THE BLOOD CODE:

UNRAVELING THE SECRETS OF OXYGEN TRANSPORT

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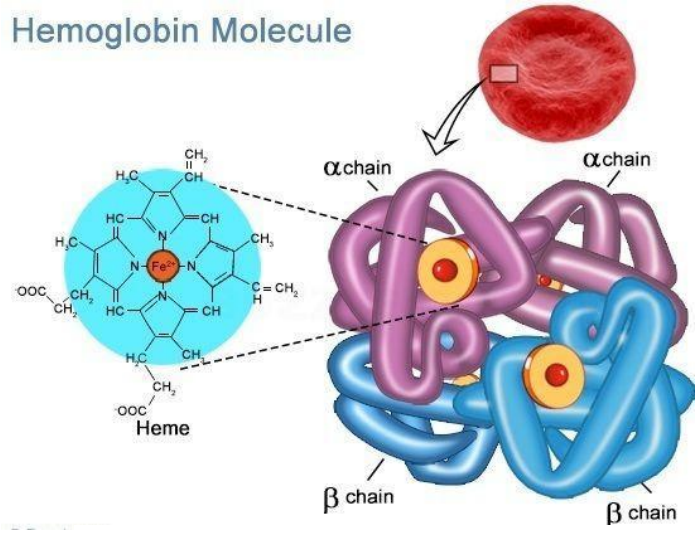
ABSTRACT

The main reason why every part of our body is constantly supplied with oxygen is blood, a red fluid made of cells and plasma. The most important part for oxygen transport are the red blood cells, which contain hemoglobin – an oxygen carrier protein. As every other protein, hemoglobin is coded in the DNA, and it contains two genes, each for a different subunit of the protein. In this project, we focused on the beta hemoglobin gene which was extracted and amplified from white blood cells of a cornsnake (*Pantheropsis guttatus*), a mouse (*Mus musculus*), and a rat (*Rattus norvegicus*). The comparison was made based on the length of the DNA fragment, as well as by using bioinformatic tools for building a phylogenetic tree.

INTRODUCTION

Blood is a bodily fluid made of blood cells and blood plasma. There are three types of blood cells: red blood cells (eritrocites), white blood cells (leukocites) and trombocites. Red blood cells, hemoglobin, more precisely, are responsible for delivering oxygen and carbon dioxide to and from every little cell in the body. White blood cells help us fight infections and diseases, while trombocites help with coagulation. Blood plasma is mostly made out of water. Red blood cells contain hemoglobin, which is a hemeprotein and metalloprotein. It is also a tetromere, which means that it contains four components: α1, α2, β1, β2. Each component has aminoacids and a heme. Our blood is red because of the iron in heme.

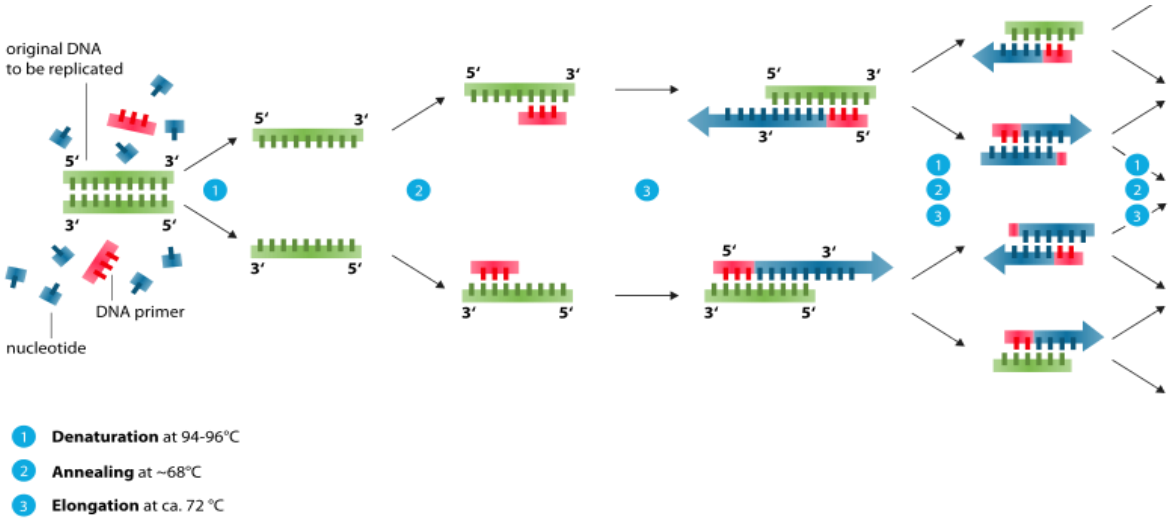


Picture 1. Hemoglobin structure

Proteins are coded in DNA, whichcarries our genes. It is located in the nucleus of cells. Its structure is double helix which is made of two DNA strings, which are connected with hydrogen bonds. Nucleotides make up DNA and they contain sugar, phosphate group and nitrogenous bases. There are four DNA bases: adenine, cytosine, thymine, guanine. DNA with histones builds chromosomes.

In this project we used PCR – polymerase chain reaction. It is a technique used in molecular biology to generate thousands to millions copies of a DNA sequence from just a

few copies or segments. This procedure is divided in three steps. In the first step ,or denaturation, DNAs double helix separates at high temperature. Then in the second step, or annealing, each strand connects with primers (short DNA fragments) at low temperature. In the last step, elongation, DNA polymerase binds to primer and keeps synthesising DNA strands.



Picture 2. PCR protocol

The goal of this project was to isolate DNA from the hemoglobin.

MATERIAL AND METHODS

- DNA isolation from fruits

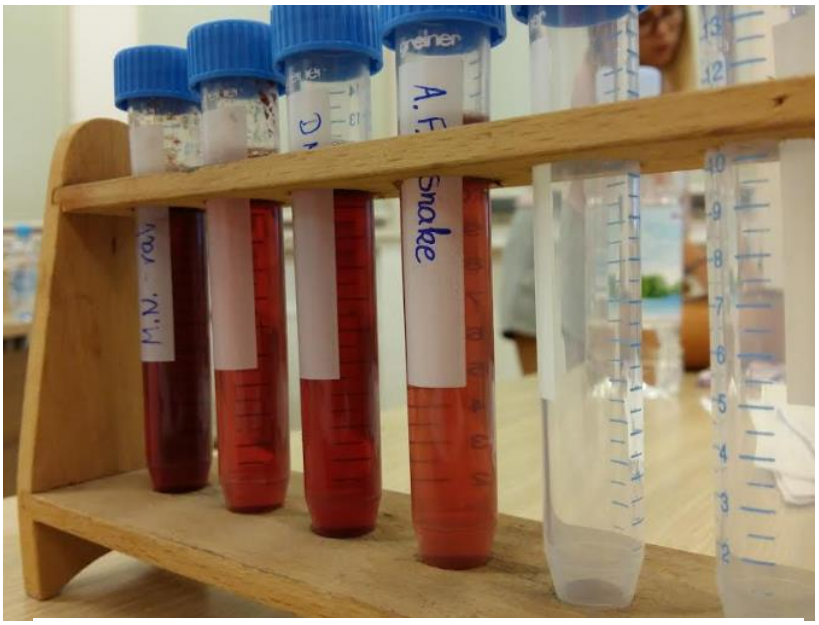
In this process strawberries were smashed until they were liquid and then mixed with a solution of cold 70% EtOH, NaCl and a detergent.

- Microscopy

Light microscope was used to visualise red blood cells from rat's, snake's and mouse's blood. Slugs blood was taken and also visualised by a microscope.

- DNA isolation from blood

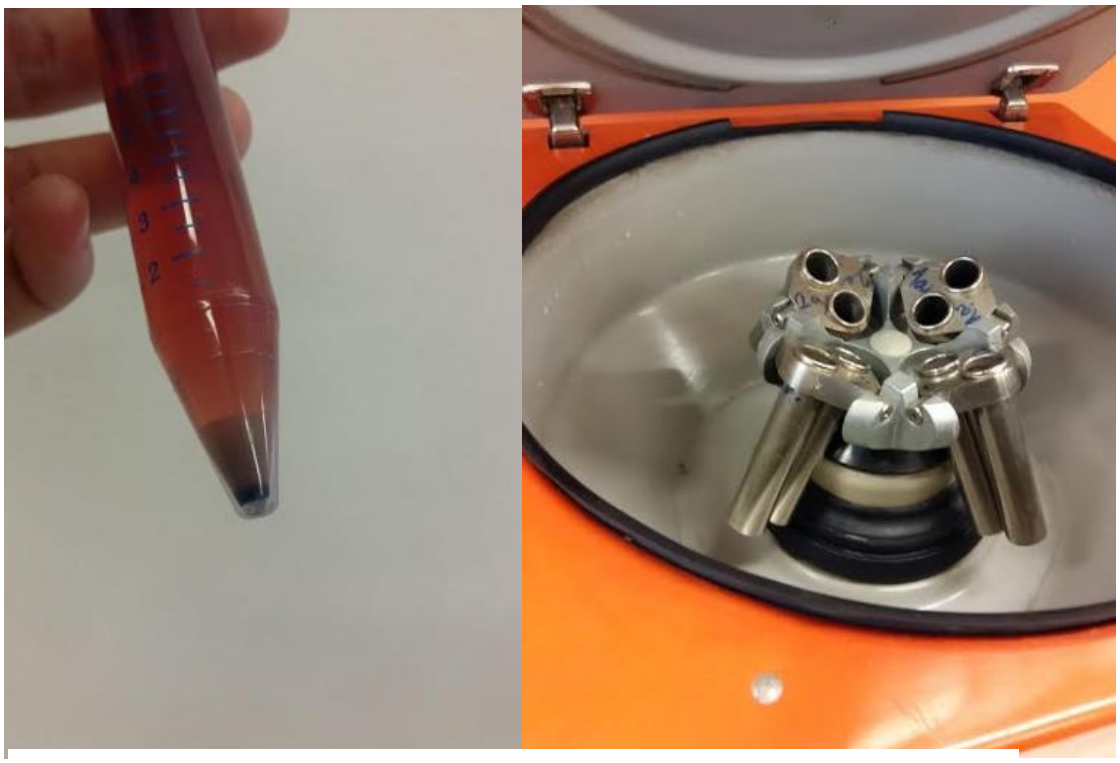
For this procedure rat's, mouse's and snake's blood was used.



Picture 3. Tubes with centrifuged blood

The 2x lysis mix and WBC was prepared beforehand.

2 mL of blood in heparin was transferred to a 15 mL tube and spun in a centrifuge at 2200 rpm for 20 minutes. Plasma was carefully removed afterwards. 15 mL of 2x lysis mix was added to the tube. It was shaken and rotated for 10 minutes. After that it was again spun in a fresco biofuge centrifuge and spun at 2500 rpm for 15 mins. The white blood cells settled down on the bottom of the tube.



Picture 4. Layered blood cells and a centrifuge

Supernatant was removed. 450 µL of WBC lysing solution, 30µl of 10 % SDS were added in the eppendorf tube. White blood cells were transferred to the eppendorf tube. Approximately 200 µL of proteinase K (10 mg/mL) was added to the white blood cells in the lysing solution and SDS and it was pipetted up and down a few times so that the lysate was vicious. The sample was incubated at 37®C overnight. An equal volume of phenol (700 µL) was added to the sample and spun in the fresco biofuge centrifuge at 3000 rpm for 5 minutes. The temperature inside the centrifuge varied between 24 and 27 degrees Celsius.

Picture 5. Centrifuge

The upper aqueous layer was removed to a clean, appropriately labeled tube and another equal volume of phenol was added and then the sample was spun in a fresco biofuge at 3000 rpm for 5 minutes. The previously mentioned step was repeated 3 times. After the last aqueous layer was removed, 1 µL of 4 M Ammonium acetate and 2x volume of 96% ethanol was added. The tube with the sample was shook gently. Tube was taken to centrifuge and was spun at 2500rpm for 5min. After that 96% ethanol was taken carefully from the tube (to not disturb the pelled DNA) and 500µl of 70% ethanol was added. The tube was shaken and putted in centrifuge. It spun at 2500rpm for 5min. 70% ethanol was again

taken from the tube so that only DNA remains. Pellet was left so that all ethanol can evaporate. Pellet was dissolved in destilled water and left for a day.

- PCR

For this project „Programmable thermal blok 2“ PCR was used.

First PCR reaction mix was made. 12,5µl of Emerald master mix, 0,5µl of forward primer, 0,5µl of reverse primer, 11µl of destilled water and 0,75µl of DNA template were added and mixed in the PCR tube. PCR tube was putted in the PCR.

PCR protocol:

98°C - 10 sec

53°C – 30 sec

72°C – 1 min/kb

(30 cycles)

72°C – 30 min

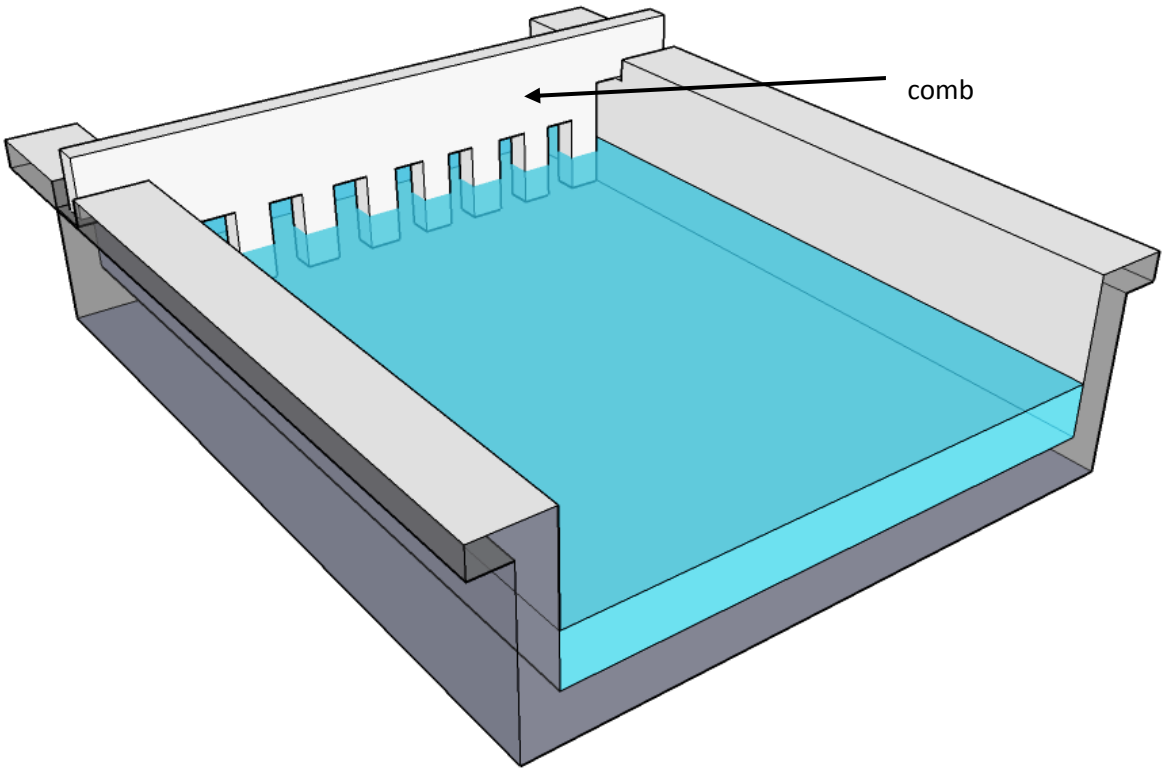
After the protocol tube was left on the temperature of 4°C.

Picture 6. PCR

- Gel electrophoresis

For the gel 0.39 g of agarose was needed. Then, agarose powder was mixed with 30ml of TAE in a microwaveable flask. Flask was put in the microwave for 1 minute. After 30 sec it was shaken and put again in the microwave. Agarose solution was let to cool down to

50°C. 0.5µg of Syber Safe was added to final concentration. Agarose was poured into a gel tray with the well comb in place.

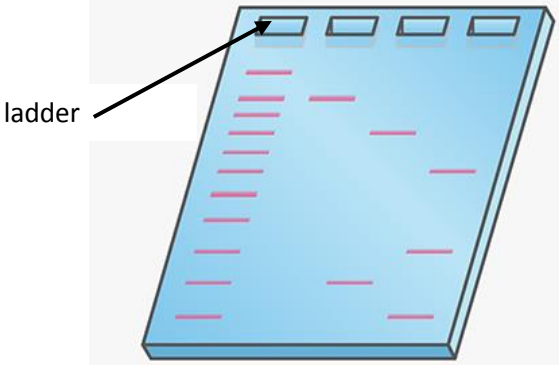


Picture 7. Electrophoresis unit

Loading buffer was added to DNA samples. When the concentration became solidified gel was placed into the gel box (electrophoresis unit).

Picture 8. Gel electrophoresis used in the experiment

Gel box was filled with TAE. Molecular weight ladder was loaded with the pipet into the first lane of the gel.



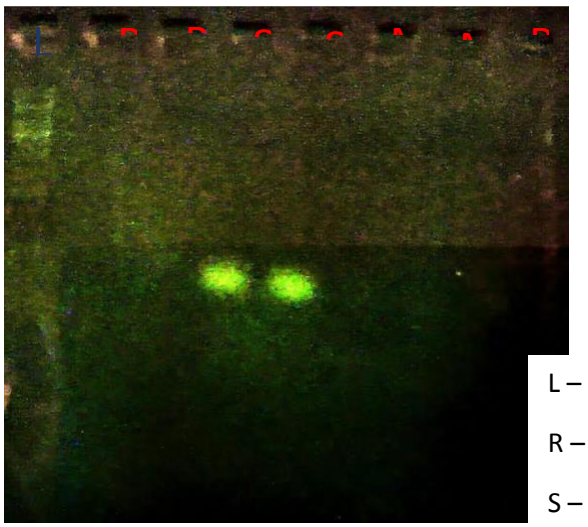
Picture 9. Example of a gel after visualisation

7µl of DNA samples were carefully loaded with the pipet into the additional wells of the gel. Gel was runned at 150 V for half hour. After that device with UV light was used for visualizing DNA fragments.

RESULTS

After running the PCR, we had enough of DNA samples to run the gel electrophoresis. After running the gel electrophoresis and using the UV light, this is what we got:

Picture 9. Gel with DNA under the UV light

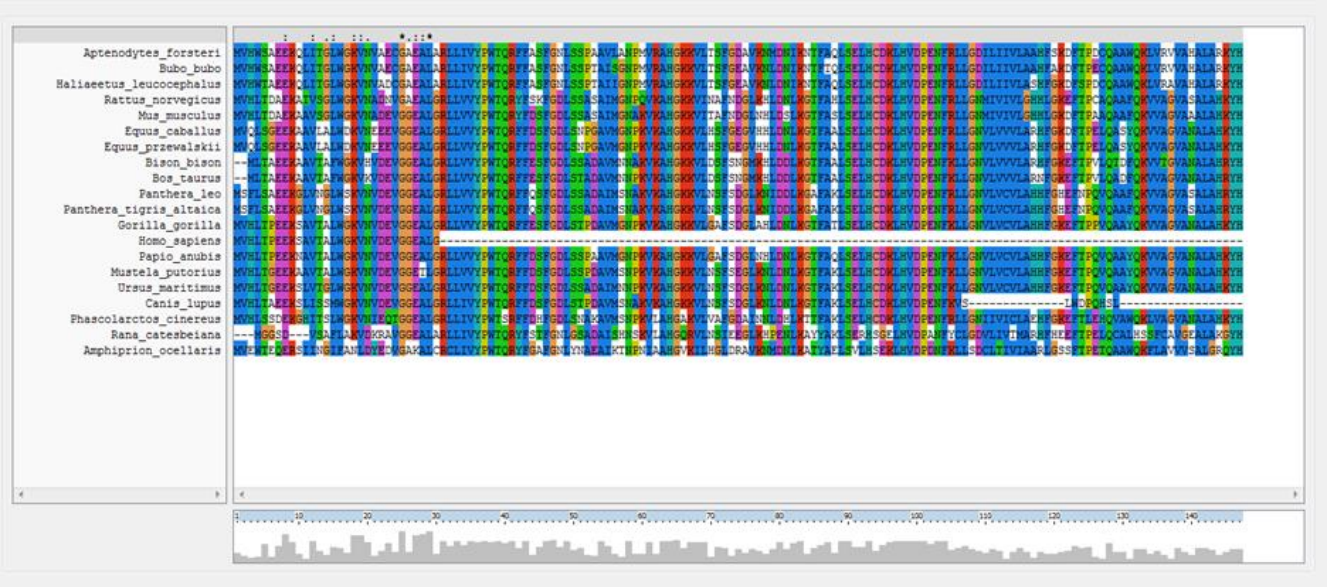


L – DNA ladder

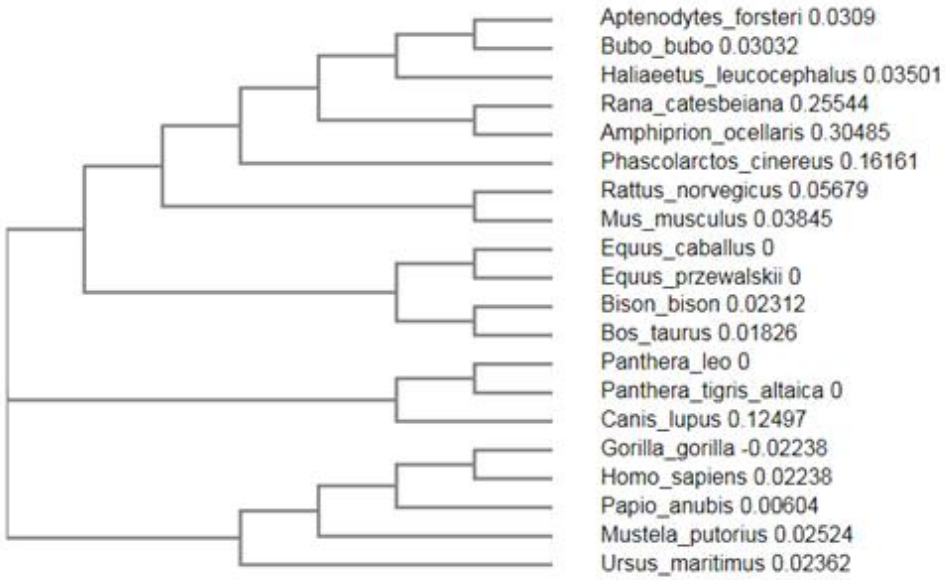
R – rat's blood

S – snake's blood

Picture 10. Gel after the visualisation and editing



Picture 11. Aligned sequences of beta hemoglobin aminoacids



Picture 12. Phylogenetic tree from the aligned sequences from Picture 11.

DISCUSSION

As seen in results, we managed to isolate the snake's and mouse's DNA. One of the possible reasons we didn't get any results for rat's blood is because the sample of rat's blood was in worse condition than other two samples. You can see the bands of the snake's DNA

best because it's probable that the snake's DNA molecules were most abundant and the sample of snake's blood was in best condition. The bands from the mouse's blood samples have travelled further down the gel because its DNA molecules are smaller, and thus, less visible.

CONCLUSION

In this project we successfully isolated DNA from snake's and mouse's blood. We concluded that rat and mouse belong to the same family of animals and that mouse's DNA molecules are smaller than rats and snakes.

ACKNOWLEDGEMENT

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