MOLECULAR BIOLOGY LAB MODULE
Location: 257 MNTL
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Analysis of DNA for Determination of Blood Type

Objective

Students will isolate DNA from cells in hair follicles. The DNA will be used as a template for PCR and the PCR product will be digested to determine the blood phenotype of each individual.

Background

Polymerase chain reaction (PCR) is a technique used in molecular biology, genetics, and clinical diagnostics. The technique amplifies small amounts of DNA to make millions of copies. DNA is this lab will come from a hair sample. The process involves the design of a label, called a primer, a buffer to carry out the reaction, and a device used for heating and cooling the mixture to amplify the sequence for detection. Using PCR of the ABO blood group phenotype with primers designed for each allele will allow us to do blood type determination without the use of a blood sample.

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.

Gel electrophoresis is a technique used to separate and view macromolecules, such as DNA, RNA, and proteins. During gel electrophoresis, the macromolecules are loaded into a gel made of agarose. Then a current is applied across the gel. The result is a separation of the macromolecules, based upon their mass, which is observable in visible bands.

ABO blood groups were first identified in 1900 and serve an important role in the medical field for blood transfusion, organ transplants and other procedures. The phenotype refers to antigens on the surface of the red blood cells, the body produces antiantibodies to the antigens which cuase an individual to have an immune reaction to blood cells of other types.

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

The ABO locus, which is located on chromosome 9, contains 7 exons that span more than 18 kb of genomic DNA. Exons 6 and 7 are the largest of the set. In the case of the O allele, when compared to the A allele, exon 6 lacks on nucleotide, which results in a lack of enzymatic activity. This is why we can identify the blood type by using only exons 6 and 7.
There are also subgroups made up of mutations and heterozygous combinations, which we can identify using PCR or

sequencing. A has two subgroups A1 and A2, B has no subgroups, and O has 2 subgroups, O1 and O2. The following combinations are possible.

	A^1, A^2	A^1, B	A^1, O^1	A^1, O^2	A^2, B	A^2, O^1	A^2, O^2	B, O^1	B, O^2	O^1, O^2
309 bp	++	++	++	++	++	++	++	++	++	++
223 bp		+			+			+	+	
204 bp	++	+	++	++	+	++	++	+	+	++
145 bp	+				+	+	+			
137 bp		+		+	+		+	+	++	+
119 bp	+	++	++	++	+	+	+	++	++	++
96 bp	++	+	++	+	+	++	+	+		+

+ indicates that one allele will produce the fragment and ++ indicates that both alleles will produce the fragment.

Obtain DNA

We will obtain DNA from hair samples,

1. Obtain 1 screwtop tube containing 200 uL of Instagene matrix plus protease and table with your initials.
2. Collect 2 hairs from yourself. Select hairs that have a noticeable sheath (cells bound to the hair surface near the base of the hair) or a root. Trim the hair leaving ~2 cm of the base of the hair. Place the trimmed hairs in the screwtop.
3. Place the tube in the floating foam tube holder in a 56C water bath for 10 minutes. At the halfway point, remove the tube and shake vigorously or vortex then place back in the bath.
4. Remove the tube after 10 minutes and shake or vortex again.
5. Spin the tube at 6,000 X g for 5 minutes (or 2,000 X g for 10 minutes).

PCR

PCR amplifies DNA. Using primers, we will be able to identify specific regions, SNPs, to determine blood type.

1. Label a PCR tube and a capless mirco-tube with you initials. Place the PCR tube in the capless tube.
2. Transfer 20 uL of DNA template (supernatant) from the screwcap with DNA into the bottom of the PCR tube. Do not transfer any beads into the PCR tube.
3. Add 20 uL of the PCR MasterMix (yellow cap in ice bath) into the PCR tube. Pipette 2-3 times to mix. Cap the tube tightly.
4. Place into the thermal cycler for 40 cycles of PCR amplification.

Prepare tubes with cDNA, primers, and supermix reagent from kit.

13 uL supermix with Taq
2 ul cDNA
1 uL 3' Primer
1 uL 5' Primer
8 uL nuclease free water

25 uL reaction

Run a separate PCR reaction with your DNA for both Exon 6 and Exon 7 primer sets – you should have 2 tubes! Program the thermal cycler according to the PCR protocol

Exon 6

1. 94C for 45 seconds
2. 53C for 45 seconds
3. 72C for 45 seconds
4. Repeat steps 1-3 for 35 cycles
5. 72C for 5 minutes (this step ensures complete synthesis of all strands)
6. 4C indefinitely

Exon 7

1. 94C for 45 seconds
2. 60C for 45 seconds
3. 72C for 45 seconds
4. Repeat steps 1-3 for 35 cycles
5. 72C for 5 minutes (this step ensures complete synthesis of all strands)
6. 4C indefinitely

Transfer to freezer for long-term storage.

Digestion

In order to determine the bands, we need to cut the PCR product using enzymatic digests.

The total mixture will look like this

	10X Buffer	PCR Product	10X BSA	Enzyme
Exon 6 (K)	1.5 ul Promega J	11.5 uL exon 6	1.5 uL	0.5 uL Kpn I
Exon 7 (H)	1.5 uL Promega A	11.5 uL exon 7	1.5 uL	0.5 uL Hpa II

Premix Solution (per 2 students)

	10X Buffer	10X BSA	Enzyme
Exon 6 (K)	6 uL Promega J	6 uL	2 uL Kpm I
Exon 7 (H)	6 uL Promega A	6 uL	2 uL Hpa II

Reagents should be added in the following order, 10X buffer, 10X BSA, enzyme

Make the digests by combining 3.5 uL of the premix for the corresponding digest enzyme and adding 11.5 uL of PCR product to the tube. Centrifuge briefly to combine. Place all of your reactions at 37C for 2 hours. Once completed, store the digest at -20C.

Electrophoresis Gel Technique

To obtain results from the PCR product, a gel must be run.

Exon 6

We will use a pre-cast 1% agarose gel in TAE buffer. The samples are prepared using
15 uL Digested Exon 6 PCR product
3 uL of Loading Buffer
The samples are loaded into the channels in the gel and run for 40 minutes at 150V.

Exon 7

We will use a pre-cast 4% agarose gel in TAE buffer. The samples are prepared using
15 uL Digested Exon 7 PCR product
3 uL of Loading Buffer
The samples are loaded into the channels in the gel and run for 100 minutes at 95V.

Run your samples in the same lane on each gel to keep track. See you instructor or TA for your lane assignment.

Quick Staining of Agarose Gels in 100x Fast Blast DNA Stain

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

1. *Prepare 100x Fast Blast DNA stain*

Dilute 100 ml of 500x Fast Blast DNA stain with 400 ml of deionized water in an appropriately sized flask and mix. Cover the flask and store at room temperature until ready to use.

2. *Stain gel (2–3 min)*
Pour 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 2–3 min, but not for more than 3 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.

3. *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.

4. *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 (in 202 lab) or gentle rock by hand for 5 min.

5. *Wash gel (5 min)*
Perform a second wash as in step 4.

6. *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. To obtain maximum contrast, additional washes in warm water may be necessary. Destain to the desired level, but do not wash the gel in water overnight.

7. *Record your results.*
Take a picture of your gel and note the ladder spacing for analysis. See your instructor or TA for help identifying your blood type using known samples. Examine Exon 6 results first, since this will split the O and A groups.

Record your bands here:
Exon 6

Sample	K digest

Exon 7

Allele	A1	A1v/A2	B	O1	O2
309 bp					
223 bp					
204 bp					
145 bp					
137 bp					
119 bp					
96 bp					