ONTARIO SCIENCE CENTRE

TEACHER PACKAGE

DNA Fingerprinting workshop

Table of Contents

DNA Fingerprinting Protocol

DNA ISOLATION TECHNIQUE:

1. Select a micro-centrifuge tube containing 300 µl of a solution of Chelex / Proteinase K (50/50) solution.
2. Wearing gloves, obtain four to five hairs – ideally with large hair-sheaths and/or hair roots, from the back of your head. You can use tweezers to do this, and you can use the magnifying glasses to better view your hair sheaths.
3. Do not place the hair on the bench.
4. Place the hair sheaths and roots in the chelex / Prot K solution. Make sure the hair sheaths and follicles are inside the solution. Use a hair tip to push the hairs into the tube.
5. Cut off the shaft of the hair with scissors. Close the lids of the tube. This will be called your chelex tube.
6. Incubate the chelex samples at 65 °C for 10 min in the thermal cycler
7. Take out chelex samples from thermal cycler. Vortex the chelex samples for 3 seconds and subsequently make sure the hair sheaths and follicles are submerged in the solution. Use a hair tip if necessary
8. Boil the chelex samples for 10 min at 100 oC in the thermal cycler.
9. Take out chelex samples from thermal cycler. Vortex the chelex samples for 3 seconds and subsequently centrifuge for 3 minutes.

PCR PREP PROTOCOL:

10. 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 in ice.
11. 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 in ice.
12. If necessary, centrifuge your tube containing the DNA, primer mix and master mix for 3 seconds.
13. 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 – Amplification

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

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

Time: Approx. 1.25 hours

GEL ELECTROPHORESIS TECHNIQUE:

14. 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.
15. Also load 5 µl of a 50 bp ladder solution into two of the wells on the gel.
16. 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.
17. Soak the gels in SYBR safe stain for 30 minutes.
18. View the gels on the UV transilluminator to visualize the bands. Check out your DNA fingerprint for the D1S80 locus.
19. 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.

Analysing 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.

DNA band size (bp)	50 bp Ladder	D1S80 allele No. of repeats (16 bp/ repeat)	Allele Frequencies
800	**800 bp**	>41	0.0040
784		41	0.0015
768		40	0.0013
752		39	0.0027
	750 bp		
736		38	0.0007
720		37	0.0030
704		36	0.0044
	700 bp		
688		35	0.0013
672		34	0.0271
656		33	0.0032
	650 bp		
640		32	0.0185
624		31	0.0619
608		30	0.0283
	600 bp		
592		29	0.0540
576		28	0.0779
560		27	0.0126
	550 bp		
544		26	0.0111
528		25	0.0491
512		24	0.2967
	500 bp		
496		23	0.0119
480		22	0.0449
464		21	0.0486
	450 bp		
448		20	0.0210
432		19	0.0049
416		18	0.1875
400		17	0.0143
	400 bp		
384		16	0.0056
368		15	0.0015
352		14	0.0002
	350 bp		

1) My **first (or only)** band is located between _____ **bp** and _____ **bp** as compared to the 50 bp DNA ladder.
My band is approximately _____ **bp** long.
My band has approximately _____ **repeats** (D1S80 allele).
The frequency of my D1S80 allele in the population is _____ (**p**).

2) My **second band** is located between _____ **bp** and _____ **bp** as compared to the 50 bp DNA ladder.
My band is approximately _____ **bp** long.
My band has approximately _____ **repeats** (D1S80 allele).
The frequency of my D1S80 allele in the population is _____ (**q**).

3) My **genotype** for the **D1S80 locus** is _____ (e.g. 22, 24). I am _____ (**homozygous, heterozygous**) for this locus.

4) Calculate your **genotypic frequency** using the Hardy-Weinberg principle

- If you are **homozygous**, the frequency of your genotype is a square of the frequency of the allele you possess, i.e. p^2
- My **genotypic frequency** is ($\mathbf{p^2}$)_____.

- If you are **heterozygous**, the frequency of your genotype is two times the product of frequencies of the alleles you possess, i.e. 2pq
- My **genotypic frequency** is (**2pq**)_____.

5) Making sense of your genotypic frequency
Convert the number to a fraction and then reduce it by dividing the denominator by the numerator.

E.g. If my genotypic frequency comes to 0.0002618

0.0002618 = [fraction] $\frac{2.618}{10000}$ = [reduced fraction] $\frac{1}{3819.7}$

This means that 1 in ~ 3800 humans have the same genotype as me

6) What do your genotypic results tell you about yourself?

DNA Fingerprinting Workshop

Morning Protocol Questions

1. **What was the source of human DNA for today's lab? _____**

2. **What role does Chelex play in the experimental protocol?**
   a. Makes the DNA come out of the hair sheath cells
   b. Removes trace metals from the hair sheath
   c. Helps to separate the sheath cells from the hair

3. **What is the function of the enzyme Proteinase K?**
   a. Makes the DNA denature into single strands
   b. Digests proteins to detach sheath cells from hair shaft and denatures DNase & RNase
   c. Makes more copies of the DNA

4. **Why incubate the Chelex sample at 65°C?**
   a. Optimal temperature for functioning of Proteinase K
   b. Optimal temperature for functioning of Chelex
   c. Makes the DNA denature into single strands

5. **Why vortex the sample after incubation?**
   a. Mixes up the contents of the tube
   b. Separates the contents of the tube into two distinct layers
   c. Acts as a mechanical shear to separate the sheath cells from the hair shaft

6. **Why is it necessary to boil the sample?**
   a. Denatures the DNA into two strands allowing replication to proceed using the DNA polymerase enzyme
   b. At this high temperature the chelex will denature, killing the sheath cells and any bacteria in the solution
   c. Phospholipid membrane breaks causing the cells to lyse and the contents of the cell to be released- including the nucleus, which also lyses releasing the DNA into the solution

7. **Why centrifuge the sample?**
   a. To separate components based on density
      - Denser molecules settle to the bottom (Chelex, cell sediment)
      - Less dense molecules on top (DNA)
   b. Mixes up the contents of the tube
   c. Acts as a mechanical shear to separate the DNA from the sheath cells

8. **What is a primer? What is its' function?**
   a. A paint product that helps DNA adhere to the cell wall
   b. Short sequence of single-stranded DNA (or RNA) that is complementary to a given DNA sequence from which DNA replication can initiate
   c. A short protein strand that is complementary to a given DNA sequence from which DNA replication can initiate

9. **Outline the functions of the following components in the Master Mix:**
   a. dNTP
   b. Taq Polymerase
   c. $MgCl_2$
   d. Buffer

10. **Why is it important to only transfer the supernatant from the chelex tube to the tube containing Master Mix and Primers?**
   a. We only want the DNA, which is in the supernatant layer
   b. We only want the cell sediment, which is in the supernatant layer
   c. We want the DNA and the cell sediment, which are in the supernatant layer

11. **What does PCR stand for?**

   _____

   a. **Which machine performs PCR?**

   _____

   b. **Explain the 3 phases of PCR (name of phase and temperature)**
      i. _____ (Temp: ____°C)
      ii. _____ (Temp: ____°C)
      iii. _____ (Temp: ____°C)

12. **Who invented PCR?**

_____

13. **From which organism did he obtain the *Taq polymerase* enzyme?**

_____

Afternoon Protocol Questions

1. **What is the purpose of gel electrophoresis?**
   a. Makes the DNA visible to the naked eye
   b. Makes DNA positively charged
   c. Separates charged molecules (DNA, RNA, proteins) based on size and charge

2. **What are the components of the loading dye that is used during electrophoresis? What is their purpose?**

   _____
   _____
   _____
   _____

3. **Why do the smaller fragments of DNA travel *faster* than the larger fragments?**
   a. Smaller fragments are negatively charged whereas larger fragments are positively charge
   b. Smaller fragments can move through the pores in the gel faster than larger fragments
   c. Larger fragments got their holiday shopping done earlier

4. **Why is it important to use a ladder during electrophoresis?**
   a. Acts as a ruler/scale to estimate lengths of DNA fragments, which enables us to determine which D1S80 allele/s we have
   b. The DNA fragments use the ladder to climb up in the gel
   c. None of the above

5. **What is used to stain the gels? What is the stain's function?**
   a. SYBR safe, which fluoresces under UV light to make the DNA bands visible
   b. Bromophenol blue, which fluoresces under UV light to make the DNA bands visible
   c. Neither, DNA is self fluorescing under UV light

DNA Fingerprinting Workshop
Answer sheet

Morning Protocol Questions

1. **What was the source of human DNA for today's lab?** Hair sheath cells

2. **What role does Chelex play in the experimental protocol?**
   a. Makes the DNA come out of the hair sheath cells
   b. ✓ Removes trace metals from the hair sheath
   c. Helps to separate the sheath cells from the hair

3. **What is the function of the enzyme Proteinase K?**
   a. Makes the DNA denature into single strands
   b. ✓ Digests proteins to detach sheath cells from hair shaft and denatures DNase & RNase
   c. Makes more copies of the DNA

4. **Why incubate the hair sample at 65°C?**
   a. ✓ Optimal temperature for functioning of Proteinase K
   b. Optimal temperature for functioning of Chelex
   c. Makes the DNA denature into single strands

5. **Why vortex the sample after incubation?**
   a. Mixes up the contents of the tube
   b. Separates the contents of the tube into two distinct layers
   c. ✓ Acts as a mechanical shear to separate the sheath cells from the hair shaft

6. **Why is it necessary to boil the sample?**
   a. Denatures the DNA into two strands allowing replication to proceed using the DNA polymerase enzyme
   b. At this high temperature the chelex will denature, killing the sheath cells and any bacteria in the solution
   c. ✓ Phospholipid membrane breaks causing the cells to lyse and the contents of the cell to be released- including the nucleus, which also lyses releasing the DNA into the solution

7. **Why centrifuge the sample?**
   a. ✓ To separate components based on density
      - Denser molecules settle to the bottom (Chelex, cell sediment)
      - Less dense molecules on top (DNA)
   b. Mixes up the contents of the tube
   c. Acts as a mechanical shear to separate the DNA from the sheath cells

8. **What is a primer? What is its' function?**
   a. A paint product that helps DNA adhere to the cell wall
   b. ✓ Short sequence of single-stranded DNA (or RNA) that is complementary to a given DNA sequence from which DNA replication can initiate
   c. A short protein strand that is complementary to a given DNA sequence from which DNA replication can initiate

9. **Outline the functions of the following components in the Master Mix:**

   a. dNTP :
   deoxynucleoside triphosphates
   Four types: dATP, dTTP, dGTP, dCTP
   Phosphate bonds provide energy for polymerase to join the premature nucleotide to the new growing strand of DNA

   b. Taq Polymerase:
   Adds free dNTPs onto the new strand of DNA

   c. MgCl2 :
   Mg^{2+} acts as cofactor for Taq Polymerase

   d. Buffer:
   Maintains pH level within the solution

10. **Why is it important to only transfer the supernatant from the chelex tube to the tube containing Master Mix and Primers?**

   a. ✓ We only want the DNA, which is in the supernatant layer

   b. We only want the cell sediment, which is in the supernatant layer

   c. We want the DNA and the cell sediment, which are in the supernatant layer

11. **What does PCR stand for?**
   Polymerase Chain Reaction

   a. **Which machine performs PCR?**
   Thermal cycler

   b. **Explain the 3 phases of PCR (name of phase and temperature)**

      i. Denaturing (Temp: 95°C)

      ii. Annealing (Temp: 65°C)

      iii. Extending (Temp: 72°C)

12. **Who invented PCR?**
   Dr. Kary Mullis

13. **From which organism did he obtain the *Taq polymerase* enzyme?**
   Thermus aquaticus

Afternoon Protocol Questions

1. **What is the purpose of gel electrophoresis?**

   a. Makes the DNA visible to the naked eye

   b. Makes DNA positively charged

   c. ✓ Separates charged molecules (DNA, RNA, proteins) based on size and charge

2. **What are the components of the loading dye that is used during electrophoresis? What is their purpose?**

   Glycerol: makes the sample dense
   Bromophenol blue: dark blue dye, runs at speed of 65 bp DNA fragment
   Xylene cyanol: light blue dye, runs at speed of 250 bp DNA fragment
   The dyes track movement of DNA through the gel
   Turning off the electricity when Xylene cyanol reaches near the bottom will ensure that D1S80 (300 – 800bp) DNA fragments are located in an area of gel that is easy to visualize and analyse

3. **Why do the smaller fragments of DNA travel *faster* than the larger fragments?**

   a. Smaller fragments are negatively charged whereas larger fragments are positively charge

   b. ✓ Smaller fragments can move through the pores in the gel faster than larger fragments

   c. Larger fragments got their holiday shopping done earlier

4. **Why is it important to use a ladder during electrophoresis?**

   a. ✓ Acts as a ruler/scale to estimate lengths of DNA fragments, which enables us to determine which D1S80 allele/s we have

   b. The DNA fragments use the ladder to climb up in the gel

   c. None of the above

5. **What is used to stain the gels? What is the stain's function?**

   a. ✓ SYBR safe, which fluoresces under UV light to make the DNA bands visible

   b. Bromophenol blue, which fluoresces under UV light to make the DNA bands visible

   c. Neither, DNA is self fluorescing under UV light

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'GTTACCCTCATGGCCATTTCCATTGGACAGAAGGCTAGCAGGTTCCAAAGGCCAGACTAGAC
3'CAATGGGAGTACCGGTAAAGGTAACCTGTCTTCCGATCGTCCAAGGTTTCCGGTCTGATCTG

ATGTACTTCAGGAAGTTAACCTGGATTTACTTCACTAGACGGTTTACTTAGGCTAGCCCAGTTA
TACATGAAGTCCTTCAATTGGACCTAAATGAAGTGATCTGCCAAATGAATCCGATCGGGTCAAT

CGGTACTGGTTCAGTAACCGCATGAACCTGTGACATTGGATTTACTACTCGGATTCGAACCGT
GCCATGACCAAGTCATTGGCGTACTTGGACACTGTAACCTAAATGATGAGCCTAAGCTTGGCA

TATAGCCATGAGTGCAGTATCGTAGCATAGCCTTATTTACTAGTTCCA 3'
ATATCGGTACTCACGTCATAGCATCGTATCGGAATAAATGATCAAGGT 5'

DNA Sequence for Suspect #2

5'CCGGCATTTGCGCCGACTGAGACACTTTCAGGTTCAGGATCCATGTCTACTCCGGATATCGA
3'GGCCGTAAACGCGGCTGACTCTGTGAAAGTCCAAGTCCTAGGTACAGATGAGGCCTATAGCT

CCTGAACTTATCCGGATCCGTGACCTTTACTACTCCGAAATCGGTAGCGCGTAGCGTACCATG
GGACTTGAATAGGCCTAGGCACTGGAAATGATGAGGCTTTAGCCATCGCGCATCGCATGGTAC

GCATGGGTACTTCTGGAGTCTTTACTCAGGTCAAGGTTTTACTAGTACGGGATACGGATTCCG
CGTACCCATGAAGACCTCAGAAATGAGTCCAGTTCCAAAATGATCATGCCCTATGCCTAAGGC

ATACTGCAGGTAGCATGACACTTTGGCTGGCAGTTCGTTTACTCAGTCAG 3'
TATGACGTCCATCGTACTGTGAAACCGACCGTCAAGCAAATGAGTCAGTC 5'

DNA Sequence for Suspect #3

5'CCGTTAATCGGAATTCATTTACTCGTGGATAATCCCGACATGTCAGGTCAGATCTCGGATCT
3'GGCAATTAGCCTTAAGTAAATGAGCACCTATTAGGGCTGTACAGTCCAGTCTAGAGCCTAGA

CCGGATACAGTTTACTGCATTGGATCCATGTAGGATCATGAATCAATGGGACTCGAGCACCTTC
GGCCTATGTCAAATGACGTAACCTAGGTACATCCTAGTACTTAGTTACCCTGAGCTCGTGGAAG

GAGCTTAGGCCTCATTTACTCGCAAATTCCGGACGTACCGAATCGATTCTACTGATCCATGCAT
CTCGAATCCGGAGTAAATGAGCGTTTAAGGCCTGCATGGCTTAGCTAAGATGACTAGGTACGTA

TTTACTGGACTCTCGACATTAGTACTGAGCCAATGGCCATTCAGGCTA 3'
AAATGACCTGAGAGCTGTAATCATGACTCGGTTACCGGTAAGTCCGAT 5'

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

DNA Sequence for Suspect #1

5'GTTACCCTCATGGCCATTTCCATTGGACAGAAGGCTAGCAGGTTCCAAAGGCCAGACTAGAC
3'CAATGGGAGTACCGGTAAAGGTAACCTGTCTTCCGATCGTCCAAGGTTTCCGGTCTGATCTG

ATGTACTTCAGGAAGTTAACCTGGATTTACTTCACTAGACGGTTTACTTAGGCTAGCCCAGTTA
TACATGAAGTCCTTCAATTGGACCTAAATGAAGTGATCTGCCAAATGAATCCGATCGGGTCAAT

CGGTACTGGTTCAGTAACCGCATGAACCTGTGACATTGGATTTACTACTCGGATTCGAACCGT
GCCATGACCAAGTCATTGGCGTACTTGGACACTGTAACCTAAATGATGAGCCTAAGCTTGGCA

TATAGCCATGAGTGCAGTATCGTAGCATAGCCTTATTTACTAGTTCCA 3'
ATATCGGTACTCACGTCATAGCATCGTATCGGAATAAATGATCAAGGT 5'

DNA Sequence for Suspect #2

5'CCGGCATTTGCGCCGACTGAGACACTTTCAGGTTCAGGATCCATGTCTACTCCGGATATCGA
3'GGCCGTAAACGCGGCTGACTCTGTGAAAGTCCAAGTCCTAGGTACAGATGAGGCCTATAGCT

CCTGAACTTATCCGGATCCGTGACCTTTACTACTCCGAAATCGGTAGCGCGTAGCGTACCATG
GGACTTGAATAGGCCTAGGCACTGGAAATGATGAGGCTTTAGCCATCGCGCATCGCATGGTAC

GCATGGGTACTTCTGGAGTCTTTACTCAGGTCAAGGTTTTACTAGTACGGGATACGGATTCCG
CGTACCCATGAAGACCTCAGAAATGAGTCCAGTTCCAAAATGATCATGCCCTATGCCTAAGGC

ATACTGCAGGTAGCATGACACTTTGGCTGGCAGTTCGTTTACTCAGTCAG 3'
TATGACGTCCATCGTACTGTGAAACCGACCGTCAAGCAAATGAGTCAGTC 5'

DNA Sequence for Suspect #3

5'CCGTTAATCGGAATTCATTTACTCGTGGATAATCCCGACATGTCAGGTCAGATCTCGGATCT
3'GGCAATTAGCCTTAAGTAAATGAGCACCTATTAGGGCTGTACAGTCCAGTCTAGAGCCTAGA

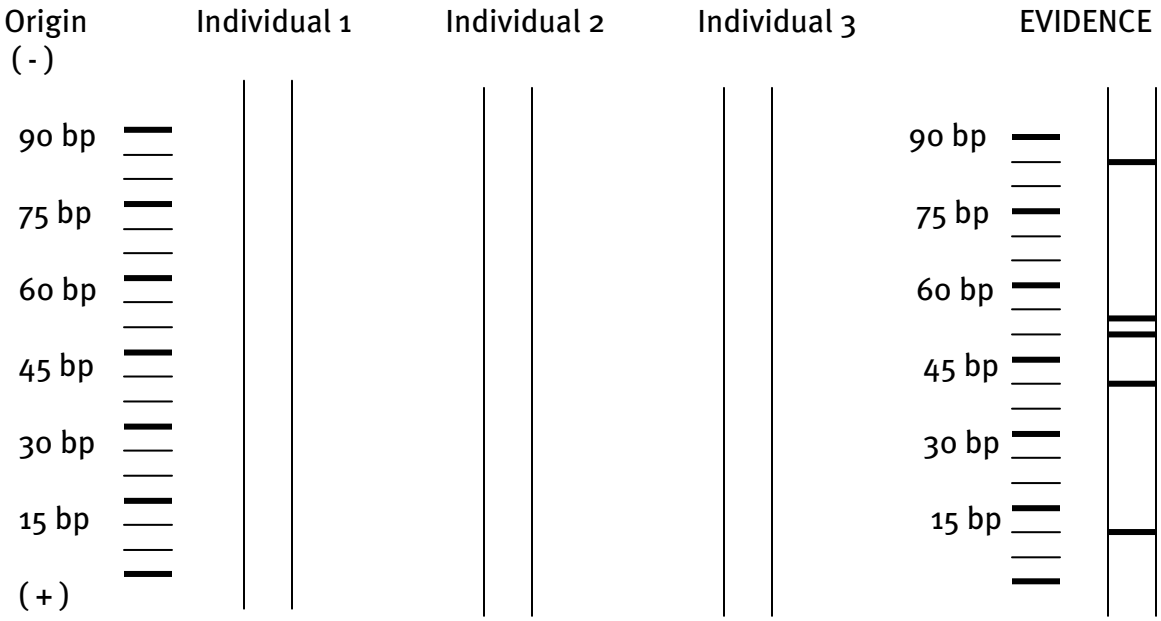
CCGGATACAGTTTACTGCATTGGATCCATGTAGGATCATGAATCAATGGGACTCGAGCACCTTC
GGCCTATGTCAAATGACGTAACCTAGGTACATCCTAGTACTTAGTTACCCTGAGCTCGTGGAAG

GAGCTTAGGCCTCATTTACTCGCAAATTCCGGACGTACCGAATCGATTCTACTGATCCATGCAT
CTCGAATCCGGAGTAAATGAGCGTTTAAGGCCTGCATGGCTTAGCTAAGATGACTAGGTACGTA

TTTACTGGACTCTCGACATTAGTACTGAGCCAATGGCCATTCAGGCTA 3'
AAATGACCTGAGAGCTGTAATCATGACTCGGTTACCGGTAAGTCCGAT 5'

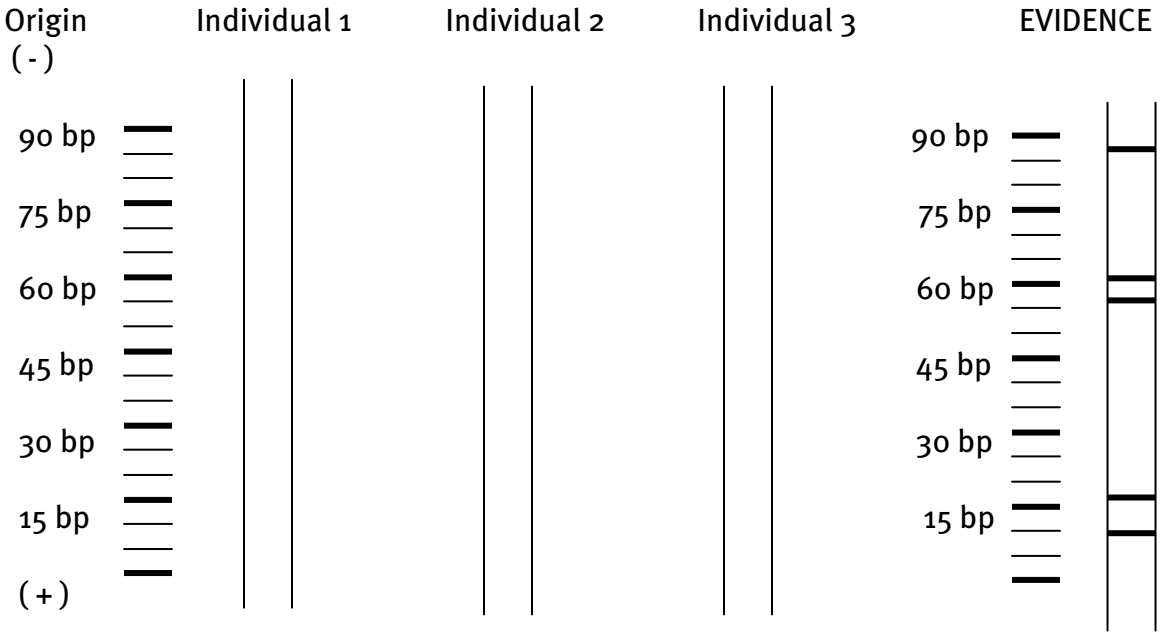
WORKSHEET

Restriction Enzyme Type #1



Match(es) to Evidence:

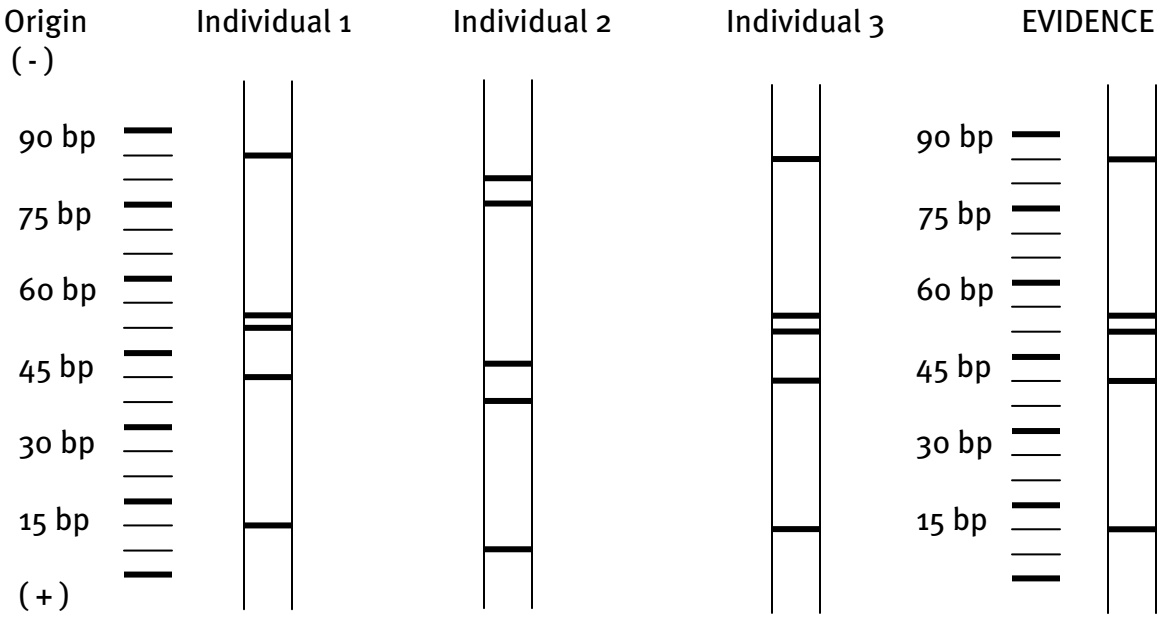
Restriction Enzyme Type #2



Match(es) to Evidence:

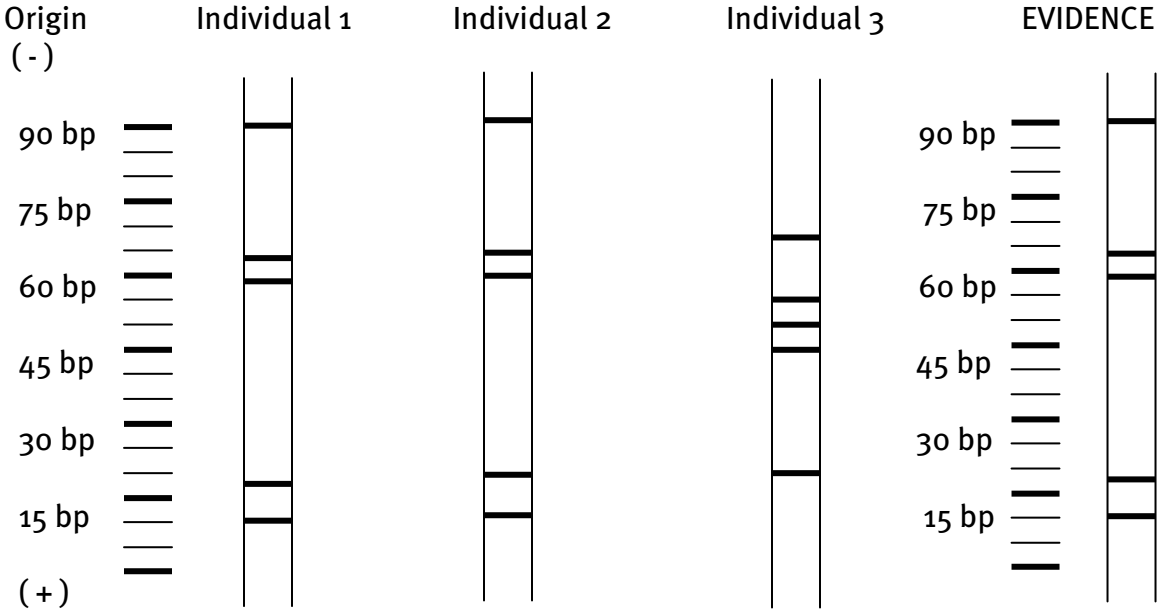
WORKSHEET ANSWER KEY

Restriction Enzyme Type #1



Match(es) to Evidence: Individuals 1 and 3

Restriction Enzyme Type #2



Match(es) to Evidence: Individuals 1 and 2