

Practical 1: “Who killed Nosey Parker?”- DNA fingerprinting to catch the killer

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Introduction

In this practical course, specific sequences in the human genome will be analysed using a polymerase chain reaction (PCR) approach. As a target, we use DNA sequences in non-coding regions that are expressed as multiple repeats and that vary greatly in number among human populations (variable number of tandem repeats, VNTRs). As a result of the PCR amplification, we will obtain a highly specific genetic profile due to individual DNA polymorphisms that will be used to identify a criminal - that is why it is called a “genetic fingerprint”. Such a genetic fingerprint can also be used to determine the paternity of children.

DNA fingerprinting is used as a routine technique to solve crimes. Using PCR technology, you will amplify VNTRs in DNA samples from suspects and one DNA sample that was found at the hypothetical “crime scene”. Gel electrophoresis will then allow you to separate the amplification products, to analyze the DNA profile and finally to come to a conclusion.

The aim of this course

DNA is a powerful tool to identify individual genetic profiles. This course provides in depth instruction about how PCR amplification is performed, how electrophoresis is used to separate, visualise and analyse DNA fragments and how the set of these techniques can be used to answer a question that has direct societal relevance.

In this activity, you can analyze four different samples of genomic DNA. One sample obtained from a hypothetical murder and three samples from potential “suspects”. Based on the results of the PCR amplification, you will be able to

draw a conclusion by matching the suspects' DNA profile to the sample found in the close vicinity of the victim.

Student's objectives

- Understand the application of genetic fingerprint in forensic medicine
- Learn, apply and analyse various scientific methods
- Become familiar with theory and practice of PCR, gel electrophoresis, result evaluation
- Estimate molecular weight of DNA fragments in gels

Experimental protocol

1) Genomic DNA from suspects:

In this workshop it is already prepared from human cell lines.

2) Polymerase chain reaction (PCR):

Materials and equipment:

- 5 PCR tubes (one for each DNA sample + negative control)
- Micropipettes
- Sterile water (H₂O)
- PCR buffer, to allow optimal function of the enzyme
- Primer pair (one forward + one reverse primer), P1 and P2, that define start sequence for Taq-polymerase
- Free Deoxy-nucleotide triphosphates (dNTPs)
- DNA samples
- Heat stable enzyme Taq polymerase
- PCR machine

You will analyse DNA samples from 3 suspects and 1 sample that was found next to the victim. In addition, you will make one negative control, in which you add all the components listed below except for DNA.

Start by labelling 5 PCR tubes, pipet into each PCR tube:

- 2,5 µl DNA sample 1-4
- 19,3 µl H₂O
- 0,125 µl primer P1
- 0,125 µl primer P2

- 2,5 µl buffer (10x concentrated)
- 0,2 µl dNTPs
- 0,25 µl polymerase

Total volume: 25 µl

It is useful to prepare a premix of all the components, without the individual DNA samples.

After addition of the enzyme (Taq-polymerase), the amplification is performed in a PCR cycler using the following programme:

| | | | |
|------------|------|-----------|--------------|
| 2 min | 94°C | | DENATURATION |
| 30 seconds | 94°C | | DENATURATION |
| 30 seconds | 55°C | 30 cycles | ANNEALING |
| 30 seconds | 72°C | | EXTENSION |
| 1 min | 72°C | | EXTENSION |

3) Gel electrophoresis:

You need:

- Ready-to-use 6% polyacrylamide gel (PAGE) (Invitrogen)
- Your amplification products
- Sample buffer (Blue buffer that allows sample to flow into the lane and helps you to follow the sample during the gel run)
- DNA Marker (DNA fragments of defined molecular weight)
- UV lightbox

- Power supply

Add 10 μ l sample buffer to each sample and load 20 μ l in each lane of the pre-prepared gel. Don't forget to load 10 μ l of DNA marker to allow comparison of bands after the gel run. Don't forget to use gloves while loading the samples! Run the gel at 100 V for 30 minutes. The results are analysed under UV light with the help of your supervisor.