Microfluidic cartridges for DNA purification and genotyping processed in standard laboratory instruments

Maximilian Focke¹, Daniel Mark², Fabian Stumpf², Martina Müller¹, Günter Roth¹, Roland Zengerle¹,², Felix von Stetten¹,²

¹ University of Freiburg, Department of Microsystems Engineering – IMTEK, Freiburg, Germany
² Institut für Mikrotechnik und Informationstechnik (HSG-IMIT), Villingen-Schwenningen, Germany

1 Introduction

We present two disposable microfluidic cartridges intended for upgrading standard laboratory instruments with automated liquid handling capability by use of centrifugal forces. Both cartridges integrate and automate laboratory protocols for analysis of nucleic acids. The first microfluidic cartridge enables purification of DNA from human whole blood and is operated in a standard laboratory centrifuge. The second microfluidic cartridge enables genotyping of pathogens by geometrically multiplexed real-time PCR. It is operated in a slightly modified off-the-shelf thermal cycler. Both solutions aim at smart and cost-efficient ways to automate work flows in laboratories.

2 DNA purification in a standard lab centrifuge

Purification of nucleic acids is an important step in sample preparation prior to DNA analysis. DNA extraction is conventionally very time-consuming and requires several manual handling steps or large, expensive automated workstations. Our disposable DNA purification cartridge integrates required liquid reagents and allows to automate all liquid handling steps starting from a lysed blood sample to PCR-ready DNA. The following steps are integrated: Binding of DNA from a lysed blood sample to a silica membrane, two consecutive washing steps, and elution of the purified DNA from the silica membrane. Unbound sample residues and washing buffers are routed into a waste chamber. The purified eluate is directed into a separate elution chamber from which it can be collected and directly used in a DNA analysis.

The extraction disk contains two glass capillaries with 100 µl of washing buffer 1 (AW1) and washing buffer 2 (AW2), respectively [1]. The glass containers are crushed manually to release the buffer solutions just before the DNA extraction starts. The final release of the liquid is then achieved by passive, time-controlled burst valves. These work by controlled delamination of the lid foil during fast rotation in the centrifuge to open a passage for the liquid [2].
The operator just adds elution buffer and a sample of lysed blood into the respective chambers. Subsequently, the DNA extraction procedure can be started. In contrast to a previously reported routing approach [3], a novel routing mechanism for separation of the eluate from waste liquids is applied in this approach: A liquid portion is directed to a venting channel to block an air vent. This prevents further filling of the waste chamber and forces the eluate to flow into the elution chamber for collection. The schematic microfluidic routing is displayed in figure 1.

Figure 1: Microfluidic principle. a) Filling levels before rotation and uncrushed glass capillaries containing washing buffers AW1 and AW2; b) break of burstable seal of AW1 and centrifugation of AW1 into the waste chamber; subsequent automatic release of AW2; c) elution step after breakthrough of burstable seal of AE and blocking the switch channel by a liquid plug. Thereby the elution buffer AE is guided into the elution chamber, after collecting the purified DNA from the silica solid phase.

All experiments were implemented on a standard laboratory centrifuge (Figure 2) (Sigma 1-15, Sigma GmbH, Osterode am Harz, Germany) that was provided by the manufacturer with an automated control option via an RS232 interface. For fixation of the disk, a hematocrit rotor is used. DNA extraction reagents and the silica extraction matrix were taken from a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Table 1 shows the protocol of rotational frequency in the lab centrifuge. In three independent experiments an average of 192 ± 30 ng DNA has been extracted from 32 µl of blood, which is a yield of 53 ± 8 %, compared to a reference extraction with the QIAamp Kit. Currently, the processing time for the complete extraction is about 66 minutes, since the opening time of the burst valves has not been optimized yet. Burst times of washing buffer AW1, AW2, and elution buffer are 50 ± 20 s, 105 ± 40 s, and 62 minutes (!) respectively, whereby the latter is to be reduced to ~6 minutes with an adapted layout. In total, three out of four experiments with the completely integrated system were successful. Due to sealing failure in the fourth experiment the air vent of the waste chamber was not blocked so that the elution buffer flows directly in the waste chamber and the measurement of DNA-yield was not possible.
Table 1: Frequency protocol of the automated DNA purification on disk after pipetting of blood and crushing of the glass capillaries containing both washing buffers

<table>
<thead>
<tr>
<th>DNA extraction step</th>
<th>Frequency /rpm</th>
<th>Time /min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation of lysed blood, AW 1 and AW 2</td>
<td>6000</td>
<td>5.0</td>
</tr>
<tr>
<td>(100 µl each) into the waste chamber</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakthrough of burstable seal of AE (190 µl)</td>
<td>6000</td>
<td>57.0</td>
</tr>
<tr>
<td>Capillary filling of the siphon</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Incubation step (optional for increased elution efficiency)</td>
<td>300</td>
<td>0.1</td>
</tr>
<tr>
<td>Elution step: centrifugation of the elution buffer in the elution chamber</td>
<td>900</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Fig. 2: Microfluidic cartridge for DNA-Extraction operated in a standard laboratory centrifuge

3 Automated genotyping in a microfluidic disk

Genotyping of DNA samples plays an important role for decision making in the fields of food or infection control, in clinical settings as well as forensic scenarios. Here, our disposable genotyping cartridge is applied to analyse isolates of the multi-resistant Staphylococcus aureus (MRSA) by real-time PCR. The cartridge is made of a microstructured polymer foil (“Lab-on-a-Foil”) [4,5]. This is for example advantageous as the thin sidewalls of this so-called blister cartridge support efficient thermocycling by fast heat transfer. Further, foil-based Lab-on-a-Chip systems suit perfectly as disposable consumables because they only require a minimum of material volume. In the future, such cartridges could be produced in fully integrated form-fill-seal production lines. Such production lines are long standard facilities for example in the field of packaging of pharmaceutical pills into blister packages.

Each foil cartridge contains four independent structures that allow to process up to four separate samples per cartridge (Fig. 3). The foil cartridge is designed to be
processed in a slightly modified off-the-shelf thermal cycler (Rotor-Gene 2000, Corbett Research Ltd., Fig. 4). The modification of the instrument refers to an additional relay to increase the rotational speed discretely from standard 6.6 to 27.2 Hz. Further, the microtube holder is replaced by a custom-made, light-weight adaptor for fixation and alignment of microfluidic disks in the device.

Fig. 3: Microfluidic cartridge for genotyping of DNA. Up to four samples can be processed independently in each disk. On the outer diameter are four separate structures comprising eight reaction wells (highlighted with a dye for better visibility) and a reservoir for waste liquid.

The master mix (1x, RealMasterMix with ROX, 5Prime, Germany) containing the DNA sample is automatically aliquoted into individual reaction wells based on a centrifugo-pneumatic valving principle [6]. Reaction volumes of 9.8 µl are aliquoted with a precision of CV 3.4 % (32 wells).

The reaction wells contained pre-stored dry reagents such as primers and probes. This allowed to evaluate the system with 44 genotyping assays containing clinical isolates of 8 different genotypes of MRSA. The evaluation proved full specificity and agreement with the reference assays in standard tubes in all 44 tests. The lower limit of detection was below 10 copies of DNA per reaction (N = 24 wells in 3 independent disks).
4 Conclusions
In conclusion, we demonstrate that microfluidic cartridges can be designed to be processed in standard laboratory instruments. This creates added value for the supplier of standard lab equipment and reduces market entry barriers for organisations developing microfluidic solutions.

5 Acknowledgements
The research leading to these results has received funding from the European Community’s Sixth Framework Programme (FP6) under contract no. 37957 (project MagRSA) as well as the MINT Sofortprogramm (Baden-Württemberg) in cooperation with the HSG-IMIT.
6 References


