Practical activity (4)

Paternity Testing

(Teacher's Guide)

I. Objectives of the experiment

To determine the biological relationship among four simulated DNA samples by means of gel electrophoresis.

II. Expected Learning Outcomes

Upon completion of the activities, students should be able to:

1. recognise the application of DNA fingerprinting;
2. outline the principle of DNA fingerprinting; and
3. perform gel electrophoresis.

III. Teaching notes:

1. Introduce the task by going through the "Background" with the students or have the students read through this part and the "Guiding questions about the design of the experiment" as a pre-class activity.
2. Discuss the design of the experiment with the students using the "Guiding questions about the design of the experiment".
3. Plan the laboratory work with reference to the "Time allocation for the experimental activities" in section IV according to the lesson time of your school.
4. Always remind the students about the safety precautions of each part of the experiment.
5. Go through the "Results" and "Discussion" with the students.

IV. Time allocation for the experimental activities

Experimental activities		**Duration**	
		In class	**Out of class** (to be done by laboratory technicians)
1	Casting of agarose gel		30–45 min
2	Electrophoresis of simulated DNA samples	45 min	
3	Gel image documentation and analysis	15 min	
Total lesson time for the experimental activities		Pre-lab session: 30 min Lab session: 60 min	

V. Equipment, materials and preparatory work for the experiments

A commercial education kit (Innovating Science™ DNA Paternity Testing Electrophoresis Lab Activity Educational Kit; Fisher Scientific; #S28494) will be needed for this experiment.

This kit has enough materials and reagents for 24 students (6 groups of 4 students). Some additional equipment or materials may be required.

Website: https://www.fishersci.com/shop/products/dna-paternity-testing-electrop/s28494

Experiment (per group) :

a) Equipment

- Electrophoresis tank × 1
- Power supply × 1
- Gel documentation device / UV lamp × 1
- Micropipette (P10) and sterile pipette tips

b) Materials

- Simulated Mother's DNA (6 µl) × 1
- Simulated Son's DNA (6 µl) × 1
- Simulated Man A's DNA (6 µl) × 1
- Simulated Man B's DNA (6 µl) × 1
- Micro-centrifuge tube rack × 1
- Agarose gel tray with a comb × 1
- Tris-Borate-EDTA (TBE) buffer (1 L) × 1
- DNA ladder (4 µl) × 1
- Parafilm

- Permanent marker × 1
- Paper towel × 1 box
- Biohazard bag × 1
- Disposal container with 10% chlorine bleach × 1

c) Preparatory work

Casting of agarose gel **(To be done by teacher/laboratory technicians before lab session)**

1. Weigh 0.2 g of agarose powder (per gel) in a conical flask.

   [Tip: Or weigh the powder on a small piece of weighing paper and pour the powder into the conical flask after weighing.]

2. Add 20 ml TBE buffer (per gel) to the conical flask. Heat the mixture in a microwave oven until all the agarose powder is dissolved and the solution becomes clear.

   [Important note: Shaking of the agarose and TBE buffer mixture should be avoided, as such shaking may carry the undissolved agarose aggregates to the sidewall of the conical flask. These aggregates may not be dissolved after heating.]

   [Tip: Ready-to-use 1X TBE buffer can be purchased from biotechnology companies. 10X TBE buffer can be purchased and diluted to 1X with distilled/deionised water before use.]

   [Important note: Completely dissolved agarose should be transparent and very clear. Sometimes small fibre-like materials may be observed in the transparent and clear agarose solution after heating, when observing at close range. These fibre-like materials are the undissolved agarose, and they indicate that further heating is needed.]

3. Cool the agarose solution slightly under running water.

   [Tip: Check whether the hot agarose solution is sufficiently cooled by placing the bottom of the conical flask on the back of the hand (with gloves on) after wiping off all the water on the conical flask. If the agarose solution feels warm, it is considered sufficiently cooled. Over-cooling may cause the gel to solidify prematurely.]

4. Add 2 µl of gel red (per gel) to the agarose solution and mix gently.

   [Important note: Traditional DNA visualising agents are carcinogenic. Modern DNA visualising agents, such as gel red and gel green, are non-toxic to humans. However, it is still suggested handling gel red with care and avoiding direct skin contact. Adding gel red into a very hot agarose solution should be avoided, as gel red may get vaporised .]

5. Place a 8-well comb into a casting tray. Carefully pour in the agarose solution. Remove any bubbles formed, with the back of a P200 micropipette tip (inverting the tips).

6. Wait for 25–30 minutes until the agarose solidifies into an opaque gel.

   [Tip: Agarose gels can be prepared a day before the lab session if they are stored in TBE buffer at 4°C.]

Aliquot of experimental materials **(To be prepared by the teacher/laboratory technicians before the lab session)**

1. Aliquot 6 µl Simulated Mother's DNA into each micro-centrifuge tube. Prepare one tube per group.
2. Aliquot 6 µl Simulated Son's DNA into each micro-centrifuge tube. Prepare one tube per group.
3. Aliquot 6 µl Simulated Man A's DNA into each micro-centrifuge tube. Prepare one tube per group.
4. Aliquot 6 µl Simulated Man B's DNA into each micro-centrifuge tube. Prepare one tube per group.
5. Aliquot 4 µl DNA ladder into each micro-centrifuge tube. Prepare one tube per group.

VI. Suggested answers to the "guiding questions about the design of the experiment"

1. What is the purpose of conducting gel electrophoresis?

   - *To separate and visualise the DNA samples of different sizes.*
   - *To compare the size of the gene loci of each individual sample.*

2. Draw the approximate position of the following DNA fragments of different sizes in the gel after electrophoresis, as shown in Fig. 2.

   Well 1: 1000 bp; Well 2: 780 bp; Well 3: 400 bp; Well 4: 800 bp.

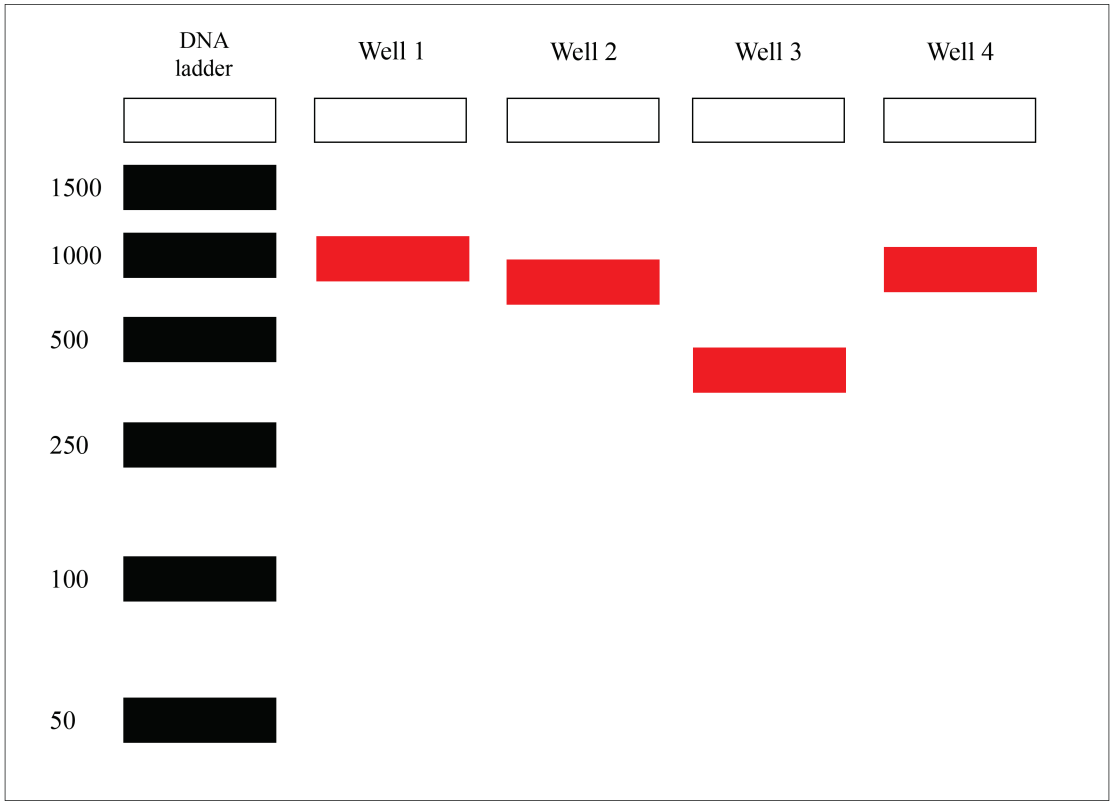
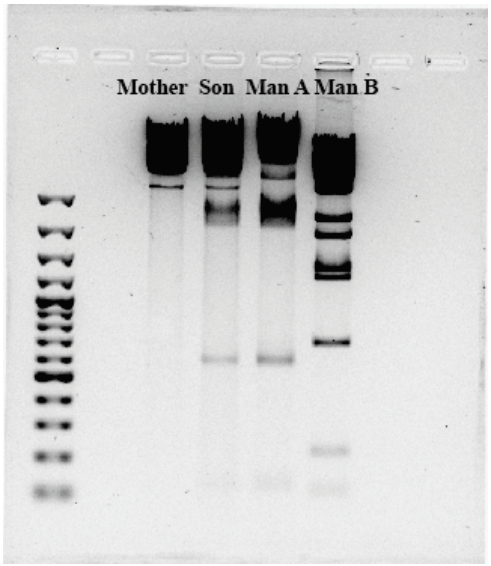


Fig. 2 A piece of gel

VII. Results

1. Paste the agarose gel electrophoresis image of the simulated DNA samples in the box below.



2. According to the gel electrophoresis result of the four simulated DNA samples, which Man (A or B) is the biological father of the Son? Briefly explain your answer.

Man A is the biological father of the Son because the Son should have the same gene pattern as his mother and father. Man B shows a very different band pattern from that of the Son.

VIII. Discussion

1. How can the biological relationship between parents and children be built from the banding pattern of electrophoresis?

   - *By comparing the allele size of multiple loci (genes), which is shown by the position of the DNA band on the agarose gel*
   - *For confirming the relationship between potential parents and children, a valid pair should have one allele of the same size in every locus tested. In other words, the size of one allele of a locus of the children should be the same as that of the mother. The size of another allele of the same locus should be the same as that of the father.*

2. Can the relationship between siblings be checked in a similar manner?

   Yes. Siblings should have at least an allele of the same size (same band pattern) in 50% of the loci tested.

3. Why should 18–20 loci be checked for paternity testing?

   To rule out the possibility of the individuals having identical alleles by chance, and thus increase the accuracy of the test.

4. What kind of body tissues can be collected as biological samples for DNA extraction for a paternity test?

   Blood, hair, bone, fingernail, muscle tissue, organ tissue, skin tissue and amniotic fluid.

5. What is the purpose of having the DNA sample extracted to undergo a PCR?

   To increase the number of copies of the DNA samples.

Extension discussion questions:

6. To perform a PCR, the following chemicals are added to the PCR tube in addition to the DNA sample. What is the function of adding each of these chemicals?

Chemical	Function
Nuclease-free water	*To provide a liquid environment and maintain the final volume for the reaction*
10× *Taq* buffer	*To provide a suitable chemical environment for Taq polymerase, e.g. an optimum pH and adequate concentration of ions*
25 mM $MgCl_2$	*To serve as a co-factor of Taq polymerase, to enhance Taq activities*
10 mM dNTP	*To provide free nucleotides for the synthesis of new DNA strands*
Forward and reverse primers	*The forward and reverse primers attach to the start point and the endpoint of the sequence of DNA to be amplified respectively. This guides the process of amplification of DNA by flanking the target region of the DNA sample to be amplified.*
Taq polymerase	*This enzyme catalyze the synthesis of a new strand of DNA from the free nucleotides.*

7. Usually, a PCR of 18–20 short tandem repeat (STR) loci are examined in a particular paternity test. To each examined locus, the length of the 2 alleles are compared. If the lengths of the 2 alleles of the locus are the same, the alleles are known as homozygous; otherwise, they are heterozygous. By comparing the size of the alleles of different loci, the biological relationship can be determined.

   (a) Search information from books and the internet. List some commonly examined STR loci in paternity testing.

   The commonly examined loci include CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11

   (b) Identify the number of repeat units [AATG] in the following STR, i.e. TPOX, which is located on human chromosome 2.
   CCACACAGGTAATGAATGAATGAATGAATGAATGCCTAAGTGCC

   The number of repeat units of [AATG] is 6.

(c) Determine whether an individual is heterozygous or homozygous for the alleles at the particular loci according to the number of short repeats of base sequence found in the alleles at the particular loci listed in the table below.

Locus	Repeat of short base sequence	No. of repeats of allele 1	No. of repeats of allele 2	Heterozygous or homozygous
STR D5S818 on Chromosome 5	AGAT	7	8	*heterozygous*
STR CSF1PO on Chromosome 5	AGAT	13	13	*homozygous*
STR D7S820 on Chromosome 7	GATA	6	12	*heterozygous*
STR D8S1179 on Chromosome 8	TCTA	10	10	*homozygous*

(d) Based on the following results, who are probably biologically related?

	Man A	Man B	Man C
Locus	No. of repeats of alleles 1 / 2	No. of repeats of alleles 1 / 2	No. of repeats of alleles 1 / 2
STR D5S818	10 / 12	7 / 8	10 / 12
STR CSF1PO	13 / 13	13 /12	13/ 13
STR D7S820	6 / 12	12 /12	6 / 12
STR D8S1179	10 / 10	8 / 10	10 / 10

Men A and C are probably biologically related because the no. of short tandem repeats of the alleles at the 4 loci are all matched.

IX. References

Eskandarion, M., Najafi, M., Akbari Eidgahi, M., Alipour Tabrizi, A., & Golmohamadi, T. (2015). Optimization of short tandem repeats (STR) typing method and allele frequency of 8 STR markers in referring to forensic medicine of Semnan Province. *Journal of Medicine and Life*, *8*(Special Issue 4), 180–185. PubMed PMID: 28316728; PubMed Central PMCID: PMCPMC5319289.

International Committee of the Red Cross. (2009). *Missing people, DNA analysis and identification of human remains: A guide to best practice in armed conflicts and other situations of armed violence*. Geneva: ICRC.

Micka, K. A., Sprecher, C. J., Lins, A. M., Theisen Comey, C., Koons, B. W., Crouse, C., et al. (1996) Validation of multiplex polymorphic STR amplification sets developed for personal identification applications. *Journal of Forensic Science*, *41*(4), 582–90. PubMed PMID: 8754568.