MOLECULAR BIOLOGY LAB MODULE
Location: 3110 DCL
Lead Instructor: Jenny Amos, Bioengineering
Lab Assistant: Kenny Long, Bioengineering

Analysis of DNA for Determination of Blood Type

Objective

This activity provides in-depth instruction about how restriction enzymes cleave DNA, how electrophoresis is used to separate and visualize DNA fragments, and how these techniques can be combined to obtain a DNA fingerprint. Principles of restriction analysis, plasmid mapping and DNA fragment size determination will also be discussed.

Background

Genetic markers are seen on DNA and can be used to identify genes, but single nucleotide polymorphisms (SNPs) can also be used to identify genotype or location of a gene that causes a specific disorder. In order to location SNPs, restriction enzymes are used.

A *restriction enzyme* acts like molecular scissors, making cuts at a specific sequence of base pairs that it recognizes. A restriction enzyme binds a DNA molecule and slides along the strand until it recognizes specific sequences of base pairs that signal the enzyme to stop sliding. The enzyme then cuts or chemically separates the DNA molecule at that *restriction site*. DNA that has been cleaved with restriction enzymes can be separated by length and observed using a process known as *agarose gel electrophoresis*.

Gel electrophoresis is a technique used to separate and view macromolecules, such as DNA, RNA, and proteins. DNA is colorless so DNA fragments in the gel cannot be seen during electrophoresis.
A sample loading buffer containing two bluish dyes is added to the DNA samples, which are then loaded into a gel made of agarose. Agarose serves as a loose mesh through which small molecules travel faster than larger molecules. When a current is applied across the gel, there is a separation of the macromolecules, based upon their mass, which is observable in visible bands. Staining the DNA pinpoints its location on the gel.

Lesson 1 Restriction Digestion of DNA Samples (~1 to 1.5 hours)

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.
2. Label one microcentrifuge tube for each of your samples: CS, S1, S2, S3, S4, S5
    a. Label should have sample name as well as Group ID and Table #
3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored microcentrifuge tubes. Make sure the sample is transferred to the bottom of the tubes.
4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube. Pipet up and down carefully to mix well.
5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.
6. Incubate the tubes for 45 min at 37°C.

7. Spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube.
8. After the incubation period place the tubes in the refrigerator until the next laboratory period.

Lesson 2 Electrophoresis of DNA Samples (~1 hr)

1. Spin the tubes in the centrifuge to bring all of the liquid into the bottom of the tube.
2. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by spinning in a centrifuge.
3. Place the agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel
4. Check that the wells of the agarose gels are near the black (–) electrode and the bottom edge of the gel is near the red (+) electrode.
5. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:
   Lane 1: S, DNA size standard, 10 µl
   Lane 2: CS, green tube, 20 µl
   Lane 3: S1, blue tube, 20 µl
   Lane 4: S2, orange tube, 20 µl
   Lane 5: S3, violet tube, 20 µl
   Lane 6: S4, red tube, 20 µl
   Lane 7: S5, yellow tube, 20 µl
6. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid of the horizontal electrophoresis chambers will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.
7. Turn on the power and run your samples at 100 V for 30 minutes.

Lesson 3: Analysis of Results (~1 hr)

In order to process the results, the gel is soaked in FastBlast. This stain will show where the bands are in the gel.

1. *Stain gel (2–3 min)*

Pour ~100 mL of 100x stain into a gel staining tray. Remove the gel from the gel tray and carefully slide it into the staining tray containing the stain. If necessary, add more 100x stain to completely submerge the gel. Stain the gel for 2 min. Using a funnel, pour the 100x stain into a storage bottle and save it for future use. The stain can be reused at least 7 times.

2. *Rinse gel (10 sec)*

Transfer the gel into a large container containing 500–700 ml of clean, warm (40–55°C) tap water. Gently shake the gel in the water for ~10 sec to rinse.

3. *Wash gel (5 min)*

Transfer the gel into a large container with 500–700 ml of clean, warm tap water. Gently rock or shake the gel on a rocking platform for 5 min. *Repeat this twice!*

4. *Analyze results*

Examine the stained gel for expected DNA bands. The bands may appear fuzzy immediately after the second wash, but will begin to develop into sharper bands within 5–15 min after the second wash. This is due to Fast Blast dye molecules migrating into the gel and binding more tightly to the DNA molecules.

References:

Biotechnology Explorer™ Forensic DNA Fingerprinting Kit Instruction Manual Catalog #166-0077EDU