A HAND-HELD DNA-TO-PROTEIN MICROARRAY TRANSLATION SYSTEM

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Summary

The "DNA Array to Protein Array" (DAPA) system [1,2] enables the production of multiple protein microarrays from one master DNA microarray. The system consists of a DNA microarray, a membrane containing a cell-free transcription / translation system for protein synthesis and a protein capture surface. We have improved the basic idea by designing a simple hand-held microfluidic device, consisting of 2 standard microscope slides with a seal forming a microfluidic chamber of ~80 µm in height. The membrane of the original DAPA system is replaced by a capillary gap filled with the cell-free expression system. Hence any mechanical damage potentially caused by the membrane touching the microarrays is prevented as only the cell free reaction mix is in physical contact with the slide surfaces. Moreover, the amount of cell free translation system needed for the protein expression is reduced significantly and the capillary priming of the microfluidic chamber leads to a defined start time of protein expression. Protein translation and protein microarray formation is performed in a standard cell incubator for 45 min at 30 °C. After disassembling the device and washing, both the DNA master array as well as the newly generated protein microarray are available for further use. Typically the DNA master array can be re-used for further protein microarray generation.

Introduction

In order to further simplify the generation of protein microarrays and to increase the accuracy of the protein expression process itself we modified the DAPA system (Fig. 1). The DAPA system as originally described has some undefined aspects. Firstly, the membrane soaked with cell-free translation system itself is non-woven fiber material and therefore undefined in structure isotropy and thickness of tolerances (I don't understand this phrase). Secondly, the time point of starting protein expression is immediately after placing the membrane in contact with the DNA array. This happens whilst the DAPA system is assembled and therefore is in principle undefined. Our microfluidic system enables a precisely defined distance between the DNA master array and the protein capture surface combined with a free to determine time point for starting the protein expression by simply filling in the cell-free translation system by capillary priming. This system is neither limited by the membrane's capacity, nor bears the risk of contamination or inhibition by substances of the membrane or damage to the slide surfaces by physical contact of the membrane with the arrays. Additionally, irregularities in the membrane may effect isotropic diffusion and lead to nonhomogeneous protein deposition.

Experimental results

Protein microarrays were produced with our hand-held DNA-to-protein translation devices, depicted in Fig. 2 The device harbours an incubation chamber that consists of two microscope slides separated by a seal, fabricated from a laser-cut self-adhesive polyester foil of ~80 µm thickness, as incubation chamber (Fig. 4a, b). The DNA master array is produced by spotting PCR-amplified DNA featuring an amino linker onto an epoxy coated PMMA or glass slide. The protein microarray slide is Ni-NTA coated to capture $(His)_6$-tagged proteins. Two different DNA sequences on the depicted DNA microarray (Fig. 3a, marked in red and green) were expressed as proteins. The resulting protein microarray was stained by different corresponding antibodies (Fig. 3b). Analysing the result of the protein expression with this setup revealed deficiencies such as intrusion of air bubbles and fluid loss by leaks. Further a certain violation of the integrity of the epoxy coating by the self-adhesive polyester foil and the laser-cutting process was noted. Therefore we modified the system by changing the design of the incubation chamber. Either a laser-cut seal of ~80 µm thickness from a spin-coated PDMS layer, transferred by a vacuum setup to the DNA

slide (Fig. 4c), or a complete PDMS inlay (Fig. 4d) with variable 3-dimensional structures moulded in a proprietary tool promise enhanced performance. Early experiments indicate a significantly better air and water tightness of the seal and no inhibitory effects on either DNA spotting or protein expression.

Conclusion

We presented a membrane-free hand-held DNA-to-protein translation device that circumvents the requirement for a membrane and thus improves the DAPA-system [1, 2]. Each of the different layouts under study replaces the membrane by a capillary gap and allows the defined priming and therefore start of protein expression. This avoids effects of membrane irregularity, allows a better and more defined expression of proteins and avoids any physical damage of either the DNA or the protein microarray.

Outlook

Ongoing optimisations of the hand-held devices will further ease the handling of this manual process for rapid generation of protein arrays and aim to increase the spot density of the microarray. Finally we aim to develop a simple disposable hand-held for daily laboratory routine use.

References

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

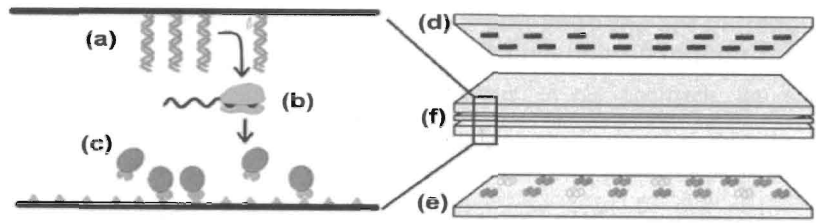

Fig. 1: Scheme of the DAPA (DNA array to protein array) system – DNA (a) is transcribed and translated (b) into proteins (c). Therefore, starting from a DNA microarray (d) a protein microarray (e) is expressed. A membrane (f) is used to carry the cell free protein expression system.

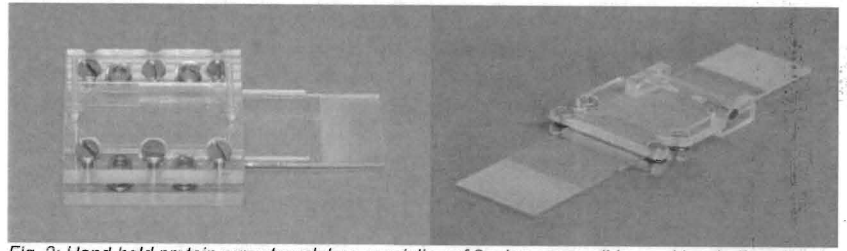

Fig. 2: Hand-held protein array translators consisting of 2 microscope slides and incubation chamber made of laser-cut self-adhesive polyester foil: prototypes for top priming with liquids (a) and lateral priming (b) of the cell free expression system.

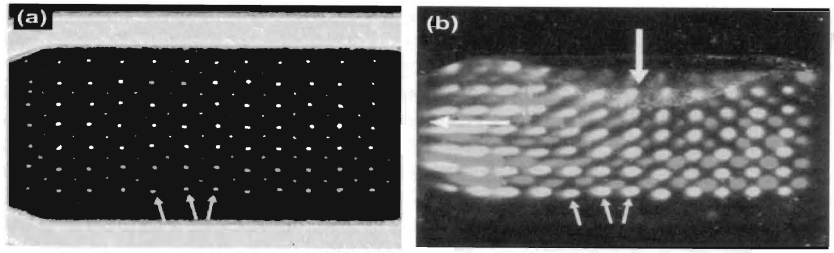

Fig. 3: (a) DNA array of 2 sequences, (b) protein array expressed by cell-free translation system – blue arrows indicate corresponding spots on DNA and protein array, yellow arrow shows affect of air bubble, white arrow depicts area of distortion due to evaporation caused by leak. The method shows both sensitivity and accuracy.

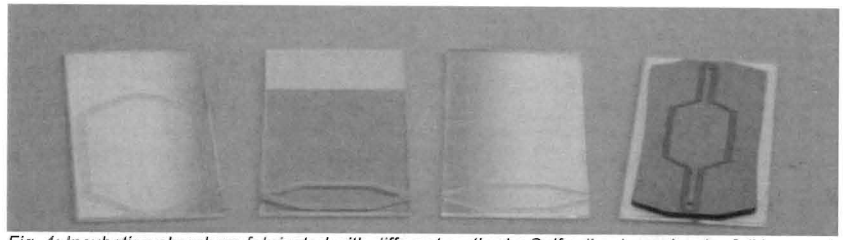

Fig. 4: Incubation chambers fabricated with different methods: Self-adhesive polyester foil laser-cut on slide with (a) 70 µl volume, top priming and (b) 12 µl volume, lateral priming; (c) spin-coated PDMS layer, laser-cut, vacuum transferred to slide; (d) moulded PDMS inlay with chamber frame.