FABRICATING DNA MICROARRAYS BY COPYING A NEXT GENERATION SEQUENCING CHIP

Jochen Hoffmann[1,2]*, Sebastian Hin[1], Felix von Stetten[1,2], Roland Zengerle[1,2,3] and Günter Roth[1,2]

[1]Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[2]HSG-IMIT - Institut für Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, Germany

[3]BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany

ABSTRACT

A novel way of manufacturing DNA microarrays based on a copy process is presented. A picowell sequencing chip [1] is used as copy template to perform thousands of parallel PCR reactions within each 76 pl well. Thereby, the DNA from 83 % of the wells is copied onto a planar slide providing the DNA microarray copy of the sequencing chip.

KEYWORDS: Next-generation sequencing, DNA microarrays, solid-phase PCR, parallelization

INTRODUCTION

DNA sequencing technology is a powerful tool for decoding the sequence of whole genomes. In the GS FLX system (Roche/454), DNA to be sequenced is immobilized onto microbeads via emPCR and decoded by pyrosequencing [1]. DNA microarrays enable a massively parallel analysis of SNPs, mRNA, or genes. Current microarrays are mainly in-situ synthesized by photolithograpy [2] or printing [3]. Such arrays bear drawbacks by the synthesis itself like limited length of DNA probes or high error rates within the sequence.

We present an ingenious way for manufacturing DNA microarrays by copying the DNA out of a sequencing chip. By combining both technologies unique characteristics can be realized. Firstly, whole genome microarrays derived from natural DNA can be generated. Secondly, low error rates within the sequences can be obtained due to biosynthesis compared to the in-situ synthesis of the state of the art. Thirdly, the length of the sequence can be adjusted within a range of 100 – 1500 bp.

FUNCTIONAL PRINCIPLE

Test DNA sequences are coupled to microbeads from the GS FLX system. DNA beads are then mixed with a PCR reaction mix. This mix is loaded into a commercial sequencing chip (PicoTiterPlate, PTP™) and sealed with a planar COP slide coated with solid phase primers [4]. This primer sequence is complementary to a generic primer sequences flanking each DNA sequence. Bead-bound DNA colonies from each well are amplified and simultaneously bound to the slide via the mechanism of solid-phase PCR [5]. After PCR, the slide is detached, washed, and stained (Figure 1). Thereby, the transfer and identity of individual DNA colonies from the wells to corresponding areas on the planar slide is visualized [6].

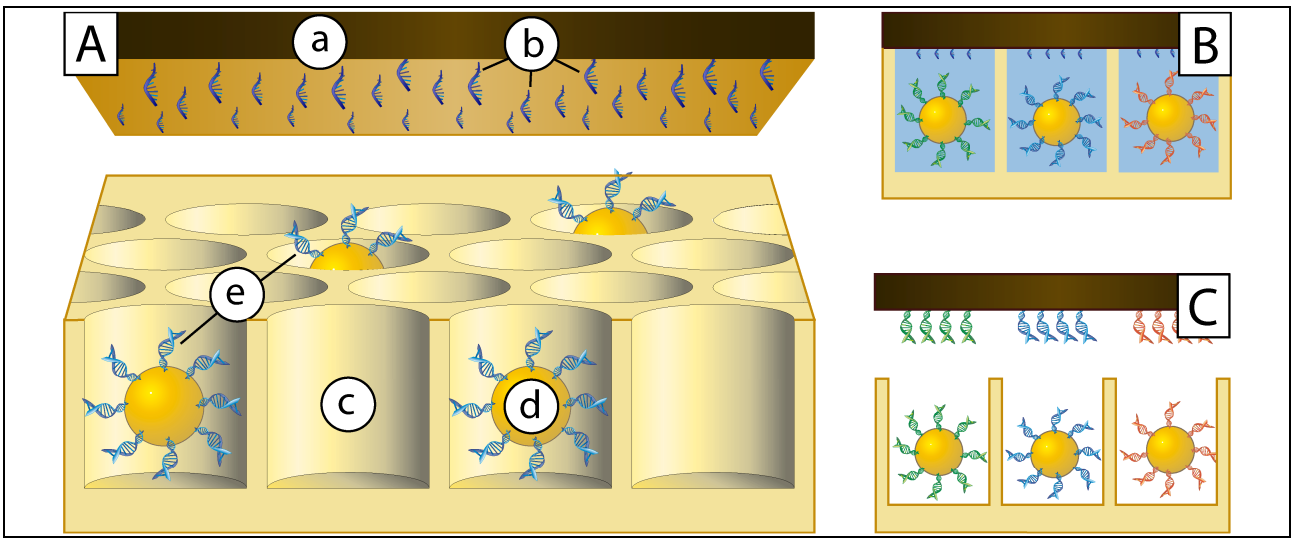

Figure 1: (A) Solid-phase primers (b) are immobilized onto a 188 µm thick COP slide (a). Wells with a volume of 76 picoliter (c) of the sequencing chip PTP™ are loaded with DNA beads (d) containing DNA fragments (e). (B) The chip is then filled with a PCR mix and sealed with the slide. (C) After solid-phase PCR, the generated product is bound to the slide and stained either unspecific by coupling streptavidin-Cy5 to incorporated biotin-dUTP molecules or specific by molecular hybridization with labeled probes.

EXPERIMENTAL RESULTS

Firstly, a PCR mix is doped with soluble 1,513 bp DNA fragments. After 50 PCR cycles, the homogeneous signal distribution on the slide shows the pattern of the sequencing chip (Figure 2 A). This demonstrates that the fundamental reaction works in each well.

Secondly, copy reaction is performed using DNA beads featuring a 100 bp DNA sequence. After 20 PCR cycles, a discrete pattern of signals is obtained on the slide, which matches the DNA bead distribution of according chip (Figure 2). After evaluation, DNA from 83 % of the beads is copied onto the slide (Figure 3).

Thirdly, a copy reaction is performed using two different DNA beads. After 20 PCR cycles and two-color hybridization, a red-green pattern of signals is obtained on the slide demonstrating leak-tightness of wells (Figure 4).

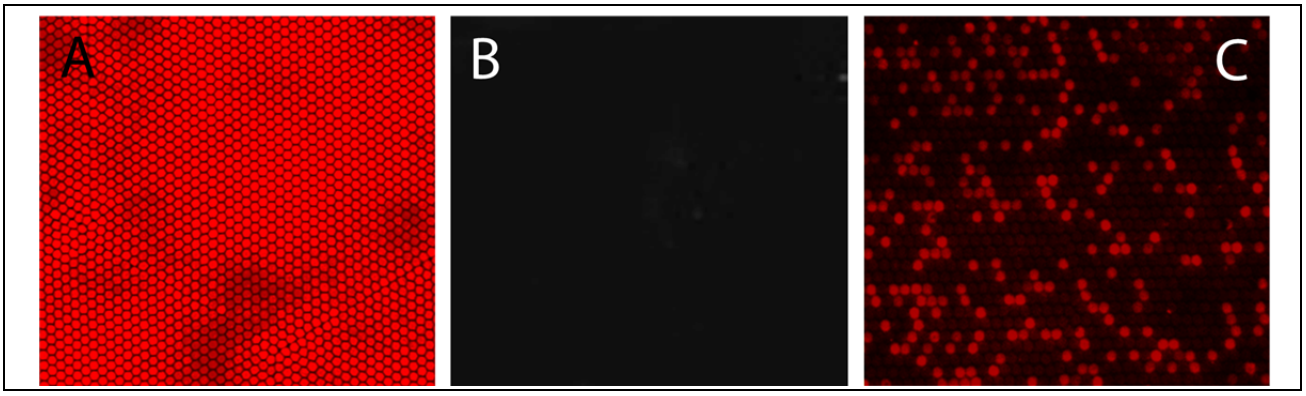

Figure 2: (A) Copy process after 50 PCR cycles with calculated 20 unbound DNA fragments per well, a slide containing a matching solid-phase primer, and staining with streptavidin-Cy5 resulted in a regular chip-like pattern of signals. This demonstrated that the copy process worked in each well in principle. (B) No signals were detectable when a mismatching solid-phase primer is used. (C) Copy process with one sort of DNA beads after 20 PCR cycles resulted in a distinct pattern of signals.

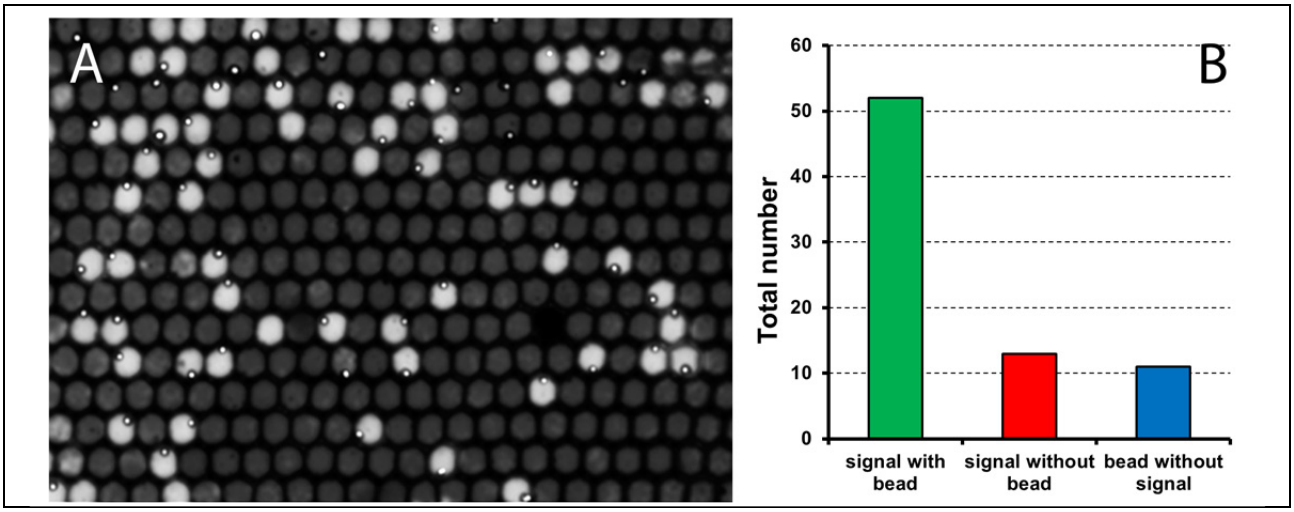

Figure 3: (A) Copy process with 5,000 DNA-beads distributed to 50,000 wells. The microscopic image of the sequencing chip after the copy process and the fluorescence image of the corresponding slide are merged. (B) Analysis of (A) revealed that at least 83 % of signals are triggered by a DNA bead. Due to washing the chip before imaging, beads may be lost in positive wells. Roughly 17 % of the beads did not generate any signal.

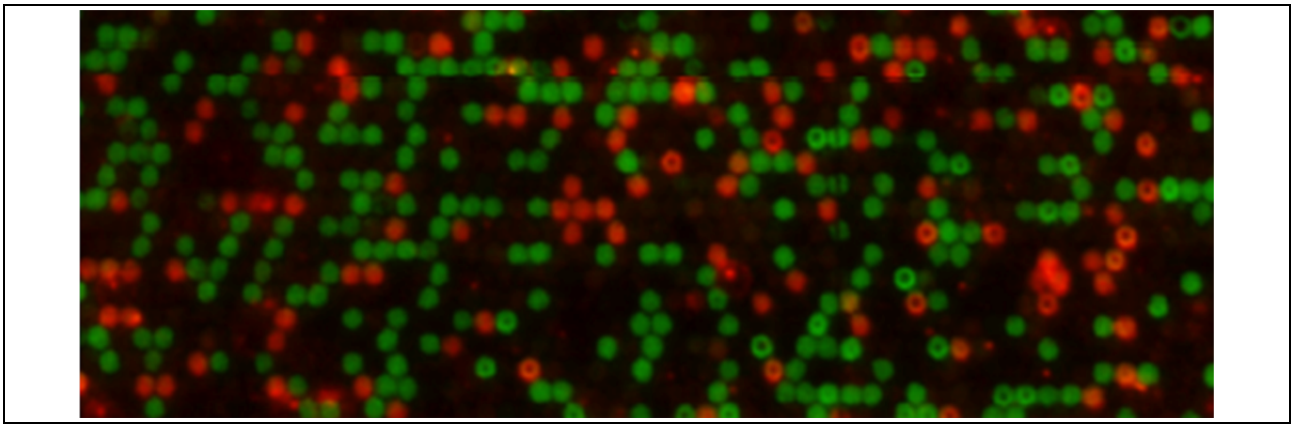

Figure 4: Copy process with two different DNA beads (5,000 each) distributed to 50,000 wells. Each sort of bead carried a unique 100 bp sequence. After PCR, two-color hybridization identified the two different DNA sequences copied onto the slide. Only green and red signals were obtained verifying that wells are entirely enclosed during the process.

CONCLUSION AND OUTLOOK

This work shows an unprecedented technique for copying the sequencing chip PTP™ into the format of a DNA microarray. We successfully implemented a massively parallel solid-phase PCR within picoliter volumes and showed for the first time solid-phase PCR on a COP surface. This copy process may enable the fabrication of next generation DNA microarrays with truly natural DNA of fully sequenced whole genomes with probe length up to 1,500 nucleotides and unmatched purity.

ACKNOWLEDGEMENTS

We gratefully thank the Hans L. Merkle Foundation for Excellency in Science and Technology and the Federal ministry for Education and Science (FKZ: 16SV3857) for financial support of this project.

REFERENCES

[1] M. Margulies, M. Egholm, W.E. Altman, S. Attiya, J.S. Bader, L.A. Bemben, J. Berka, M.S. Braverman, Y.J. Chen, Z.T. Chen, S.B. Dewell, L. Du, J.M. Fierro, X.V. Gomes, B.C. Godwin, W. He, S. Helgesen, C.H. Ho, G.P. Irzyk, S.C. Jando, M.L.I. Alenquer, T.P. Jarvie, K.B. Jirage, J.B. Kim, J.R. Knight, J.R. Lanza, J.H. Leamon, S.M. Lefkowitz, M. Lei, J. Li, K.L. Lohman, H. Lu, V.B. Makhijani, K.E. Mcdade, M.P. McKenna, E.W. Myers, E. Nickerson, J.R. Nobile, R. Plant, B.P. Puc, M.T. Ronan, G.T. Roth, G.J. Sarkis, J.F. Simons, J.W. Simpson, M. Srinivasan, K.R. Tartaro, A. Tomasz, K.A. Vogt, G.A. Volkmer, S.H. Wang, Y. Wang, M.P. Weiner, P.G. Yu, R.F. Begley, J.M. Rothberg: Genome Sequencing in Microfabricated High-Density Picolitre Reactors. *Nature*, 437 (2005) 376-380.

[2] A.C. Pease, D. Solas, E.J. Sullivan, M.T. Cronin, C.P. Holmes, S.P.A. Fodor: Light-Generated Oligonucleotide Arrays for Rapid Dna-Sequence Analysis. *Proc. Natl. Acad. Sci. U. S. A.*, 91 (1994) 5022-5026.

[3] T.R. Hughes, M. Mao, A.R. Jones, J. Burchard, M.J. Marton, K.W. Shannon, S.M. Lefkowitz, M. Ziman, J.M. Schelter, M.R. Meyer, S. Kobayashi, C. Davis, H.Y. Dai, Y.D.D. He, S.B. Stephaniants, G. Cavet, W.L. Walker, A. West, E. Coffey, D.D. Shoemaker, R. Stoughton, A.P. Blanchard, S.H. Friend, P.S. Linsley: Expression Profiling Using Microarrays Fabricated by an Ink-Jet Oligonucleotide Synthesizer. *Nat Biotechnol*, 19 (2001) 342-347.

[4] J. Hoffmann, S. Hin, F. von Stetten, R. Zengerle, G. Roth: Universal Protocol for Grafting PCR Primers Onto Various Lab-on-a-Chip Substrates for Solid-Phase PCR. *RSC Advances*, 2 (2012) 3885-3889.

[5] C. Adessi, G. Matton, G. Ayala, G. Turcatti, J.J. Mermod, P. Mayer, E. Kawashima: Solid Phase DNA Amplification: Characteristics of Primer Attachment and Amplification Mechanisms. *Nucleic Acids Research*, 28 (2000) e87.

[6] J. Hoffmann, H.M. Trotter, F. von Stetten, R. Zengerle, G. Roth: Solid-Phase PCR in a Picowell Array for Immobilizing and Arraying 100,000 PCR Products to a Microscope Slide. *Lab Chip*, DOI:10.1039/C2LC40534B (2012).

CONTACT

*J. Hoffmann, tel: +49-761-203 73234, jochen.hoffmann@imtek.de