PCR Analysis

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BIO 1120 – Section 03

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04/16/2025

Introduction

In this lab, we explored the process of analyzing human DNA using molecular biology techniques. The experiment walked us through DNA collection, extraction, amplification via Polymerase Chain Reaction (PCR), and visualization through agarose gel electrophoresis.

We began by isolating DNA from our cheek cells as a simple, non-invasive way to collect a personal sample. The focus of the experiment was to amplify a specific genetic marker called a Variable Number Tandem Repeat (VNTR). These regions differ between individuals and can be used for genetic fingerprinting (Jeffreys, Wilson, & Thein, 1985).

PCR was the key technique used in this experiment. It enables scientists to generate millions of copies of a specific DNA segment from a small sample (Mullis & Faloona, 1987). Following amplification, we ran the DNA through agarose gel electrophoresis to separate fragments by size, allowing us to visualize genetic differences (Lee et al., 2012).

Overall, this experiment offered a hands on introduction to tools commonly used in genetics, forensics, and biotechnology.

Methods and Materials

We began by collecting cheek cells through a saline mouth rinse. The rinse was transferred into a microcentrifuge tube, and Chelex resin was added to lyse the cells and protect the DNA. The mixture was then incubated at a high temperature and centrifuged to isolate purified DNA.

For PCR, we transferred 2 µL of our DNA into a PCR tube containing the master mix. The thermal cycler was programmed to run the following cycles:

* Denaturation (94–96°C): separated the DNA strands,
* Annealing (-68°C): allowed primers to bind to specific sequences,
* Elongation (72°C): extended DNA using polymerase.

After PCR, we added loading dye to the samples and pipetted them into a pre-made agarose gel. We ran the gel at 120 volts for 45 minutes. A 1kb DNA ladder was used to estimate the fragment sizes.

Accurate pipetting was crucial at every step to ensure reliable results and prevent contamination. We wore gloves and sanitized our workspace consistently.

Result

A black and white image of a dna strand

AI-generated content may be incorrect.

**Fig: An image of DNA fragments**

After running the gel electrophoresis, we were able to see clear bands under the transilluminator. Here’s what we observed:

* Sample 1&4: A single band likely homozygous at the VNTR locus.
* Samples 2 & 3: Two distinct bands, indicating heterozygosity.
* Positive control: Showed clear bands as expected.
* Negative control: No bands appeared, confirming no contamination.

We used the DNA ladder to estimate band sizes. Then, to calculate the number of VNTR repeats, we used the formula:

VNTR repeats= (Total base pairs−145) /16

Example calculations:

* Band at 285 bp: (285 - 145) / 16 = 8.75 =9 repeats
* Band at 205 bp: (205 - 145) / 16 = 3.75 = 4 repeats

These values give insight into individual genetic variation at the targeted locus.

Discussion

This lab helped solidify our understanding of DNA analysis techniques. Using PCR to amplify the VNTR region and visualizing the results on a gel gave us direct insight into genetic variability. Seeing my own DNA result a single band suggested I was homozygous at that locus. Meanwhile, classmates with two bands were likely heterozygous.

The positive and negative controls were crucial: they confirmed our reagents worked correctly and that no contamination occurred. I learned that even small errors in pipetting or sample handling could lead to failed amplification or unclear results.

Though this lab didn't test a specific hypothesis, it successfully demonstrated core techniques in molecular biology. The application of VNTR analysis extends to fields like forensics, paternity testing, and genetic ancestry.

**Additional Background**

VNTRs are short DNA sequences that repeat multiple times and differ between individuals. Their high variability makes them ideal for identification purposes (Jeffreys et al., 1985). PCR allows for the exponential amplification of these regions, making analysis possible even from minimal DNA quantities (Mullis & Faloona, 1987). Gel electrophoresis then enables us to visualize and interpret these differences (Lee et al., 2012).

These methods, used together, form the foundation for DNA fingerprinting, a powerful tool in modern genetics.

References:

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