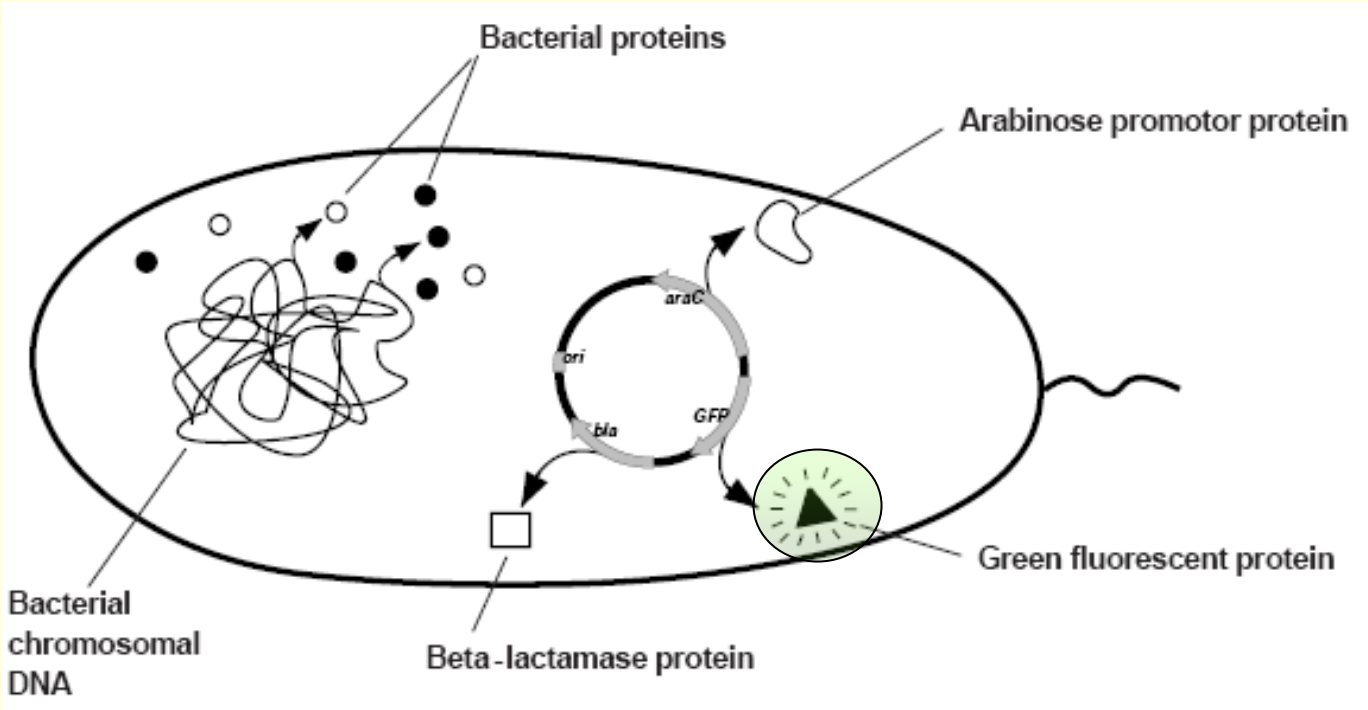
Purification

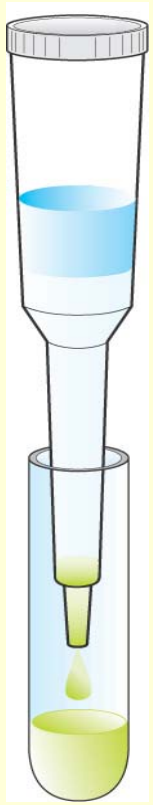
The art of chromatography

Purify a single recombinant protein of interest from over 4,000 naturally occurring *E. coli* gene products.



Column Chromatography

- A separation method in which different components of a mixture travel through the resin of a column differently.



Protein purification is based on chemical properties

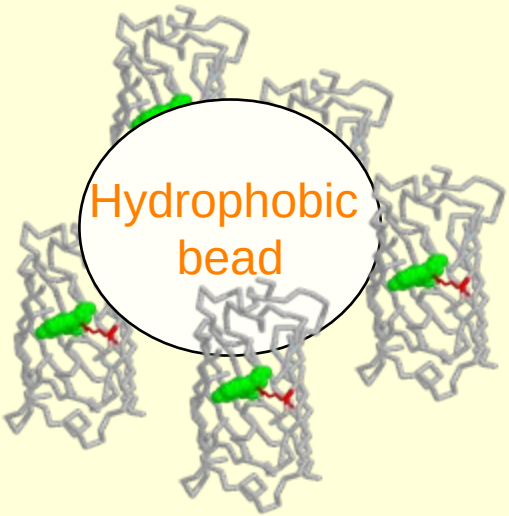
- Proteins can be separated based on:
  - Size
  - Charge (ion exchange)
    - Cation Exchanger (anions on resin bind positively charged protein)
    - Anion Exchanger (Cations on resin bind negatively charged protein)
  - Specific binding affinity (resin coupled w/antibody specific to protein of interest)
  - Hydrophobicity (Hydrophobic Interaction Column).

Hydrophobic Interaction

- Hydrophobic (water hating) substances do not mix well with water
- Some amino acids of proteins are very hydrophobic
- In salt water, these parts of the protein stick tightly to other hydrophobic substances (causes conformational change so hydrophilic regions are protected).

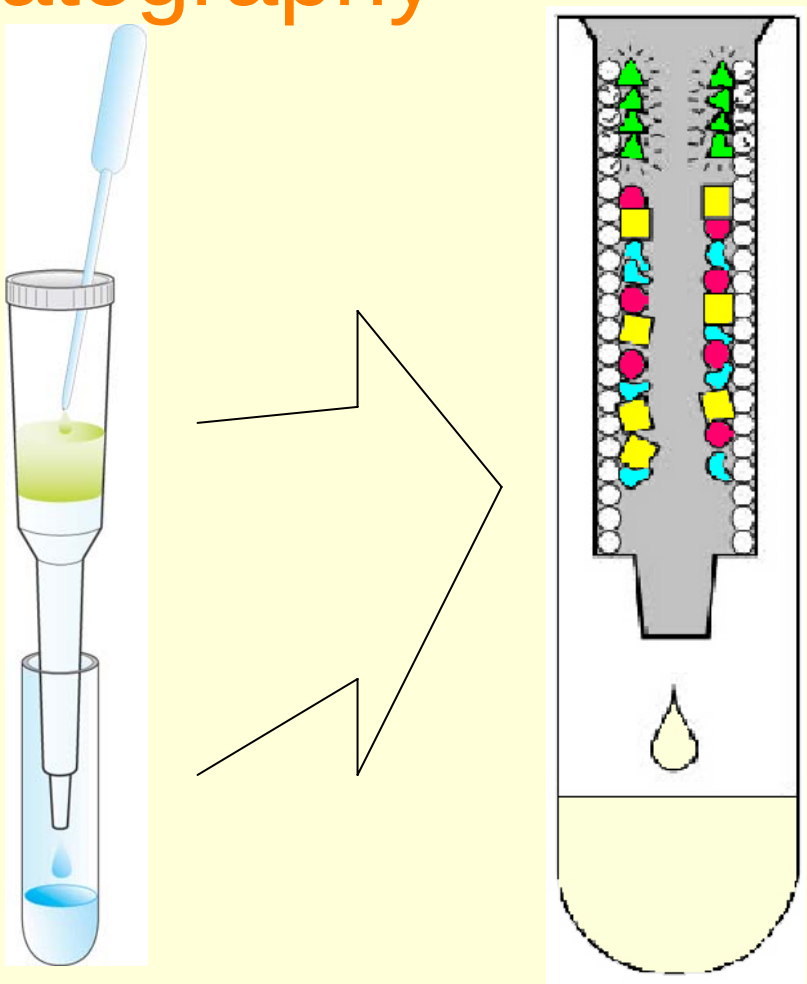
HIC Column

- Hydrophobic amino acids of protein bind to a support (gel matrix) in the column, which contains immobilized hydrophobic groups (phenyl).



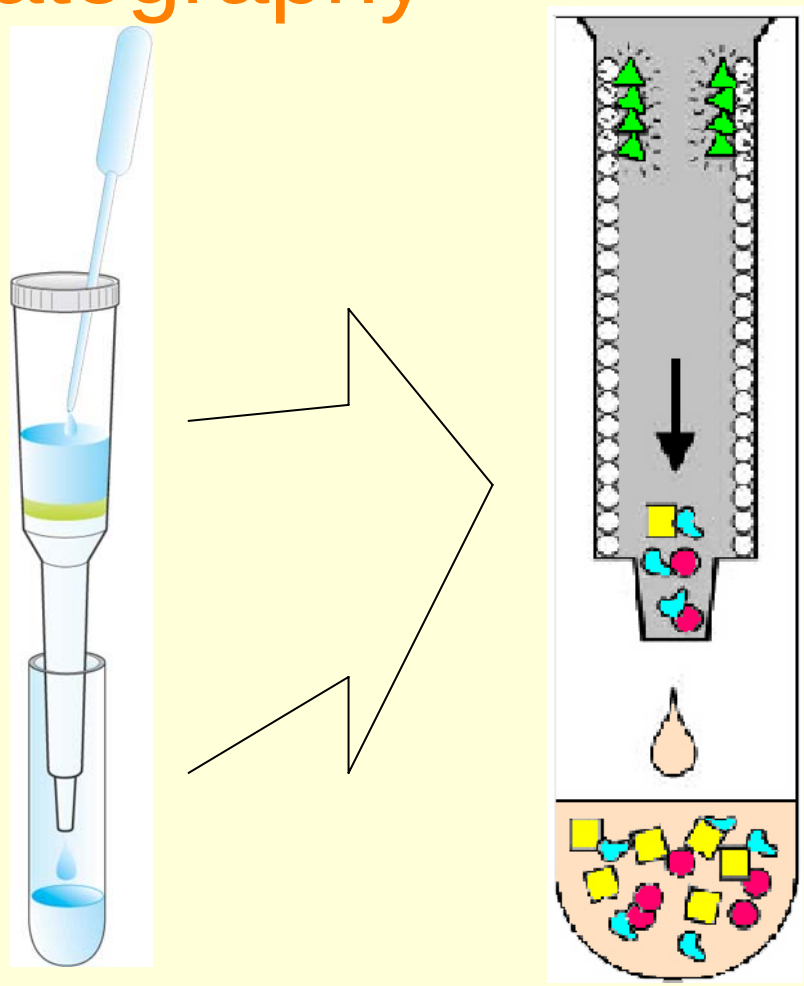
Step 1:
Hydrophobic Interaction Chromatography

- Add bacterial lysate to column matrix in high salt buffer
  - Hydrophobic proteins interact with column



Step 2:
Hydrophobic Interaction Chromatography

- Wash less hydrophobic from column with low salt buffer
  - Less hydrophobic E. coli proteins fall from column
  - GFP remains bound to the column



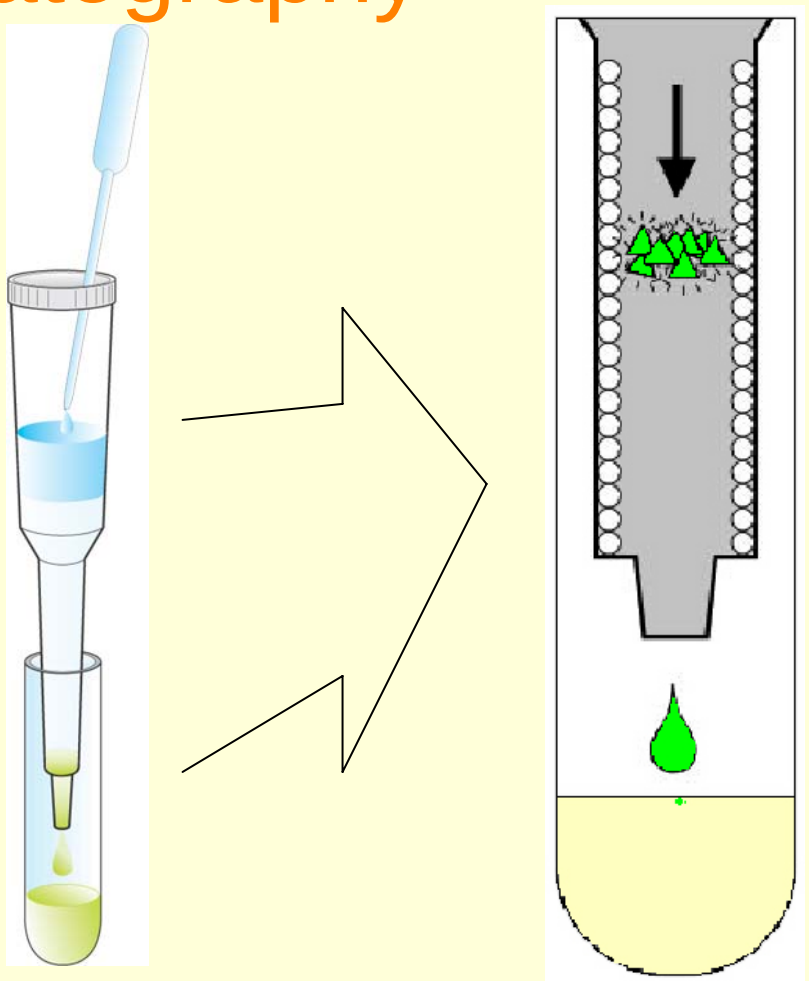
http://www.bio-rad.com/LifeScience/docs/Official_pGLO_GFP_powerpoint_Spring_2005.ppt

Step 3:
Hydrophobic Interaction Chromatography

- Elute GFP from column by adding no salt buffer

GFP

  - Released from column matrix
  - Flows through the column



Biopharmaceutical Manufacturing

- **Controlled Process**
  - **Reproducible**
  - **Consistent**
  - **Robust**
  - **Aseptic**
- **Product**
  - **Safe**
  - **Pure**
  - **Potent**
  - **Stable**

Protein Purification

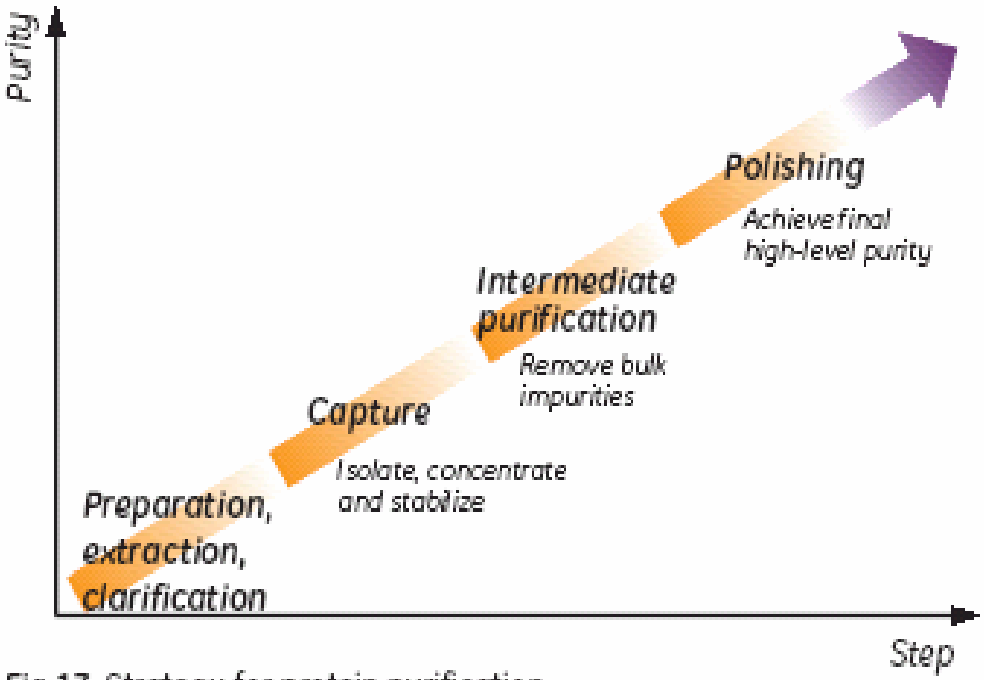


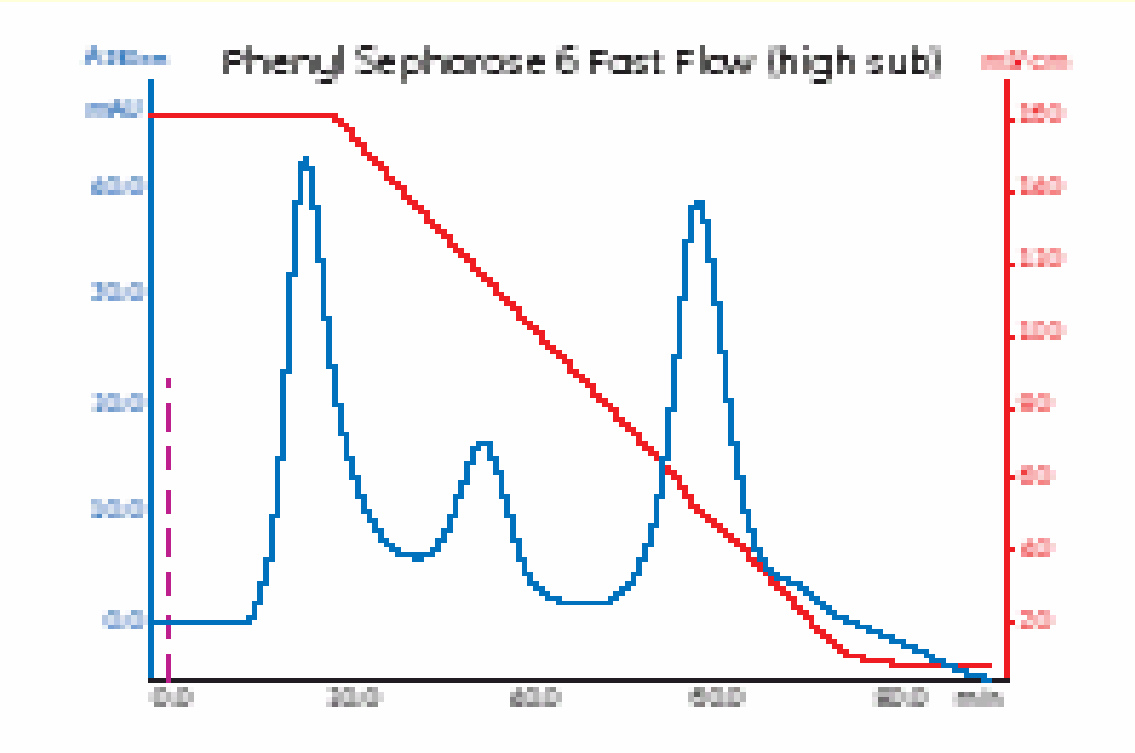
Fig 17. Strategy for protein purification.

http://www4.amershambiosciences.com18102229AD.pdf

http://www.pharmaceutical-technology.com/contractors/contract/neo/

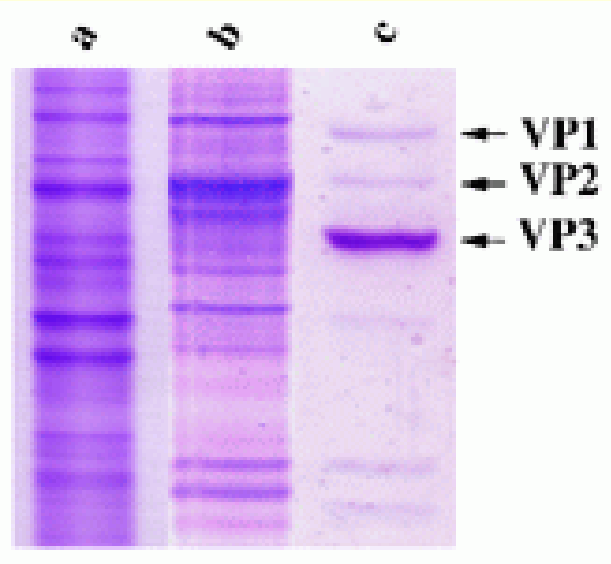
Center of Excellence for Regenerative Health Biotechnology

Chromatogram



http://www4.amershambiosciences.com18102229AD.pdf

Purity



http://www.genedetect.com/rave.htm

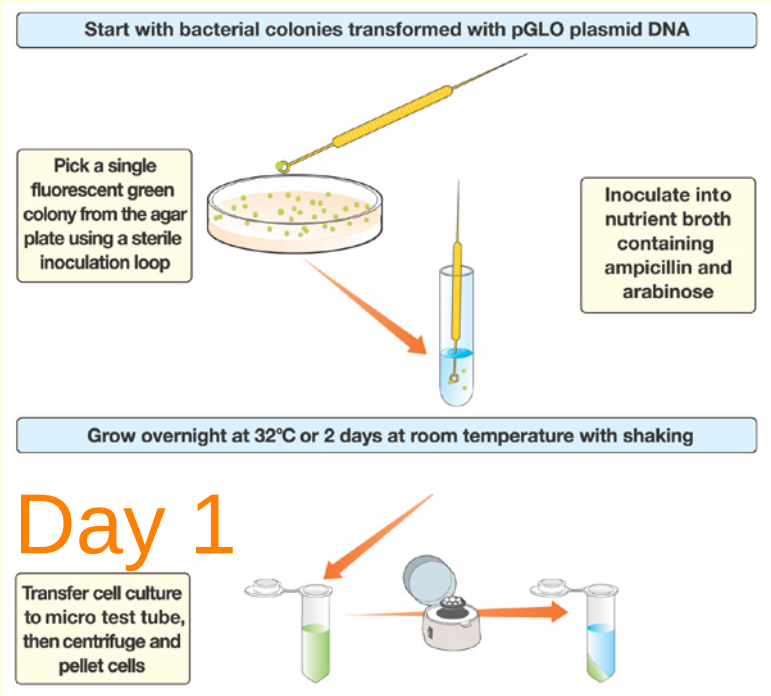
Process Development

1. Identification of target protein w/therapeutic value
2. Identification of target gene
3. Isolation of the target gene
4. Insertion of the target gene into a host cell (such as *E.coli*) & express protein
5. Purification: Separation of the target protein from the host cell protein

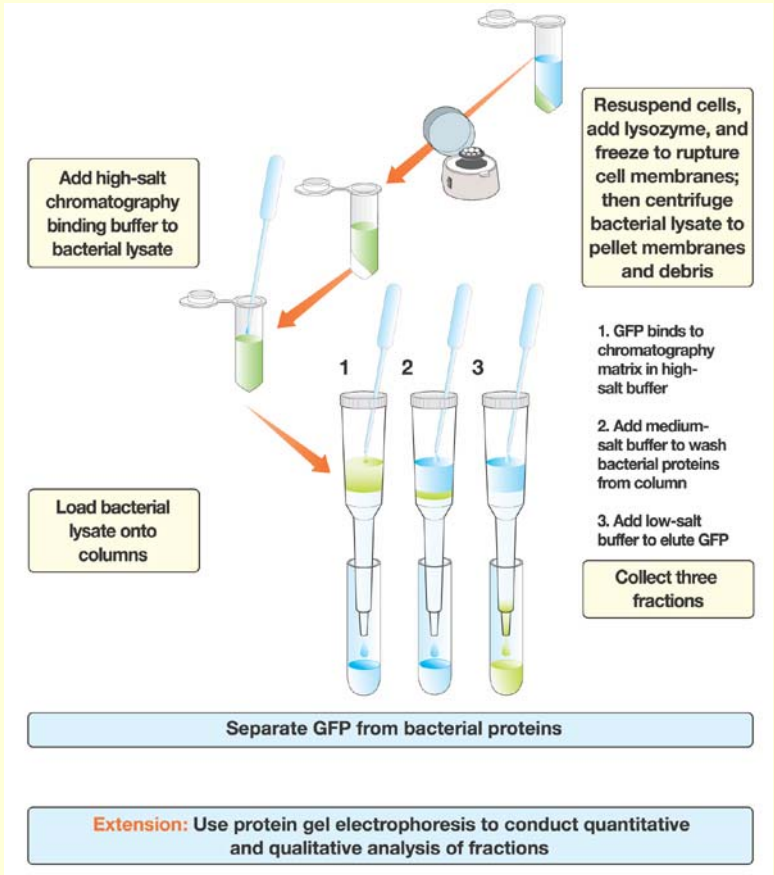
Process Development cont.

6. Large scale production of the target protein (under controlled manufacturing conditions)
7. Formulate
8. Testing for safety and efficacy
9. Marketing of a new medicine

GFP Purification Procedures



Day 2



Day 3

http://www.bio-rad.com/LifeScience/docs/Official_pGLO_GFP_powerpoint_Spring_2005.ppt

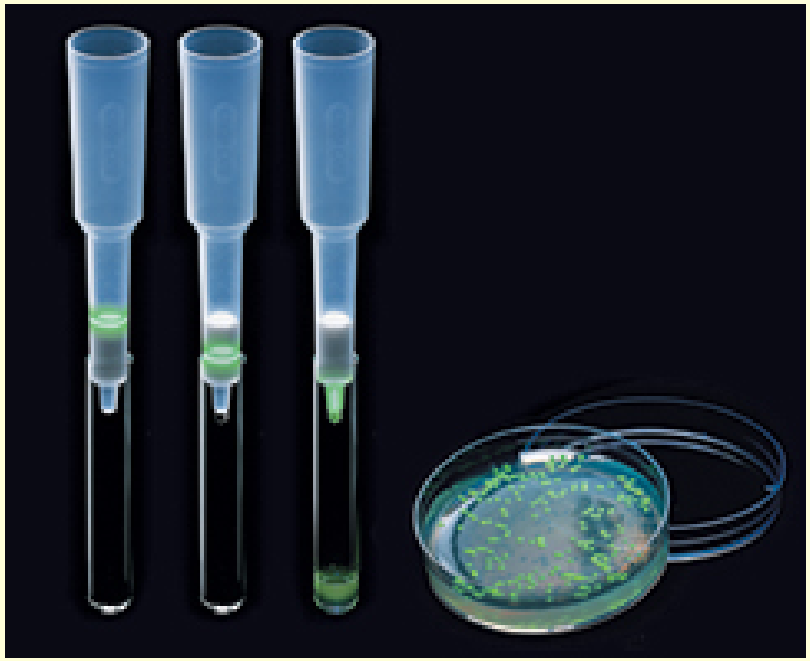
Hydrophobic Interaction Chromatography:

Steps 1–3

1. Add bacterial lysate to column matrix in high salt buffer

2. Wash less hydrophobic proteins from column in low salt buffer

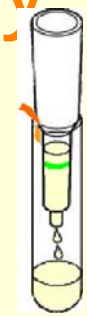
3. Elute GFP from column with no salt buffer



http://www.bio-rad.com/LifeScience/docs/Official_pGLO_GFP_powerpoint_Spring_2005.ppt

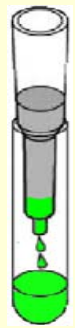
Helpful Hints:
Hydrophobic Interaction Chromatography

- Add a small piece of paper to collection tube where column seats to insure column flow



- Rest pipette tip on side of column to avoid column bed disturbance when adding solutions

- Drain until the meniscus is just above the matrix for best separation



This material is based upon work supported by the National Science Foundation under Grant No. 0438229.