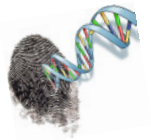
The Genetic Fingerprint



Some theory:

The genetic fingerprint is for example applied for definite identification of persons in criminal prosecution or paternity tests. Only with twins, this process fails, since twins have identic genes.

In case of a crime, the genetic fingerprint provides evidence of the alleged criminal's identity. More precise information about features of persons (e.g. colour of hair or eyes, character, etc.) cannot be obtained with this process.

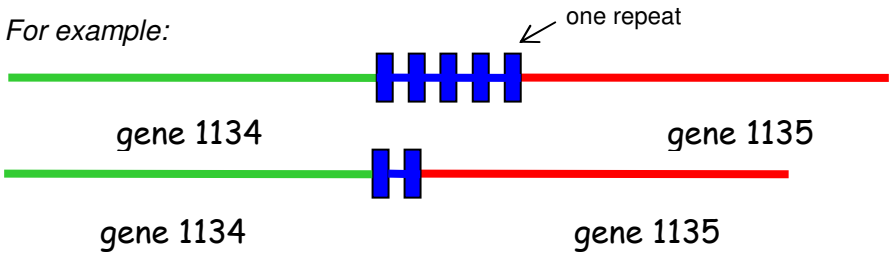
Smallest traces of DNA, for example a streak of blood on broken glass, a root of hair or saliva and cells on a cigarette, are enough to perform a genetic fingerprint successfully.

But how does a genetic fingerprint work?

Every human being has 46 chromosomes, 23 from its father, 23 from its mother. Our complete DNA contains about 3 billion base pairs, but only a small part of that builds the construction plan of our body. The rest is just "filling" of unknown function. In this filling, there are sites, which appear in every human being, but may differ from other individuals in length. If one takes a look at a couple of these sites, a highly accurate fingerprint of a human being can be constructed by differences in length of these sites.

Today, we want to take a look at only *one* site and its length of our DNA. This site is called **D1S80 locus.** The D1S80 locus is situated on chromosome 1 and consists of DNA sequences, which are of variable repeat length in different persons.

For example:



The variable repeats cause differences in length. Such a site of repeats is called **microsatellite-locus**. The DNA sequence around the D1S80 locus is known and identical in all persons.

That's enough. Let's start!!

Important: Always label your Eppis carefully, since you won't retrieve your test

To the pipettes:

1000s pipette (blue): please use blue tips and only pipette 100-1000 µl
200s pipettes (yellow): please use yellow tips and only pipette 20-200 µl
20s pipette (yellow): please use yellow tips and only pipette 2-20 µl

Instructions Additional information Questions Checkbox

You should be able to answer the following questions after this experiment:

1) *Which fields of application do you know for PCR?*
2) *If you had to describe the DNA isolation in four steps, which steps would you name?*
3) *Why do we do a PCR?*
4) *Please comment briefly on the three steps of a polymerase chain reaction.*
5) *Which components are necessary for a PCR?*
6) *What is so special about Taq-polymerase?*
7) *What is the name of the method that visualizes DNA fragments after PCR? Which parameters are used for the analysis of the fragments afterwards?*
8) *Can you imagine a figurative expression of an agarose gel? Why are smaller fragments able to pass through it much faster than longer ones?*

Part 1: Getting the samples

Step 1: Sampling from oral mucosa

- [ ] Rinse out your mouth vigorously with **8 ml** water for 2 minutes and fill the solution into a plastic tube (spitting out).
  The water contains cells from the oral mucosa, enzymes and whatever you have in your mouth. We want to get hold of the cells.

- [ ] Add **2 ml of this solution** into an Eppendorfgefäß („Eppi“) with a pipette and put it into a centrifuge for 2 minutes at 3200rpm.
  During centrifugation, the cells move outwards due to their weight. After this process you can see a small white spot at the bottom of your Eppi, called pellet. These are your cells!

- [ ] Pour away the supernatant (liquid). Please repeat the steps b) to c) at least two times.
  We want to guarantee that you have enough cell material.

  Please explain the functionality of a centrifuge!

Step 2: Lysis of the cells

- [ ] Add 500 µl lysis buffer L to the pellet with the blue pipette. Snip your finger against the Eppi until the pellet has disappeared
  One ingredient of the lysis buffer *is a detergent, which dissolves the cells.*

  How does the lysis buffer *work in detail? Think about the bodywork of cell membranes to answer this question.*

Step 3: Precipitation of proteins

- [ ] Afterwards, add 100µl precipitation buffer F, shake the Eppi well and put in on ice for 5 minutes. Observe, what happens to the solution!
  The precipitation buffer contains salts (potassium acetate) with which proteins are precipitated.

- [ ] After that, the Eppi is centrifuged for 15 minutes at maximum speed (20000rpm) to pelletize the precipitated proteins.

Step 4: Precipitation of DNA

☐ ▶ Transfer ca. 400µl of the supernatant in a new, labeled Eppi. Add 360µl cold Isopropanol. If you are able to transfer more that 400µl of the supernatant you have to add more Isopropanol according to this. After this, you have to put the eppi back on ice for a few minutes.

Take care that you don't transfer anything of the pellet to the new tube!

DNA is insoluable in alcohol and precipitates. .

☐ ▶ Shake well and centrifuge again (20000rpm, 15 min).
The centrifugation causes pelleting of DNA at the bottom of the Eppi.

Step 5: Washing the DNA

☐ ▶ Carefully pour away the supernatant and add 500µl 70% cold ethanol and put it back in the centrifuge (20000rpm, 5min).

After pouring away again, you might (!) see a small white pellet at the bottom of the tube.

The pellet is then dried at 60°C in a heating block (leave the eppi open).
In the 70% EtOH the DNA is also insoluable. In the 30% of water residual potassium acetate is dissolved.

? Why can we say about washing, if we add 70% ethanol? _____

_____

Step 6: Solution of DNA in water

☐ ▶ Dissolve the pellet in 30µL UV-water.
UV-water has been irradiated with ultraviolet light. Any DNA that may have been in the water has been destroyed

☐ ▶ To cause a better solution of the DNA in water, the Eppi is put on the heating element again (this time, close the eppi).
*The new eppi only contains **your isolated DNA**, solved in UV-water!*

Part 2: Amplification of the DNA-section "D1S80" using PCR

During the polymerase-chain-reaction (PCR) the designated DNA-section is copied and amplified.

☐ We prepare the **Master-Mix** together. The Mastermix contains all necessary components for a successful PCR. Afterwards, it is filled in special PCR tubes (45 µL each) that fit to the thermocycler. Everyone has to add 5 µL of his or her sample of isolated DNA.

***DO NOT** snip your finger against the tubes after filling, because we are not able to centrifuge these small eppis. Otherwise the PCR might not work with your sample! Please take great care during the Mastermix preparation because you have to pipette very small quantities!*

Master-Mix		
UV-water	32 µL	
PCR-buffer with $MgCl_2$	5 µL	*The incubation buffer causes optimal conditions for the Taq-polymerase.*
dNTP-mix	5 µL	*The dNTP mix contains all components necessary for DNA synthesis (= the four nucleotides).*
D1S80 primer-mix	2 µL	*The primer mix consists of two primers (short, single-stranded DNA-sections) that are used as starting points by the Taq-polymerase.*
Taq-polymerase	1 µL	*The heat stable Taq-polymerase is an enzyme that attaches to the primers and that synthesizes a new DNA strand. It copies the DNA.*
DNA in aq.	5 µL	
	50 µL	

☐ Then your work is done for the time being. You have to put your Eppi into the PCR machine.

PCR program:

	step	temperature	time	comment
	Denaturation	95°C	5 min	*We want to make sure that the target-DNA is separated into single strands.*
32 cycles	Denaturation	95°C	60 sec	*The double-stranded DNA is separated into single strands.*
	Annealing	63°C	60 sec	*At this temperature the primers bind to the single stranded DNA.*
	Extension	72°C	60 sec	*The Taq-polymerase extends the primers by adding nucleotides. A double-stranded copy of the target DNA is formed.*
	Extension	72°C	5 min	*The final extension time helps to finish the elongation of some PCR-products.*

Part 3: Agarose gel electrophoresis

Detachment and visualization of DNA fragments created during PCR.

Step 1: Producing agarose gel

☐ 2 g agarose (pro 100 mL) are filled into a beaker. 1x TBE buffer is added to make an agarose suspension of 2%. The suspension is boiled at 250°C (or in a microwave oven if available). To prevent the solution from boiling over, it is agitated with a magnetic stirrer at approx. 500rpm. In hot water, the agarose is dissolved.

Agarose is a powder from the Agar-Agar plant, which produces a gel when cooled down. The puffer is required to keep the pH value on a constant level.

☐ ▶ The solution has to cool down a bit before you pour it into the **electrophoresis chamber,** where it will finally solidify. After **hardening** (ca. 30 min later), remove the ridges (spacers) and the comb carefully. Add 1 times concentrated TBE buffer into the chamber, until the gel is completely covered.

The comb forms pockets in the gel, into which you can fill your samples.

Step 2: Filling of the gel

☐ ▶ Fill 10 µl of your sample in a new Eppi, add 5 µl loading buffer and mix it.

*The **loading buffer** contains:* **glycerol**.This is heavier than the 1x TBE buffer, and your sample will sink to the bottom of the pocket. Two **blue dyes** (Bromophenolblue and Xylencyanol) are in the loading buffer so that you can easily control where you pipet the sample. IMPORTANT: These dyes do not stain the DNA. **Ethidium-Bromid** or **SYBR-Green** are dyes that intercalate in the DNA double helix. They can be used to visualize the DNA, because they fluoresce under UV light but are invisible in daylight.

☐ ▶ Pipet your 15 µl sample into a pocket of the gel.

*The first pocket is filled with a **marker**.*
***IMPORTANT** Take a note in which pocket your DNA was loaded! Otherwise you will not be able to identify your sample later!*

Step 3: Start of gel electrophoresis

☐ ▶ Apply electricity (ca. 100 V – max. 120 V), electrophoresis begins. It should be let run for 30-45 minutes. After this, we take the gel out of the puffer and put it onto the **Dark-Reader**. Now the bands can be uncovered.

*The DNA fragments (results of the PCR) are separated by length. **During the whole process they are invisible!!!** You can take a first look at the gel now, but you will also get a picture of it**!***