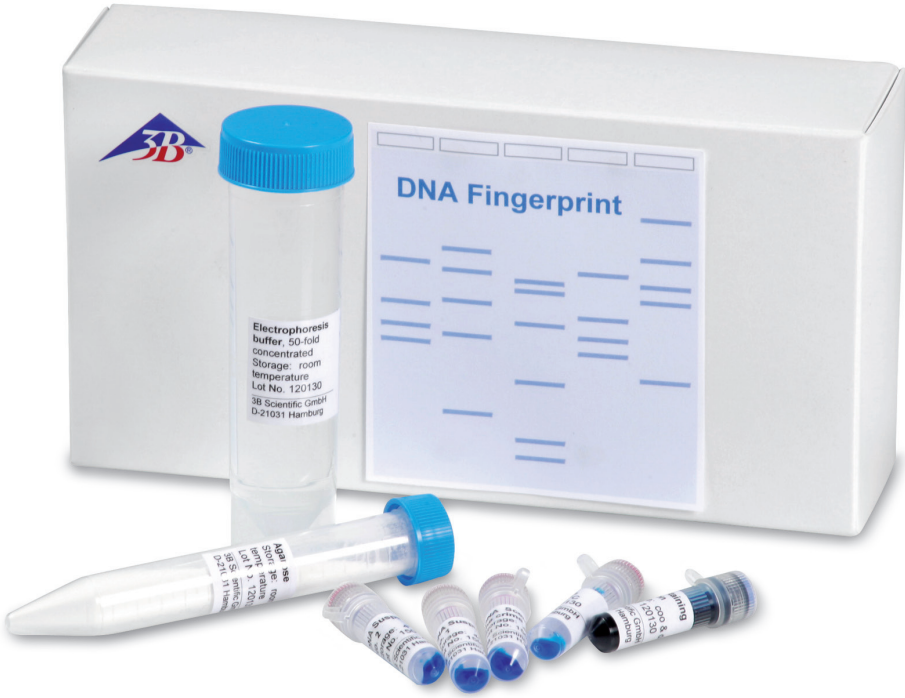
...going one step further



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Genetic Fingerprint

English

I. Introduction

This manual explains the method of genetic fingerprinting as it is applied today. The present experimental kit contains various DNA samples, which can be separated by size in the gel electrophoresis. In this way individual DNA pattern can be displayed and, according to these DNA pattern, individuals can be identified and distinguished without ambiguity. The DNA segments included in this kit were generated before by the PCR (Polymerase Chain Reaction) procedure. The generation is not part of this experiment.

II. Theory of the Genetic Fingerprint

Genetic fingerprint or genetic fingerprinting is a term used to describe the DNA profile of an individual, displayed in form of a barcode. The DNA profiling technique was first developed in the middle of the 1980s by the British molecular geneticist Alec Jeffreys at the University of Leicester. For a genetic fingerprint, polymorph DNA areas, i.e. areas that show significant differences, are compared.
These so-called “repeats”, repeating sequences of two or more nucleotides, can be found in uncoded DNA regions. The number of repeats varies from one individual to the other and can therefore be used for identification of individuals.

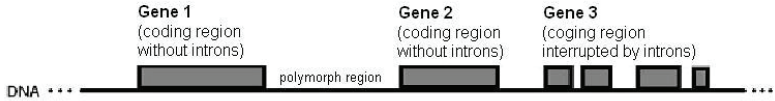

Fig. 1: Schematic illustration of polymorph DNA regions.

Various terms exist for these polymorph regions in literature, some of them are synonyms, others, however describe different characteristic forms of polymorphism. The most important terms will be briefly discussed in the following:
VNTRs (Variable Number Tandem Repeats) describes repeats of sequences between 10 to 150 nucleotides. If repetitive sequences are shorter (2 to 7 nucleotides), they are called STRs (Short Tandem Repeats).
Nowadays polymorph DNA regions are usually amplified via PCR by using conserved DNA sequences on the left and on the right of these polymorph regions for the binding of PCR primers. As a result, you receive PCR fragments of various lengths, which are narrowed on the left and on the right by the primer pair that had been applied. Those pairs are practically unique for every single individual (monozygotic twins are the only exception).

Strictly speaking, for one primer pair PCR usually reveals 2 individual DNA fragments per region of reproduction, as the DNA is normally diploid in body cells. This is related to the fact that the homologue areas of a polymorph DNA region are frequently heterozygous. If both alleles are equal (homozygoty), they form equally long PCR products for these positions (see fig. 2).
With a single set of chromosomes, e.g. in traces of sperm, there is only one allele type per locus. Therefore, when duplicating the DNA of this region, you will get only one fragment length as a result since there is only one allele.

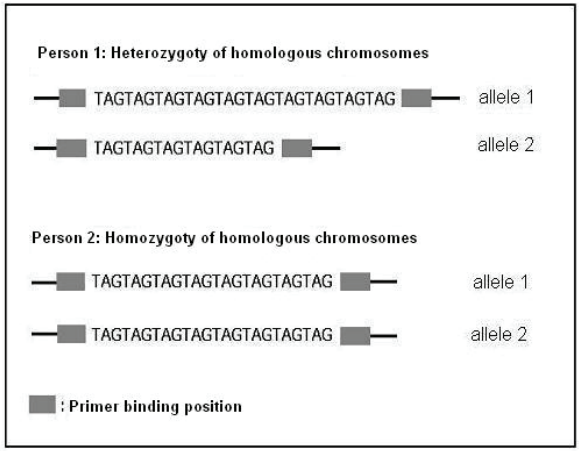

Fig. 2: VNTR loci of two people, each allele is illustrated in single-stranded form.

When duplicating several of these DNA areas by applying different PCR primer pairs, many PCR products with different lengths are formed. After separation according to size during the electrophoresis process these PCR products result in a kind of DNA barcode (see fig. 3).
By default, 10 to 13 primer pairs are used to provide a higher level of security for the genetic analysis. As an example, figure 3 shows the PCR for four DNA regions. If each DNA region was duplicate available (diploidy) and if there was hereozygoty for all four loci, the DNA profile below would have a more complex structure and would be composed of eight DNA fragments.

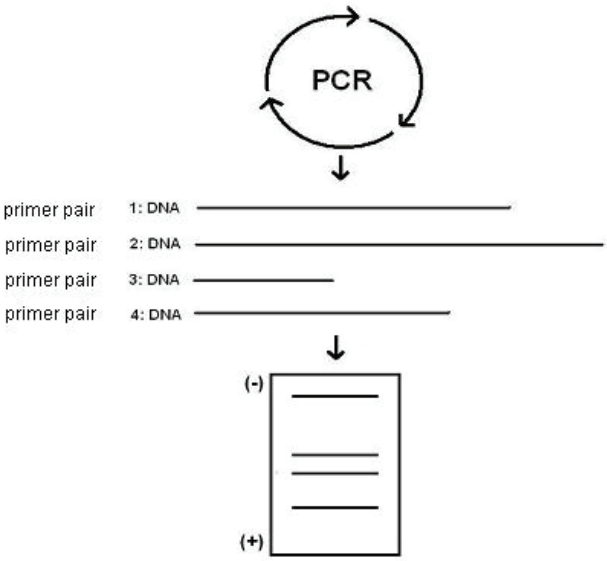

Fig. 3: Amplification of various regions of polymorph DNA results in PCR fragments with different lengths. The separation by electrophoresis of these PCR fragments results in a DNA barcode.

III. Components of the Experimental Kit

DNA of the victim, 120 µl
DNA obtained at the crime scene, 120 µl
DNA of suspect no. 1, 120 µl
DNA of suspect no. 2, 120 µl
Electrophoresis buffer, 50-fold concentrated, 50 ml
Agarose, 6 g
DNA-staining solution, 200-fold concentrated, 1.5 ml

Note: The DNA samples are ready to use and can be applied directly for gel electrophoresis. For long term storage, they maybe kept in the freezer at –18°C.
The DNA samples contain the dye bromophenol blue (BPB) so that it is possible to observe the progress of gel electrophoresis. The staining of the DNA fragments should be carried out after gel electrophoresis by using the staining solution included in this experimental kit.

The DNA samples included in this kit are not of human origin and only serve to simulate the findings of a real investigation.

IV. Equipment and Solutions Required

Electrophoresis chamber incl. power supply
Microliter pipette incl. pipette tips
Protective clothing (lab coat, protective goggles, gloves)
Microwave and Erlenmeyer flask (for the production of the agarose gel)

V. Preparation of the Experiment

Electrophoresis buffer
Dilute the 50-fold concentrated electrophoresis buffer (or a part of it) with distilled water to a one-fold concentration. This one-fold concentrated electrophoresis buffer can then be used for the experiment. It is possible to use it several times.

Casting the agarose gel
For the electrophoresis of the DNA fragments we recommend a 1% agarose gel. Depending on the electrophoresis chamber in use you will require differing gel volumes. You can find out the amount required, along with the directions for use of the equipment, by checking the operating instructions for the specific electrophoresis chamber in use.
Many electrophoresis chambers allow castingof the gel 1-2 days in advance of the actual experiment. This can be an advantage for reasons both of time and organization.

Note: For the production of the agarose gel weigh the necessary amount of agarose and place into an Erlenmeyer flask. Add the equivalent volume of electrophoresis buffer and seal it gently with a cotton plug. Before heating in a microwave take note of the total weight of the Erlenmeyer flask (flask incl. contents). This is useful in order to be able to set off the evaporation losses after dissolving the agarose solution with distilled water. In this way you can make sure that you reach the percentage of the agarose gel that you actually require. The gel should not be casted too thickly as this would be unfavourable for the staining of the DNA fragments after the gel electrophoresis process. Ideally, a 3-4 mm thick gel should be used for this experiment.

Prepare the DNA-staining solution
Dilute the 200-fold concentrated staining solution with distilled water so that you receive a one-fold staining solution. This means 1 volume of 200-fold concentrated staining solution and 199 volumes of distilled water, to produce a staining solution ready to use. You can store the solution in the fridge at 4°C, protected from light. The DNA staining solution may be used several times.

VI. Experiment

Note: Experience shows that pipetting with the microlitre pipettes causes initial difficulties. Therefore practising the application of the samples into the gel pockets of the agarose gel before carrying out the actual experiment by using a solution made of 2 volumes of blue ink and 1 volume of glycerine is recommended. This solution has approximately the same viscosity as the included DNA samples and is therefore well-suited for purposes of exercise.
Frequently occurring errors: In the beginning, you might tend to hit the gel pockets badly or fill them up too much. It also might be the case that you insert the pipette too deeply into the gel pocket so that the bottom of the pocket is damaged. Another error may occur by pulling the pipette tip out too quickly so that part of the sample is spilt outside the pocket . Take care that you have fully released the contents of the pipette and do not suck any of them back-up again when removing the tip..

Electrophoresis of the DNA samples
Using a pipette place the DNA samples into the pockets of the agarose gel in the following order:

- DNA – victim
- DNA – crime scene
- DNA – suspect no. 1
- DNA – suspect no. 2

When working with the staining solution enclosed in this kit, use 12 µl of DNA for each gel pocket. However, if you use a more sensitive dye for staining, 8 to 10 µl of each DNA sample may be sufficient. Care must be taken to ensure that the bottom of the gel pockets is not damaged. This can happen if you insert the pipette tip too deeply into the gel pocket during the pipetting process.

Start the gel electrophoresis immediately after the application of the DNA samples. The direct voltage that you have to set depends on the electrophoresis chamber in use. As a rule of thumb, 5 volts/ cm of electrode gap should be set. Stop the electrophoresis procedure when the bromophenol blue dye within the DNA samples reaches the lower edge of the agarose gel.

Staining of the DNA bands
After the gel electrophoresis you should transfer the agarose gel carefully into a suitable bowl for staining. You can use either a plastic or glass bowl, for transferring the gel into the bowl use a spatula.
In the next step, coat the gel with the staining solution and stain in for approximately 10-15 mins. whilst moving the bowl gently back and forth to ensure that the DNA will be equally stained. After this, pour the staining solution back into the protective flask and rinse the gel with tap water until the background is sufficiently de-colourised and the bands become visible. Ideally, take a picture of the gel with transmitted light on a light box.
You can wrap the gel with household foil and store it in the fridge. Usually, one can see the DNA bands even more clearly after storing the gel over night in a fridge.

VII. Evaluation

When applying the DNA samples in the following order, from left to right

- DNA of the victim
- DNA of the crime scene
- DNA of suspect no. 1
- DNA of suspect no. 2

the DNA shows under optimum conditions for electrophoresis and an optimum staining the following fragment lengths (in base pairs, bp):

DNA crime scene	DNA victim	DNA suspect no. 1	DNA suspect no. 2
21.200	23.100	23.100	21.200
7.400	21.200	9.400	7.400
5.800	9.400	6.500	5.800
5.600	7.400	4.300	5.600
4.800	6.500	2.300	4.800
3.500	5.800	2.000	3.500
	5.600	560	
	4.800	120	
	4.300		
	3.500		
	2.300		
	2.000		
	560		
	120		

With a gel length of ca. 8 cm, when staining with the methylenblue dye that is included, you get the following DNA pattern (see fig. 4).

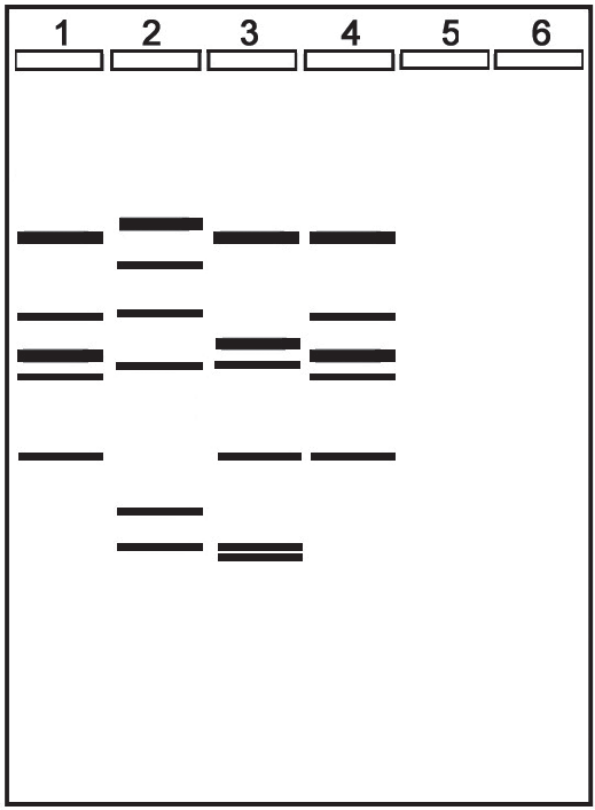

Fig. 4: DNA fragments after gel electrophoresis and staining of DNA. Lane 1: DNA from the crime scene, lane 2: DNA of the victim, lane 3: suspect no. 1, lane 4: suspect no. 2.

Frequently the small DNA fragments are not visible because the staining with the dye provided does not result in a high enough sensitivity to be able to display such relatively small fragments.
In circumstances such as too short a separation path, DNA fragments with similar lengths cannot be separated from each other and double bands may occur.
However, both effects mentioned above are irrelevant for the evaluation and the principal understanding of the methodology.
As the DNA fragment pattern shows, there is a clear match of the DNA pattern of suspect no. 2 and the DNA pattern of the DNA obtained at the crime scene.

VIII. Suggestions and Questions for Integration into Teaching

Preparatory tasks for the lesson

1. Do some research on the topic ”genetic fingerprint.” Alternatively a lecture on the subject might be prepared and given by a student or by the teacher.

2. Discuss with the students the social, ethical and legal aspects of DNA analysis. If applicable, what possible effects or risks would there be if genetic analyses were used by state authorities, insurance companies or employers?

3. What about the reliability of the procedure? Is the genetic fingerprint really individual?

4. Does the DNA of a suspect obtained at a crime scene prove that the suspect had been there?

5. How does the agarose gel electrophoresis work?

Tasks during the lesson

1. The pipetting procedure and the application of the samples into the gel pockets of an agarose gel might be practised in advance by using a solution made of blue ink and glycerine. For the preparation of such a solution, add 2 volumes of blue ink and 1 volume of glycerine. This solution has approximately the same viscosity as the enclosed DNA samples and is therefore well-suited for practising pipetting.

2. Read experiment manual carefully, clarify any questions with your neighbour.

3. Carry out the experiment and evaluate it.

4. Check results you did not expect by checking for possible errors in the experimental procedure. Please read the manual again, if necessary, run the experiment again.

IX. Safety and Disposal

The safe handling of chemicals and laboratory equipment requires a certain amount of basic knowledge and safety measures. During the experiment, lab coats and protective goggles should be worn. Gloves must be provided by the teacher and should be worn if necessary. For the production of the agarose gel wear insulated gloves to avoid burning or scalding of hands.
Familiarise yourself with all equipment and check that you know how to use it correctly and safely; pay particular attention to the dangers of electricity. All connectors, power cords, the work area including your hands must be dry before touching electrical devices.

Further health and safety measures: tie up long hair, do not wear any jewellery, and wear tight-fitting sleeves, so that no unwanted contact with equipments, chemicals, etc., occurs.

All waste should be disposed of in accordance with the instructions and local regulations.

English

Hazard Classification of the Kit Components

DNA samples
The DNA samples contain an amount of 10% glycerin as well as the bromophenol blue dye at a concentration of 0.25%.
Classification according to Regulation (EC) No. 1272/2008: not harmful.
Classification according to Directive 67/548/EEC and Directive 1999/45/EC: not harmful.

Electrophoresis buffer, 50-fold concentrated
The following information refers to the concentrated electrophoresis buffer and does not necessarily apply to the diluted buffer (working solution).
Classification according to Regulation (EC) No. 1272/2008:
Safety instructions
H315: causes skin irritation.
H319: causes eye irritation.
H335: may cause irritation to the respiratory system.

Safety instructions
P280: Wear protective clothing and eye protection.
P261: Avoid inhalation.
P302+P352: In case of accidental contact with your skin, wash your skin thoroughly with soap and water.
P305+P351+P338: In case of contact with eyes, rinse the eyes gently with water for several minutes. Remove contact lenses if necessary and continue rinsing.

Classification according to Directive 67/548/EEC and Directive 1999/45/EC:
Hazards
R36+R37+R38: Irritating to eyes, respiratory system and skin.

Safety instructions
S26: In case of contact with eyes, rinse immediately with water and seek medical advice.
S28: After contact with skin, wash immediately with plenty of water.
S37+S9: Wear suitable protective gloves and goggles/ face protection.

Agarose
Classification according to Regulation (EC) No. 1272/2008: not harmful.
Classification according to Directive 67/548/EEC and Regulation 1999/45/EC: not harmful.
Suggestion: Wear gloves and protective goggles; avoid contact with skin and eyes. Avoid generation of dust, do not inhale agarose.

DNA-staining solution, 200-fold concentrated
According to Regulation (EC) No. 1272/2008 the watery solution is not harmful.
According to Directive 67/548/EEC and Directive 1999/45/EC: not harmful.
Suggestion: Wear protective gloves and goggles. Avoid contact with skin and eyes.

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