College of the Canyons: Introduction to Biotechnology

Protein Gel Electrophoresis: Post Lab Write Up

1. Name three reasons why vertical gel rigs give better resolution than agarose gels.
   Vertical gel rigs often have polyacrylamide at a % much higher than agarose (25% vs. 4%), Vertical gel rigs can be stratified (4% onto 6%, etc.), Easier to load, Gravity works with gel loading etc.

2. Describe how a PAGE gel can be stratified.
   The PAGE gel can be poured in a series with different amounts or polymers, cross linker agents, etc. One gel is poured to fill about 30 % of the cartridge and allowed to solidify. After that another, and another layer is added. The result is a stratafied gel.

3. What is the maximum concentration of agarose gels? PAGE gels? Why is a greater concentration possible in PAGE gels?
   Agarose is dissolved and the gel forms when cooled. You can easily dissolve up to about 4% (4 gram/100mls), PAGE gels are polymerized and the monomers are very soluble (up to 30%), so when you polymerize in the rig (the box that hold the gel). Some agaroses can exceed 4 %, but the gel itself starts to get opaque, causing difficulty when it comes to seeing the gel bands.

4. Why are the gel wells with the Triton 100 Tris/ Glycine buffer placed in the middle of the gel? What does this tell us about the charge on proteins?
   The triton 100 only solubilizes proteins. The charges of the proteins are unaffected so the direction of migration is variable. Hence the comb is placed in the middle to accommodate for + or – charged proteins.

5. Often proteins can change their "chemistry" in the gel box. This is due to the electrophoretic field altering the pH. Show what functional groups are pH sensitive and how the shift in pH can alter a protein's migration pattern (diagram, doodle, etc. ***hint***, look at old pH quiz..). Show two such examples…. For instance, at the negative electrode, electrons are coming out and these electrons can reduce functional groups, which could affect charges. For example: COO- could become COOH or NH2 could become NH3 +. Since these two functional groups as the basis of amino acids, they are very numerous in proteins. As a result. The pH shifts between the negative and postive pole. Buffers help stop this shift, but in some cases, buffers are NOT used. This allows for a process called "isoelectric focusing" to be conducted. See the text for more details on isoelectric focusing.

6. Revisit the two detergents and cite their specific chemical structure. How does this structure impart the properties that are associated with either Triton Gels or SDS gels? A diagram and/or doodle may be very useful. Triton is non polar with only a weak charge disparity, As a result, the detegent in not strongly amphipathic. As part of the Triton dissolves in the protein (non-polar parts in the non-polar domains), the slightly polar part faces outwards towards the water. Since this parts lack a strong charge, the protein's charge is unaffected. The SDS will act like a phospholipid. The non polar part inserts itself into the non-polar parts of the protein. The sulfate then sticks outward and gives the protein a strong, negative charge. SEE BELOW DIAGRAMS:

7. When loading a sample into the vertical gel rig, why must you be careful to stops the plates from separating?
   Do not force the pipet tip between the plates. This will cause sample to spill in the gap between gel and plates. As a result, it will effectively run "outsid" the gel, beween the gap created between the plate and the poly-acrylamide.

8. With a gel of protein diversity, how can you determine what different varieties of fish the fake “Krab, AKA surimi” is made of? Name (do not describe if gory detail) three pieces of information the gels provide
Compare lanes for presence/absence of bands. Look also at band intensity, number, distance migrates, etc.

9. Assume that the fake “ Krab” is made from a processed fish. This processing often involves cooking the fish. What effects should this have on the fish? After learning what fish is the basis of fake Krab (ask the instructor) revisit the gel information and see if this makes sense. What fish is the basis of fake Krab?
Does the PAGE gel support this? The baked fish (surimi), typically has fewer bands. Most surimi is processed from cod, so compare this to the cod sample provided in the gel samples.

The Triton gels? Same idea as PAGE gel idea above, just few bands seen with the Triton sample.