# Genetic fingerprinting: lab-protocol



The protocol consists of 5 steps:

1. A sample (blood, skin, sperm, saliva etc.) is collected from the crime scene (handed out by the teacher).
2. DNA is extracted from the sample (the samples you get from the teacher has already been extracted)
3. The ***Polymerase Chain Reaction (PCR)*** multiplies certain DNA-sequences, which means that you will be able to compare these sequences between the victim, the perpetrator and the suspects.
4. ***Gel electrophoresis*** separates the multiplied DNA-sequences according to their length
5. The gels are **prepared,** **photographed** and **interpreted.** Some of the suspects can be set free and hopefully the perpetrator can get a sentence.

**NOTE:**

The teacher will hand out the extracted DNA (**step 1** and **2**), you will then complete **step 3,4** and **5** yourself in the lab.

## 3) Multiplying of DNA by the Polymerase Chain Reaction (PCR)

**Materials**

At the workplace (at the table):

* Rack with micropipettes
* Wastebin (tripod with plastic bag)
* Ice bath and foam-floater
* Permanent tusch
* Rack with the following:
	+ 5 PCR-tubes, each with one reaction pellet
	+ 1 eppendorftube with primer mix (120

In common:

* DNA-samples
* PCR-thermocycler

**Protocol:**

|  |  |
| --- | --- |
| 1. First step will be an introduction to the micropipettes!
2. Mark the 5 PCR-tubes with the permanent tusch: CS (Crime Scene), 1, 2, 3, 4 (numbers of suspects) on top of the lid.
3. Place the PCR-tubes in a ”foam-floater”, and put them on ice in an ice bath
4. Add 20 µL primer mix to each PCR- tube (Operate the micropipette very precisely, there is only enough primer mix for exactly 5 tubes).
5. Add 5 µL DNA from each DNA sample to the PCR-tube that corresponds with it (CS -> CS, 1->1, etc.)**REMEMBER:** change the tip of the micropipette for each tube (if not you could ruin the experiment).
6. Mix carefully by tapping the PCR-tube with your fingernail, so that the pellet will dissolve
7. Place the PCR-tubes on ice in the ice bath
8. When all groups are ready, put the PCR-tubes into the PCR thermocycler and turn it on.
 | **Explanations:** |
| *Reaction pellet* contains:-*salts*, that keep DNA into solution and keep the pH stable-*Taq-polymerase*: the enzyme, that has to copy the DNA-string-*Nucleotides*, that will be put on the DNA-string (A, T, G, C) |
| Primermix: contains primers, which will stick to the DNA-singlestring at the right sequences, so that the copying process can take place |
| *Icebath:* DNA will disintegrate if the temperature is too high |

PCR-thermocycler is programmed with a cyclic program as following:

|  |  |  |
| --- | --- | --- |
| Firstdenaturation | 94 °C 3 min | *Denaturation*: The dobble strandet DNA-string is separated to two single stranded DNA-strings due to the heating. |
| 30 cycles (repeats) | 94 °C 30 sek |
| 55 °C 65 sek | *Annealing*: because of the cooling the opposing DNA-string will join together again. And the primer will bind to the opposing DNA sequence. |
| 72 °C30 sek | *Polymerisering /copying*: DNA-polymerase will operate and place nucelotides on the single standet DNA-string beginning from the primer (from 5’ to the 3’ end). In this manner DNA will be copied from the primer and forward.For each copying the number of DNA strings in the tube will be doubled. |
| Terminal copying | 72 °C 4 min |

The PCR-thermocycler will run for approximately 1½ hour.

***Meanwhile there will be other tuition.***

## 4) Gel electrophoresis of the multiplied DNA samples

 **Materials**

At the workplace:

* Ice bath
* 10x gel load-solution
* Rack with micropipettes
* Test-gel (plasticgel) for test-loadning of gels (practicing)
* Vessel for test-gel.
* Methylenblue-solution for loading test-gels (prøve-loadning af geler
* Wastebin (glass)

In common:

* Heating block (56 °C)
* Vessel for gel electrophoresis
* Gel-casting-tray
* 1 % Agarose for molding a gel
* Concentrated (50x) electrophoresis buffer
* 1x electrophoresis buffer
* DNA-ladder

**Protocol:**

1. Get your tubes with DNA-samples from the thermocycler and place them on ice in your ice bath
2. Add 5 µL 10x gelload-solution to each of the 5 tubes. **Use a new micropipette tip for each tube.**
3. Now you have to **practice how to load a gel** by using the plastic test-gels placed at the workplace. Load them with the methylenblue-solution also placed at the workplace. Place the test-gel in a vessel and fill with water to submerse the gel (maybe you have to place something heavy on the gel to keep it down). **Add 7 µL methylenblue-solution into each slot**.

You must keep practicing until you are able to fill the slots in the test-gel without any trouble.

Then you rinse the test-gels under demineralized water.

1. **Casting a gel:** to cast a gel first you have to seal the ends of the gel-casting-tray with the rubber end caps. Then you place the comb in the appropriate notch.



Figur 1: A gel-casting-tray with one black end-cap placed on the tray and one removed. The comb is placed in one of the notches

Preparing the agarose gel: pour **1,0 ml concentrated electrophoresis buffer** into a 100 ml flask, then add **49,0 ml demineralized water**. Now add **0,5 g agarose** and mix. Finally, you heat the solution in the microwave for 1 min. Carefully remove the flask from the microwave and mix by swirling the flask. Continue to heat the solution in 15 sec. bursts until the agarose is completely dissolved (it should be clear like water).

Let the agarose cool to 60 °C with carefully swirling to promote even dissipation of heat.

**Pour the cooled agarose solution into the prepared gel-**casting tray.

Now **pour the agarose into the prepared gel-casting-tray**. Within 15-20 min. it will be solid Then **remove the end caps** and. Finally **remove the co**mb very carefully to prevent damage to the gel.

1. The gel (still placed on the gel-casting-ray) is placed into the gel electrophoresis chamber and the diluted (1x) electrophoresis buffer is poured into the chamber until the gel is submersed.
2. The gel is loaded with the DNA-ladder in lane no. 1. **Load 25 µL ladder into the slot.**
3. The gel is loaded with the groups DNA-samples for CS-DNA and suspects no. 1-4 in lane no. 2 to 6.

(If your group share the gel with another group, you have to write down which number of lane you place which sample into…..to remember 😊) **Load 20 µL sample into each slot.**

1. When the gels are loaded we will run the electrophoresis at 100 volts.

***While we wait for the gels to run there will be other tuition.***

***Explanation:*** DNA is negatively charged. Therefore it will move from the negative pole to the positive pole. The shorter the DNA string is, the faster it will move. That is why the DNA string will place themselves in bands down the lanes. The gel-load solution contains a blue dye that wander a bit faster than the DNA. When the blue dye approach the end of the gel we cut of the power.

The DNA-ladder in lane no. 1 is af known sample of DNA-strings wit the sizes: 200, 400, 600, 800, 1000 basepairs (bp). The bands that shows in this lane will therefore be markers that will show the size of all the other bands in the gel. The shortest DNA-strings travel the longest road. So the 200 bp-band will be seen longest down the gel.

## 5) Staining the gels and interpretation of the results

***SECURITY:***

* ***Use gloves when you work with the staining or with the stained gel.***
* ***UV-light can cause burns and mutations. Therefore you must only be exposed to the light in very short time. Use googles.***

**Materials**

In common

* SYBR-Safe-DNA-stain (75 mL per gel)
* Vessel for staining and plastic foil
* UV-transilluminator with a camera

**Protocol:**

1. The gel is cafefully placed in a staining vessel
2. The SYBR-Safe-DNA-stain is poured over until it is submersed.
3. It is covered with the plastic foil and left for 10-15 min.
4. The gel is then placed on the UV-transilluminator and photographed. The photos are distributed to the students somehow. If the bands are hard to see you can try staining for 10-15 min. more.
5. The gels are interpreted.

***Explanation:*** SYBR-Safe-DNA-stain is a dye which binds to DNA. When you put UV-light on it, it glows fluorescent. That’s why you can now see the DNA bands from the DNA samples.

## Interpretation

1. Compare the lanes on the gel. Whom of the suspects seems to match the DNA we found on the crime scene? Write down an explanation.
2. Consider how safe this test is: can we be sure that we have found the perpetrator? How can we make the test even more safe in terms of pinpointing the perpetrator?
3. Who are homozygotic (same alleles on both chromosome), and who are heterozygotic (different alleles on the two chromosomes)?
4. Compare the bands location with the bands in lane no. 1 (the DNA-ladder). Which length do the multiplied DNA-strings have (approximately)?