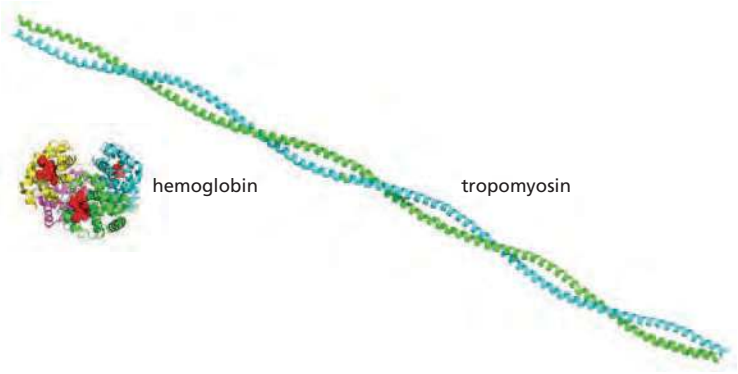
BIO-205-9

Discuss this in pairs

Isolation of cells from tissues, luorescence-activated cell sorting, and laser-capture microdissection are just a few of the ways for generating homogeneous cell populations. Why do you suppose it is important to have a homogeneous cell population for many experiments?

Tropomyosin, at 93 kd, sediments at 2.6S, whereas the 65-kd protein, hemoglobin, sediments at 4.3S. (The sedimentation coefficient S is a linear measure of the rate of sedimentation: both increase or decrease in parallel.) How is it that the bigger protein sediments more slowly than the smaller one? Can you think of an analogy from everyday experience that might help you with this problem?



Distinguish among ion-exchange chromatography, hydrophobic chromatography, gel-iltration chromatography, and affinity chromatography in terms of the column material and the basis for separation of a mixture of proteins.

Multiple choices

1. Which statement(s) are correct for *in vitro* studies?
   a. Transformed cell lines can be used only for short period of time, e.g. 10 passages max.
   b. Immortalized cell lines require to have overexpressed telomerase enzyme.
   c. Proteolytic enzymes facilitate the detachment and singularization of the cells.
   d. All cells require tissue culture dishes coated with extracellular matrix components.

2. Which of the following is the correct order to purify and identify your protein of interest from the cells?

a. Harvest the cells by using proteolytic enzymes, lyse the cells, centrifugation, column chromatography, mass spectrometry
b. Lyse the cells, column chromatography, harvest the cells by using proteolytic enzymes, mass spectrometry, centrifugation
c. Centrifugation, mass spectrometry, column chromatography, lyse the cells, harvest the cells by using proteolytic enzymes
d. Harvest the cells by using proteolytic enzymes, lyse the cells, column chromatography, centrifugation, mass spectrometry

3. Researchers use model organisms…

a. To understand the structure and functions of a gene/protein
b. To decipher the mechanisms of fundamental questions in biology, e.g. cell cycle.
c. To mimic human disease and discover or develop pharmaceutical applications
d. To reduce the amount of time and cost for the experiments

4. Which of the followings are INCORRECT about the methods to resolve the protein structures?

a. NMR spectroscopy is a suitable technique for the small proteins.
b. X-ray crystallography offers high resolution atomic position for larger protein structures.
c. Proteins should be crystallized for nuclear magnetic resonance (NMR) spectroscopy.
d. Cryo-EM makes it possible to work with proteins which are hard to crystallize.

5. You want to know the interaction partners of your protein of interest, let's call it "SHINE" and the size of your protein is 70 kDa. You have 1 possible candidate protein (45kDa in size), which you hypothesized that it might be interacting with SHINE. You want to test whether your candidate protein interacts with SHINE. You tagged your candidate with a green fluorescent protein (GFP) (25kDa) because you only have GFP antibody to detect your protein. Which method would you use to reveal the interaction of SHINE and GFP-tagged candidate protein? And, which size you expect to see your protein complex, if they interact with each other?

A. Immunoprecipitation and southern blot. Expected size of protein complex would be 70 kDa.
B. HPLC and western blot. Expected size of protein complex would be 140 kDa.
C. HPLC and SDS-PAGE. Expected size of the protein would be 45 kDa.
D. Immunoprecipitation and western blot. Expected size of the protein complex would be 140 kDa.

6. What are the differences between primary and secondary cell cultures?

    a. Primary cultures have infinite number of subculturing, while secondary cultures go through senescence much faster.
    b. Primary cultures are more heterogeneous than secondary cultures.
    c. Primary cultures resemble the *in vivo* conditions more than secondary cultures.
    d. Primary cultures are isolated directly from a tissue or organ, secondary cultures are derived from the primary culture by subculturing.

7. What is the primary application of hybridoma cell lines?

    a. Production of vaccines for viral infections
    b. Synthesis of recombinant proteins
    c. Large-scale production of monoclonal antibodies
    d. Generation of pluripotent stem cells

True or False

1. Monoclonal antibodies recognize different epitopes of a specific protein.

2. Basic Local Alignment Tool (BLAST) allows us to compare the protein sequences across different species and it gives information about protein structure and function across different species.

3. X-ray crystallography is one of the methods that we can utilize to reveal the structure of a protein of interest.

4. Sodium dodecyl sulfate and B-mercaptoethanol help to denature the protein structure for them to run through the PAGE properly.

5. Southern blot is used to detect proteins of interest, whereas western blot allows the researchers to visualize RNA.

6. Affinity chromatography is based on the ability of a protein to bind small molecules.

7. The cells can grow indefinitely on a tissue culture dish coated with extracellular matrix components.