Think about the following problems and discuss them in pairs

You are set to run your irst SDS polyacrylamide-gel electrophoresis. You have boiled your samples of protein in SDS in the presence of mercap- toethanol and loaded them into the wells of a polyacrylamide gel. You are now ready to attach the electrodes. Uh oh, does the positive electrode (the anode) go at the top of the gel, where you loaded your proteins, or at the bottom of the gel?

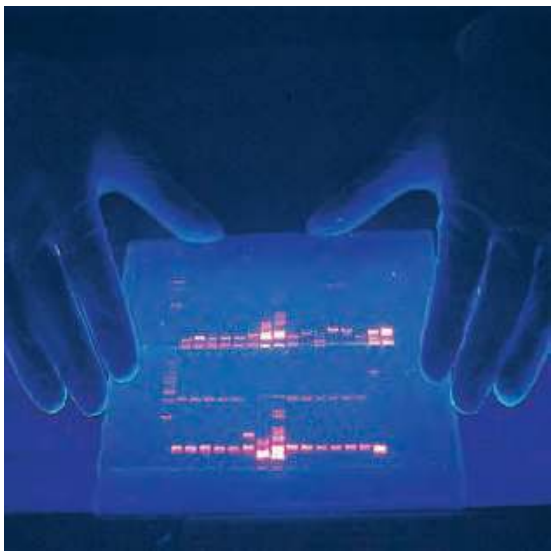
The detergent SDS carries a negative charge so that when it binds, the proteins become highly negatively charged. Because they are negatively charged, the proteins will move towards the positive electrode (the anode). Thus, it is critical that you place the positive electrode at the bottom of the gel. (Nearly everyone who has ever run a gel has attached the electrodes incorrectly, but usually once is enough.)

Hybridoma technology allows one to generate monoclonal antibodies to virtually any protein. Why is it, then, that genetically tagging proteins with epitopes is such a commonly used technique, especially since an epitope tag has the potential to interfere with the function of the protein?

Although it is invaluable, hybridoma technology is labor intensive and time consuming, requiring several months to isolate a hybridoma cell line that produces a monoclonal antibody of interest. Also, there is no guarantee that the cell line will produce a monoclonal antibody with the speciic properties you are after. It is much simpler—a few days' work—to add an epitope tag to your protein, using recombinant DNA technology, and use a commercially available, well-tested antibody to that epitope. he possibility that the tag may alter the function of the protein is a critical concern, but you can add the epitope easily to either the N- or C-terminus and test for the efect on the protein's function. In most cases, a tag at one or the other end of the molecule will be compatible with its function.

The figure shows a picture of DNA fragments that have been separated by gel electrophoresis and then stained by ethidium bromide, a molecule that fluoresces intensely under long-wavelength UV light when it is bound to DNA. Such gels are a standard way of detecting the products of cleavage by restriction nucleases. For the DNA fragment shown below, decide whether it will be cut by the restriction nucleases EcoRI (5′-GAATTC), AluI (5′-AGCT), and PstI (5′-CTGCAG). For those that cut the DNA, how many products will be produced? (8-59)

5′-AAGAATTGCGGAATTCGAGCTTAAGGGCCGCGCCGAAGCTTTAAA-3′ 3′-TTCTTAACGCCTTAAGCTCGAATTCCCGGCGCGGCTTCGAAATTT-5′



EcoRI will cleave the DNA once, generating two products; AluI will cleave the DNA twice, generating three products; and PstI will not cleave the DNA at all.

Multiple Choice Questions

1. Which of the following statements are **correct**?
    a. Fluorescence and Chemiluminescence are both triggered by light
    b. Excitation requires a smaller wavelength than the emitted fluorescence.
    c. FRET requires an overlap between the Emission Spectra of the donor and acceptor
    d. FRET requires an overlap between the Excitation Spectra of the donor and acceptor

Chemiluminescence is triggered by chemical reaction (slide 9), b) is True (slide 10), FRET requires an overlap between the donor's emission and the acceptor's excitation wavelength.

2. Mark the **wrong** statements:
    a. A combination of different techniques is often necessary to identify interacting proteins
    b. Using tandem affinity purification requires tagging of only one target protein through genetic modification
    c. Using tandem affinity purification requires tagging a target protein and its suspected binding partners through genetic modification
    d. After TAP-TAG the sample contains the TEV protease due to the elution step

The slides about CnoX illustrate how an array of methods can be combined. c) and d) are incorrect, the specific binding partners are not tagged (slide 6). d) most of TEV is washed out in the second affinity purification step.

3. When identifying interacting proteins using the yeast two-hybrid system with Gal4 BD and Gal AD:
    a. Binding between prey and bait is identified if the transcription of the reporter gene is stopped
    b. Binding between prey and bait is identified if the reporter gene is transcribed
    c. An antibody against the bait is essential
    d. The protein expressed by the reporter gene is purified to assess the outcome of the assay

Slide 7: If there is an interaction between prey and bait, GAL4 BD and Gal4 AD are in close proximity and bind the promotor region, thus activating transcription → b is correct. d) is false, the reporter gene may encode a protein that causes a visible color change (e.g. ß-galactosidase) or one that is essential for the yeast growth on deficient media.

4. You want to study a target protein X. Which of the following are true?
    a. The quickest way to solve the protein's structure is by crystallography
    b. Similarity between protein X and other proteins can be explored for free online
    c. There is no need to genetically modify protein X if you choose to use co-IP
    d. Protein X could dissociate from its interaction partners if samples are handled incorrectly during analysis

b), c), and d) are true

5. Which of these statements are correct?
    a. Next-generation sequencing has advanced beyond Sanger sequencing by employing nucleotides that stop elongation
    b. In Illumina sequencing, the different nucleotides dATP, aTTP, dGTP, dCTP are each washed off after meeting the target DNA so that they are never present at the same time.
    c. Next-generation sequencing relies on gel electrophoresis
    d. For Illumina sequencing the DNA sample is fragmented and each fragment is isolated on a bead, subsequently the addition of nucleotides to a single fragment on a beat is detected as the release of PP_i causes an optical signal

a) is false because Sanger sequencing relies on dideoxy nucleotides that terminate elongation. b) is true, c) is false, Next gen sequencing relies on fast readouts like the optical readout in Illumina sequencing. d) is false because each fragment is amplified and many copies are detected simultaneously on each bead.

6. Mark the wrong answers:
    a. Gene annotation is the process of genetically or chemically modifying a protein of interest to carry a tag for its identification among other proteins
    b. When reconstituting a genome, next generation Illumina sequencing is the best choice since it is large scale
    c. In PCR the copy number of the amplified DNA grows exponentially with each cycle.
    d. Between different people the repeat number of the same STR varies significantly

A is false, Gene annotation is not about modifying proteins; it's the process of identifying and describing genes within a genome sequence (slide 35). B) is also false, because long-read technologies are more suitable for the application.

7. What is the purpose of using SDS and Proteinase K used in DNA extraction?
    a. To precipitate the DNA
    b. To denature and degrade cellular proteins
    c. To separate DNA from RNA
    d. To neutralize the phenol-chloroform mixture

Slide 37. b is correct

<u>True or false</u>

1. FRET can be used to measure the distance of molecules if they are less than 10 µm apart

FALSE, FRET can identify if molecules are at very close distance. (Slide 8: <10 nm)

2. The binding partner of CnoX was identified based on the position of its band in SDS-PAGE

FALSE, Mass spectrometry was used to identify which protein caused the other band on the SDS-PAGE (Slide 23)

3. Co-immunoprecipitation can only be done if you already have an antibody that binds the protein you want to study

TRUE, slide 5

4. DNA precipitates in the presence of phenol-chloroform

FALSE, slide 37

5. During the pandemic PCR tests were used to directly amplify the RNA of SARS-CoV-2 to diagnose people

FALSE, in PCR tests the RNA was reverse transcribed and the cDNA was amplified

6. We are testing a suspect's DNA in a murder case and look at 6 different STRs. After PCR we expect to see 12 bands in gel electrophoresis.

TRUE, slide 44

7. In PCR, the annealing temperature is typically higher than the denaturation temperature.

FALSE