

# DNA EXTRACTION, USING CARRIER RNA, INTEGRATED WITH AGAROSE GEL-BASED POLYMERASE CHAIN REACTION IN A MICROFLUIDIC DEVICE

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## ABSTRACT

This presentation reports an integrated DNA extraction and amplification device based on electro-osmotic pumping (EOP). Solid-phase DNA extraction is performed on a thermally activated silica-based monolith and carrier RNA is included in the chaotropic salt solution to increase the efficiency of the DNA extraction process. The extracted DNA is then transferred by EOP into the DNA amplification chamber where polymerase chain reaction (PCR) reagents, encapsulated in agarose, have been pre-loaded. Integration of DNA extraction and PCR amplification without the need for hydrodynamic pumping is achieved as well as long-term storage of the PCR reagents in the microfluidic device.

**KEYWORDS:** Carrier RNA, DNA extraction, PCR, Electro-osmotic pumping

## INTRODUCTION

Extraction of DNA from biological samples is critical for the success of downstream processes such as PCR. The use of a solid-phase extraction methodology, as presented here, has the added advantage that it facilitates pre-concentration of the DNA, important when limited sample material is available. While carrier RNA has been shown to increase DNA extraction efficiency in Qiagen DNA extraction kits [1], the same principle has not yet been applied to microfluidic solid-phase DNA extraction systems. Although hydrodynamic pumping allows accurate control of flow rates there are problems associated with interfacing the mechanical requirements to the microfluidic device. In order to produce fully portable microfluidic devices for 'point-of-need' applications there would be substantial benefits to using EOP and on chip storage of reagents.

## THEORY

Solid-phase DNA extraction relies on the binding of DNA to a silica support in the presence of a chaotropic salt at  $\text{pH} \leq 7.5$ ; this is below the  $\text{pK}_a$  of the surface silanol groups and so reduces the negative charge at the surface thereby decreasing electrostatic repulsion and facilitating DNA adsorption [2]. It is believed that the addition of carrier RNA increases the partitioning coefficient, thus increasing the likelihood of the DNA binding to the silica monolith. EOP creates a bulk movement of solution across a solid support due to the potentials applied and can be used to elute the DNA from the monolith into the amplification chamber containing PCR reagents. PCR reagents are afforded a degree of protection by encapsulation within

a gel matrix and the amplification of specific target loci of the DNA is subsequently achieved using well established PCR methodology.

## EXPERIMENTAL

All glass microfluidic devices were prepared using standard photolithographic techniques and wet-etching. Monoliths were produced in the DNA extraction chamber by thermally curing a mixture of potassium silicate and formamide. Next PCR reagents for amplification of the Amelogenin locus, including bovine serum albumin, poly(vinylpyrrolidone) and Tween-20 for dynamic passivation, were added to a molten solution of low-melting temperature agarose and injected into the PCR amplification chamber and stored at 4°C until required.

DNA was added to 5M guanidine hydrochloride solution, with or without poly-A carrier RNA, and injected onto the monolith. Potential contaminants of downstream applications, e.g. haem protein from blood, were then removed using an EOP-based ethanol wash by applying positive potential to electrode B and a negative potential to electrode C, creating bulk electro-osmotic flow (Figure 1). Finally, DNA was eluted into the amplification chamber by water using EOP by applying a positive potential to electrode A and a negative potential to electrode D. Once the DNA had been transferred into the pre-loaded PCR gel, thermal cycling was achieved using a Peltier heating system. PCR products were analysed off-chip using standard capillary electrophoresis.

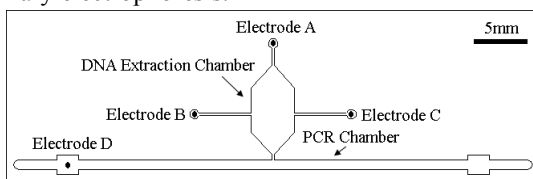


Figure 1: Schematic of microfluidic device showing integrated DNA extraction and amplification chambers and location of electrodes (● for EOP).

## RESULTS AND DISCUSSION

The use of carrier RNA led to increased DNA extraction efficiency (Figure 2).

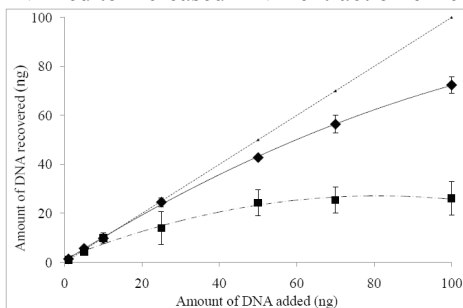


Figure 2: Amount of DNA recovered from the monolith during the elution step compared with the amount of DNA initially added. Samples with carrier RNA (◆) (ratio 50:1, RNA:DNA) were compared to those with no carrier RNA (■) added ( $n=3$ ). The theoretical 100% recovery is shown by the 45° line.

When carrier RNA is used the DNA extraction efficiency follows the ideal 100% theoretical recovery up to 25ng of DNA added compared to only 5ng when no carrier RNA is added to the system. At higher quantities of DNA, the presence of carrier RNA continues to give higher yields of DNA during the elution step. The use of agarose gel to encapsulate the PCR reagents has no adverse effects on DNA amplification but offers the advantage of long-term storage of the reagents on the microfluidic device at 4°C for at least 8 weeks. Examples of the PCR products generated using this system are shown in Figure 3.

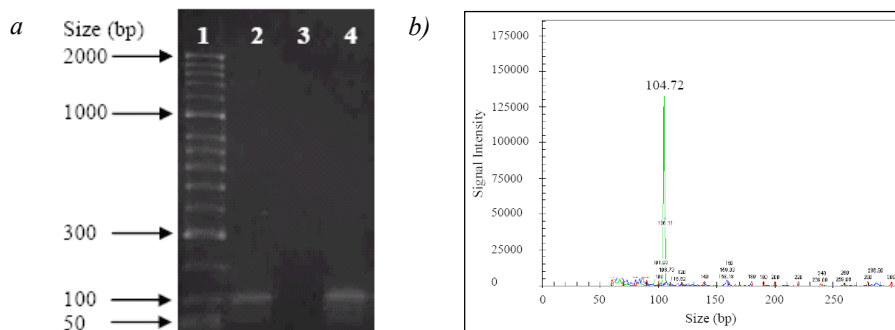


Figure 3: (a) Agarose gel showing: Lane 1 – DNA size ladder, Lane 2 – Positive control, Lane 3 – Negative control, Lane 4 – PCR product from gel-based amplification in microfluidic device. (b) Amelogenin PCR product analysed by capillary electrophoresis for accurate sizing.

## CONCLUSIONS

The addition of carrier RNA to the DNA binding solution increases the DNA extraction efficiency of thermally activated silica monoliths. The use of agarose gel to encapsulate the PCR reagents allows them to be stored on the microfluidic device. This, along with the use of EOP rather than hydrodynamic pumping, offers considerable advantages for portable applications and reduced potential for contamination.

## ACKNOWLEDGEMENTS

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## REFERENCES

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