ABSTRACT

We present a novel method for integrated magnetic bead based DNA purification on a centrifugal microfluidic platform ("disk"). The system is characterized by purifying DNA from lysed E. coli cultures. Advantages over existing solutions [1-4] include operation at low centrifugal frequencies (≤8 Hz), modular design using one repeating unit operation, short processing time and yield close to 100% vs. manual reference purification. During purification process, magnetic beads are moved between different liquid filled reservoirs by an interplay of centrifugal and magnetic forces. PCR ready DNA of 3 different dilutions (from $1.5 \times 10^4$ CFU up to $1.5 \times 10^8$ CFU per sample) of an E. coli lysate was purified within ~6 min without manual interference.

KEYWORDS: Microfluidic, DNA purification, DNA extraction, Lab-on-a-Chip

INTRODUCTION

During the last years, automated systems for nucleic acid analysis gained more and more importance e.g. for in-vitro diagnostics. Therefore the “on-disk” integration of DNA purification is crucial for the development of fully integrated nucleic acid analysis based systems [3]. However, current state-of-the-art solutions reveal several disadvantages. High and bidirectional rotational frequencies are required as well as active liquid valving, requiring the implementation of additional add-on modules such as lasers or servo motors [1-4]. Furthermore, all mentioned systems occupy a significant amount of space in radial direction (liquid flow direction under centrifugation) limiting the possibilities for an integration of further downstream processing steps e.g. PCR [5, 6]. In contrast, the presented mechanism applies a mobile solid phase and stationary liquid reagents, resulting in lower requirements of space in radial direction.

FUNCTIONAL PRINCIPLE

The laboratory process of DNA purification with magnetic silica beads had to be represented by a microfluidic structure on a rotating disk. The laboratory protocol requires that the beads are consecutively brought into contact with the mixture of sample and binding buffer (binding of DNA to the beads), washing buffer (removing PCR inhibitors), and finally elution buffer (re-dissolving the purified DNA). To realize those specifications, a transport mechanism was developed, that is able to transport magnetic beads, between fluidically separated chambers on the disk using only one stationary permanent magnet. This magnet is mounted approx. 1 mm above the disk, generating a magnetic field gradient that is able to attract and manipulate the magnetic beads in the liquid (Fig 1).

The presented microfluidic structure consists of three independent inlets for preloading the required reagents. Each inlet is connected to a processing chamber via a channel. Centrifugal forces created by disk-rotation can route the liquid reagents from the inlets (Fig 2: a, b, c) to the processing chambers (Fig 2: A, B, C) where an air-gap between adjacent chambers guarantees a diffusion-free liquid separation.

Fig 1: Foil disk mounted to centrifugal test set up. For development purposes, the permanent magnet is attached to a flexible positioning unit enabling the user to position the magnet in arbitrary azimuthal and radial positions above the disk.

Fig 2: 3D image showing a complete DNA purification structure. Each inlet (a, b, c) is connected to a chamber via a channel, where (A) is the binding chamber, (B) the washing chamber and (C) the elution chamber. The fluids are kept separated while the chambers are connected via an air-gap.
The single steps of the purification and bead transport process are depicted in Fig 3: All reagents, the sample and the magnetic beads are loaded into the corresponding inlets (1), then the disk is accelerated to 8 Hz. The centrifugal forces route the liquids and beads into the processing chambers allowing DNA from the sample to bind to the beads (2). After incubation, the disk is stopped at a defined position relative to the external magnet (3) where the beads are attracted by the magnet in such a way, that they can overcome the liquid air interface (4). Next, the disk is moved in 0.5° increments, while the beads in the air-gap are fixed by the magnet (5). Spinning the disk at 8 Hz again transports the beads into the next process chamber due to centrifugal forces (6). Steps (3) – (6) can be repeated to transport magnetic beads in further processing chambers.

EXPERIMENTAL

Preparation of sample stock solution was conducted by growing E. coli in LB medium at 37° C overnight to a bacterial count of 6.6·10⁸ CFU mL⁻¹. Subsequently bacteria were lysed with a standard combined lysis-binding buffer (Buffer ML, Qiagen GmbH, Hilden, Germany). For digestion of remaining cell debris proteinase K (Roche Diagnostics GmbH, Mannheim, Germany) was added. Two separate dilutions of the stock lysate (1:100 and 1:10000) were prepared by adding adequate volumes of lysis-binding buffer. All samples were stored at -18° C.

For DNA extraction, the disk was mounted onto the centrifugal test set up, the inlets were loaded with respective buffers, sample and beads and afterwards sealed with adhesive tape: inlet a: 230 µL sample (comprising the lysed equivalent of 1.5·10⁹ CFU for undiluted sample) and 10 µL of bead-suspension (Mag Attract Susp. G, Qiagen GmbH, Hilden, Germany); inlet b: 130 µL washing buffer (Buffer AW2, Qiagen GmbH, Hilden, Germany); inlet c: 130 µL elution buffer (DI-H₂O). Afterwards, the frequency protocol was started. The magnetic beads were automatically transported into chamber A together with the sample. Here, the DNA can bind to the beads for ~70 sec. According to the transport mechanism described in Fig 3, the beads were then transferred out of the sample through the air gap into the washing chamber B where the beads were incubated for 60 sec. in the chamber allowing the washing buffer to remove PCR inhibitors. Subsequently the beads were transported into the elution chamber C, where the DNA is released from the beads for another 70 seconds. Since the bead transfer between two adjacent chambers takes ~80 seconds, the whole purification process was conducted in ~6 minutes.

For quantification, the eluate was taken from the disk and analysed by real time PCR to determine the amount of E. coli pal gene copies. As reference, the DNA was also purified in standard tubes using identical amounts of sample, buffers and bead volumes. Instead of transporting the beads actively from tube to tube, the beads were fixed to the tube with a permanent magnet while the supernatant was discarded.
RESULTS AND DISCUSSION

DNA purification was performed with 230 µL of undiluted, 1:100 and 1:10,000 diluted lysate containing 1.5 \(10^8\) CFU, 1.5 \(10^6\) CFU and 1.5 \(10^4\) CFU respectively always on different disks (n = 5 for each dilution). As reference, purification was performed in standard tubes according to bench top protocol (n = 3 for each dilution). Purified copies from “on-disk” extraction and reference were compared by detecting \textit{E. coli} pal gene via real-time PCR. Purification from disk and tube yielded comparable results (Fig 4).

\[
\begin{array}{c|c|c|c|c}
\text{Absolute Number of Bacteria in Sample [CFU]} & \text{Recovered Number of DNA Copies} \\
\hline
10^3 & 10^4 & 10^5 & 10^6 & 10^7 & 10^8 \\
\hline
\end{array}
\]

\[ \text{On-Disk} \] \[ \text{Reference} \]

Fig 4: Results from DNA purification. Numbers of pal gene copies from undiluted, 1:100 diluted and 1:10,000 diluted sample. Blue triangles show results from on-disk purification while black circles represent results from reference purification. Furthermore, DNA was reconcentrated from 230 µL sample volume to 130 µL elution buffer volume.

CONCLUSION

We demonstrated a compact and modular system for the automatic bead based purification of DNA on a centrifugal microfluidic platform at very low rotational frequencies. The arrangement of the microfluidic components leaves sufficient space for further processing of DNA in downstream unit operations such as Real-Time PCR [5]. The geometry of the system is compatible to a standard PCR cycler (Qiagen Rotor-Gene) providing the means for complete sample-in answer-out capability on a cheap and disposable foil disk processable in standard lab equipment. The bead transport mechanism may also be applicable to bead based immunoassays. In future work, we plan to link the DNA purification structure to a PCR structure, for microfluidic integration of complete diagnostic assays.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from the federal state of Baden-Württemberg (Baden-Württemberg Stiftung – MST II 17). We also want to express our gratitude to Qiagen (Qiagen GmbH, Hilden, Germany) for supplying us with all reagents and beads for DNA extraction.

REFERENCES


CONTACT

* D. Mark, Tel: +49-761-203-7538; daniel.mark@hsg-imit.de