Teacher Reference Guide

DNA Fingerprinting workshop
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DNA Fingerprinting Workshop schedule

9:00 a.m. Class arrives at the Ontario Science Centre (OSC)
9:15 a.m. School group check-in - meet the educator on Level 2 near the group check-in desk
9:30 a.m. DNA isolation / PCR preparation
10:50 a.m. Break - 10 min.
11:00 a.m. Lesson - Morning protocol, DNA structure and Polymerase Chain Reaction, PCR activity (hands-on)
12:00 p.m. Lunch / explore OSC
1:00 p.m. Electrophoresis explanation / Assemble gel boxes and load mock samples
1:45 p.m. Gel loading and K'NEX model building at student benches
2:30 p.m. Lesson - Electrophoresis theory, D1S80 locus, and applications of DNA fingerprinting in the justice system, staining and visualization
3:15 p.m. Gel staining and visualization (educator) (students on break - 30-45 min.)
4:00 p.m. Results, analysis and conclusion
4:30 p.m. End of workshop
4:00 p.m. OSC closes to the public

PLEASE NOTE: This schedule is not flexible due to the time involved in the PCR process which cannot be reduced. Failure to arrive on time for the workshop may preclude the students from being able to see their results before they leave. If it is impossible for the group to see their results by 4:30 p.m., we will forward the photographic results to your school.
DNA Fingerprinting Protocol

DNA ISOLATION TECHNIQUE:

1. Select a micro-centrifuge tube containing 300 µl of a solution of Chelex / Proteinase K solution.
2. Wearing gloves, obtain a cheek swab from the inside of your cheek (scrub for 10 seconds).
3. Do not place the cheek swab on the bench.
4. Place the cheek swab in the Chelex / Prot K solution.
5. Twirl the cheek swab for 10 seconds.
6. Incubate the Chelex samples at 65 °C for 10 min in the thermal cycler.
7. Boil the Chelex samples for 10 min at 99°C in the thermal cycler.
8. Take out Chelex samples from thermal cycler. Vortex the Chelex samples for 3 seconds and subsequently centrifuge for 3 minutes.

PCR PREP PROTOCOL:

9. Label a new tube with your number and add 20µl of the primer mix (tube marked P) and 20µl of reaction mix from the tube marked M (contains dNTPs, Taq polymerase, MgCl2 and buffer). Keep your tube on ice.
10. Dispense 12 µL of the supernatant (top, clear liquid) from your Chelex samples into your tube containing the Master mix and Primers. Take care not to draw up any Chelex and cell sediment. Keep your tube on ice.
11. If necessary, centrifuge your tube containing the DNA, primer mix and master mix for 3 seconds.
12. Close the mixture and place in the thermal cycler for 30 cycles of PCR.

Thermal cycler settings

a) 30 cycles of:
   - 30 sec – 95 °C – Template denaturation
   - 45 sec – 65 °C – Primer annealing
   - 30 sec – 72 °C – Extension

b) 10 min – 72 °C – Final extension to maximize results

c) Soak File – 4 °C – Default temperature to keep samples from degrading

Time: Approx. 1.25 hours
GEL ELECTROPHORESIS TECHNIQUE:

13. Following the PCR cycles, load 20 µl of your DNA sample into the wells of a 6% polyacrylamide gel after mixing them with a loading dye.
14. Also load 5 µl of a 50 bp ladder solution into two of the wells on the gel.
15. Subject the gels to 250 V for 42 minutes to allow the DNA fragments to separate. The loading dye will allow you to visually track the movement of the samples through the gels.
16. Soak the gels in SYBR safe stain for 30 minutes.
17. View the gels on the UV transilluminator to visualize the bands. Check out your DNA fingerprint for the D1S80 locus.
18. Take a picture of the gel and analyse the DNA fragments to estimate their size and the D1S80 alleles. Calculate your genotypic frequency for the D1S80 locus.
Analyzing Your Electrophoresis Results

Procedure
1. Obtain a print out of the gel image containing your DNA band(s).
2. Locate your well and your DNA bands
3. Using the D1S80 ladder comparison chart and your gel image, determine the approximate location and size (in bp) of each of your DNA bands.

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Analyzing Your Electrophoresis Results

1. How many bands do you see? _______
   Does this mean that you are homozygous or heterozygous? (circle one)

2. i. My first (or only) band is located between: _______ bp and _______ bp
   ii. Band size according to the 50bp DNA ladder: _______ bp long (______ repeats)
   iii. D1S80 allele frequency in the population (p): _______

3. i. My second band is located between: _______ bp and _______ bp
   ii. Band size according to the 50bp DNA ladder: _______ bp long (______ repeats)
   iii. D1S80 allele frequency in the population (q): _______

4. My genotype for the D1S80 locus is (____, ____).
   Example: (22, 24) means that your first band has 22 repeats, and the other band has 24 repeats

5. Calculate your genotypic frequency using the Hardy-Weinberg principle.

   If you are homozygous: If you are heterozygous:

   The frequency of your genotype is the square of your single allele frequency. \((p^2)\) or \((p \times p)\)\)
   The frequency of your genotype is one allele frequency multiplied by the other allele frequency, times two. \((2pq)\) or \((2 p \times q)\)

   Your genotypic frequency = _______

6. Making sense of your genotypic frequency. Perform the following calculations.
   Example:
   If my genotypic frequency comes to 0.0002618
   Step 1 \(1 \div 0.0002618 = 3819.7\)
   Step 2 This means that 1 in \(~3820\) humans have the same genotype as me

7. What do your genotypic results tell you about yourself? Do you have a rare or common combination? Does this mean that you are more or less special than your classmates?
DNA FINGERPRINTING
DNA EXTRACTION QUESTIONS

STEP 1: Remove cheek cells from your cheek.
Identify 2 other sources of DNA from the human body.

STEP 2: Place your cheek swab in a tube containing Chelex and Proteinase K.

Why do you need Chelex in your tube?

Why do you need Proteinase K in your tube?

STEP 3: Heat your cheek cell tube to 65°C.

Why did you heat your tube to this temperature?

STEP 4: Heat your cheek cell tube to 99°C.

What happens inside your tube at this temperature?

STEP 5: Vortex!

What's the point?

STEP 6: Centrifuge your tubes

What does centrifugation do to the contents of your tube?

DNA extraction is complete!

What's in the top layer?

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DNA FINGERPRINTING
POLYMERASE CHAIN REACTION QUESTIONS

- **Primers**
  Why does the PCR need primers?
  
- **Master Mix [dNTPs + Taq Polymerase + other goodies]**
  What is the function of the dNTPs?
  What is the function of Taq Polymerase?
  
- **20 µL**
- **12 µL**
- **Your DNA [top layer]**

- 30 - 40 cycles

- **95°C** → **65°C** → **72°C**

- **Denaturation**
  What happens at this temperature?
  
- **Annealing**
  What happens at this temperature?
  
- **Extension**
  What happens at this temperature?

**DNA Fingerprinting**

**Gel Electrophoresis/Staining Questions**

- **Your amplified DNA**
- **Gel goes in gel chamber**
- **Gel electrophoresis**
  - Why does the DNA move down the gel?
  
  - DNA ladder
  - Why do we use it?

- **SYBR Safe**
  - What are the 2 properties of SYBR Safe that give it the ability to make DNA glow?

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### DNA Fingerprinting

**DNA Extraction Answers**

**STEP 1:**
Remove cheek cells from your cheek.

Identify 2 other sources of DNA from the human body:
- Blood
- Hair

**STEP 2:**
Place your cheek swab in a tube containing Chelex and Proteinase K.

Why do you need Chelex in your tube?
- Removes trace metal ions from your sample.

Why do you need Proteinase K in your tube?
- This enzyme helps to digest cheek cells and to denature DNA in the cell.

**STEP 3:**
Heat your cheek cell tube to 65°C.

Why did you heat your tube to this temperature?
- Temperature for optimal function of Proteinase K.

**STEP 4:**
Heat your cheek cell tube to 95°C.

What happens inside your tube at this temperature?
- The cell and the nucleus lyse.
- The DNA is released into the solution.

**STEP 5:**
Vortex!

What’s the point?
- Shakes content of tube, dislodging the DNA from the sheath cells.

**STEP 6:**
Centrifuge your tubes.

What does centrifugation do to the contents of your tube?
- Separates the contents of the tube based on density.
- The DNA goes to the top.

**DNA extraction is complete!**

What’s in the top layer?
- Purified DNA
DNA FINGERPRINTING
POLYMERASE CHAIN REACTION
ANSWERS

**Primers**

Why does the PCR need primers?
Act as a beacon, telling Taq Polymerase where to start adding DNA.

**Master Mix [dNTPs + Taq Polymerase + other goodies]**

What is the function of the dNTPs?
The building blocks used to make new DNA molecules.

What is the function of Taq Polymerase?
Enzyme that builds new DNA strands.

20 µL

Your DNA [top layer]

12 µL

20 µL

30 - 40 cycles

95°C → 65°C → 72°C

**Denaturation**

What happens at this temperature?
DNA strands separate.

**Annealing**

What happens at this temperature?
The primers bind to their complementary sequences.

**Extension**

What happens at this temperature?
Taq Polymerase starts building DNA strands.
**DNA Fingerprinting**

**Gel Electrophoresis/Staining ANSWERS**

1. Your amplified DNA goes in the gel chamber.
2. Gel electrophoresis
   - Why does the DNA move down the gel? DNA is negatively charged (due to its phosphate groups) and so moves towards the positive electrode.

3. After 20 minutes
   - DNA ladder
     - Why do we use it? The DNA ladder is used as a reference to determine how big the DNA bands are.

4. After 20 more minutes
   - SYBR Safe
     - What are the 2 properties of SYBR Safe that give it the ability to make DNA glow?
       1. SYBR Safe glows green when exposed to UV light.
       2. SYBR Safe binds to DNA.
RFLP Student Activity

Introduction

Restriction enzymes cut DNA at specific sequence locations (restriction sites). This results in the formation of a number of DNA fragments of various lengths. These fragments can be separated according to the differences in length using gel electrophoresis. In this technique, the DNA is placed at one end of a gel and an electric current is applied across the gel. Since DNA contains negatively charged phosphate groups, the fragments will be attracted to the positive electrode at the opposite end of the gel with the smallest fragments moving the fastest and, therefore, migrating the farthest along the gel.

Different individuals have restriction sites at different locations in the non-coding sections of their DNA. Because of this, when subjected to restriction enzymes, everyone’s DNA produces different numbers and lengths of fragments. This is known as RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) and is used to identify individuals.

Purpose

To compare the RFLP banding patterns of three suspects with that of evidence found at the scene of the crime.

Materials

- DNA sequence from three individuals
- Restriction enzyme #1 (5'-CA/TG-3')
- Restriction enzyme #2 (5'-TTT/ACT-3')
- Worksheet for predicting the banding patterns of suspects
- The banding pattern generated from evidence found at the crime scene

Method

1. Scan each suspect’s DNA sequence looking for restriction site #1.
2. Measure the length of the resulting fragments by counting the base pairs of each.
3. Mark these lengths on the worksheet.
4. Repeat steps 1 to 3 for restriction site #2.

Conclusion

State if there is a match.
Restriction Site #1 (5'-CA/TG-3')

DNA Sequence for Suspect #1

5’GTTACCCTCATGGCCTTTCCATTTGACAGAAGGCTAGCAGGGTTCCAAAGGCAGACTAGAC
3’CAATGGGAGTACCCGGTAAAGGTAAACCTGTCTTTCGATCCACTGCTCAGTCTGCT

ATGTACTTCCAGGAGTTAACCTCTTGAATTCTACTACTACGTAGCCATGTAAT
TACATGAAGTCTTAATGTGACACACTGTAGGCTAGCCATGTAAT

CGGTACTGGTTCACTAAACCAGTGAACCTGTGACATTTGAGTTTCTACTACTCGGAGTGAAGC
GCCATGACCAAGTCTTTGGCCTACCTGCAACTGTAACCTATTGAATGTAGAGCTCTAAGCTT

TATAGCCATGAGTGCAGTATCGTACGTAGCTGCTTTACATTAGTTCCA 3’
ATATCGGTACTCAGTCACTAGCATTCAAAATGATCAGGT 5’

DNA Sequence for Suspect #2

5’CCGGCATTTTGCCTCGGACTGAGACATCTTCTAGGTTCACTATCATTCCGGATATCGA
3’GGCCGTAACCCGGCCTAGCTGACCTACTACCGAATCTGAGGTAGCCTAGTACAGATGAGGGTACTAT

CCTGAACCTTATGCAGGTACTCTTAACCATCGGAAATGCTGAGGTGCTCAGTACATAGGCTAGCCAT

GGACTTTGAAATAGGTGGCCAATGCAGTGGCGTTTGCTCTATTGCCTACCCGCATCGCATGCTTGAT

GCATGGGATCTTTCTGGAGTCTTTACTACAGGTCAGGGATACGCGATTCG
CGTACCCTAGAAGACTCTCAGAAATGATGCTAGCCAGTTCCAAATGACGTACAGCCTATGCTAAGGC

ATACTGCGAGGTAGTACAGCACATTTTGCTGGCAGTCTGCTTTACTACGTACGG 3’
TAGACGTGCTACATGTACTGTGAAACCAGCCGCTGAAGCAGAATGAGCTAGTCGTC 5’

DNA Sequence for Suspect #3

5’CCGTTAACCTGGAAATTCATTTACTCCTGGATAATCCCGACATGTCCGGATCTCTCGGATCT
3’GGCAATTAGCTTTAAGTAAATGGACACCTATTAGGGGCTGTACAGTCCAGTCTAGGCTAGCGTAC

CCGGATACAGTTTAATCAGGATCTGGATATGCAATCTGGAATTTGGACTGGACACTTCC
GGCCTATGCAATTGACGTAAACCTAGGTACATCGACTTACTTACTTACCCTGAGCTGGAG

GAGCTTAGGCCTACTTACTCGCAGAATCTGGAATTTCCAGGATCGTTACTGACATGCAT
CTCAGATCCGGAGTAAATGAGCCTTATCCCTGACTAGGCTTACGTAGTACGT

TTTACTGGAATCTCTCGACATTAGTACTGAGGCAATCGGCTTTCCAGGCTTA 3’
AAATGACCTGAGAGCTCTGAATCAGTACGTCTGGTTACCGTGTAAGTGGCTTACGGT 5’
Restriction Site #2 (5'TTT/ACT-3')

DNA Sequence for Suspect #1

5'GTTACCCCTATGGCCCATTTCCATTTGGAAGCTAGCAGGTTCCAAAAGGCCAGACTAGAC
3'CAATGGGGAGTACCGGTAAAGGTAACTCTCTTGTTCCGATCGTCAAGGTTCTTCGGTGCTGTG
ATGACTTCCAGGAAGTTACCCTGGAATTTACTCTACCTAGACGGTTTACTAGGCTAGCCAGTTA
TACATGAAATCTCTTCATTGGAACCTAATGGAAGTGATCTGCTCCAATGAATCCGATCTGGTGTAAT
CGTACTGGTTGAGTAAACCAGTAAACCTGGACTAGCAGTTACTACTCGGAATTCGAACGT
GCCATGACCAAGCTATGTGGCCATCTTTGACACTGTTCAAGCTTGAACATGAAATCCTAAGGGCTAAGC
TATAAGCCATGAGTGCAGTATGCTAGCATAGCCTATTTACTAGTTGCA 3'
ATATCGGTACTCGACGTCATGCTAGCGCTATCCGAATAAAATGATCAAGGT 5'

DNA Sequence for Suspect #2

5'CCGGCATTTTGCGCCGACTGAGACATTCCTTACGTTAGGATCCATGTCTACTCGGATATCGA
3'GGCCGTAACGCACGCGTCACTGTAAATTGATGGAATGCTAAAGTGCTGATTTAGCTAAGGGCTATAGCT
CCTGAACTTATCAGGGATCCGTTACCTTACTACCTCCAAAATTGAGCGCGCTAGCGTACATG
GGAATTTACGACGCTGTGGCAGATGTGATTCGGAGCTTTACGATTACGGGATACGGAATTCG
CGTGACCATGACCAAGCTACCTGAGAAATGAGTCTCCAGTTCAAAGATCATGGCCCTATGCTAAAGGC
ATACTGCAGGATGACATGACACTTTGGCTGGCAGTTCTGCGTATTACTCAGTCAG 3'
TATGACGTCCATCGTACTGTAAGGCAACCCGGTCAGCAATGAGTTCAGTC 5'

DNA Sequence for Suspect #3

5'CCTTTAATCGGAATTCATTACCTGATGTTGGATAATCCCGACATGTCAGGTCGATCCTCGGATCT
3'GGCAATTGACGCTTAAATGGAACCTAATGACGCTTACAGTCCAGTCATAGCGCTAGCTA
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# WORKSHEET

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**Match(es) to Evidence:**

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**Match(es) to Evidence:**
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<td>(+)</td>
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**Match(es) to Evidence:** Individuals 1 and 2