

HIGH-PERFORMANCE FLOW-THROUGH DNA PURIFICATION ON A MICROFLUIDIC CHIP

M. Karle^{1*}, G. Czilwik¹, J. Miwa², N. Paust^{1,2}, G. Roth^{1,2,3}, R. Zengerle^{1,2,3} and F. von Stetten^{1,2}

¹HSG-IMIT, Wilhelm-Schickard-Straße 10, 78052 Villingen-Schwenningen, Germany, ²Laboratory for MEMS Applications, Department of Microsystems Engineering – IMTEK, University of Freiburg, Georges-Koehler-Allee 106, 79110 Freiburg, Germany, ³Centre for Biological Signalling Studies (bioSS), University Freiburg, Germany

ABSTRACT

We present a significant increase in performance for flow-through purification of nucleic acids by continuous microfluidic processing. After binding to superparamagnetic beads the nucleic acids are sequentially transported across the phase-interface of co-flowing laminar streams of purification reagents. The entire purification procedure is performed within only 2 minutes. Compared to classical batch-wise purification in test tubes, $150 \pm 50\%$ of total DNA have been recovered in on-chip purifications over a DNA concentration range of 7 orders of magnitude. With appropriate surface modification of the magnetic beads the chip is also suggested for the implementation of other continuous biomolecular purification tasks.

KEYWORDS: Nucleic Acids Purification, Magnetophoresis, Continuous Processing, Flow-Through Assay

INTRODUCTION

The phase transfer magnetophoresis platform presented here serves as module for continuous DNA-extraction and paves the way to continuous flow monitoring of cell growth in bioreactors. The novel module closes the gap between the already existing systems for continuous cell lysis [1] and continuous DNA amplification and detection [2, 3]. The three crucial steps in DNA purification are separation of cell debris, washing and elution of DNA. The presented system realizes these unit operations by the interplay of continuous laminar flow of the purification reagents and a time-varying magnetic field for manipulating superparamagnetic beads which serve as mobile DNA carrier.

WORKING PRINCIPLE

A mixture of cell lysate, binding buffer, and superparamagnetic beads binding the DNA is injected into the chip. The microfluidic channels are arranged circularly around a central rotating permanent magnet (Fig. 1). At channel junctions different buffer solutions are brought into contact to form laminar flow interfaces. The permanent magnet enables controlled transfer of the magnetic beads between the different reagent streams attracting the beads towards the inner channel wall. Thus the magnetic beads and DNA respectively are transferred across the laminar flow interfaces.

Furthermore, the rotating permanent magnet facilitates transportation of the beads along the microfluidic channels by inducing a rolling motion of self assembled magnetic bead filaments, which align along the magnetic field lines.

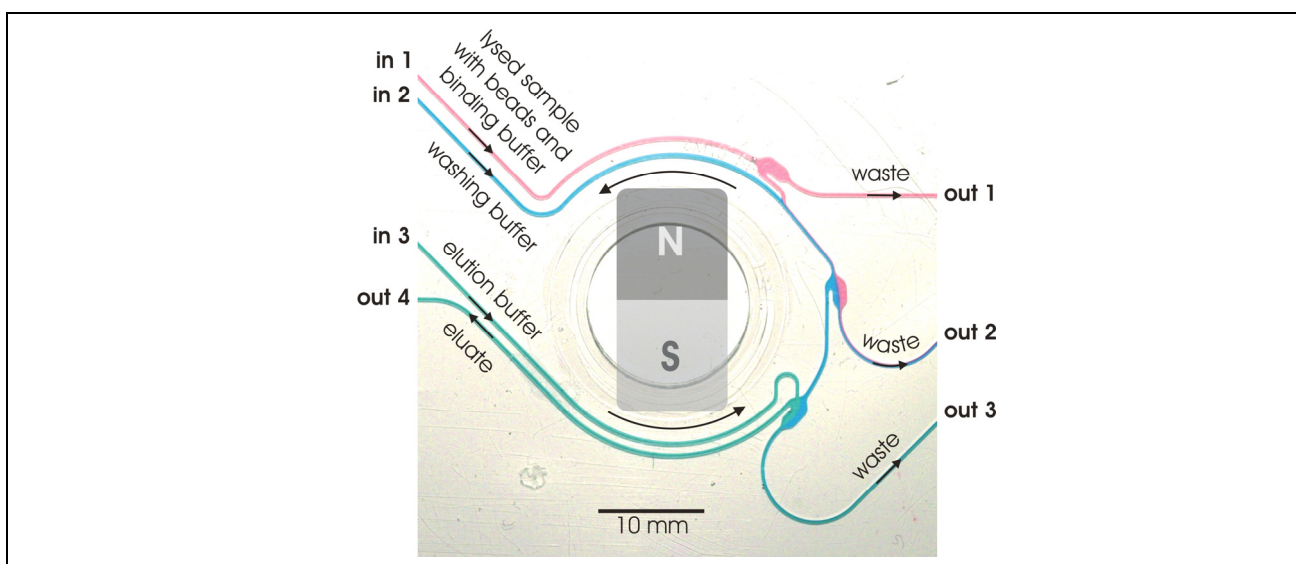


Figure 1: Photograph of the chip that was used for the purification experiments. The microchannels have been milled directly into a polycarbonate substrate (blank DVD). To illustrate the purification process DI water dyed with ink has been injected into the chip. The different colours denote the different buffer solutions for the purification process. Red: lysis and binding buffer including the cell sample and the magnetic beads, blue: washing solution, green: elution buffer.

In sum, the motion of the magnetic beads is governed by an interplay of fluidic drag and magnetically induced actuation. Using this working principle, the three essential purification steps: separation, washing, and elution can be performed in continuous flow on a single chip.

CHIP PRODUCTION

The purification chips were fabricated by micromilling the channel structure into blank polycarbonate DVDs (Sonopress GmbH, Gütersloh, Germany) according to a 2D layout designed with CAD software. The standard channel width was set to 300 μm and the channel depth to 210 μm (compared to 400 μm x 400 μm of a former design [4]). After the milling process the chips were cleaned with DI-water and isopropanol. To seal the microfluidic channels adhesive PCR foil (676070, Greiner Bio-One GmbH, Frickenhausen, Germany) was applied.

MATERIALS AND METHODS

For DNA purification a commercially available DNA extraction kit (AJ Innuscreen GmbH, Berlin, Germany) was implemented into the platform. Following the kit's extraction protocol *E. coli* DH5 α Z1 cells [5] have been lysed. A dilution series of the lysate covering 7 orders of magnitude in sample DNA concentration was prepared. DNA was purified on-chip from each dilution (Fig. 2). For this, the sample and the purification reagents supplied with the kit were continuously injected into the chip with precision syringe pumps (neMESYS, Cetoni, Korbußen, Germany) at a flow velocity of 11.9 $\text{mm}\cdot\text{s}^{-1}$ (compared to 12.5 $\text{mm}\cdot\text{s}^{-1}$ before [4]). The DNA yield of the extraction experiments was determined by quantitative PCR (qPCR) analysis. As a reference, the lysate samples were also processed off-chip in a batch-wise manner using the same extraction chemistry as on chip.

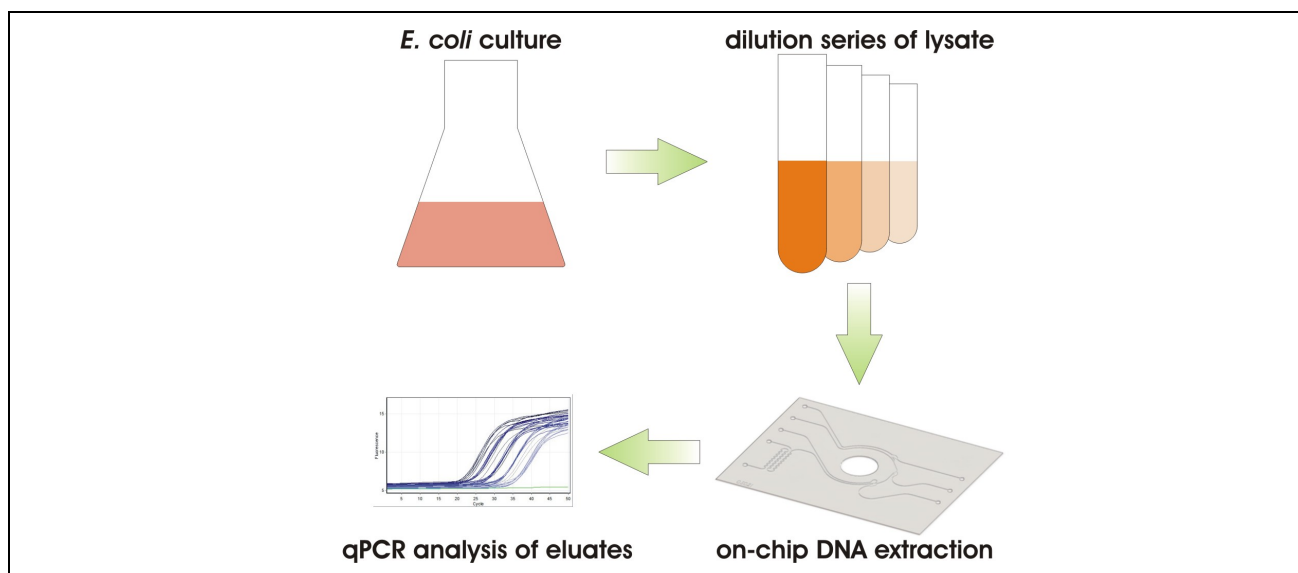


Figure 2: Schematic procedure of the experiment. An *E. coli* culture was lysed and a dilution series of the lysate was prepared. From each dilution DNA was extracted on the microfluidic chip. After the purification the DNA content of each eluate has been determined using qPCR.

RESULTS

In on-chip DNA purification experiments, an applied inlet flow velocity of 11.9 $\text{mm}\cdot\text{s}^{-1}$ (corresponding to a flow rate of 0.75 $\mu\text{l}\cdot\text{s}^{-1}$) led to an average bead velocity of 0.7 $\text{mm}\cdot\text{s}^{-1}$ and thus a sample transition time of approximately 2 minutes. The purified DNA was successfully amplified off-chip via qPCR, indicating sufficient removal of PCR inhibitors.

The on-chip DNA purification showed linearity over 7 orders of magnitude from saturation at very high DNA concentrations down to the single molecule level. Compared to the reference purifications in test tubes, 150 ± 50 % of total DNA has been recovered on-chip (Fig. 3).

CONCLUSION

Compared to previous results (25 % [4] and 80% [6] efficiency) we have greatly increased the DNA yield of our continuously working chip through changes in channel geometries and flow-rates. The continuous on-chip DNA purification outperforms the batch-wise procedure in test tubes by an average of 50 %. As the microfluidic DNA purification chip closes the gap between continuous cell lysis and continuous DNA amplification and detection it paves the way to fully integrated and continuously operating microsystems for monitoring of cell growth in bioreactors.

In addition to the purification of nucleic acids, the field of application of the presented system can be extended using appropriate buffer solutions and surface modifications of the beads, allowing other targets to bind to the beads. This would enable continuous protein purification, immunoassays, or cell-based assays.

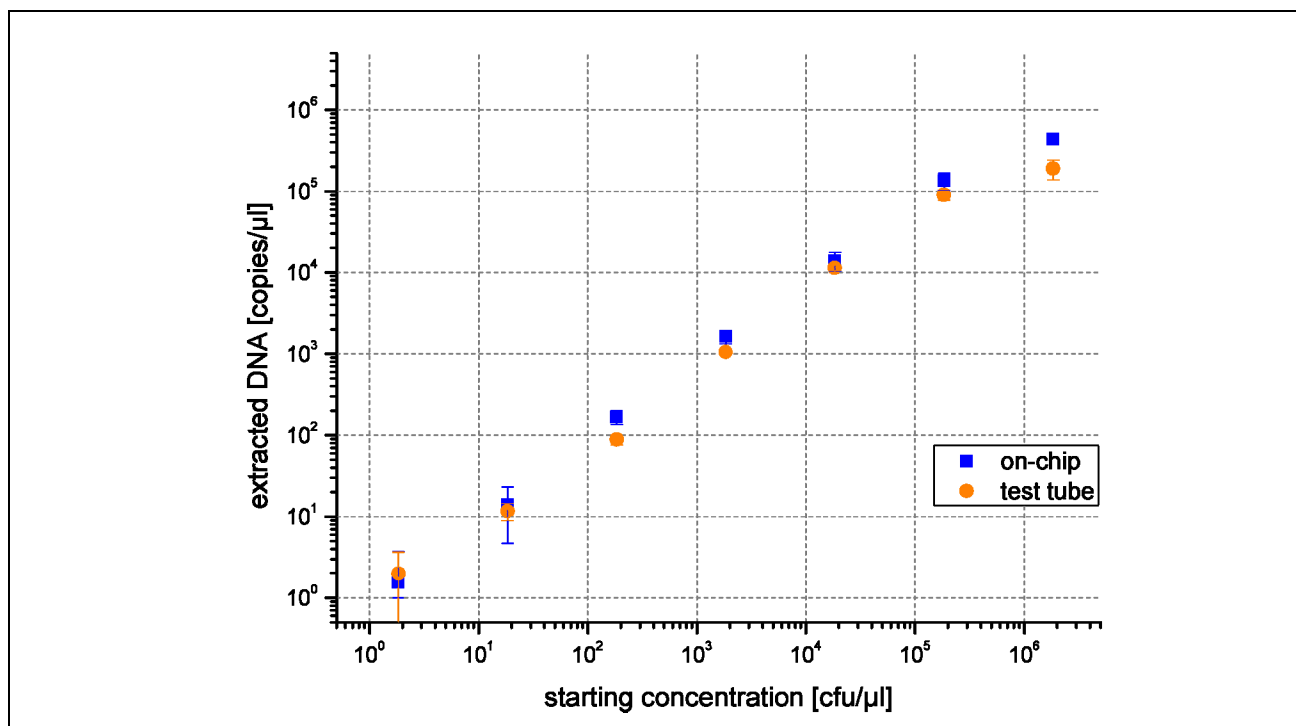


Figure 3: DNA purification yield. The amount of DNA extracted was determined by real-time PCR (genomic *E. coli* DNA, *Pal* gene, 1 copy per genome). Every data point represents three purification experiments, which have been analyzed in triplicate qPCR runs each. The standard deviation is depicted as error bars.

ACKNOWLEDGEMENTS

The present study is part of the Minamed project and is funded by the German Federal Ministry of Education and Research (16SV3528).

REFERENCES

- [1] P. Sethu, M. Anahtar, L. L. Moldawer et al., *Continuous row microfluidic device for rapid erythrocyte lysis*, Analytical Chemistry, vol. 76, no. 21. pp.6247-6253, (2004).
- [2] H. Nakano, K. Matsuda, M. Yohda et al., *High-Speed Polymerase Chain-Reaction in Constant Flow*, Bioscience Biotechnology and Biochemistry, vol. 58, no. 2. pp.349-352, (1994).
- [3] J. Felbel, A. Reichert, M. Kielpinski et al., *Technical concept of a flow-through microreactor for in-situ RT-PCR*, Engineering in Life Sciences, vol. 8, no. 1. pp.68-72, (2008).
- [4] M. Karle, J. Miwa, G. Roth et al., *A Novel Microfluidic Platform for Continuous DNA Extraction and Purification Using Laminar Flow Magnetophoresis*, Proceedings of the 22nd IEEE International Conference on Micro Electro Mechanical Systems, pp. 276-279, (2009).
- [5] R. Lutz and H. Bujard, *Independent and tight regulation of transcriptional units in Escherichia coli via the LacR/O, the TetR/O and AraC/I-1-I-2 regulatory elements*, Nucleic Acids Research, vol. 25, no. 6. pp.1203-1210, (1997).
- [6] M. Karle, J. Miwa, G. Roth et al., *Kontinuierlich arbeitende Mikrofluidik-Plattform zur Aufreinigung von Biomolekülen* Tagungsband Mikrosystemtechnik-Kongress, Berlin, 12.-14. Oktober., pp. 134-137, (2009).

CONTACT

*Marc Karle, tel: +49-761-203-7329; marc.karle@hsg-imit.de