

1. Does genome size equal the number of genes?  
In prokaryotes ONLY.  
It does NOT in eukaryotes
2. What is a microarray?  
Microarrays are used to detect the expression of many genes.
  - a. How does it work?
    - a. A microarray consist of DNA probes fixed to a solid support, such as a glass slide
    - b. Each spot has a different DNA probe
    - c. RNA is extracted from cells and reverse transcribed into cDNA and fluorescently tagged
    - d. The tagged cDNA will pair with any complementary probe
    - e. After hybridization the color of the dot indicates the relative amount of mRNA in the samples
    - f. A microarray can be constructed with thousands of different DNA probes
  - b. Why do you use extracted RNA?  
So you can detect the actively transcribed genes that are expressed rather than any junk DNA.
3. What is the function of mass spectrometry (“mass spec”)? Explain the technique.  
Mass spectrometry is used to identify a protein.  
A protein is treated with the enzyme trypsin, which breaks it into short peptides. The peptides are analyzed with a mass spectrometer, which determines their mass-to-charge ratio. A profile of peaks is produced. A computer program compares the profile with those of known and predicted protein. A match identifies the protein.
4. Another method of determining proteins is a 2D-PAGE. What does 2D-PAGE stand for and how does it work?  
2D-PAGE – two dimensional polyacrylamide gel electrophoresis  
Proteins are separated in one dimension by charge, separated in a second dimension by mass and then stained.
5. What is affinity capture?  
Affinity capture is when a specific antibody is made that binds to a specific protein. That protein can then be filtered out. The protein can then be determined using mass spec.
  - a. What is the interactome?  
The protein-protein interactions. Affinity capture can be used to identify the interactome.
6. What is a protein microarray?  
Instead of having probes bound to the glass slide, antibodies with fluorescents that will light up when bound are found in each spot.  
A collection of proteins from a tissue sample will be washed on the slide. If the protein binds the antibody fluorescence will light up indicating the expression of that protein.
7. What are the structural proteomics techniques that Dr. Riddle mentioned?
  - a. X-ray crystallography- diffraction patterns of the x-ray bombardment are used to determine the structure.
  - b. NMR- nuclear magnetic resonance with provides info on the position of specific atoms within molecules using magnetic properties.

8. Fill in the laboratory techniques we have learned about that detect for DNA expression and protein expression.

DNA Expression

Shot-gun sequencing

Microarray

Protein Expression

mass spectrometry

Protein microarray

Affinity capture

2D-PAGE

Structural proteomics