

Lab report electrophoresis assignment



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Other factors that can affect the mobility are electric-field strength, matrix pH, and ionic buffer strength of the electric field. Because there are so many factors involved in analyzing proteins during electrophoresis, it is unusual for two different proteins to have the same relative mobility (G?? meg, 1998). In this experiment, *Drosophila melanomas*, *Drosophila virile*, as well as a marker strain (mutant strain of *D. Melanomas*) were used to examine the genetic variation.

Electrophoresis followed by the staining of the proteins will cause the enzymes, allayed sides, alcohol dehydrogenate, and emulate dehydrogenate, to become visible, appearing as a set of different banding patterns. The banding patterns will dependent on the molecular form of the enzyme, indicating the genetic variation that can exist between strains (Biology Department, 2014). For this experiment, hypothesize that I will see differences at the biochemical level due to the morphological differences that exist within these three strains.

My prediction to test my hypothesis is that the banding patterns between the strains will be different. Materials and Methods allowed the preparation and the extraction procedure according to the lab manual (Biology Department, 2014). Results observed that all the three different enzymes Emulate dehydrogenate (MAD), Allayed oxides (AAA), and Alcohol dehydrogenate (ADD) displayed different banding patterns. Figure 1: Cellulose Acetate gel plate stained with Emulate dehydrogenate (MAD) showing banding patterns.

The photo on the left shows the plate with the stain and the photo on the right show the plate with the banding pattern after the stain is washed off.

Figure 2: Cellulose Acetate gel plate stained with Allayed oxides (AAA) showing ending patterns. The photo on the left shows the plate with the stain and the photo on the right show the plate with the banding pattern after the stain is washed off. Figure 3: Cellulose Acetate gel plate stained with Alcohol dehydrogenate (ADD) stain is washed off. The order of my banding patterns started first with the wild type (*Drosophila melanomas*), marker strain, and then *D. Rills*. I observed the wild type (first band) to be the same in all three enzymes, appearing as a single band that was quite dark. For the marker strain, it was different for all three enzymes. For MAD, there was just a single band (Fig 1). For AAA, it appeared as 2 bands with the top band being slightly darker than the bottom band (Fig 2). And lastly for ADD (Fig 3), I observed three bands, with the one of the bands being darker than the other two. For the *D. Virile*, two bands were observed for AAA, a dark single band was seen for ADD, and then another dark single band was observed for the MAD enzyme.

In general, for all three enzymes, the banding patterns appeared as a setoffs 12 columns, with the banding pattern repeating itself after the first three columns. Discussion A few assumptions can be made for this experiment. One is that the difference in mobility between enzymes is a reflection of the genetic variation that exists within the amino acid sequences of the genes encoding for the proteins (Gomez, 1998). I can assume from observing the varying banding patterns between the enzymes, that these variations are due to the genetic differences.

The second assumption is that of the occurrence of codename with a dominant and recessive allele appearing together. This is one of the

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advantages of this system because it allows us to confirm heterozygous. The net charge of a protein is based on its amino acid sequence. To study the genetic variation existing in organisms, the DNA sequences, or the proteins coded for by the DNA can be examined (Biology Department, 2014).

However the method we are using does not always reveal all the variability present. All nucleotide variants in a gene does not result in changes to the proteins net charge (Gomez, 1998).

An example of this is silent mutation. Silent mutation happens when a single nucleotide is substituted by another base, resulting in a different code that still codes for the same amino acids. Therefore, even if amino acid sequence remains unchanged, the actual DNA sequence changes. Also, different banding patterns can occur from non-genetic changes such as post-transcriptional modification, enzyme degradation, denaturation or activation, oxidation of sulfhydryl groups, or even aggregation of a protein (Gomez, 1998).

Change in pH can alter the net charge of a protein. A protein's mobility within the gel changes due to its net charge and therefore changing the pH used in the electric field may show more variability. If genetic variation does exist within the strains, I would think that by running these strains at different pH levels, unique reactions of the enzymes may occur, allowing more variability to be seen. The banding patterns I observed depend on the quaternary structure of the protein, that is, the number of polypeptide subunits it is composed of (Gomez, 1998).

The enzymes looked at in this experiment are dimmers. Dimmers are when an enzyme consists of two polypeptide chains. At this locus, homozygous appear as a single band, and the heterozygous have 3 bands (Biology Department, 2014). As mentioned above, since codename is assumed to be occurring, the appearance of three bands presents an allocate designated for a fast allele or an allocate designated for a slow allele, as well as a fast/slow allele (middle band) (Laurie & Steam, 1988).

For ADD, the wild type single band represents homozygous; the three bands observed for the marker strain represent heterozygous at the demerit loci; and the single band for D. Virile again represents homozygous. For MAD, all three strains appeared as single bands indicating homozygous for all of them. Lastly, for AAA the wild type strain appeared as a single band representing the characteristic homozygous, and the marker strain and D. Rills had two bands indicating heterozygous at the monomer loci.

The expected ratio of banding patterns for a dimer is 1: 2: 1 with the “ 1” representing the splitting of the Homeric bands into two, and “ 2” representing the heterocyclic band (fast/ slow allele), giving a total of three bands (Micelles & Bonded, 1995). The deviation seen with AAA can be a result of instances where a polypeptide will not contribute equally to the activity of the enzyme due to either: a slower rate of synthesis, low stability, or a tendency to break down before it can be assembled into the final enzyme. This can result in not seeing the expected ratios (Micelles & Bonded, 1995).

For the AAA enzyme, there is one allele for the D. Melanges (homozygous single band) and two alleles for both the marker strain and D. Virile (heterozygous multiple bands). For the ADD enzyme, there is one allele present for both the wild type strain and D. Virile; and two alleles present for the marker strain. Lastly, for MAD, there is one allele present for each strain. In conclusion, as previously stated if different banding patterns are observed, I can relate this to my hypothesis in that the three strains must be different at the biochemical/ electorol level.