

Droplets and DNA – Extracting and Purifying DNA in a Magnetic Droplet Manipulation System

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We present a microdroplet-based system for the on-chip extraction and purification of DNA from lysed cells. The setup of the droplet manipulation system is schematically shown in figure 1. A reservoir, placed on a Printed Circuit Board (PCB) with four levels of square coils, serves as platform for the experiments. The hydrophobic Teflon bottom of the reservoir carries several hydrophilic spots created via local oxygen plasma treatment. The reagents necessary for the desired bioanalytical protocol can thus be immobilized over the PCB, while magnetic particles are passed through them [1].

In the experiment described here, we implemented a macroscopic protocol for the extraction and purification of DNA into the magnetic droplet manipulation system. We are able to use the same set of reagents, while using only a fraction of the volume usually required [2]. First experiments show, that we are able to capture the DNA in an immobilized droplet of 10 μ l at a high rate and with a good reproducibility (figure 2). Thus we are able to extend the processing steps performed on the chip. Figure 3 presents the on-chip extraction and purification procedure, which consists of a lysis and binding step containing Guanidinium thiocyanate, three washing steps containing an ethanol solution and two final elution steps in deionized water [3]. Thus the DNA is selectively attached to the magnetic silica particles [4], cleaned from cell debris and proteins and finally again eluted from the particles to be either quantified or amplified in a off-chip Polymerase Chain Reaction (PCR) step. The result of a fluorescent DNA quantification for different amounts of Jurkat cells is presented in figure 4. Due to the small sample volume the influence of the viscous cells debris in the binding buffer needs to be considered for larger amounts of cells, since the increased viscosity inhibits the movement of the magnetic particles and thus decreases the amount of bound DNA. In addition to the fluorescent quantification using PicoGreen, we examined the purity and intactness of the DNA in a PCR amplification step. Since this type of reaction is easily inhibited by impurities such as proteins, a successful amplification is a sign for the quality of the purified DNA. Figure 5 shows that the DNA obtained in the presented system is sufficiently pure to be amplified via PCR. In the electropherogram we can additionally see differences between the columns of the experiments. A low cell and thus DNA content results in a smaller intensity of the signal for the amplified stands as well as in the comparatively large amount of remaining primer (lowest band).

Using a magnetic droplet manipulation system we demonstrated successfully the miniaturization of a standard lab-bench bioanalytical protocol. With only fractions of the reagents usually required we are able to extract, purify and measure the DNA of as little as 10 cells.

References:

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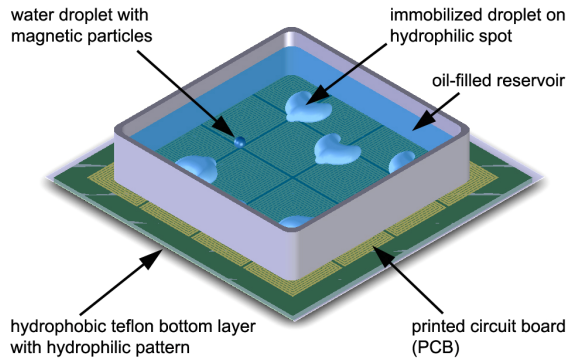


Figure 1: Schematics of the magnetic droplet manipulation system

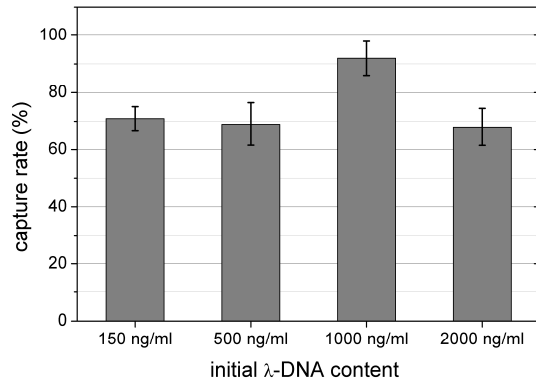


Figure 2: DNA capture rates for different concentrations of DNA in the sample droplet

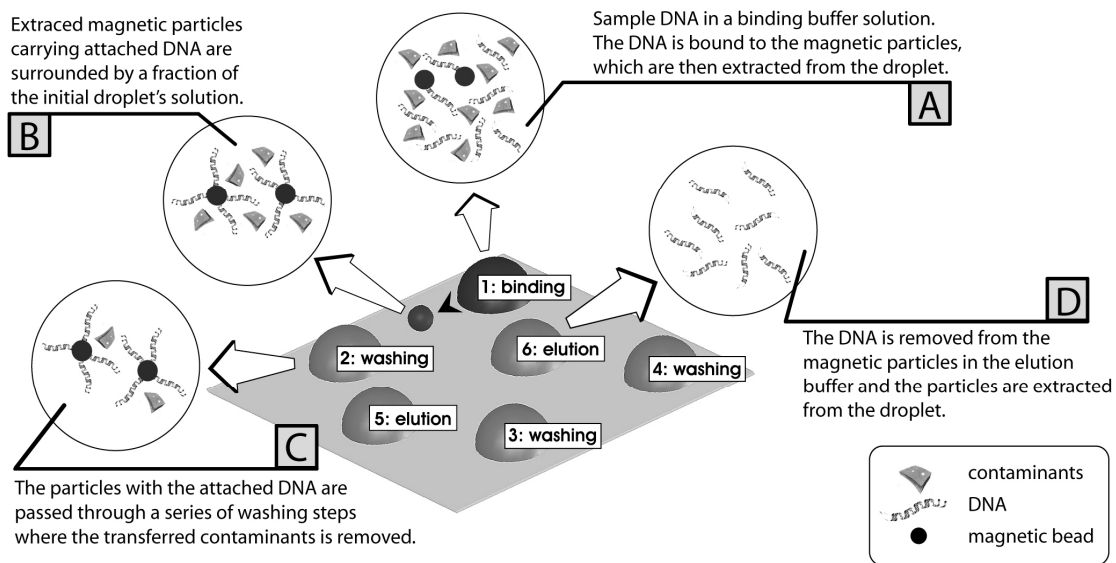


Figure 3: Schematic principle of the on-chip DNA purification protocol. The sample in the binding buffer solution is injected onto the chip and mixed with a droplet of magnetic particles. These are extracted and wash in three stages, before the DNA is again eluted in the steps 5 and 6.

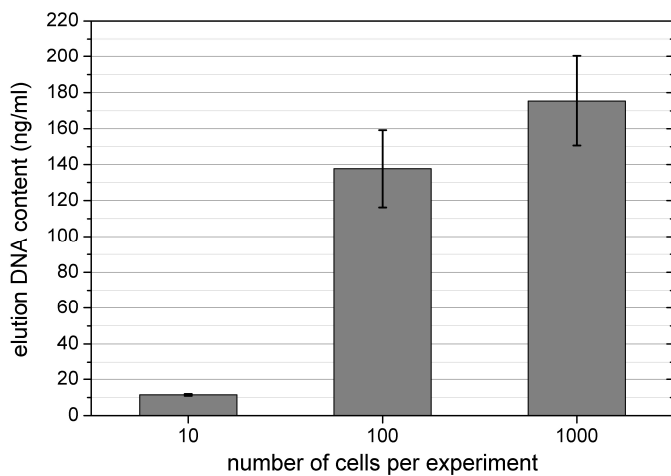


Figure 4: Measured DNA content for different amounts of jurkat-cells in the binding buffer solution. The measurement was done off-chip.

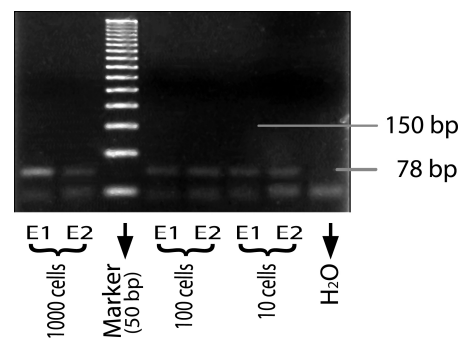


Figure 5: Agarose gel electropherogram of human β -actin after 35 cycles of PCR for different initial amounts of cells. The off-chip amplification started from 3 μ l of eluated sample.