Genetic fingerprinting (Item No.: P8110300)

Curricular Relevance

Area of Expertise:	Education Level:	Topic:	Subtopic:	Experiment:
Biology	Age 16-19	Genetics	Molecular Genetics	Genetic fingerprinting

Difficulty	Preparation Time	Execution Time	Recommended Group Size
Difficult	20 Minutes	50 Minutes	2 Students

Additional Requirements:

- Water bath vessel for the Erlenmeyer flask (for example a sufficiently large beaker glass DURAN®) or a microwave
- For exact length determination of the DNA fragments: DNA length marker with color marker bromphenol blue

Experiment Variations:

Keywords:

chromosome, DNA profiles, enzyme, gel electrophoresis, genetics, minisatellite DNA, polymerase, polymerase chain reaction, recombination, VNTR, inheritance, genetic fingerprint, homologous agarose

Teacher information

Introduction

These instructions describe the DNA fingerprinting procedure as it is used today. The present experiment kit includes several different DNA samples that will be separated based on their respective sizes by way of gel electrophoresis. This leads to individual DNA profiles which are then used to discriminate between, and to identify, individuals. The DNA sequences that are used here have been generated by way of the DNA amplification method "polymerase chain reaction" (PCR). This first part of the analysis is not part of this experiment kit.

Theoretical background of DNA fingerprinting
A DNA (or genetic) fingerprint is the DNA profile of an individual, represented in the form of a barcode pattern. The method was discovered or rather developed in the mid-1980s by the British geneticist Alec Jeffrey.
The DNA regions that are used for DNA fingerprinting are highly polymorphic, i.e. they are very different in unrelated individuals. They are located in non-coding DNA regions. These regions often include repetitive sequences of two or more nucleotides that are known as repeats. The number of repeats differs from individual to individual so that it can be used for identification.

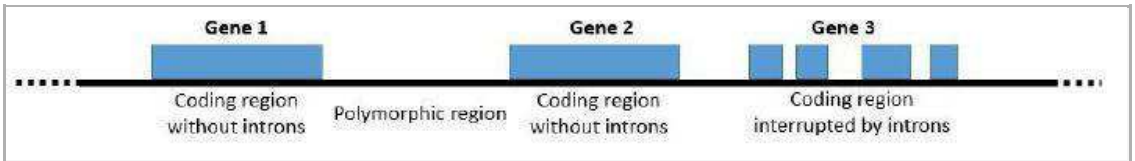

Fig. 1: Schematic representation of polymorphic DNA regions

The specialised literature uses various terms for these polymorphic regions. Some of them are synonyms, but others also describe different types of polymorphism. These are the most important terms:
VNTRs (Variable Number Tandem Repeats) are repetitive sequences of 10 to 15 nucleotides. If the repetitive sequences are shorter (2 to 7 nucleotides), they are called STRs (Short Tandem Repeats).
Today, polymorphic DNA regions are usually amplified by polymerase chain reaction (PCR). During this process, conserved DNA region on the left and right of such a polymorphic region are used t o created PCR primers. This leads to PCR fragments of different lengths that are limited on the left and on the right by the primer pair that is used and that are basically unique for every individual (exception: identical (monozygotic) twins).
To be precise, in most cases 2 individual DNA fragments per amplification site result for every primer pair, since the DNA in body cells is usually diploid. This is due to the fact that the homologous sites in the polymorphic DNA regions are often heterozygous. If both alleles are identical (homozygosity), the results are PCR products of identical length for these positions (Fig. 2).
If there is only one single set of chromosomes, e.g. in sperm traces, there is, of course, only one type of allele per locus. This

means that the amplification of DNA of this region will result in only one DNA fragment length, since there is only one allele.

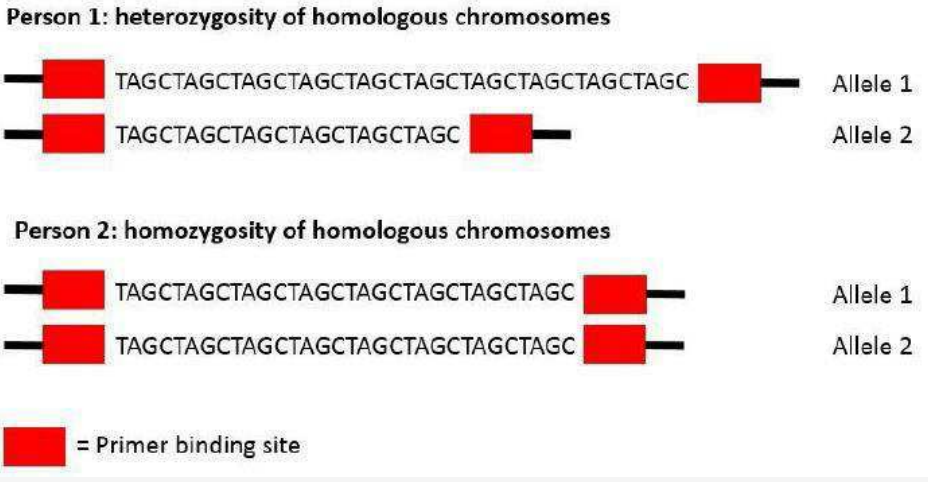

Fig. 2: VNTR loci of two individuals with every allele being represented in a single-stranded form

If several of these DNA regions are amplified by using different PCR primer pairs, the result will be a corresponding number of PCR products with different lengths. Following their size-based separation by way of electrophoresis, these PCR products lead to some kind of DNA barcode (Fig. 3).
As a standard, 10 to 13 primer pairs are used in order to ensure a high level of reliability for the genetic fingerprint. Figure 3 shows the PCR for 4 DNA regions as an example. If every DNA region would exist twofold (diploidy) and if all 4 loci would fall under heterozygosity, then the DNA profile that is shown below would be more complex and consist of 8 DNA fragments.

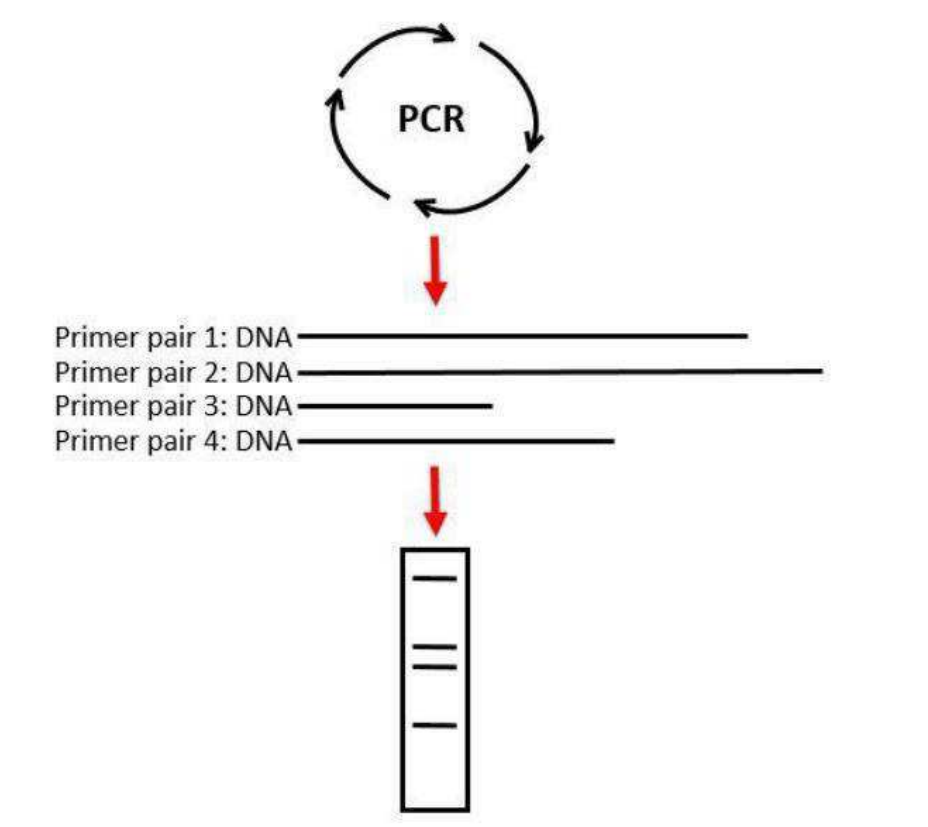

Fig. 3: When several regions of polymorphic DNA are amplified, PCR products with different lengths result. Following their size-based separation by way of electrophoresis, these PCR products lead to some kind of DNA barcode.

Suggestions and questions for the integration into the lesson
Tasks for preparing the lesson
1. Conduct some research concerning the topic "DNA fingerprinting". Alternatively, a student and teacher lecture concerning this topic can be prepared.
2. Discuss the social, ethical and legal aspects of DNA analyses with your students. What are the potential consequences or risks that must be feared if governmental institutions, insurance companies or employers use genetic analyses?
3. What about the reliability of the procedure? Is the genetic fingerprint really so unique and, if so, what are the reasons?
4. Does the fact of discovering the DNA of a suspect at a crime scene prove that the suspect has been present at that crime

scene?
5. How does the agarose gel electrophoresis work?

Tasks during the lesson
1. The pipetting and transferring of the samples into the gel pockets of the agarose gel can be trained by way of a solution of blue ink and glycerine. Take 2 volumes of blue ink and add 1 volume of glycerine. This training solution has approximately the same viscosity as the supplied DNA samples which makes it particularly suit-able for training the pipetting process.
2. Thoroughly read all of the experiment instructions. If you have any questions, dis-cuss them with the student sitting next to you.
3. Perform the experiment and evaluate it.
4. Check any unexpected results by trying to identify any mistakes that might have occurred during the execution of the experiment. Read the experiment instructions again. If necessary, repeat the experiment.

Material

Position No.	Material	Order No.	Quantity
1	Microliterpipette 2-20 µl	47141-10	1
2	Microliterpipette 20-200 µl	47141-11	1
3	Pipette tips, 2-200 µl, 1000 pcs	47148-01	1
4	Electrophoresis chamber, horizontal	KLA-530-200	1
5	Spatula, steel, l=185mm	46952-00	1
6	Spoon, nickel-plated, 180 mm	33392-00	1
7	Protecting glasses, clear glass	39316-00	1
8	Staining dish, UV permeable, PETG	35023-20	1
9	Electrophoresis power supply 100V/200V	65966-93	1
10	Hotplate magnetic stirrer with connection for electronic contact thermometer, 3 ltr., 230 V	35760-93	1
11	Erlenmeyer flask, narrow neck, 500 ml	36121-00	1
12	Graduated cylinder, high ,PP, 500 ml	46288-01	1
13	Magnetic stirring bar, 50 mm, cylindrical	46299-03	1
14	Cotton wool, white 200 g	31944-10	1
15	Water, distilled 5 l	31246-81	1
16	Rubber gloves, size S (7)	39325-00	1
17	Kit: Forensic DNA Fingerprinting (crime scene)	KLA-530-120	1

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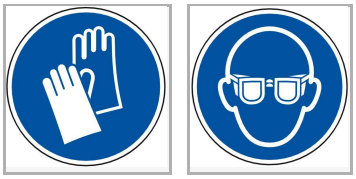
Safety and disposal



Wear gloves and safety goggles!
The safe handling of laboratory equipment and chemicals requires a certain level of fundamental knowledge and safety measures. As a general rule, you should wear a laboratory coat and safety goggles during the experiment. Gloves should also be provided and worn as required. When preparing the agarose gel, wear insulated gloves in order to avoid burning or scaling your hands. The handling of the equipment and the risks that are involved should be known. Particular attention must be paid to the electricity hazards. Ensure that all of the connect-ors, mains power cables and work surfaces (and your hands) are dry prior to operating the electrical equipment. Further health and safety measures: Tie your hair back, do not wear any jewellery and wear clothes with tight-fitting sleeves in order to avoid any unwanted contact with the equipment, chemicals, etc. Waste must be disposed of in accordance with the instructions and with the local rules and regulations.

Potential hazards of the components of the kit
DNA samples
The DNA samples include 10% glycerine as well as the dye bromophenol blue with a concentration of 0.25%. In accordance with the directive (EC) 1272/2008, the substance or mixture has not been rated as hazardous. In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.

Electrophoresis buffer, 50x concentration
The following information refers to the concentrated electrophoresis buffer. This means that it does not necessarily apply to the diluted buffer (working solution).
Rating in accordance with the directive (EC) 1272/2008:

Hazard information H315:
Causes skin irritation
H319: Causes serious eye irritation
H335: May cause respiratory irritation

Safety information
P280: Wear protective clothing and eye protection.
P261: Avoid breathing dust/fume/gas/mist/vapours/spray.
P302+P352: If on skin: Wash with plenty of water and soap.
P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.

Agarose
In accordance with the directive (EC) 1272/2008, the substance has been rated as non-hazardous. In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous. Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes. Avoid the formation of dust. Do not breathe the agarose.

DNA staining solution (200x concentration)
In accordance with the directive (EC) 1272/2008, the aqueous solution has not been rated as hazardous.
In accordance with the directive 67/548/ECC or 1999/45/EC, the substance or mixture has not been rated as hazardous.
Recommendation: Wear gloves and protective goggles. Avoid contact with the skin and eyes.

Results

After the application of the DNA samples from left to right as follows:

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

and under optimum electrophoresis conditions and optimum staining of the DNA, the following fragment lengths can be observed (values stated in base pairs, bp):

DNA crime scene	DNA vic-tim	DNA suspect 1	DNA suspect 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		

In the case of a gel length of approximately 8 cm, staining with the supplied methylene blue dye typically leads to the following DNA pattern in the agarose gel (Fig. 4).

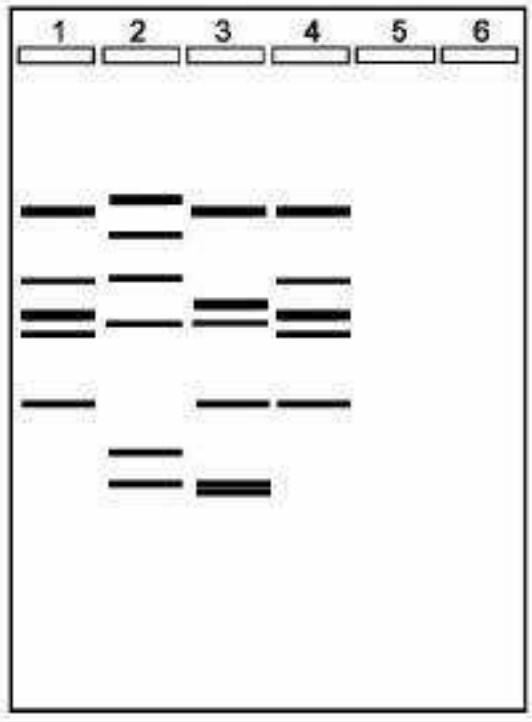


Fig. 4: DNA fragments after the gel electrophoresis and staining of the DNA. Lane 1: DNA from the crime scene; lane 2: DNA of the victim; lane 3: suspect 1; lane 4: suspect 2.

Small DNA fragments are often not visible, since the supplied dye that is used for staining is not sensitive enough in order to stain relatively short DNA fragments.
If the migration distance is too short, it may be impossible to separate DNA fragments of a similar length, resulting in double bands.
However, both effects are insignificant for the evaluation and fundamental comprehen-sion of the methodology.
As the DNA fragment pattern shows, the DNA sample of suspect no. 2 clearly matches the DNA sample taken from the crime scene.

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Genetic fingerprinting (Item No.: P8110300)

Introduction

DNA profiling is a forensic technique used to identify individuals by characteristics of their DNA, represented in the form of a barcode pattern.

DNA fingerprinting is a routine forensic tool to convict an offender if she leaves DNA traces at the crime scene

The DNA regions that are used for DNA fingerprinting are highly polymorphic, i.e. they are very different in unrelated individuals. They are located in non-coding DNA regions.
Minisatellite DNA sequences, the variable number tandem repeats (VNTRs), are non-coding DNA segments composed of repetitions of a particular sequence of bases. The number of repetitions, i.e. the length of the minisatellite, is variable to a limited extent.
The minisatellites are inherited by the parents. Since, however, homologous recombination occurs within the chromosome pairs in the parental germline, equivalent sections of the grandparent chromosomes are randomly redistributed to the germ cells (oocytes and sperm cells) an individual composition of the genome in every human being results.

In order to find these differences, the PCR (polymerase chain reaction) in this experiment is used. Using this method, DNA sections can be amplified so they can be visualized in gel electrophoresis by separating their respective sizes.

Equipment

Position No.	Material	Order No.	Quantity
1	Microliterpipette 2-20 µl	47141-10	1
2	Microliterpipette 20-200 µl	47141-11	1
3	Pipette tips, 2-200 µl, 1000 pcs	47148-01	1
4	Electrophoresis chamber, horizontal	KLA-530-200	1
5	Spatula, steel, l=185mm	46952-00	1
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Safety and disposal

Wear gloves and safety goggles!

Please follow the directives of your instructor in respect to electrical instruments (power supply and electrophoresis instrument) as well as how to handle chemicals. Please also follow the regulations concerning disposal of chemicals.

The safe handling of laboratory equipment and chemicals requires a certain level of fundamental knowledge and safety measures. As a general rule, you should wear a laboratory coat and safety goggles during the experiment. Gloves should also be provided and worn as required. When preparing the agarose gel, wear insulated gloves in order to avoid burning or scaling your hands. The handling of the equipment and the risks that are involved should be known. Particular attention must be paid to the electricity hazards. Ensure that all of the connectors, mains power cables and work surfaces (and your hands) are dry prior to operating the electrical equipment.
Further health and safety measures: Tie your hair back, do not wear any jewellery and wear clothes with tight-fitting sleeves in order to avoid any unwanted contact with the equipment, chemicals, etc.
Waste must be disposed of in accordance with the instructions and with the local rules and regulations.

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Set-up and procedure

The kit includes the following:

DNA of the victim: 120 µl
DNA from the crime scene: 120 µl
DNA of suspect no. 1: 120 µl
DNA of suspect no. 2: 120 µl
Electrophoresis buffer (50x concentration): 50 ml
Agarose: 6 g
DNA staining solution (200x concentration): 1.5 ml

Note: The DNA samples are ready for use and can be directly used for the gel electrophoresis. If the DNA samples need to be stored for a longer period of time, they should be deep-frozen at -18°C.
The DNA samples include the dye bromophenol blue (BPB). This dye is used to monitor the progress of the gel electrophoresis. After the gel electrophoresis, the DNA fragments are stained with the aid of the supplied DNA staining solution.
The DNA samples that are included in the kit are of non-human origin. They are intended solely for the simulation of the results of real analyses.

Required devices, ancillary equipment and solutions:
Electrophoresis chamber, including a power supply unit Microlitre pipette, including pipette tips Protective clothing (laboratory coat, protective goggles, gloves) Microwave oven and Erlenmeyer flasks (for preparing the agarose gel)

Tip: Experience has shown that pipetting with a microlitre pipette can be difficult for beginners. This is why the pipetting process and the filling of the samples into the gel pockets of the agarose gel should be trained prior to the experiment. A suitable training solution is a solution of blue ink (2 volumes) and glycerine (1 volume). This training solution has approximately the same viscosity as the supplied DNA samples which makes it particularly suitable for training purposes.

Common problems: It can be difficult to hit the gel pockets precisely and sometimes the pockets are overfilled. In other cases, the tip of the pipette may be inserted into the gel pocket too deep so that the bottom of the gel pocket is damaged. Another common problem is that the tip of the pipette is withdrawn from the gel pocket too quickly so that part of the sample, which has just been dispensed into the pocket, will also be dragged out of the pocket. The pushbutton of the micropipette does not remain pushed down when the tip of the pipette is withdrawn so that the sample will be drawn back into the pipette.

Preparation of the experiment:

Electrophoresis buffer
Dilute the electrophoresis buffer (50x concentration) (or part of it) with distilled water until a 1x concentration is reached. This electrophoresis buffer (1x concentration) will then be used for the experiment. It can be reused.

Casting the agarose gel
We recommend using a 1% agarose gel for the electrophoresis of the DNA fragments. Depending on the electrophoresis chamber that is used, different gel volumes are re-quired. The quantities are stated in the handbook of the electrophoresis chamber.
The same applies to the correct utilisation of the electrophoresis chamber. Many elec-trophoresis chambers permit the gel to be cast 1-2 days before the actual experiment. This can be useful for reasons of time and organisation.

Tip: In order to prepare the agarose gel, weigh the required quantity of agarose, fill it into an Erlenmeyer flask, add a corresponding volume of the electrophoresis buffer and seal the flask slightly by way of a cotton-wool stopper. Prior to heating the material in the microwave oven, note down the total weight of the Erlenmeyer flask (flask in-cluding the content) so that - after the dissolution of the agarose solution - the loss due to boiling can be compensated for with distilled water. This ensures that the desired percentage of the agarose gel is reached. The gel should not be cast too thick, since this would have a negative effect on the staining of the DNA fragments after the com-pletion of the gel electrophoresis. Gel with a thickness of 3 to 4 mm is ideal for this experiment.

Preparation of the DNA staining solution
Dilute the staining solution (200x concentration) with distilled water so that a staining solution with 1x concentration results. This means that 1 volume of the staining solution with 200x concentration must be added to 199 volumes of distilled water in order to produce the ready-for-use staining solution. Store in a dark place at 4°C in a refrigera-tor. The DNA staining solution can be reused.

Procedure
Tip: Experience has shown that pipetting with a microlitre pipette can be difficult for beginners. This is why the pipetting process and the filling of the samples into the gel pockets of the agarose gel should be trained prior to the experiment. A suitable training solution is a solution of blue ink (2 volumes) and glycerine (1 volume). This training solution has approximately the same viscosity as the supplied DNA samples which makes it particularly suitable for training purposes.
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The pushbutton of the micropipette does not remain pushed down when the tip of the pipette is withdrawn so that the sample will be drawn back into the pipette.

Electrophoresis of the DNA samples
Transfer each of the DNA samples into the gel pockets of the agarose gel by way of a microlitre pipette in the order that is stated below:

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

If the staining solution that is included in the kit is used, 12 µl of DNA are required per gel pocket. If a more sensitive dye is used for staining, DNA samples of 8 to 10 µl may be sufficient.
Ensure that the bottom of the gel pockets is not damaged, i.e. do not insert the tip of the pipette too deep into the gel pockets.
Start the electrophoresis immediately after the application of the DNA samples. The direct voltage that is to be adjusted depends on the electrophoresis chamber that is used. As a rule of thumb, a voltage of 5 Volts/cm (distance between electrodes) should be used.
Stop the electrophoresis when the dye bromophenol blue in the DNA samples has reached the lower edge of the agarose gel.

Staining of the DNA bands
After the electrophoresis, transfer the agarose gel carefully into a suitable staining dish. The staining dish can be made of plastic or glass. Use a commercially available kitchen spatula or a wide putty knife, for example, for transferring the gel.
Overlay the gel with the staining solution and stain the gel for approximately 10 to 15 minutes. Shake the dish slightly during this process in order to ensure homogeneous staining. Then, pour the staining solution back into the storage bottle and destain the gel with tap water until the background has been sufficiently destained so that the DNA bands are visible. The gel should then ideally be photographed with transmitted light on a light box.
The gel can be wrapped into cling film and stored in a refrigerator for a few days. Fol-lowing overnight storage in a refrigerator, the DNA bands are often more visible with a higher contrast than the day before.

Evaluation
The goal of the experiment is the documentation of the gel (e.g. photograph) and to determine the suspect by comparing the migration pattern of the different samples.

Note: Small DNA fragments are often not visible, since the supplied dye that is used for staining is not sensitive enough in order to stain relatively short DNA fragments.
If the migration distance is too short, it may be impossible to separate DNA fragments of a similar length, resulting in double bands. However, both effects are insignificant for the evaluation and fundamental comprehension of the methodology.

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