Workshop: PCR and DNA Fingerprinting

Protocol by iGEM 2018 Team Vilnius-Lithuania

In this tutorial we will introduce tools and experiments from the DIY (Do It Yourself) workshop. Our used toolkit includes: a thermocycler for polymerase chain reaction, a microliter pipette, a power supply, a UV light source, agarose gel chamber and other tools needed for the introduced experiments. The provided DIY tools help to look at the genetic code and to diagnose mutations in a genome. While mutations could vary and result in different diseases, we decided to focus on a gene sequence, which is responsible for coding lactase enzyme, that is breaking down lactose disaccharide. The introduced experiments will give us an answer if the inspected person tolerates lactose which can be found in milk and other dairy products.

We did two experiments:

- a polymerase chain reaction;

- and a DNA fingerprinting.

Experiment number 1:

Polymerase chain reaction

Polymerase chain reaction, also called PCR, will amplify a specified segment of DNA. In this experiment we will amplify the *LCT* gene's regulator.

<u>In order to prepare 50 microliters of the sample, we will need:</u>

- Sample of saliva – 1.25 µL;
- Phusion DNA polymerase, which is included into "Phusion Human Specimen Direct PCR Kit (Catalog number: F150BID)" from Thermo Scientific™- 1 µL;
- 2x Phusion buffer from the "Phusion Human Specimen Direct PCR Kit" – 25 µL;
- Forward and reverse primers for specifying the LCT gene's regulator – each 2.5 µL of 10 µM working solution;
- Distilled water – 17.75 µL;
- Paraffin or other mineral oil – 10 µL;
- A thermocycler with a power supply and an Arduino microcontroller.
- Eppendorf 1.5 milliliter tubes.
- Eppendorf 0.2 milliliter tube.
- A powerful drill and a DremelFuge.
- A 1 to 10 microliter pipette.

- Pipette tips.
- Computer with the Arduino and Python 3 software preinstalled.

Primers used for this experiment:

LacFw: GTTGAATGCTCATACGACCATG (Tm = 59 °C; CG = 45.45%)

LacRv: TGCTTTGGTTGAAGCGAAGATG (Tm = 61 °C; CG = 45.45%)

- To begin with the experiment, we will need to connect thermocycler with the Arduino, in order to control it, and the power supply to provide voltage to it. Connect your computer with USB cable to the Arduino microcontroller. If not yet done, install Arduino drivers and copy the provided Python code onto your computer. Open Arduino software and check what port it is connected to. Add the port in the provided Python code. The port on Mac OSX will probably be similar to '/dev/cu.wchusbserial1410' and on Windows 'COM4.'
- Set the temperature cycles in the Python code according to the used reagents. If Phusion polymerase is used, set the start temperature at 98º C and let it for a minute. At this temperature the polymerase will be activated. Set the cycles: 98º C for DNA denaturation (we will need 1 second), 63º C for letting the primers bind (5 seconds), and 72º C for polymerase to complete the DNA sequence (20 seconds). Repeat this cycle for 40 times, keep the end temperature of 72º C for one minute and leave your samples at a refrigerator temperature of 4º C until you are ready to store the samples in freezer. Run the provided Python code on the terminal and see if there are no errors. Terminate the running program by pressing “CNTRL+C” keys.
- Mount the provided DremelFuge onto the drill.
- Collect some saliva into one of the provided 1.5 microliter Eppendorf reaction tube.
- Take Eppendorf 0.2 milliliter tube. Add 1.25 µL saliva, 25 µL 2x Phusion buffer, 2.5 µL of each forward and reverse primers, 17.75 µL distilled water. Finally add 1 µL of Phusion polymerase.
- Mix the tube gently and insert into the DremelFuge (use particular adapters if necessary).
- Add on top 10 microliter of mineral oil.
- Place your Eppendorf reaction tube into a thermocycler and start it by executing the provided Python code. The thermocycler will run for about an hour with Phusion polymerase.

DNA Electrophoresis

Gel electrophoresis is used for separation and analysis of macromolecules and their fragments, based on their size and charge. This experiment will tell if the target sequence of *LCT* gene's regulator was amplified to the sufficient amount in order to proceed with the analysis.

For the experiment we will need:

- An amplified DNA sample with the mineral oil on top;
- Agarose – 1 g (for 1 % gel);
- 50x TAE buffer – 4 mL;
- Distilled water – 200 mL;
- "SERVA DNA Stain G" – 8 µL;
- Invitrogen™ 100 bp DNA Ladder 15628019 – 5 µL;
- Thermo Scientific™ DNA Gel Loading Dye (6x) R0611 – 2 µL;
- An empty Eppendorf 0.2 milliliter tube;
- A power supply;
- A gel electrophoresis chamber;
- A 1 to 10 microliter pipette;
- Pipette tips;
- An electric stove or a microwave;
- A knife;
- A 250 milliliter glass flask or an alternative;
- UV light;
- Filter for the UV light;
- Precision scales.

- For the beginning prepare a 1 % agarose concentration gel. Pour 98 milliliter of distilled water into a glass flask, 1 g of agarose, 2 milliliters of 50x TAE buffer. Heat it up until it boils. The agarose should be completely dissolved. Wait until dissolved agarose cools down to approximately 60º C. Add 4 µL "SERVA DNA Stain G" and mix thoroughly to spread it (this dye is needed in order to exhibit the molecules under the UV light). Then pour the agarose solution into the provided plastic container. Use the time to wash the glass flask – we will need it again later. Place on the top of the container a plastic comb – it will make wells within the gel. If the comb doesn't hold well, use a paper clip or alternative to attach it to the side of the container. Wait until your gel thickens. In order to accelerate the process, we will place our container into a fridge.
- Take the container out of the fridge and cut approximately 2 centimeters of the gel next to the shorter sides of the plastic container. Throw out the cut outs. Place the

electrodes into the cut outs and attach the wires to the power supply. Attach the red cable to yellow and black – to blue cable. This combination will output 100 V of electric potential. Do not turn on the power supply yet!

- Pour 98 milliliters of distilled water to a glass flask and add 2 mL of the 50x TAE buffer. And 4 µL of “SERVA DNA Stain G”. Pour the buffer on top of the gel. Be sure that the buffer floods the electrodes and the gel.
- Pipette 5 µL of the DNA ladder into one of the gel pockets.
- Take an Eppendorf tube with a DNA sample and carefully take 10 µL of the sample. Pipette into a new Eppendorf tube. Add 2 µL of the 6x loading dye. Mix the solution gently. Pour the solution into a well of the gel next to the DNA ladder.
- Turn on power supply and let the electric potential flow through the chamber for about 40-50 minutes.
- Inspect the results with the UV light. You might need a filter in order to see the fluorescent molecules. The fluorescent molecules should be concentrated next to the measurement of the ladder with about 390 base pairs.

Experiment number 2:

DNA Fingerprinting

DNA fingerprinting is method when specific restriction endonucleases are used, which cut DNA at specific sequences. This experiment will take a look at single nucleotide mutations in a *LCT* gene’s regulator. DNA fingerprinting uses electrophoresis method described in a previous experiment. There are additional steps at the beginning and at the end of the experiment.

<u>In order to run the experiment, we will need:</u>

- An amplified DNA sample with the mineral oil on top;
- An empty Eppendorf 0.2 milliliter tube;
- Agarose – 2 g (for 2% gel);
- 50x TAE buffer – 4 mL;
- Distilled water – 200 mL;
- “SERVA DNA Stain G” – 8 µL;
- Invitrogen™ 100 bp DNA Ladder 15628019 – 5 µL;
- Thermo Scientific™ DNA Gel Loading Dye (6x) R0611 – 2 µL;

- Restriction enzyme NEB "CviK1-I" – 1 µL;
- 10x NEB Cut Smart buffer – 2 µL;
- Distilled water – 7 µL;
- A gel electrophoresis chamber;
- A 1 to 10 microliter pipette;
- Pipette tips;
- An electric stove or a microwave;
- A knife;
- A 250 milliliter glass flask or an alternative;
- UV light;
- Filter for the UV light;
- Precision scales.
- A thermocycler with a power supply and an Arduino microcontroller;
- Computer with the Arduino and Python 3 software preinstalled;

- To begin with the experiment, we will need to connect thermocycler with the Arduino, in order to control it, and the power supply to provide voltage to it. Connect your computer with USB cable to the Arduino microcontroller. Set the temperature cycles in the Python code so it incubates the sample at 37º C for one hour. In order to do so, we will set the start temperature at 37º C and let it run for more than an hour. Leave the rest code untouched.
- Take an Eppendorf tube with a DNA sample and carefully take 10 µL of the sample. Pipette it into a new Eppendorf tube. Take a new tip and add to the sample 2 µL of 10x NEB Cut Smart buffer. Add to the solution 1 µL of the CviKI-1 restriction endonuclease. Add to the solution 7 µL of the distilled water. Mix gently. Add to the solution 10 microliter of the mineral oil.
- Place the Eppendorf tube into the thermocycler and run the Python code in the terminal while executing the command "Phyton3 ThermocyclerController.py" from the directory where the file is located. After one hour terminate the running program by pressing "CTRL+C" keys.
- Prepare a 2 % concentration agarose gel. Pour 100 milliliter of distilled water into a glass flask, 2 g of agarose, 2 milliliters of 50x TAE buffer. Heat it up until it boils. The agarose should be completely dissolved. Wait until dissolved agarose cools down to approximately 60º C. Add 4 µL "SERVA DNA Stain G" and mix thoroughly to spread it (this dye is needed in order to exhibit the molecules under the UV light). Then pour the agarose solution into the provided plastic container. Place on the top of the container a plastic comb – it will make wells within the gel. If the comb doesn't hold well, use a paper clip or alternative to attach it to the side of the container. Wait until your gel thickens. In order to accelerate the process, we will place our container into a fridge.

- Take the container out of the fridge and cut approximately 2 centimeters of the gel next to the shorter sides of the plastic container. Throw out the cut outs. Place the electrodes into the cut outs and attach the wires to the power supply. Attach the red cable to yellow and black – to blue cable. This combination will output 100 V of electric potential. Do not turn on the power supply yet!
- Pour 100 milliliters of distilled water to a glass flask and add 2 mL of the 50x TAE buffer. And 4 µL of “SERVA DNA Stain G”. Pour the buffer on top of the gel. Be sure that the buffer floods the electrodes and the gel.
- Pipette 5 µL of the DNA ladder into one of the gel pockets.
- Take an Eppendorf tube with a DNA sample and carefully take 20 µL of the sample. Pipette into a new Eppendorf tube. Add 4 µL of the 6x loading dye. Mix the solution gently. Pour the solution into a well of the gel next to the DNA ladder.
- Turn on power supply and let the electric potential flow through the chamber for about 40-50 minutes.
- Inspect the results with the UV light. You might need a filter in order to see the fluorescent molecules. The fluorescent molecules should be concentrated as 4 or 5 individual lines (depending on the case if DNA sample has a particular mutation or not) next to the measurement of the ladder. CviK1-I restriction endonuclease cuts multiplied fragment of DNA into several pieces. If person is lactose intolerant, 4 fragments can be seen after electrophoresis. In other case, 5 lines should be visible in agarose gel.