One-step single cell solid-phase PCR

M. Trotter[1*], F. Stumpf[1*], F. von Stetten[1,2], J. Hoffmann[2], R. Zengerle[1,2,3], and G. Roth[1,2,3]

1 Institut für Mikro- und Informationstechnik (HSG-IMIT)
Georges-Koehler-Allee 103, 79110 Freiburg, Germany, www.hsg-imit.de
2 Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering,
University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany
3 BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany
* contributed equally

Summary

Cells resuspended in PCR-mix are distributed in about 104,000 19 pL wells of a PicoTiterPlate (PTP, Roche). The sealed plate is thermocycled to release DNA from single cells and perform solid-phase PCR (SP-PCR) in one step. Immobilized PCR products are accessible for downstream-analysis.

Motivation

Single cell analysis aims to discriminate rare cell variants, often related to diseases. However, for sensitive and significant results, high numbers of cells need to be analyzed in parallel.

Here, a novel approach is presented to analyze genomic information from single cells by massively-parallel SP-PCR without requiring additional extraction steps. Parallelization of reaction is achieved by using the wells of a PTP as reaction chambers. SP-PCR allows the subsequent analysis of the generated amplification products.

Principle

The procedure of one-step single cell SP-PCR is depicted in Figure 1. Cells from a culture are counted and washed before resuspension in PCR reaction mix. Loading of the PCR reaction mix to the wells of the PTP leads to a statistical distribution of cells. During SP-PCR, a distinct target sequence of the released gDNA is amplified and immobilized to the sealing lid. After disassembling, the lid features distinct spots of amplified DNA comparable to microarray.

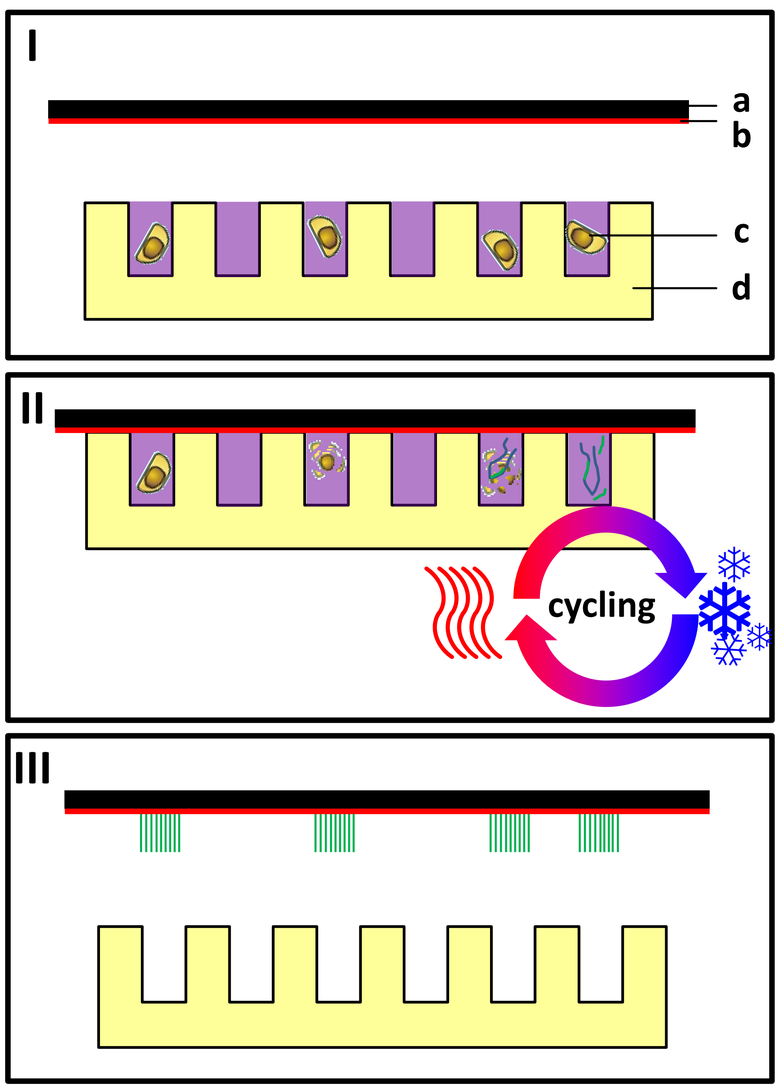

Figure 1: Procedure of single cell solid-phase PCR. **I:** PCR primers (b) are immobilized to the lid (a) [1]. Cells resuspended in PCR reaction mix (c) are randomly distributed to the wells of a PTP (d) by centrifugation. Statistically, each well contains less than 1 cell. The wells are sealed with a sealing lid (a) that features PCR primers (b) immobilized on its surface. **II:** The sealed PTP heated to 95 °C for 5 min. for cell lysis and polymerase activation followed by 50 thermocycles for SP-PCR. **III:** The PCR products that were immobilized during SP-PCR to the sealing lid can be stained with hybridization probes after disassembly.

Results

One-step single cell SP-PCR is performed using B-cells from a culture. As counted from the culture, 10,000 and 50,000 cells are resuspended in PCR reaction mix and distributed to the wells of PTPs. Instead of the expected 9 % and 38 % positive signals, 6 % and 26 % of the detected signals are positive, respectively (Figure 2). This corresponds to a detection rate of about 66 %.

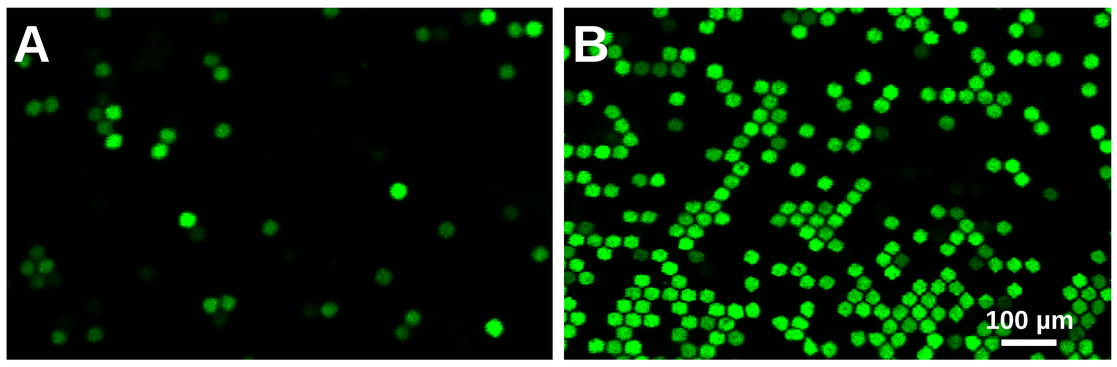

Figure 2: Fluorescence signals on the sealing lid upon single cell SP-PCR and staining with Cy3 hybridization probes. Definable fluorescent spots indicate successful amplification in distinct wells. Image processing results in 6 % (**A**) and 26 % positive signals (**B**).

Conclusions

Amplification of DNA released from single cells present in 19 pL wells was successfully demonstrated. The immobilized DNA is accessible for downstream analysis like hybridization as described with the potential to be used to screen for mutations in cell populations.

The method is also amenable to duplexing as demonstrated with cell-free DNA of up to 1,5 kbp length [2] (Figure 3 A). Furthermore, also the surface of the wells can be used for immobilization of PCR products [3] (Figure 3 B). This may enable to use wells as reaction chamber for further downstream analysis.

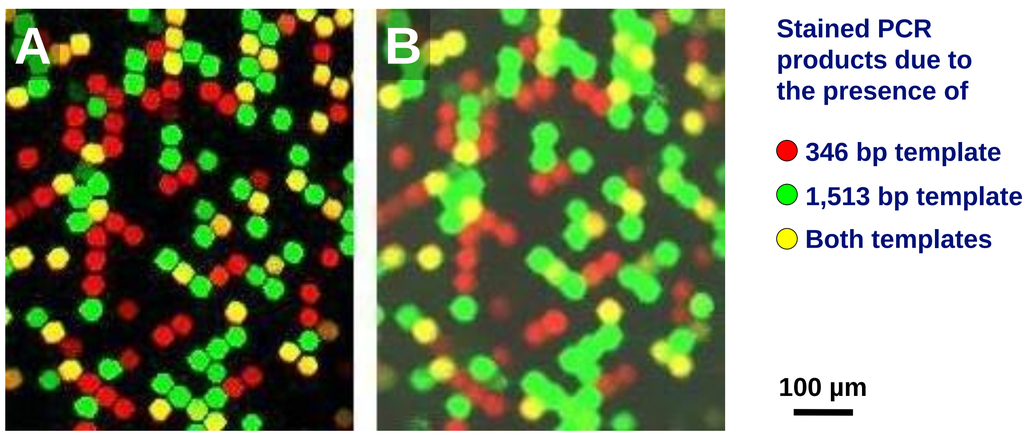

Figure 3: Fluorescence signals after SP-PCR in a PTP with two different targets of cell-free DNA. Amplification products are immobilized to the sealing lid (**A**) as well as to the surface of the wells (**B**).

Acknowledgements

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References

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