Genome “dactyloscopy” (DNA finger-printing) and gene expression: Polymerase Chain Reaction (PCR) and Real Time Polimerase Chain Reaction (RT-PCR) in action

Attention!!! This project available only for students of The Third stage of the International Student Practice (10 - 30 September 2017)

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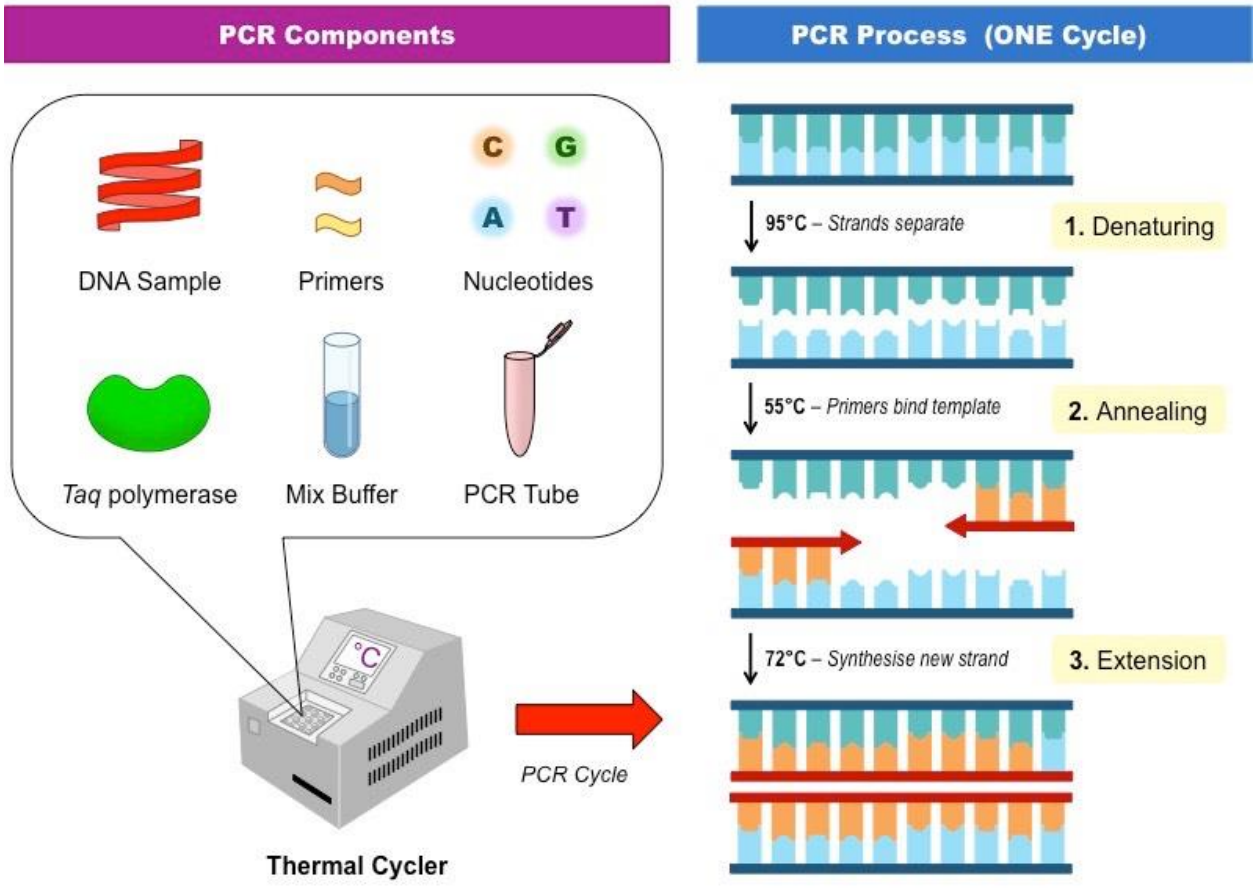
This project is dedicated to the study two basic molecular biological techniques and apply them in practice. The first of them - Polymerase Chain Reaction (PCR) - is a great method developed by Kary Mullis in the 1980s. PCR is used to make multiple copies of a segment of DNA. PCR is very precise and can be used to amplify, or copy, a specific DNA target from a mixture of DNA molecules. First, two short DNA sequences called primers are designed to bind to the start and end of the DNA target. Then, to perform PCR, the DNA template that contains the target is added to a tube that contains primers, free nucleotides, and an enzyme called Taq DNA polymerase, and the mixture is placed in a PCR machine. The PCR machine increases and decreases the temperature of the sample in automatic, programmed steps. Initially, the mixture is heated to denature, or separate, the double-stranded DNA template into single strands. Then the mixture is cooled so that the primers anneal, or bind, to the DNA template. At this point, the DNA polymerase begins to synthesize new strands of DNA starting from the primers. Following synthesis and at the end of the first cycle, each double-stranded DNA molecule consists of one new and one old DNA strand. PCR then continues with additional cycles that repeat the aforementioned steps. The newly synthesized DNA segments serve as templates in later cycles, which allow the DNA target to be exponentially amplified millions of times (1).



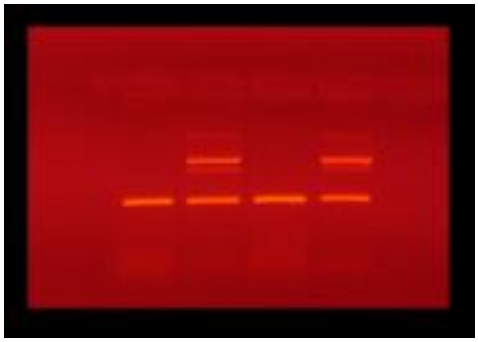
1. Polimerase chain reaction (from https://catalog-02.ru/)

Genome "dactyloscopy" (DNA fingerprinting) is a method of personal identification based on DNA analysis. The difference in DNA structure of different persons can act as genetic markers that can be identified with PCR. This ensures opportunity to make unique genetic passport for each person. This genetic passport is as unique as a fingerprint. Several genetic markers can also be used as markers of predisposition to various diseases.

The first part of work consists of the following steps:

- DNA extraction from epithelium cells with glass beads
- PCR with different sets of primers which provide a genomic identification and determine predisposition to several diseases (you can extract your own DNA and work with it or with anonymous laboratory samples)
- gel electrophoresis (is used for separation of the amplified genetic product according to its molecular size)

At the end we get something like the picture below, and discuss the results. I hope that your own DNA analysis will tell you a lot about yourself.



2. Electrophoregram of PCR-products from different DNA-templates

The second part of the Project is devoted to real-time polymerase chain reaction (Real-Time PCR), also known as quantitative polymerase chain reaction (qPCR). This is the most powerful tool for quantitative nucleic acids analysis.

In real-time PCR the amount of product formed is estimated during the course of the reaction by monitoring the fluorescence of dyes or probes introduced into the reaction that is proportional to the amount of product formed, and the number of amplification cycles required to obtain a particular amount of DNA molecules is registered. Assuming a certain amplification efficiency, which typically is close to a doubling of the number of molecules per amplification cycle, it is possible to calculate the number of DNA molecules of the amplified sequence that were initially present in the sample. The typical use of real-time PCR includes pathogen detection, gene expression analysis and single nucleotide polymorphism (SNP) analysis (2).

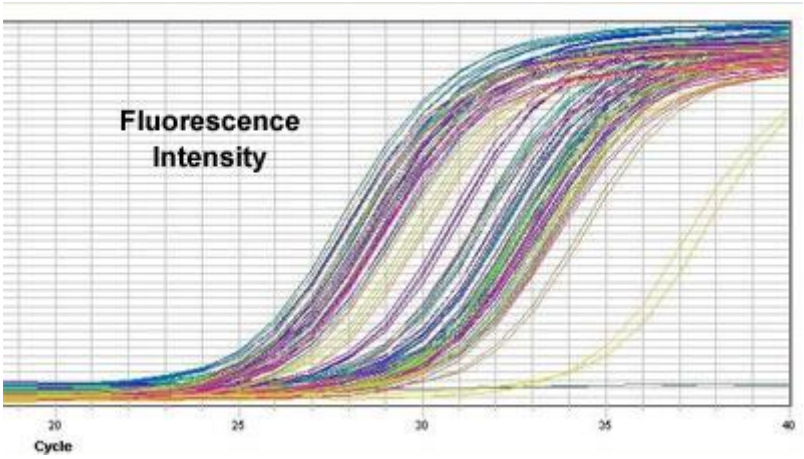
The goal of this part of the Project is to estimate the expression levels for the *yellow* gene *Drosophila melanogaster* at different stages of fly development.

In this experiment, we will try to compare the *yellow* gene expression (mRNA production) between different stages of fly development and to discover the stages at which the gene begins and ends the work or state that the gene expresses constitutively. Both results are interesting.

The second part of work consists of the following steps:

- RNA extraction
- RNA quantity and quality estimation
- reverse transcription
- RT-PCR with non-specific fluorescent dyes
- data analysis

At the end we get something like the picture below, and discuss the results.



From http://www.assay-protocol.com/molecular-biology/real-time-pcr

Requirements for students:

- biological background is desirable

- basic knowledge of DNA structure, mechanisms of replication, gene expression required

- accuracy and attentiveness

1. Scitable by Nature education
2. Mol Aspects Med. 2006 Apr-Jun;27(2-3):95-125. The real-time polymerase chain reaction. Kubista M1, Andrade JM, Bengtsson M, Forootan A, Jonák J, Lind K, Sindelka R, Sjöback R, Sjögreen B, Strömbom L, Ståhlberg A, Zoric N.