a lot of rare codons.

- If the protein has problem in folding, you can either refold from inclusion body or use **GroEL** or DnaK supplemented strain. Sometimes using special vector with thioredoxin can help.

# 6. Protein Purification

## Why purify a protein?

- To study its function
- To analyze its physical properties
- To determine its sequence
- For industrial or therapeutic applications

# Steps in Recombinant Protein Purification

1. Design expression plasmid, transform, select
2. Grow culture of positive clone, induce expression

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1. Lyse cells
2. Centrifuge to isolate protein-containing fraction
3. **Purify**
4. Assess purity on SDS-PAGE

# A Systematic Approach to Purification Development - Summary

- Develop assay methods
- Set the aims (purity and quantity)
- Characterize the target protein
- Use different separation principles
- Use few steps
- Limit sample handling between purification steps
- Start with high selectivity - increase efficiency
- Remove proteases quickly
- Reduce volume in early step
- Keep it simple!

# Protein Purification

*"To Study Proteins and their Functions,*
*you must first Produce, Extract, and Purify Them."*

**Produce:**

1. Culturing Cells under conditions that maximize *expression*.
2. Harvest cells by centrifugation or filtration.

**Extract:**

3. Resuspend cells in buffer – cell lysis: disruption, reagent, sonication.
4. Obtain extract by centrifugation or filtration – remove debris.

**Purify:**

5. Differential solubility (Ammonium sulfate / Acetone / PEG)
6. "Column" chromatography
   - gel exclusion chromatography (size)
     dextran (Pharmacia - Sephadex)
     polyacrylamide (Bio-Rad - Bio-Gel P))
     agarose (Pharmacia -Sepharose; Bio-Rad – Bio-Gel A)
   - ion exchange chromatography (charge: cation ; anion )

| | | |
|---|---|---|
| (+) | CM Sephadex | carboxymethyl – |
| | P-Cellulose | phosphate – |
| | AG-50 | sulfonic acid (Polystyrene) |
| (-) | DEAE-Sephadex | diethylaminoethyl – |
| | QAE-Sephadex | diethyl-(2-hydroxypropyl)-aminoethyl |
| | AG 1 | tetramethylammonium (Polystyrene) |

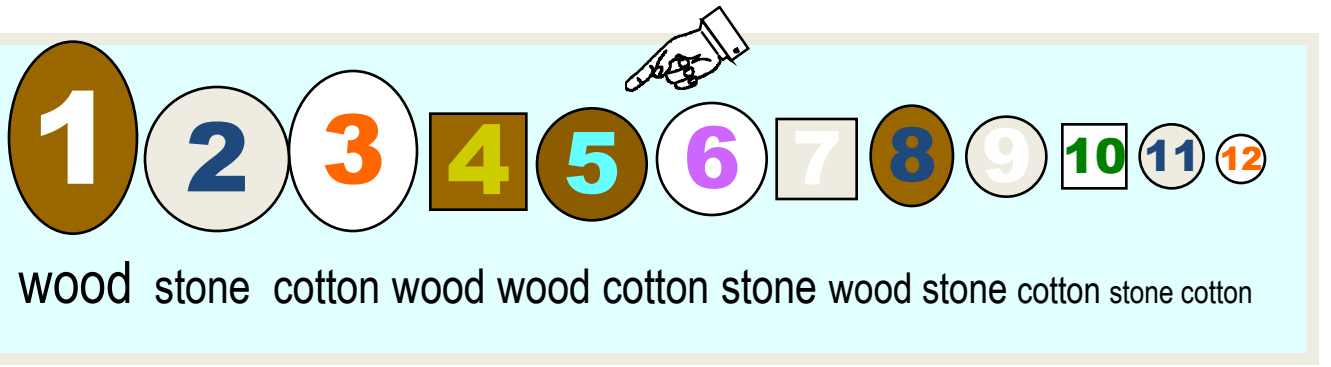
   - affinity chromatography (ligand)
     $NAD^+$ / ATP / PLP / 6 x His
     - IMAC = Immobilized Metal Affinity Chromatography
       - TALON Sepharose bead with tetradentate to $Co^{2+}$
       - Ni-NTA (Ni – nitrilotriacetic acid) resins (QIAGEN)
7. HPLC (High-Performance Liquid Chromatography)
   - stationary support with small particles of large surface area
   - improved speed with high pressure to improve solvent flow

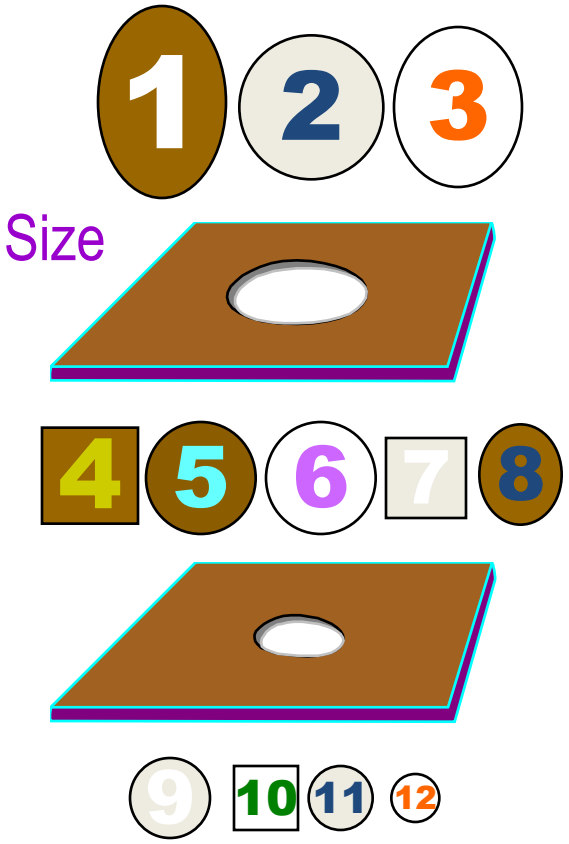
# Properties exploited to purify a protein

- **Size**
  - size exclusion (gel filtration) chromatography
- **Charge**
  - Ion exchange chromatography
- **Affinity**
  - Affinity chromatography
- **Solubility**
  - Salt-in/salt-out
- **Hydrophobicity**
  - Hydrophobic chromatography
- **Thermostability**
  - Boiling experiment

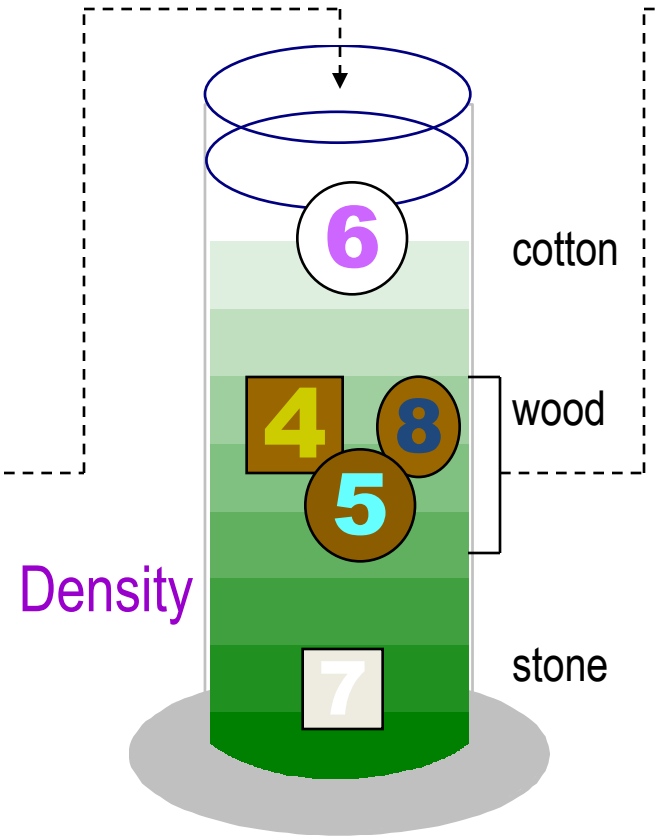
# How to Separate These Objects

Shape
Size
Density

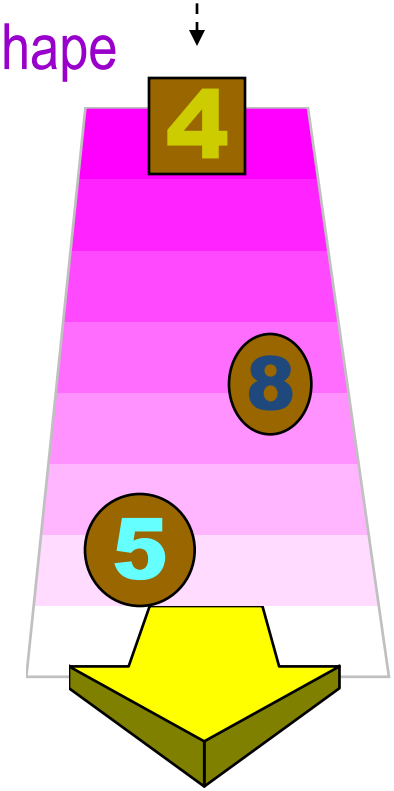



Sieving different sizes




Different sedimentation




Different rolling speed

Juang RH (2004) BCbasics

# Basic Principles of Protein Purification


| Small molecule | Macromolecule | | | | Cell Debris |
|---|---|---|---|---|---|
| Amino acid, Sugar, Nucleotides, etc | Nucleic Acid | Protein | Carbohydrate | Lipid | |

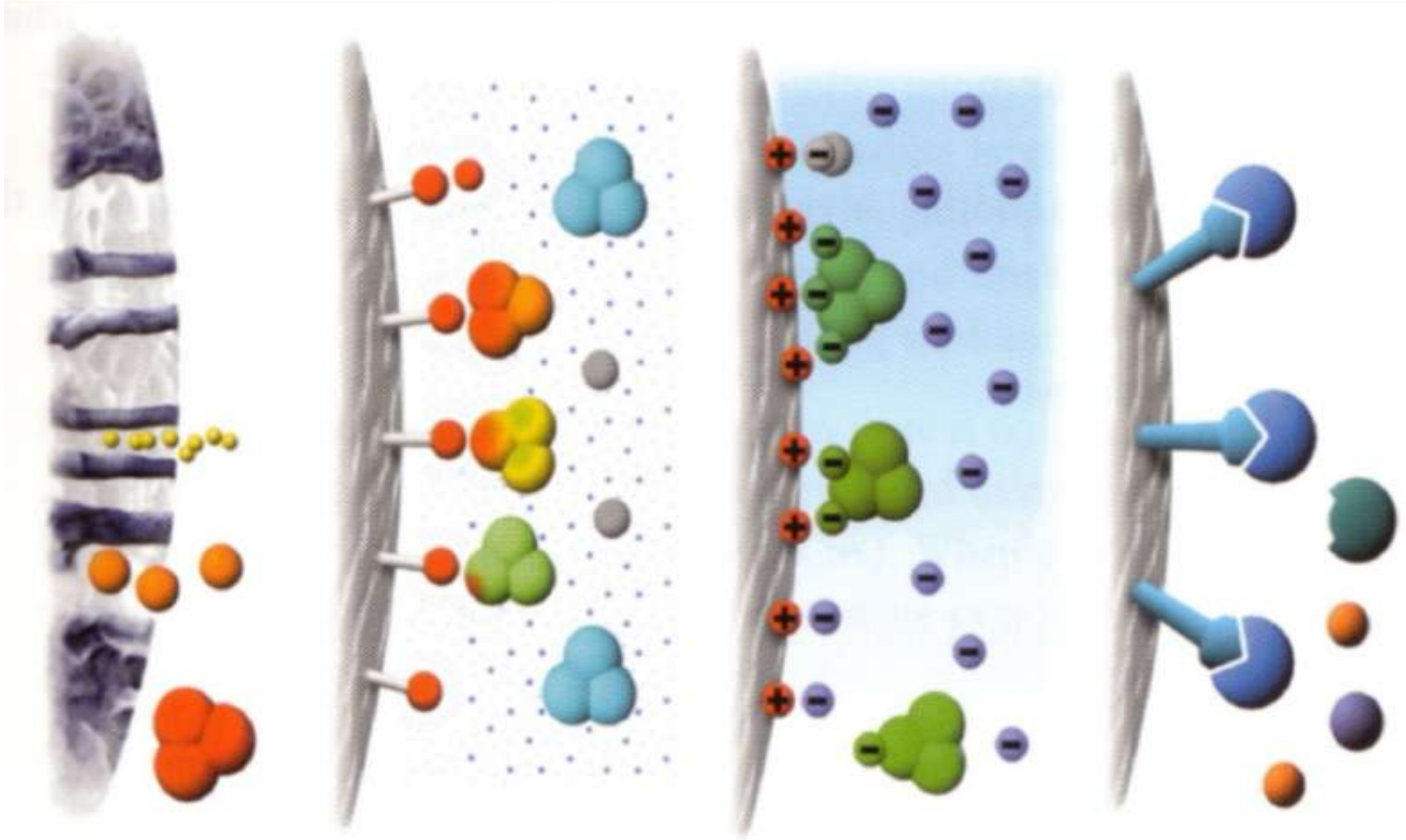
**Ammonium sulfate fractionation**

*The following techniques listed in each box can be used to separate proteins based upon the following characteristics:*

| Size (GF) | Charge (IEX) | Polarity (HIC) | Affinity (AC) |
|---|---|---|---|
| Gel filtration, SDS-PAGE, Ultrafiltration | Ion exchange, Chromatofocusing, Disc-PAGE, Isoelectric focusing | Reverse phase chromatography, HIC, Salting-out | Affinity chromatography, Hydroxyapatite |

Juang RH (2004) BCbasics

# Chromatography



# Protein Expression, Purification and Analysis

In **olden days**, protein purification used to start with a source (organ tissue, plant source, bacteria line) known to be rich in the protein of interest. Often this would involve obtaining many pounds of organ tissue directly from a slaughter house or growing large (30 or 60L) batches of bacterial culture in order to isolate sufficient material for further studies. Purification based on solubility and charge.

*Typical **protocol** for isolation of a mammalian protein (after procedure for chicken heart LDH – H4, Nathan Kaplan et al., JBC, 239: 1753-1761 (1964):*

**Step 1:** Obtain 25 lbs of fresh chicken hearts on ice from local slaughter-house.

**Step 2:** Cut tissue in small sections, grind tissue in a commercial meat grinder, extract with 14L cold distilled water.

**Step 3:** Filter cell and connective tissue debris from extract by passing thru double layer of cheese cloth.

**Step 4:** Centrifuge using 1L containers and large bucket rotor International centrifuge at 1300 x g for 30 minutes. Pool supernatant. Assay.

**Step 5:** Adjust pH to 7.0, add solid ammonium sulfate to give 70% saturation of AS, maintain pH ~ 7 by adding dilute ammonium hydroxide as needed. Let stand for 2-4 hours, filter overnight on fluted filter paper. Scrape ppt. off filter paper and suspend in 1 L of cold distilled water. Centrifuge. Assay.

**Step 5:** Adjust pH to 7.0, add solid ammonium sulfate to give 70% saturation of AS, maintain pH ~ 7 by adding dilute ammonium hydroxide as needed. Let stand for 2-4 hours, filter overnight on fluted filter paper. Scrape ppt. off filter paper and suspend in 1 L of cold distilled water. Centrifuge. Assay.

**Step 6:** Dialyze soluble fraction against two 20 L changes of 0.005M Tris buffer, pH 7.0 for a total of 12 hours. To the solution, add solid AS to 25% saturation. Centrifuge, remove ppt. Add more AS to 60% saturation, leave standing for 1 hour, spin at 18,000 x g for in a Sorvall refrig. Centrifuge, save pellet. Dissolve pellet in 300 mL of 0.005 M Tris buffer, pH 7.6. Dialyze against two 20 L changes of 5 mM Tris, pH 7.0 for 8 hours each, centrifuge.

**Step 7:** Add precooled (-15 deg) acetone slowly to dialysate to ppt protein at 25%/(v/v). After 10 minutes, cent. – discard ppt. Add more acetone to 50% (v/v), leave for 20 minutes at 0°C. Collect ppt. At 18,000 x g for 30 min., then extract with 200 mL cold Tris buffer, pH 7.6, cent., discard ppt.

**Step 7:** Add supernatant to DEAE cellulose column. Elute with a gradient of 2 L each of 0.005 M Tris, pH 7.0 and same buffer with 0.20 M NaCl. Combine fractions with LDH activity. Assay. Expected yield 50-60 %.

**Step 8:** Re-crystallization - Ppt. with saturated AS, redissolve in a minimum of buffer, centrifuge, add AS slowly with stirring to 30% saturation. Wait about 1 day, centrifuge to harvest enzyme. Assay. Yield ~ 30%, 100X purification.

# Enzyme Assay / Enzyme purification

Enzyme Activity (units)

1 International Unit = amt. of E which cat. $\frac{1\ \mu mol\ S \rightarrow P}{min}$

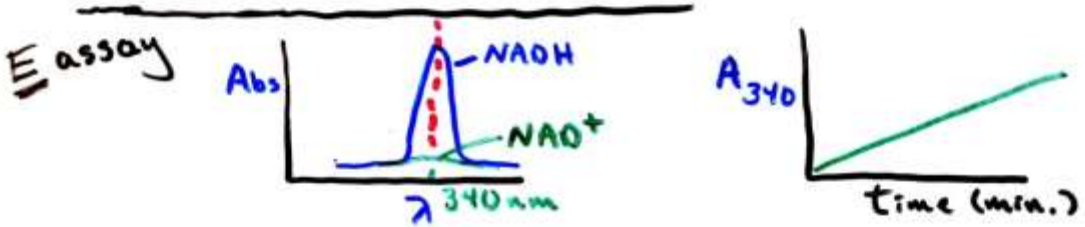
Specific Activity = $\frac{\mu mol\ S \rightarrow P / min}{mg\ protein}$ at 25°C or IU/mg

(measure of Enzyme purity)

Turnover number = $V_{max} / [E]_{tot} = k_{cat}\ (min^{-1})$

## Purification of Hypothetical Enzyme

$$S + NAD^+ \xrightarrow{E} P + NADH$$




| Step | Vol. | Protein | E Act. | Spec. Activity | % Y |
|---|---|---|---|---|---|
| 1. Cell extract | 1400 mL | 10,000 mg | 100,000 | 10 | 100 |
| 2. $(NH_4)_2SO_4$ ppt. | 280 | 3,000 | 96,000 | 32 | 96 |
| 3. DEAE col. | 90 | 400 | 80,000 | 200 | 80 |
| 4. Gel "Filtration" (size-exclusion) | 80 | 100 | 60,000 | 600 | 60 |
| 5. Affinity chrom. | 6 | 3 | 45,000 | 15,000 | 45 |

1500 x

# Protein Purification Schemes

Also, each step of the purification should be monitored by gel electrophoresis.

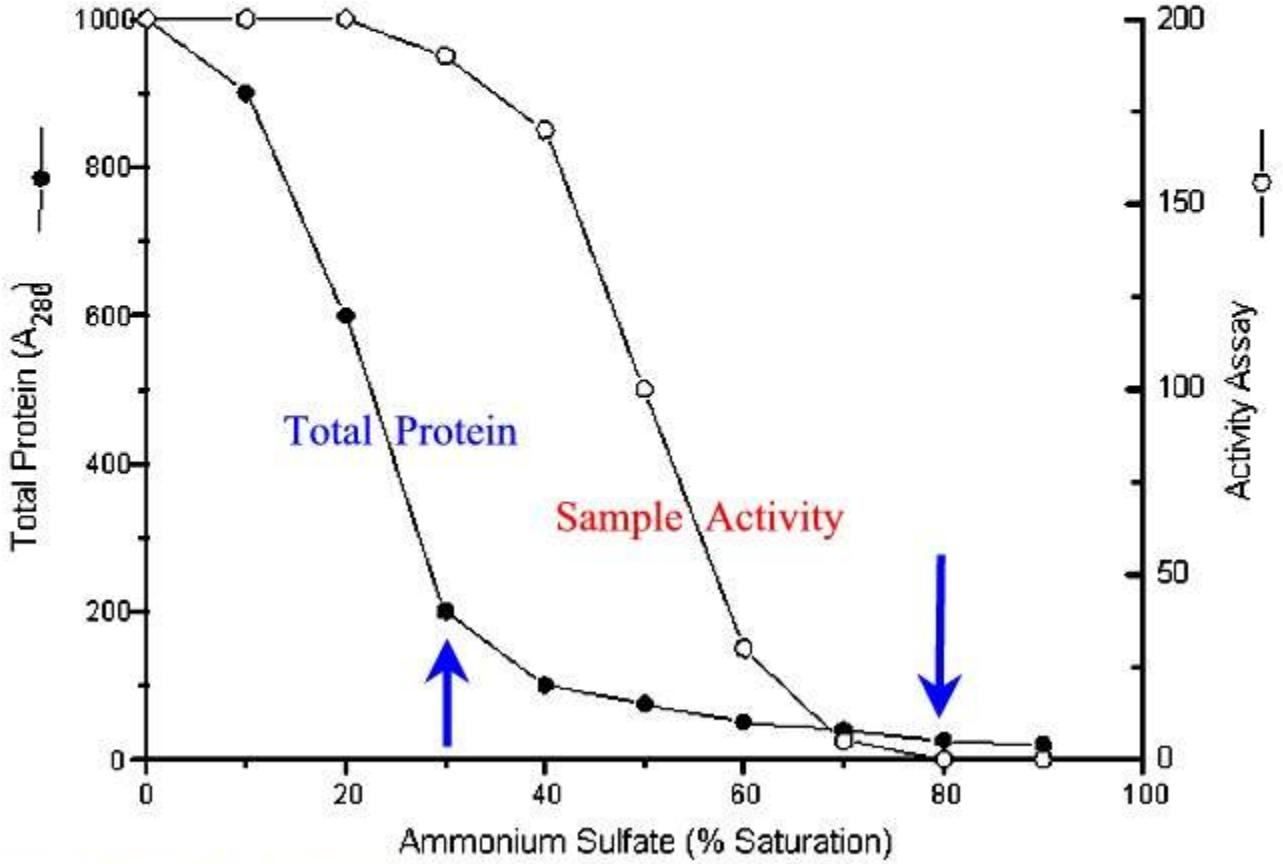
- In the initial stages of purification we will probably see a variety of bands, of various molecular weights, on our gel.
- After the different purification steps, we should see the disappearance of certain bands concomitant with the increasing concentration of a certain band (or bands) representing our protein.
- If we have successfully purified our protein (and if it is a single polypeptide) we should arrive at a constant specific activity and *a single band on a gel*.
- Analytical methods like HPLC or densitometer scanning of a stained gel can give us a quantitative idea of the purity of our final sample.

The following chart represents the typical data one would monitor during a purification:

| Step | Total protein (mg) | Total activity (units) | Specific activity (units/mg) | Purification | % Yield |
|---|---|---|---|---|---|
| Crude cell lysate | 5500 | 6600 | 1.2 | | |
| 30-70% Ammonium sulfate cut | 1020 | 5910 | 5.8 | 4.8 | 89.5 |
| DEAE Sephadex pool | 187 | 5070 | 27.1 | 4.7 | 85.8 |
| CM Sephadex pool | 102 | 4420 | 43.3 | 1.6 | 87.2 |
| Phenyl Sepharose pool | 56 | 3930 | 70.2 | 1.6 | 88.9 |
| Gel Filtration pool | 32 | 2970 | 92.8 | 1.3 | 75.6 |
| Affinity resin type #1 pool | 5.8 | 2520 | 434.5 | 4.7 | 84.8 |
| Affinity resin type #2 pool | 5.3 | 2390 | 450.9 | 1.0 | 94.8 |
| Total purification | **376** | | | | |
| Total yield (%) | **36** | | | | |

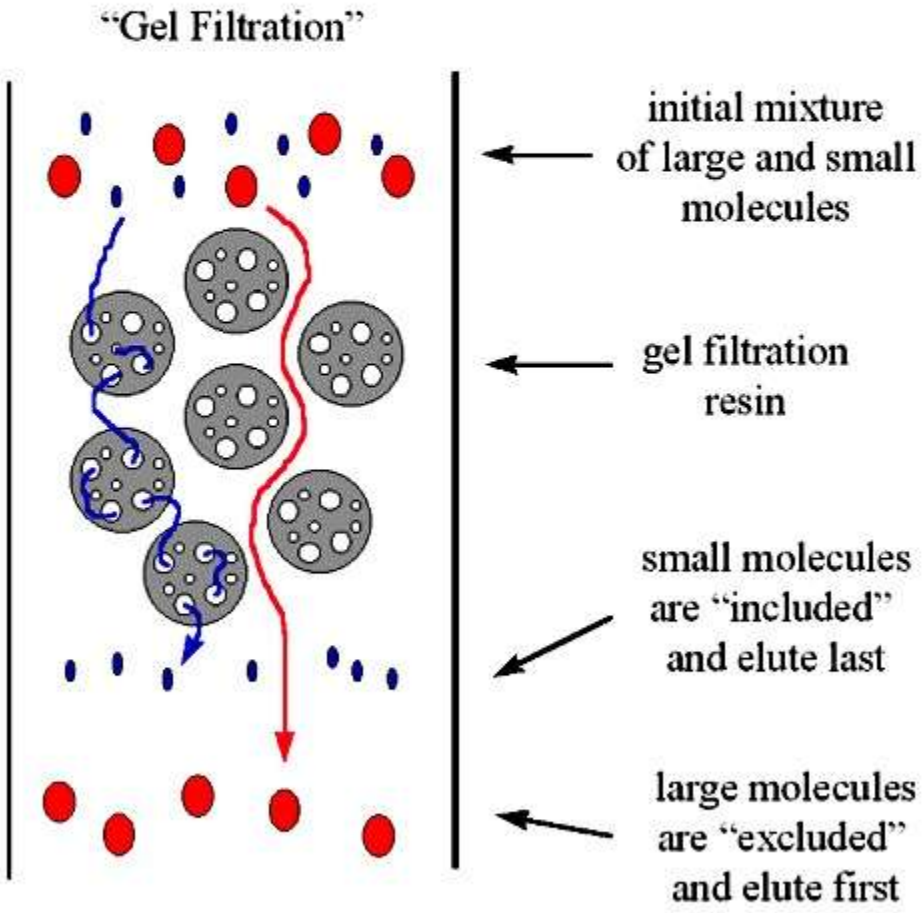
| Ammonium Sulfate (% saturated) | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample $A_{280}$ | 1000 | 900 | 600 | 200 | 100 | 75 | 50 | 40 | 25 | 20 |
| Activity assay(units) | 200 | 200 | 200 | 190 | 170 | 100 | 30 | 5 | 0 | 0 |

# Solubility and Protein Purification - AS Fractionation



# GEL FILTRATION / SIZE EXCLUSION CHROMATOGRAPHY

- Gel filtration resin can be thought of as beads which contain pores of a defined size range.
- Large proteins which cannot enter these pores pass around the *outside* of the beads.
- Smaller proteins which can enter the pores of the beads have a longer, tortuous path before they exit the bead.
- Thus, a sample of proteins passing through a gel filtration column will *separate based on molecular size*: the big ones will elute first and the smallest ones will elute last (and "middle" sized proteins will elute in the middle).

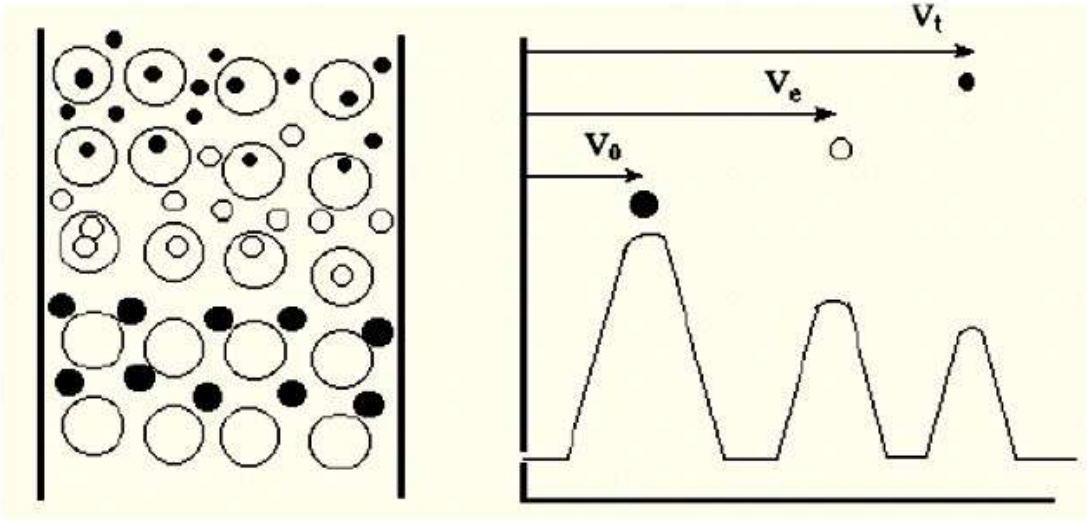


# Gel Filtration Chromatography

Vo = Void Vol; Ve = Elution Vol; Vt = Total Vol

The sample is applied in a narrow band at the top of the column and then it is washed through the column by the mobile phase. Large molecules in the sample that cannot pass through the pores of the beads are excluded from the beads and are restricted to the outer voids. They elute from the column after an amount of the mobile phase equal to $V_0$ has passed through the column. Molecules that are much smaller than the pores equilibrate with the entire liquid volume and elute at a volume equal to $V_t$, which is the sum of $V_0$ and $V_i$. Molecules that are small enough to pass through some of the pores of the beads, however, elute at various volumes ($V_e$), depending on how small they are and what fraction of the pores of the beads are accessible to them. Such molecules are referred to as partially included. Molecules in the sample can be separated in order of their size by collecting fractions as the mobile phase is eluted through the column, with the largest molecules eluting first and the smallest last.

The figure below represents the principle of gel-filtration chromatography.

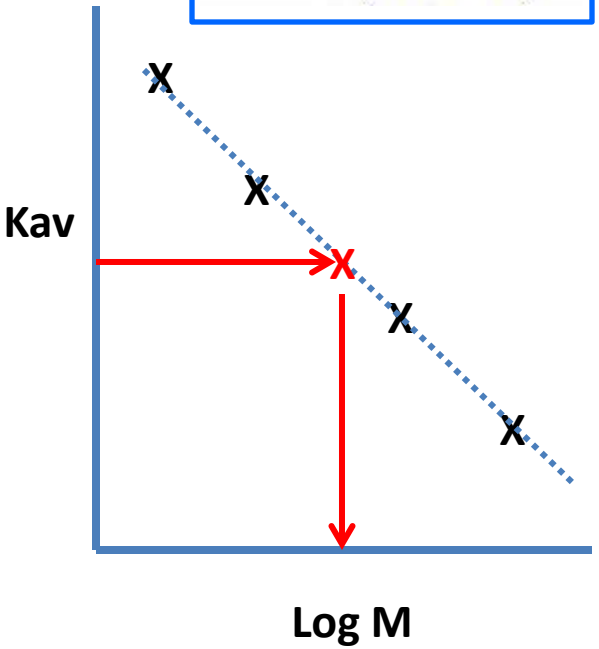



A partition coefficient can be calculated from the values above using the equation $K_{av} = (V_e - V_0)/(V_t - V_0)$.

$$Kav = \frac{(Ve - Vo)}{(Vt - Vo)}$$

A semilogarithmic plot of the relationship of $K_{av}$ to molecular weight can elucidate the efficiency of separation of molecules. Assuming that all molecules are of similar shape, the separation of molecules on the basis of molecular weight will be greatest in the linear range of the curve.

$$Kav = \frac{(Ve - Vo)}{(Vt - Vo)}$$

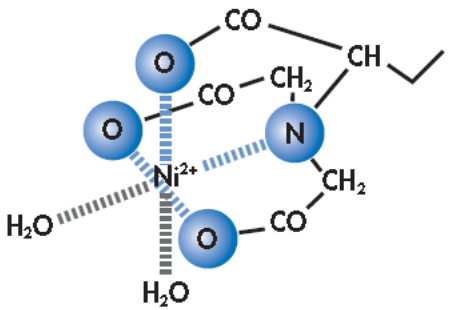


# Ni-NTA for the highest purity proteins

Purification of His-tagged proteins with Ni-NTA follows — as usual for QIAGEN purification products — a simple bind-wash-elute procedure (Figure 5). Due to the structure of NTA and its tight binding of nickel ions (Figure 6) proteins can be eluted in high yields with high purity (Figure 7). You can run several cycles without stripping and reloading the resin, which reduces also $Ni^{2+}$ waste. The purification process itself is very robust and allows the use of a broad range of reagents, meaning you can use the specific conditions your proteins need to stay active to purify them (Table 5).

**Ni-NTA — the world's most cited affinity purification resin**

- Highest purity of protein after a single purification step (Figure 7)
- Minimal nickel leaching resulting in highly pure proteins and avoiding recharging and toxic waste (Figure 6)
- High yields of up to 50 mg/ml Ni-NTA resin or up to 2 µg/µl magnetic bead suspension (Figure 8)
- Broad reagent compatibility with the only resin that is compatible to 10 mM DTT (Table 5)




Ni-NTA

**Figure 6. Ni-NTA gives you highly pure proteins.** In Ni-NTA resins nickel ions are bound via four coordination sites. Nickel ions bound to IDA-based resins are only bound via three coordination sites and can be lost more easily. Empty charged coordination sites will function like ion-exchange matrices that can bind un-tagged contaminating proteins.

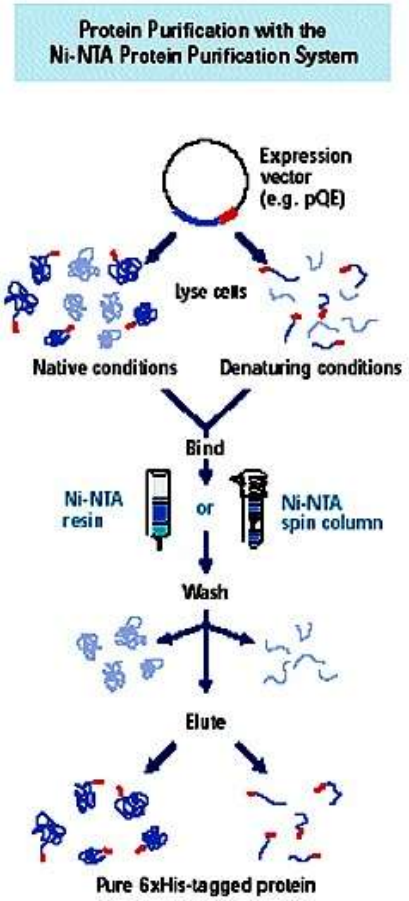
**Cell lysate or cell-free expression reaction**

# Purification of 6xHis tagged Proteins

Ni-NTA Protein Purification System is based on the selectivity of patented Ni-NTA (nickel-nitrilotriacetic acid) resin for proteins containing an affinity tag of six consecutive histidine residues — the 6xHis tag.

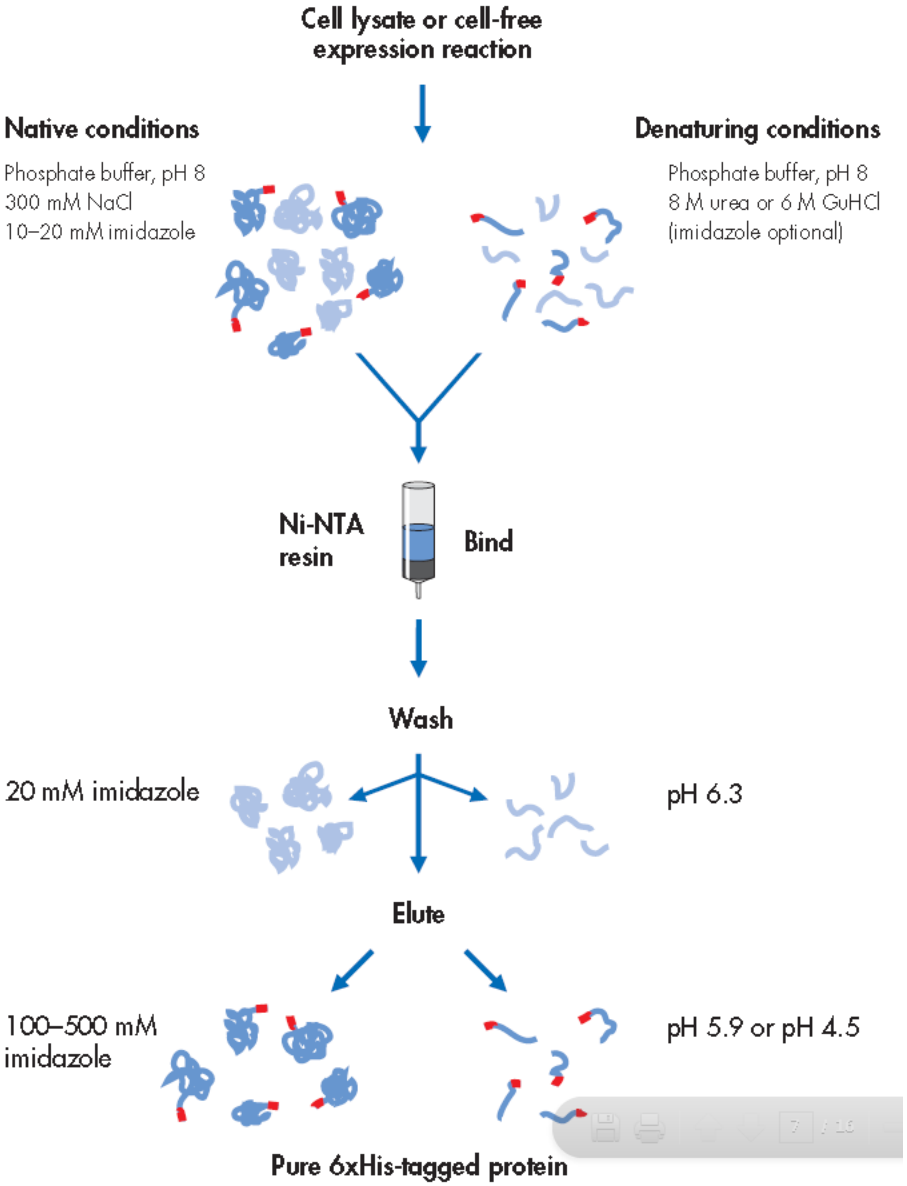
## Procedure (from - www.qiagen.com)

The purification of 6xHis-tagged proteins consists of 4 steps: cell lysis, binding, washing, and elution (see flowchart). Purification of recombinant proteins using the QIAexpress system does not depend on the 3-dimensional structure of the protein or 6xHis tag. This allows one-step protein purification under either native or denaturing conditions, from dilute solutions and crude lysates. Strong denaturants and detergents can be used for efficient solubilization and purification of receptors, membrane proteins, and proteins that form inclusion bodies. Reagents that allow efficient removal of nonspecifically binding contaminants can be included in wash buffers (see table "Reagents compatible with the Ni-NTA–6xHis interaction"). Purified proteins are eluted under mild conditions by adding 100–250 mM imidazole as competitor or by a reduction in pH.

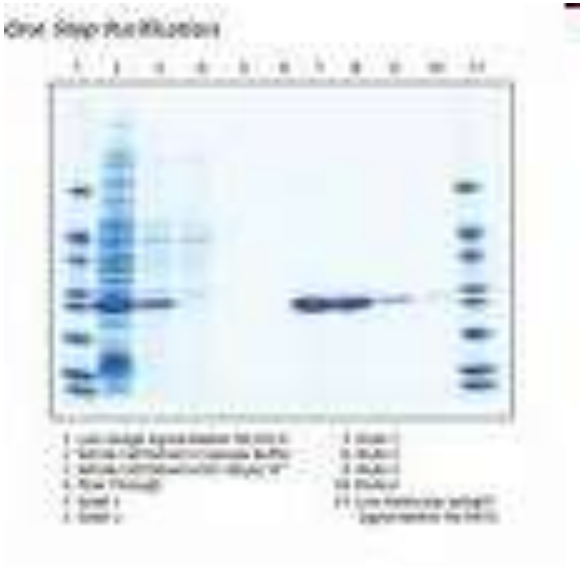



**Reagents compatible with the Ni-NTA–6xHis interaction***

| | |
|---|---|
| • 6 M guanidine HCl | • 50% glycerol |
| • 8 M urea | • 20% ethanol |
| • 2% Triton X-100 | • 2 M NaCl |
| • 2% Tween 20 | • 4 M MgCl2 |
| • 1% CHAPS | • 5 mM CaCl2 |
| • 20 mM β-ME | • ≤20 mM imidazole |




- **His tag**
  - Most commonly used.
  - Small
  - Fast and tight binding
  - Resin cost effective and reusable
  - But doesn't help folding



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# Comparison of affinity tags for protein purification

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Table 1
Comparison of affinity tag technologies

| Tag | Size (aa) | Resin | Eluting agent | Source | Capacity | Cost | Cost/10 mg |
|---|---|---|---|---|---|---|---|
| MBP | 396 | Amylose | Maltose | Biolabs | 3 mg/ml | $105/10 ml | $12 |
| HIS | 6 | Talon | Imidazole | Clontech | 5–14 mg/ml | $220/25 ml | $18 |
| | | Ni–NTA | Imidazole | Qiagen | 5–10 mg/ml | $257/25 ml | $21 |
| GST | 218 | GSH–Sepharose | Glutathione | Amersham | 10 mg/ml | $396/25 ml | $36 |
| CBP | 28 | Calmodulin affinity | EGTA | Stratagene | 2 mg/ml | $227/10 ml | $114 |
| STR (Strep II) | 8 | Strep-Tactin–Sepharose | Desthiobiotin | IBA | 50–100 nmol/ml | $1100/25 ml | $293 |
| FLAG | 8 | Anti-FLAG M2 MAb agarose | FLAG peptide | Sigma | 0.6 mg/ml | $1568/25 ml | $1045 |
| HPC | 12 | Anti-Protein C MAb matrix | EDTA | Roche | 2–10 nmol/ml | $299/1 ml | $4983 |
| CYD | 5 | InaD | DTT | N/A | >0.2 mg/ml | N/A | N/A |

Capacity for the fusion protein and retail cost information were obtained from manufacturer's catalogs and websites, except for the InaD resin for which the capacity was estimated from our experiments. The unit cost was calculated for purification of 10 mg of a 30 kDa protein fused to the appropriate tag, and the values for the MBP and GST fusions take into account the large size of those affinity tag. Since the affinity resins listed are reusable, actual costs may be less.

## Proteases *(recognize specific sequence)* used to remove tags

- **Thrombin**
  - Thrombin is a serine protease (uses serine  cleave the peptide).
  - Designed to perform the specific cleavage needed to activate fibrinogen, without digesting all the other important proteins in the blood.
  - Highly specific.
  - Cleavage site (Leu-Val-Pro-Arg-Gly-Ser)

- **Tev protease**

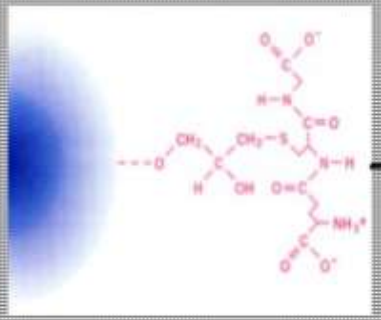
- **PreScission -** the new fashion, 3C protease
  - A genetically engineered fusion protein of glutathione S-transferase (GST) and human rhinovirus (HRV) type 14 3C protease (1) with a molecular weight of approx 46kD.
  - Enables the low-temperature cleavage of fusion proteins containing the PreScission™ Protease recognition sequence.
  - Recognition site:

Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro.

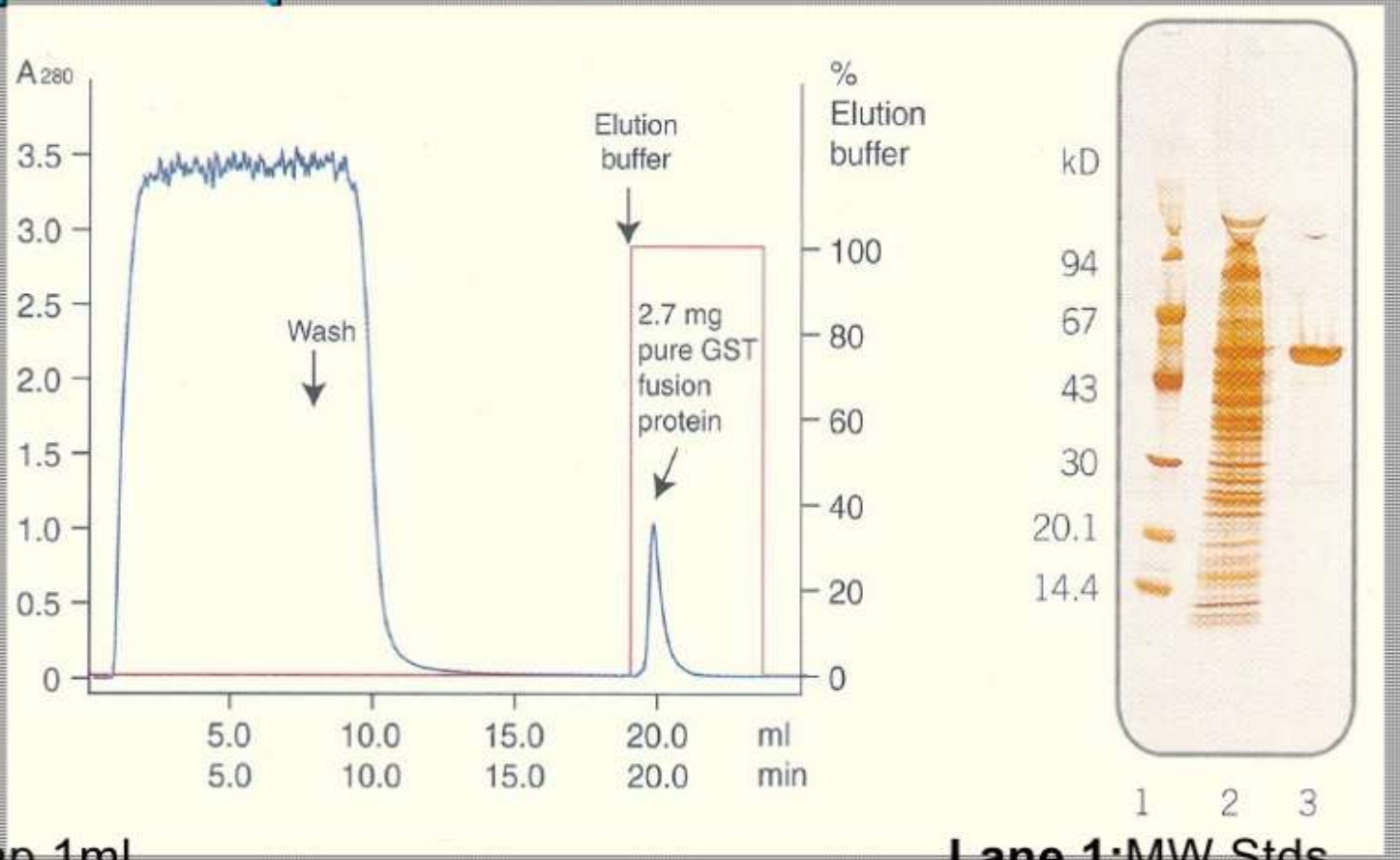
# Cleavage Sites Table for Fusion Proteins

| PROTEASE | COMPANY | Protease Capture |
|---|---|---|
| Thrombin<br>Leu-Val-Pro-Arg▼Gly-Ser | Amersham-Biosciences, Novagen, SIGMA, Roche | Benzamidine-Agarose |
| Factor Xa<br>Ile-Glu/Asp-Gly-Arg▼ | Amersham-Biosciences, New England Biolabs, Roche | Benzamidine-Agarose |
| Enterokinase<br>Asp-Asp-Asp-Asp-Lys▼ | New England Biolabs, Novagen, Roche | Trypsin Inhibitor-Agarose |
| TEV protease<br>Glu-Asn-Leu-Tyr-Phe-Gln▼Gly | Invitrogen – Life Technologies | Ni-NTA (6His recomb. TEV) |
| PreScission<br>Leu-Glu-Val-Leu-Phe-Gln▼Gly-Pro | Amersham-Biosciences | GSTrap for GST fusion enzyme |
| TAGZyme<br>His-tag removal by Exoproteolytic Digestion | Quiagen | Ni-NTA (6His recomb. enzyme) |
| Intein Site<br>dithiothreitol cleavage | New England Biolabs | DTT elimination by dialysis |
| HRV 3C Protease<br>Leu-Glu-Val-Leu-Phe-Gln▼Gly-Pro | Novagen | Ni-NTA (6His recomb. enzyme) |
| SUMO Protease<br>recognize the tertiary structure of the ubiquitin-like (UBL) protein, SUMO | Invitrogen – Life Technologies, LifeSensors | Ni-NTA (6His recomb. enzyme) |

# GST fusion protein purification



**glutathione sepharose**







**Column:** GSTrap 1ml

**Sample:** 8 ml cytosolic extract from *E.coli* expressing a GST fusion protein

**Binding buffer (BB):** PBS, pH 7.3

**Elution buffer (EB):** 50 mM Tris-HCl, pH 8.0 with elution

10 mM reduced glutathione

**Lane 1:** MW Stds

**Lane 2:** *E.coli* cytosolic extract

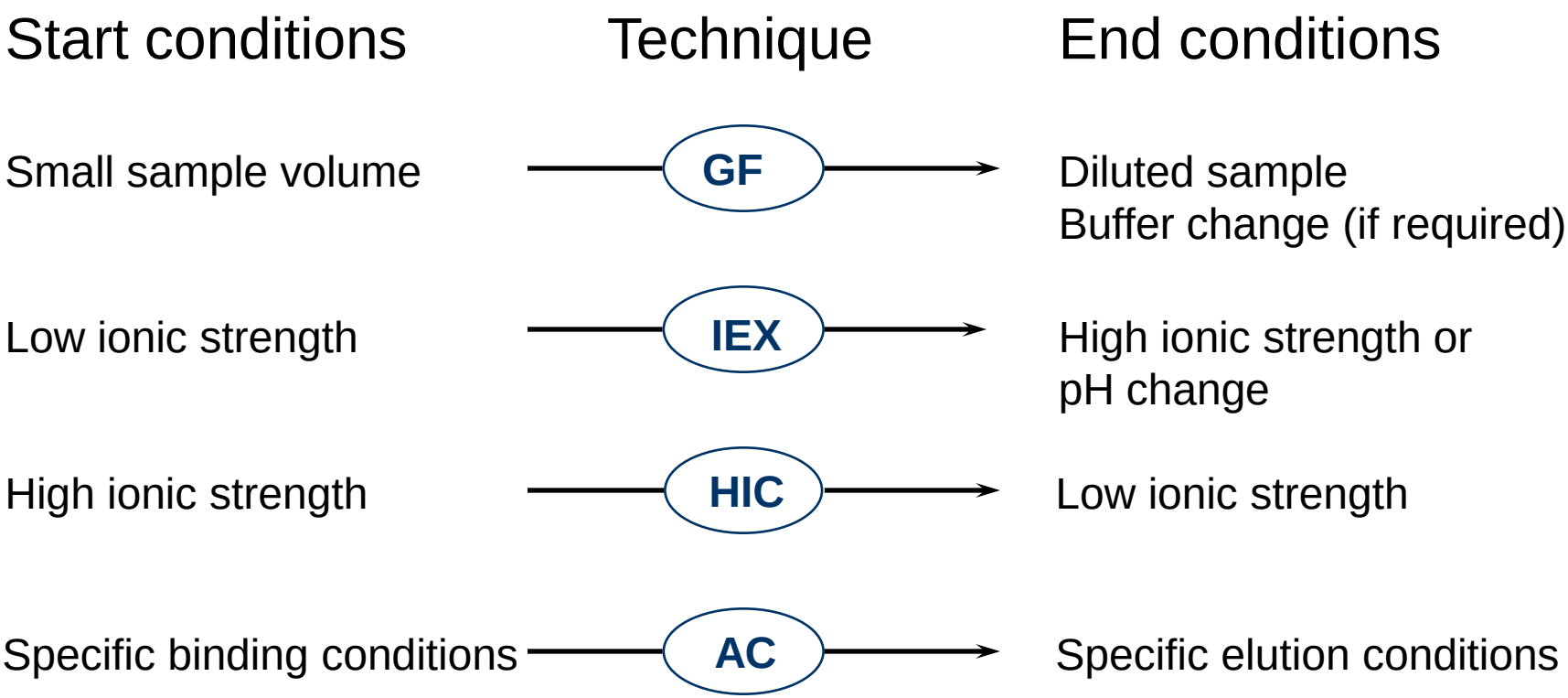
**Lane 3:** GSTrap

# Linking Chromatography Techniques into a Purification Protocol - General Rules

- Combine techniques with complementary selectivities (e.g. IEX, HIC and GF).

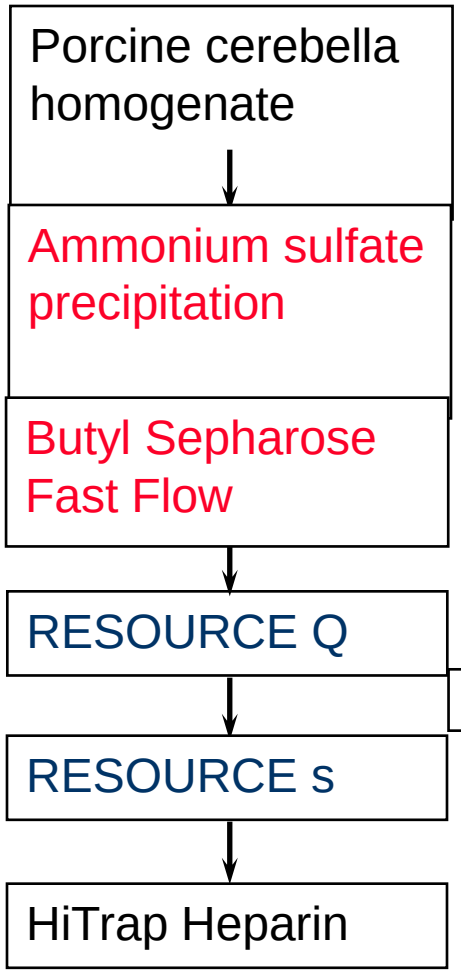
- Minimize sample handling between purification steps (e.g. concentration, buffer exchange).

# Linking Chromatography Techniques



# G Protein Receptor Kinase Purification

A. Tobin et al. (1996)
J. Biol. Chem. 271, 3907-3916




| Purification factor | Comment |
|---|---|
| 7 | • All buffers contain protease inhibitors |
| 20 | • All purifications done at +4° C |
| 2408 | • Removal step, main contaminant is bound<br>• Elution buffer is used as starting buffer for next column |
| 18647 | • 10 mg homogenous protein obtained |