Inspiration

- Cancer is very often discovered too late. To be diagnosed in time means more time for the patients, less suffering during the treatment and larger probability to be cured. Unfortunately, these kinds of treatments are very expensive, hard to access, and, on top of that, they are often harmful to the environment, since they produce a lot of waste. To summarize, there is a space on the diagnosis market for new methods that are less costly, and, if possible, more environmentally friendly.
- In our project, we wanted to develop a tool for diagnosing cancer that is faster, cheaper and more eco-friendly than the current ones.
- Moreover, we found the 2017 iGEM project of City School London for Boys thought-provoking and it served as a starting point for our idea. We really liked the concept of detecting cancer based on the biomarkers the tumours produce. The structure of their project provided an example for us, but we ended up not using any hairpin structures in our ssDNA constructs.

Description

1) What we would like to solve:

a) Inexpensive solution for miRNA detection - GP/Public Clinics + Low GDP countries

Our project aimed to provide a more straightforward, faster, cheaper and more eco-friendly way to diagnose any type of cancer. To fulfil this goal, we designed a tool, that can detect one of the biomarkers of a given cancer, which is presented at a much higher concentration in the body fluids of those suffering from it, than a healthy individual. A body fluid sample must be collected from the patient to complete this medical examination. This product could be utilised extensively in the Third World Countries with low GDP, due to its accessibility, rapid usage and results. This solution is yet to be utilized in tumour diagnostics, but due to its non-invasive nature, it is highly sought after. Our project primarily targets physicians, emergency physicians, pathologists and indirectly patients as a social target group.

b) Environmentally more friendly usage of chemicals used

We recycled the magnetic beads protocol to make it more environmentally friendly. We incubated the used magnetic beads at 95 °C for 30 minutes, since research suggests that at that temperature the beads are no longer able to immobilize DNA. This step allows us to reuse the beads multiple times to detect RNA, so it significantly decreases the price of our method, given that the magnetic beads are the most expensive component of the detection.

2) Our proposed solution: A device in which ssDNA coated magnetic beads are used for spectroscopically evaluated measurements.

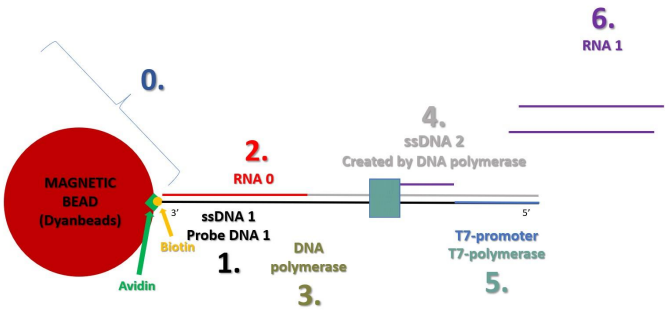
- We detected the amplified RNAs by SYBR Green II dyes. We placed the prepared samples into a PCR tube holder stand that was printed out with a three-dimensional printer. In this way, we can measure the fluorescence of a hundred times larger amount of samples than if we measured in cuvettes.

3) Scientific background: RNA detection by RNA amplification

- The detection is based on the amplification of the RNA itself. We used a ssDNA with a complementary sequence on its 3'-end. Therefore the RNA hybridizes to this "probe"-DNA. After hybridization had occurred, we applied a second strand synthesis using the RNA as a primer. This way a small number of dsDNA was created. The non-complementary part of the DNA contains a T7-RNA polymerase promoter in backward direction. After the dsDNA primer had been synthesized, the T7-polymerase created the copy RNAs, which are different from the original. These RNAs were presented in the reaction mixtures now in much larger quantities. After some loops we could reach a point where the detection starts. We detected the amplified RNAs by SYBR Green II dyes.
- We also designed and constructed a fluorimeter which could work with one single PCR-tube.
- As we intentionally use random RNAs and DNAs which were checked not to interfere with the human genome, we expect that these initial experiments won't mean any serious hazard to any person or to the environment.

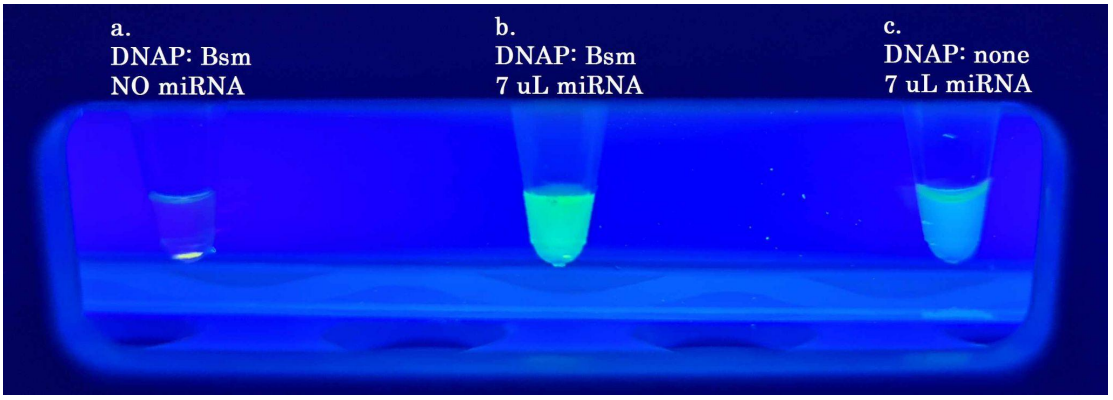
4) Bioinformatics

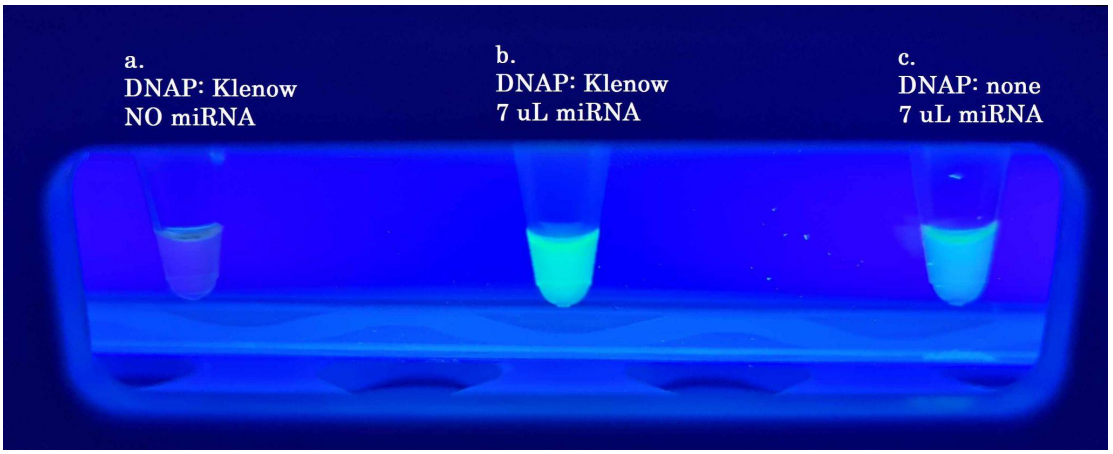
- We successfully designed the DNA part collection for qualitatively detecting a miRNA in a human sample.
- The designed DNA sequences should be used as ssDNA coated magnetic beads.
- The aim of our part collection is to produce a large amount of RNA molecules which can be detected based on their fluorescence with the intercalating dye SYBR Green II.
- During the bioinformatics part of our project we used a random sequence generator and a blast option to avoid any substantial similarity with the human genome.
- We designed the DNA sequences to
    - ensure the optimal and exclusive operation of a DNA polymerase and the T7 RNA polymerase in the same solution.
    - avoid the 3’→5’ exonuclease activity of the DNA polymerases - we bound the magnetic beads by their 3’ end to fulfil this criteria.



5) Experimental success - Results in short terms

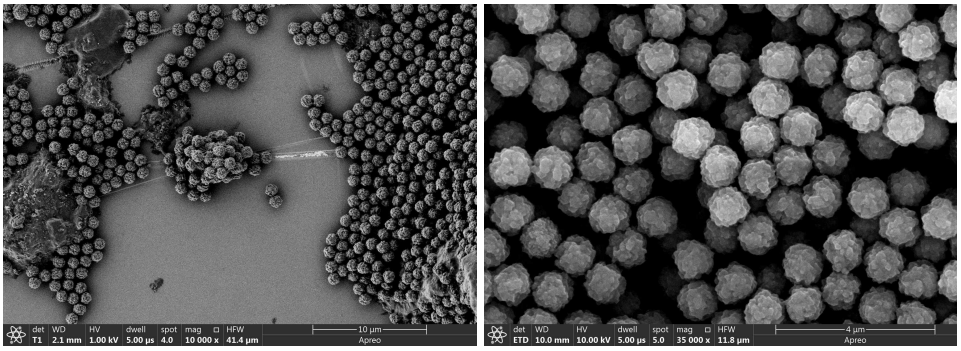
a) As we want to develop a possible qualitative detection method for miRNA, we are proud to present the figures below, which clearly show that our project works. The product of our reactions under UV light clearly demonstrate the amplification of RNA molecules in our positive samples both with a Bsm and the Klenow fragment DNA polymerases.





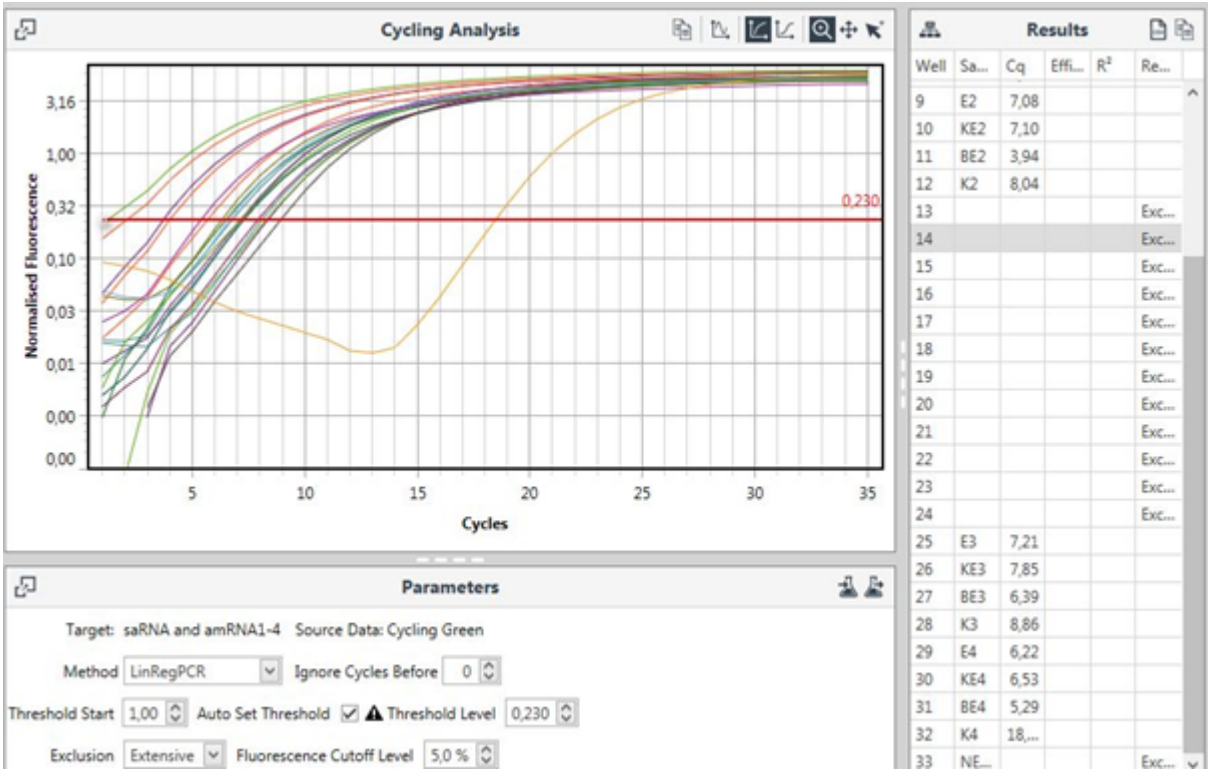
b) Imaging the beads with SEM

One of our goals was to get some photos of the Dynabeads, which can help us to make a non-expert audience understand our procedure better. Therefore we asked a researcher at the local University to create us a SEM image of the beads. As it appeared later it was essential to understand a certain buffer's problematic effect on the beads, too.



c) rtPCR reaction results

We successfully carried out a reverse transcriptase reaction with the experimental mixtures and a real time PCR was also performed. The results clearly show that a large amount of new RNA molecules were produced.



6) Engineering success:

We achieved improvements during our project in the following three areas. For details, please see our Engineering success page.

a) **Designing the DNA probe collection**

We mainly faced three problems, of which two were solved by using magnetic beads as a carrier of the DNA probes, and the third problem's solution was a simple rearrangement in the DNA sequences.

b) **Detecting the amplification product**

We originally planned to detect dsDNA by the SYBR Green I dye. We found that the originally planned restriction cut doesn't work properly, probably due to steric proximity of the beads and the restriction site. Therefore we had to change the orginal plan to detect the amplified RNA mass instead of the DNA.

c) **Storing the DNA coated beads**

The overnight storage of the beads in the reaction buffer caused severe instability on the surface of the beads, which was confirmed by SEM imaging. Therefore we had to change the storing solution.

7) Software

- We made a software to generate all the needed ssDNA sequences to carry out the experiment with a randomly selected miRNA.
- The Software is a really important part of our project. For anyone who needs it, it is an usable, fast and easy to understand assistance. In addition to all of these, we improved the limitations of the project by giving the ability to build more DNA strands in the reaction. We archived this by using c# programming language and Unity development environment. (see the Software for further info)

8) Hardware - 3D printing

- The spectrophotometer that we are using, the **Vernier Spectrovis**+, which can be found in 20.000 secondary schools. It is created for 1cm × 1cm × 3 cm cuvettes, but our samples aren't big enough to fill one of those. To solve this problem, our task was to design the model of a cuvette holder, which we can print in 3D. (see the Contributions for further info)

Our goal

- To create an equipment which is able to detect small RNAs in a natural fluid sample (soil, water, body fluids, culture medium, etc.) without using rtPCR/qPCR. Therefore we have used a random RNA sequence (which was checked not to interfere with the human genome) and amplified it using our own methods (see our Notebook for further info). The usage of the method can be useful in many research areas.