Developing a novel, inexpensive method to detect small RNAs in biological samples

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 diagnostic market for new methods that are less costly, and, if possible, more environmentally friendly.

Our project aimed to provide a more straightforward, faster and cheaper way to diagnose any type of cancer. To fulfill 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 in a healthy individual's.

To prove the universality of our idea, we used short RNA sequences to simulate the human microRNAs. We designed the DNA sequences to both ensure the optimal and exclusive operation of a DNA polymerase and the T7 RNA polymerase in the same solution and to avoid the 3'→5' exonuclease activity of the DNA polymerases - the ssDNAs were bound to magnetic beads by their 3' end.

The technique is based on the amplification of the RNA itself. The ssDNA (which we also refer to as the "probe DNA" in this text) designed by us contained a complementary sequence to the target RNA on its 3'-end. Therefore, the target RNA could hybridize to this region of the probe DNA. After hybridization occurs, second strand synthesis begins using the target RNA - probe DNA bundle as a primer. As a result, a number of dsDNAs are created. The designed ssDNA also included a T7-RNA polymerase promoter in backward direction. After the dsDNA was synthesized, the T7-polymerase could create copy RNAs. These newly synthesized RNAs differ from the original target RNA, they are presented in the reaction mixtures in much larger quantities. The copy RNAs start the process again and again, thus producing a high amount of dsDNA detected by SYBR Green II intercalating, fluorescent dyes. We calibrated a simple fluorimeter and proposed a self-designed and 3D-printed adaptor for PCR tubes as inexpensive hardware for qualitative analysis.