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Microfluidic hand-helds for DNA to protein microarray replication by cell-free systems

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Introduction

Proteins perform almost all cellular functions, are the major elements of most cellular structures, and are targets of drugs and treatments. Therefore cost and time efficient analysis of protein-protein interactions using protein microarrays is of significant impact for both medical research and pharmaceutical applications. We introduce two novel microfluidic hand-held devices for the replication of protein arrays from DNA arrays as improvements of the DAPA-System [1, 2] These devices enable to produce several protein microarray copies from one original master DNA microarray. The hand-helds allow an easy time-saving robust handling.

Methods and Results

Our hand-held devices consist of a PDITC [3] or epoxy coated microscopic glass or PDMS slide carrying the DNA microarray, a spacer of ~ 60 µm with microfluidic inlet / outlet structures, and a microscopic glass slide with a Ni-NTA surface (Fig. 1+2) catching His-tagged proteins. Protein synthesis starts after priming the microfluidic gap with ~ 20 µl of *E.coli* cell-free transcription / translation systems (RINA RTS 100 / RINA EasyXpress). Protein microarray formation takes place by diffusion 90 / 30 minutes at 30 °C (Fig. 3). T tiny microfluidic gap of ~ 60 µm ensures the transfer of the proteins by diffusion with nearly no spot broadening, compared to the original DNA spots. Ton. Hence each protein spot can be assigned to its progenitor DNA spot. After disassembling the hand-held and washing the carrier slides, both the DNA master array as well as the newly generated protein microarray are available for further use - typically the DNA master array for further protein microarray generation, the protein array for protein-protein interaction studies.

Conclusion

We realized robust and easy to use microfluidic hand-held devices for the DNA microarray to protein microarray replication by cell-free synthesis. The devices can be assembled within a few minutes and the aa protein microarray can be realized within 30 minutes. This meets requirements of daily lab work considering both time and costs for protein analysis.

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References

[1] M. He, O. Stoevesandt, E. A. Palmer, F. Khan, O. Ericsson, M. J. Taussig, Printing protein arrays from DNA arrays, Nature Methods, 5: 175-177, 2008

[2] O. Stoevesandt, M. Vetter, D. Kastelic, E.A. Palmer, M. He, M.J. Taaussig, Cell free expression put on the spot: advances in repeatable protein arraying from DNA (DAPA), New Biotechnology 2010, in press

[3] Hoffmann,J.; Hin,S.; von Stetten,F.; Zengerle,R.; Roth,G. Enabling DNA-microarrays in polymeric lab-on-a-chipsubstrates for multiplexed target analysis via solid-phase PCR, Proceedings of the 15th International Confernce on Miniaturized Systems for Chemistry and Life Sciences (µTAS): 1840-1842, 2011

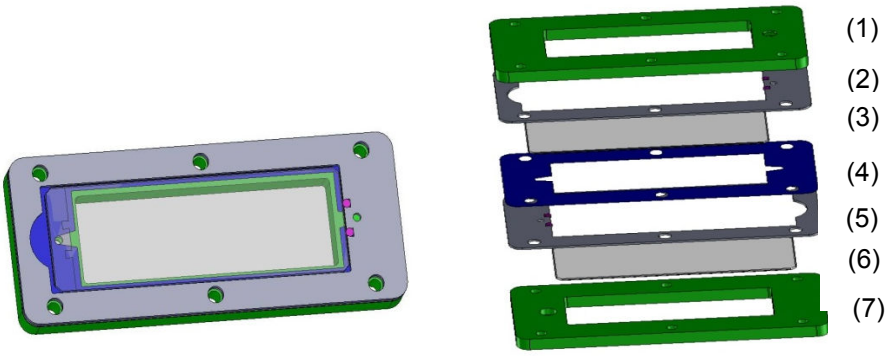

Fig. 1: Design of microfluidic hand-held using 2 standard microscope slides as carriers of the DNA and protein microarrays: left side - top-view of assembly onto upper glass slide sealed towards the inlet by sealings (purple) and kept at a distance of ~ 50 µm to lower glass slide by spacer (blue) with inlet/outlet and elastic, hydrophobic surface; right side – single parts making up the assembly consisting of outer plates (1,7), glass slides with frames (2,3,5,6), spacer (4).

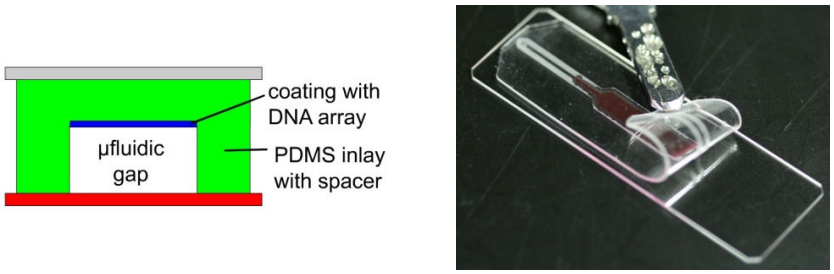

Fig. 2: Design of microfluidic hand-held using 1 standard microscope glass slide as carrier of the protein microarray and one PDITC-coated PDMS slide for the DNA microarray: left side – schematic of device; right side – simple setup showing microfluidic gap filled with red dye.

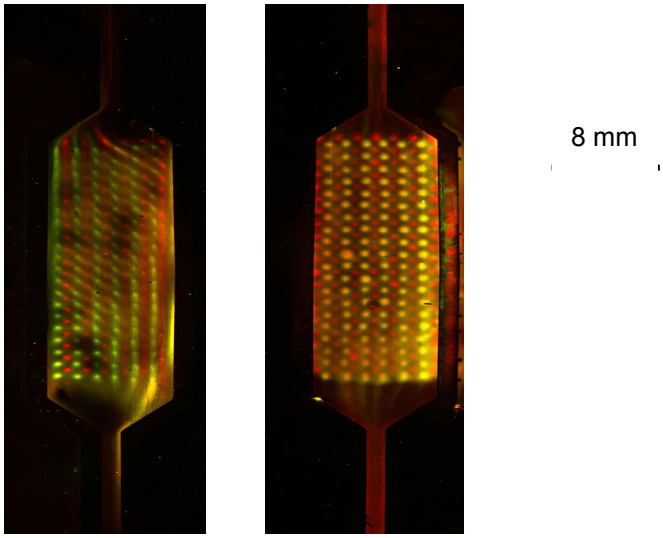

Fig. 3: Protein microarrays in-situ expressed of p53 / notch DNA microarrays immobilized on PDMS slides (see Fig. 2) and labelled by Cy3-/ Cy5-marked antibody: left side - expressed by RINA RTS 100 system, 90 minutes, 30 °C; right side – expressed by RINA easyXpress system , 30 minutes, 30 °C.

Keywords: Protein microarray, Cell-free protein expression, Microfluidic protein array translation, DNA to protein microarray