Western blotting essay sample

Abstract

Western Blotting can be used to detect the Myosin actin light chain in different species of fish and is used to distinguish from different species based on variation, commonality, or evolutionary divergence. First, proteins are extracted from the tissue and loaded into a gel matrix. The matrix will separate the proteins according to size using an electric current. Proteins that are separated after are blotted from the gel and onto a paper membrane. An antibody is then added to the membrane paper and causes a colored reaction. Following the reaction, the results help detect and quantify a single protein among hundreds of other proteins in the sample. Western blotting is used during this procedure to test that proteins can be indicators of genetic and evolutionary similarity. Results show that the different species of fish contain the myosin light chains that are equivalent in molecular mass, which then means they are similar in their evolutionary relationships.

Western Blotting is used to Identify a Subunit of Myosin Light Chain in the Proteins of All the Different Fish. Introduction

Western blotting is a technique in biological research that allows scientists to identify and quantify specific proteins among a protein mixture. The method that is used is a “ protein mixture is applied to gel-electrophoresis in a carrier matrix (SDS-PAGE) to separate a protein by size and charge. Next, the separated protein-bands are transferred into a carrier membrane. The proteins are then accessible for anti-bonding in order to detect them” (Antibodies-online. com, 2012). Myosin is a muscle protein that is essential to animals for survival and has remained stable over time. The myosin light chain can be compared from different species for evolutionary divergence

and similarities. The objective of this study is to test that proteins can be markers to help relate genetics and evolution within a species of fish. Western blotting is used to identify a myosin light chain from the proteins containing muscle tissues of different kinds of fish and tests that proteins are indicators of genetic and evolutionary likeness (Department of Biological Sciences, 2013).

Method

The lab will begin with preparing muscle protein extracts by removing proteins from the muscle tissues of different fish. First, muscle tissues from different species of fish are added to five 1. 5 ml fliptop micro tubes that are labeled. Laemmli sample buffer is then added to each tube. After, the micro tubes were flicked for a numerous amount of time to agitate the tissue in the sample buffer. The next step was put the tube in the incubator for five minutes at room temperature. Once it cooled down the buffer was extracted from the tube, leaving the fish sample still in there. The fish sample was then heated at 95°C for five minutes.

The second part of the lab is to separate the proteins using SDS-PAGE. The first step is to set up the apparatus that will be used in the experiment; which this is the polyacrylamide gel electrophoresis. A Ready Gel cassette is prepared and is placed into the electrode assembly. The electrode assembly is then placed in the inner chamber of the tank and filled with TGS gel running buffer. Once this is complete, heat the fish samples and actin and myosin standards for 2-5 minutes at 95°C and then load the gel. Run the electrophorese for about 30 minutes at 200 V. When the time is up remove

the gel from the cassette and transfer it into a container with 25 ml Coomassie stain and let it stain for one hour with gentle shaking.

The previous two steps were extracting the proteins and separating them by their size. The remaining of the lab deals with the usage of antibodies to help identify the myosin light chain in the tissue. First, chop off the bottom and top of the gel and balance the gel in blotting buffer for 15 minutes on a rocking platform. Next, soak the fiber in the blotting buffer. Following that, a blotting sandwich is made by adding blotting buffer to the container and inserting the plastic cassette. Lay a wet fiber pad on the black side of the cassette. On top of that lay a wet blotting paper on the fiber pad and then roll a pen lightly across to get the air bubbles out. Place the gel on top of the previous layer and then lay a wet nitrocellulose membrane on the gel. Another wet blotting paper and fiber pad are placed on top of the gel. Once this is complete, close the cassette and set up the Mini Trans-Blot module. Fill with blotting buffer up to the top and then blot for 2. 5 hours at 20V.

The last part of this procedure, antibodies will be used to detect one specific protein from others on the membrane. Incubate the membrane with 10 ml of primary antibody for 10-20 minutes and then place on a rocking platform. Rinse the membrane quickly after with wash buffer on a rocking platform. After three minutes is up, discard the wash and incubate the membrane with 10 ml of secondary antibody for 5-15 minutes on the platform. Rinse the membrane again shortly after and wash the membrane for three minutes. Next, discard the wash and add 10 mL of HRP color detection reagent. Incubation will occur after this for 10-30 minutes. Once it is done, rinse the membrane twice with distilled water and blot dry.

Results

According to Figure 1, all of the protein bands of the different fishes migrated to identical positions as the actin and myosin. Each band in every row represents a specific protein. The distance the bands moved are dependent on the size and molecular weight of the proteins. Smaller proteins move farther and faster through the gel than larger proteins. Figure 2 and 3 demonstrates that the different fish proteins migrated around the same distances and have similar molecular masses of myosin.

Figure 1: Picture of the gel after Western Blotting that is used to detect the myosin light chain. Molecular weights of the myosin light chain were blotted for comparison. The molecular weight standard is indicated in kilodaltons.

Figure 2: A standard curve used to measure and record the distances of the protein bands. The graph is used to determine the molecular masses of different myosins by comparing the mobility of unknown proteins with protein standards that are in the same gel. The molecular mass is plotted on the y-axis against the distances migrated for each protein on the x-axis. SAMPLE NAMETunaCatfishPollockSalmonTilapiaMahi-MahiCodLake PerchA/M| Estimated Mr from Blot252020202025252525| Distance Migrated, mm75 mm78 mm78 mm80 mm78 mm72 mm74 mm74 mm74 mm| Calculated Mr1. 3981. 301. 301. 301. 301. 3981. 3981. 3981. 398| Distance Migrated (mm)

Distance Migrated (mm)

Protein Molecular Mass (kD)

Protein Molecular Mass (kD)

Figure 3: A table showing the different fish samples and a calculated Mr for each one. The table can be used to plot a graph to compare molecular weight vs. the distance the protein migrated.

Discussion

Using western blotting actin myosin can be identified from a mixture of proteins by using antibodies. During the procedure of the gel electrophoresis, the proteins are negatively charged from the SDS in the Laemmli sample buffer. SDS is a negatively charged molecule that sticks to the polypeptide chain and adds a negative charge to allow the proteins to migrate through the gel according to their molecular mass. The smaller that the myosin light chain is, the faster it will migrate because smaller proteins can move through the gel matrix more quickly. Blotting is the next step in the procedure and the applied electric charge causes the proteins to travel out of the gel and onto the membrane. “ The protein-bands are bounded to the membrane and are now available for the identification of specific antibodies” (Antibodies-online. com, 2012). e

Western Blotting and other protein calculations helped concludr6e that all the proteins from the different types of fish contained the myosin actin light chain. The like bands show that the antibodies used in this experiment recognize a “ specific acid sequence common to most myosin light chain proteins that are in all sorts of animals” (Cell Signaling Technology, 2012). The variation in the proteins, display the evolutionary relationships between the different species of fish. The protein profiles of the fish, shows that the myosin light chains of each fish are similar and all of the species are closely related. “ The primary structure of the protein subunits all remain relatively

the same because changes in the structure can affect the function of myosin. This could decrease the chance of survival for that animal or provide evolutionary advantages” (Department of Biological Sciences, 2012).

References

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