Paternity test (Item No.: P8110400)

Curricular Relevance

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

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:

DNA profile, genetic fingerprint, inheritance, agarose gel electrophoresis, PCR, forensic medicine

Teacher information

Introduction

These instructions describe the genetic procedure of a paternity test 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 sev-eral individual DNA patterns that can be used to analyse parental relationships.
The DNA samples that are used here for the gel electrophoresis have been generated beforehand by the DNA amplification process PCR (polymerase chain reaction) so that this first part of the analysis is not part of this experiment kit.

Theoretical background

Normally, every human being has 46 chromosomes. 23 of them are inherited from the mother and 23 from the father. The 2 chromosomes in every pair of chromosomes carry identical genetic information, but often with small variations. This means that a large part of the genetic information is present in different alleles. 2 of the 46 chromo-somes are the so-called sex chromosomes (allosomes) X and Y, with women having the combination XX and men having the combination XY.

Small variations in the genes and, in particular, differences in the uncoded regions of the DNA lead to the fact that the DNA profile of a human being is an individual profile (exception: identical (monozygotic) twins). These non-coding DNA regions that display particularly extensive individual differences are also referred to as polymorphic DNA regions.

Non-coding DNA regions often include repetitive sequences of two or more nucleotides that are known as repeats. 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). The number of repeats differs from individual to individual so that it can be used for individual DNA profiling.
These DNA profiles enable the identification of individuals (genetic fingerprint), for example, but also the analysis of parental relationships, e.g. for paternity testing.

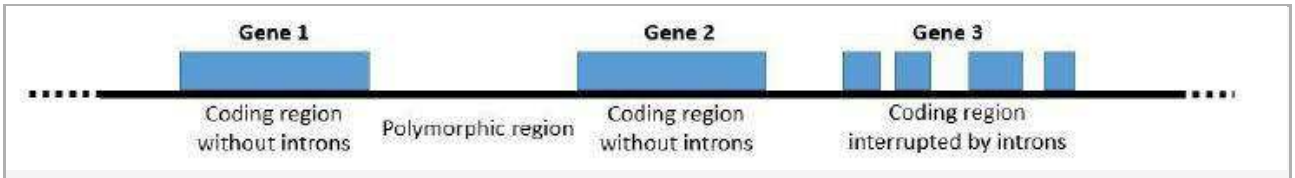


Fig. 1: Schematic representation of polymorphic DNA regions

Polymorphic DNA regions that are known to include these VNTRs or STRs are amplified by way of a polymerase chain reaction

(PCR).
As the binding sites for a primer pair, conserved DNA regions on the left and right of such a highly variable DNA region are used. 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 site will result in only one DNA fragment length.

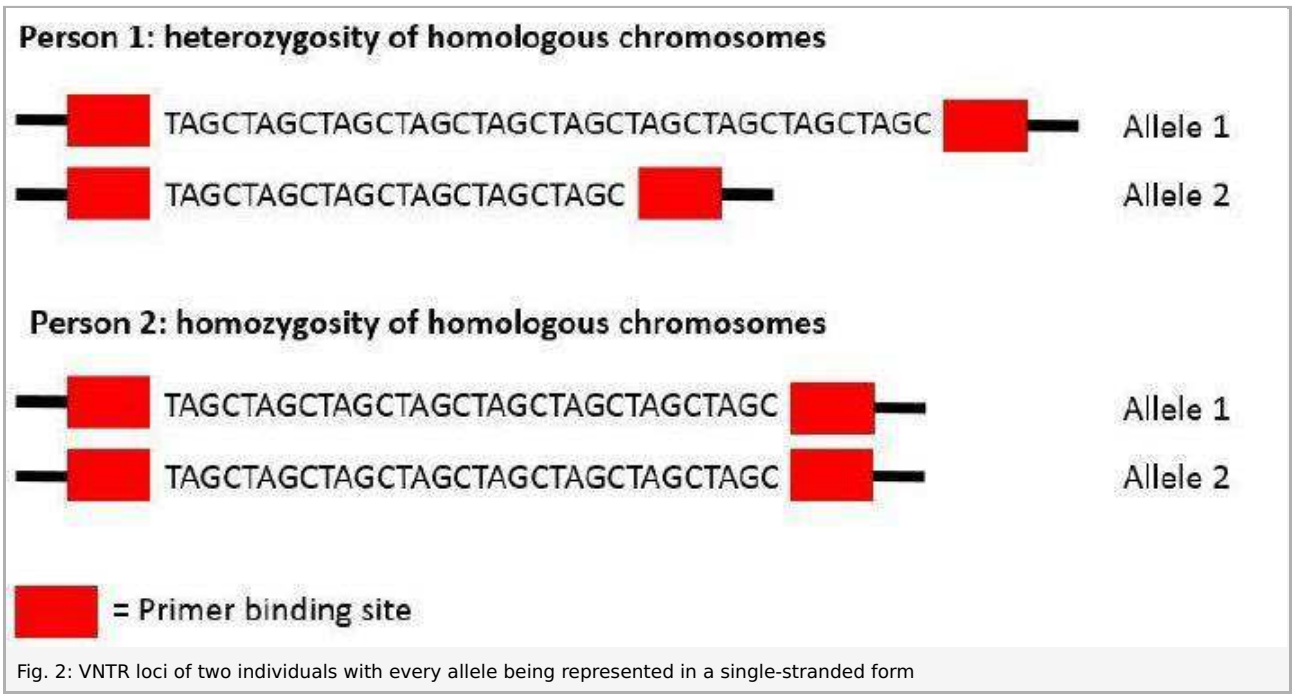


Fig. 2: VNTR loci of two individuals with every allele being represented in a single-stranded form

If several of these repeat-containing 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.
As a standard, 10 to 13 primer pairs are used in order to ensure a high level of reliability for the genetic analysis. Figure 3 shows the PCR for 4 DNA regions
as an example. If every DNA region existed twofold (diploidy) and if all 4 loci fell under heterozygosity, then the DNA profile that is shown below would be
more complex and consist of 8 DNA fragments.

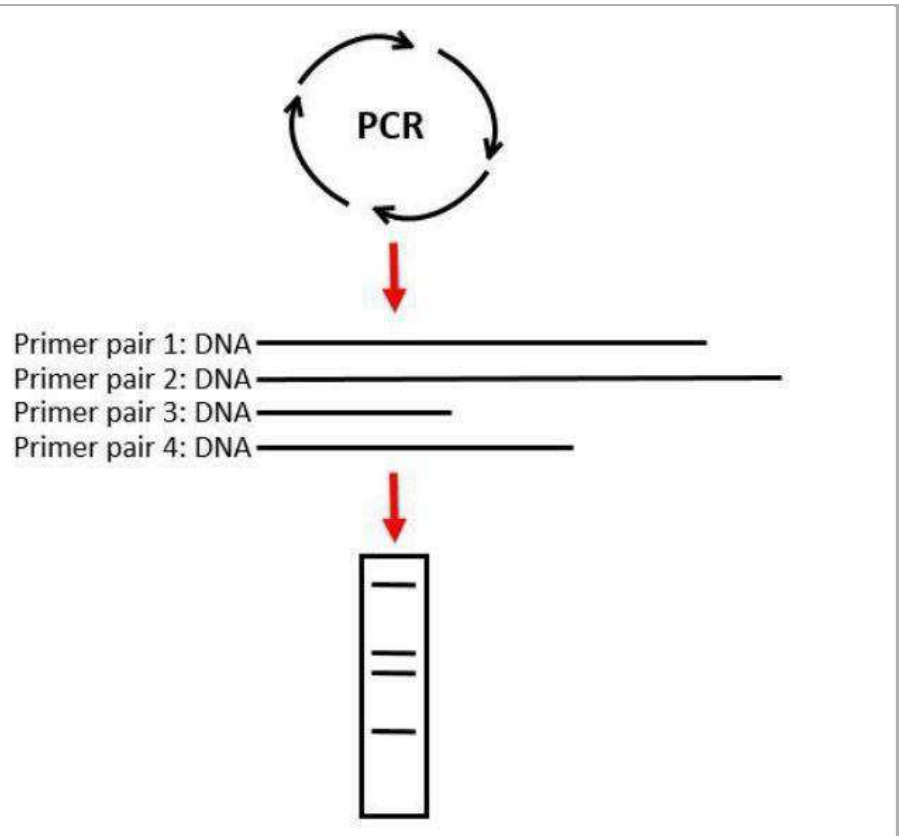


Fig. 3: When several regions of polymorphic DNA are amplified, PCR products with different lengths result. Following their size-based separation by way of electrophoresis, these PCR products lead to some kind of DNA barcode.

Suggestions and questions for the integration into the lesson

Tasks for preparing the lesson

1. Conduct some research concerning the topic "paternity test, paternity analysis". Al-ternatively, 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 that must be feared if governmental institu-tions, insurance companies or employers use genetic analyses?
3. What about the reliability of the procedure? Is the genetic paternity test really so clear and, if so, what are the reasons?
4. 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.

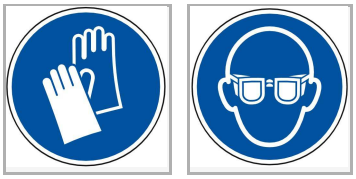
Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com

Material

Position No.	Material	Order No.	Quantity
KLA-530-130	Kit: Paternity test of a DNA profile by gel electrophoresis	KLA-530-130	1
KLA-530-200	Elektrophoresis Chamber „Mini“	KLA-530-200	1
1	Hotplate Magnetic Stirrer with connection for electroniccontact-thermometer, 3 ltr., 230 V	35760-93	1
2	Electrophoresis power supply 100V/200V	65966-93	1
3	Microliterpipette 2-20 µl	47141-10	1
4	Microliterpipette 20-200 µl	47141-11	1
5	Staining dish, UV permeable, PETG	35023-20	1
6	Grad.cylinder,high,PP,500ml	46288-01	1
7	Water, distilled 5 l	31246-81	1
8	Pipette tips, 2-200 µl, racked	47148-11	1
9	Protecting glasses, clear glass	39316-00	1
10	Erlenmeyer flask,narrow n.,500 ml	36121-00	1
11	Spoon, nickel-plated, 180 mm	33392-00	1
12	Cotton wool, white 200 g	31944-10	1
13	Rubber gloves, size S (7)	39325-00	1
14	Spatula, steel, l=185mm	46952-00	1
15	Magnetic stirring bar 50 mm, cylindrical	46299-03	1

Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com

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 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.

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 in 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.

Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com

Results

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

- DNA – mother
- DNA – child
- DNA – potential father

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 mother	DNA child	DNA potential father
21.200	23.100	23.100
7.400	21.200	9.400
5.800	9.400	6.500
5.600	7.400	4.300
4.800	6.500	2.300
3.500	5.800	2.000
	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 usually leads to the following DNA pattern in the agarose gel (Fig. 4). 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.

Fig. 4: DNA fragments after the gel electrophoresis and staining of the DNA. Trace 1: DNA sample of the mother; trace 2: DNA sample of the child; trace 3: DNA sample of the potential father.

In the present case, the child has all of the alleles of the mother and of the potential father. This indicates that the potential father is in fact the biological father of the child.

Robert-Bosch-Breite 10
D - 37079 Göttingen

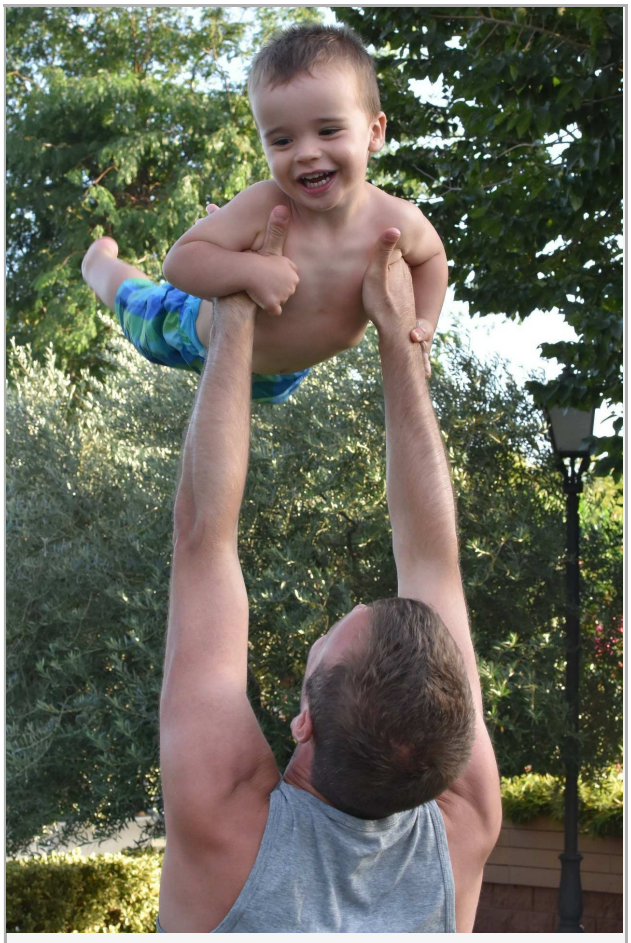
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107

info@phywe.de
www.phywe.com

Paternity test (Item No.: P8110400)

Introduction

DNA paternity testing is the use of DNA profiling (known as genetic fingerprinting) to determine whether two individuals are biologically parent and child.



Not all fathers embrace their offspring with the full of their hearts but must be convinced first of their fatherhood

The current techniques for paternity testing are using the gel electrophoresis for separating DNA samples on their respective sizes and polymerase chain reaction (PCR) for the DNA amplification.

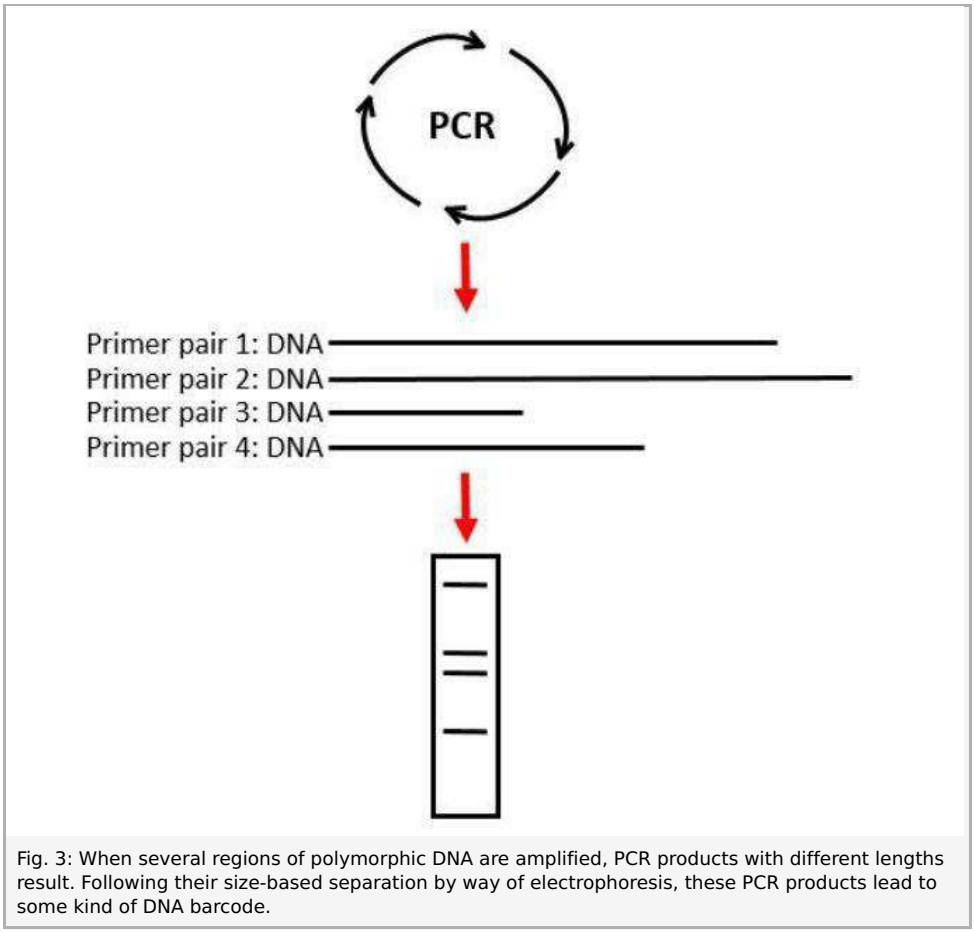


Fig. 3: When several regions of polymorphic DNA are amplified, PCR products with different lengths result. Following their size-based separation by way of electrophoresis, these PCR products lead to some kind of DNA barcode.

The human genome is packed into 46 chromosomes, including 2 sex chromosomes. Half of them are from the chromosomes of the ovum and from the chromosomes of the ovum that fertilizes the egg. The chromosomes of the sperm and the oocyte are homologous, except for sex chromosome in men. They contain genetic information relating to the same characteristics. (For example the blood group: in humans of each parent, the blood group 0, A or B can be inherited. Combinations 00, A0, AA, AB, BB and B0 are then possible.) A and B are dominant over 0).

This means that during fertilization, the haploid, oocyte and sperm are fused into a diploid cell with 46 chromosomes.
Thus, in a DNA examination, half of the genome of the child can only be identical with that of the biological mother, and the other half only with that of the biological father.

However the genome of all humans is over 99% identical, because it encodes important proteins or contributes to correct packaging of the DNA in the chromosomes

There are individual differences in very specific DNA sequences: the minisatellite DNA (VNTRs for variable number tandem minisatellites are non-coding DNA sections composed of repetitions of a very specific sequence of bases, especially minisatellite DNA is that the number of repetitions, the length of the mini satellites, varies within a certain frame and is not the same for every human being.

Using gel electrophoresis, the samples are compared to the characteristic composition of minisatellite DNA sequences. Each sequence of the child must be found with one of the parents. If this is not the case, a biological relationship can be excluded.

Set-up and procedure

The kit includes the following:

- DNA of the mother, 120 µl
- DNA of the child, 120 µl
- DNA of the potential father, 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 pipetted into the gel pockets of the electrophoresis gel. 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 indicator 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.
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.

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 electrophoresis 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 refrigerator. 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 whichmakes 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.

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 – mother
- DNA – child

- DNA – potential father

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 homogene-ous 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.
Following 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 fatherhood 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.

Robert-Bosch-Breite 10
D - 37079 Göttingen
Tel: +49 551 604 - 0
Fax: +49 551 604 - 107
info@phywe.de
www.phywe.com