Microarray Copying – on the way to Next Generation Microarrays

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Motivation

Microarrays are versatile tools for high throughput screening to analyze even whole genomes, transcriptomes and proteomes [1]. Until now, the generation of microarrays is by itself their major drawback. Even if two completely different approaches for microarray synthesis exist, they are not solving the problem of having high quality molecules at affordable prizes. One approach is the *in-situ* synthesis of molecules on the microarray surface, the other is the mainly recombinant bio-synthesis of the molecules of interest followed by harvesting, purification, and final transfer by a dispensing device onto the microarray surface. Unfortunately *in-situ* synthesis lacks of purity and probe length, whilst recombinant synthesis delivers high quality and full-length probes are simply to expensive (e.g. 1360 € for one single ProtoArray [2]).

Therefore, some believe that Next Generation Sequencing (NGS) will replace microarrays completely [3], especially driven by the fact that NGS became cheaper, less error prone and easier to handle. But if it comes to binding kinetics and binding specificity, enzyme activity or rate constants, microarrays are unmatched and can not be replaced by NGS.

There is a great desire for cheaper and easy accessible microarrays, best from any kind of species and whole length probes of DNA, RNA as well as protein.

Some facts

- Life is based on replication and nature provides therefore all enzymes needed to synthesize and replicate DNA, RNA or proteins as mere copies from an original DNA strand.
- NGS sequencing chips contain DNA in a highly ordered space resolved manner, quite similar to a microarray, but are easier in production.

Some questions

- Why not bridge the world between NGS and microarrays?
- Why not taking the DNA from a NGS chip and transfer it to a surface to generate a microarray as exact copy of the NGS chip?

Our answer

Yes, lets do so. We intend to copy DNA from a NGS sequencing chip in quite the same way like nature synthesizes DNA, RNA or even proteins to generate according microarrays [4]. And furthermore, we like to raise the vision of a future ‘microarray copier’ (fig.1) comparable to a photocopier in the secretary but capable to copy any DNA microarray or NGS chip. The outcome of the copying process in terms of DNA, RNA or protein microarray is defined by the used enzyme mix and the surface chemistry, only.

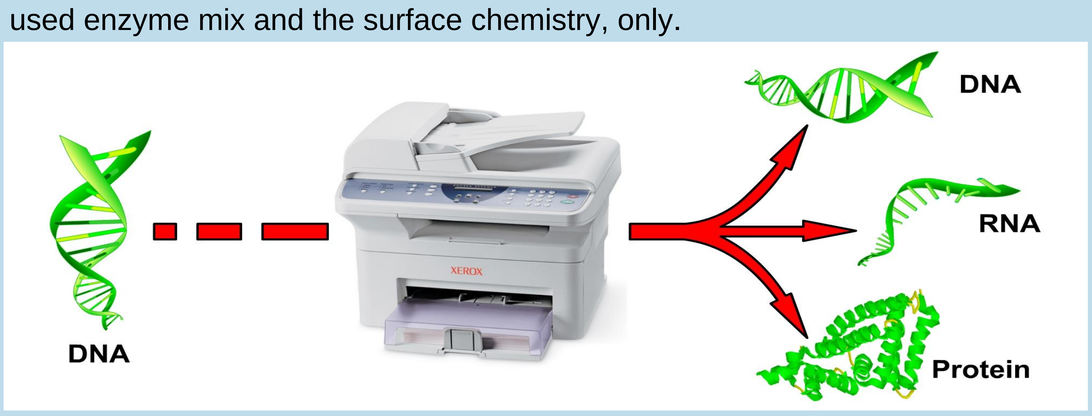

Fig.1: The microarray copier shall be capable to copy DNA microarrays into DNA, RNA an protein microarrays by combining copier technology with standard enzymes like RNA and DNA polymerases or cell free protein synthesis.

Acknowledgments and references

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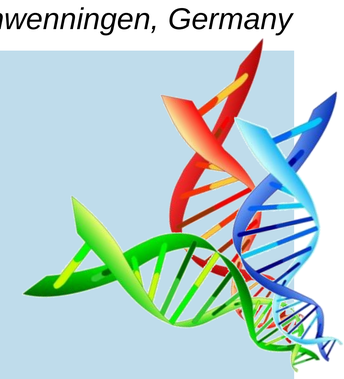
DNA-to-DNA copying [5]



To copy a NGS chips DNA is basically to perform a PCR reaction (fig.2). The important trick here is to provide a primer on the surface onto which the array has to be copied. A holder was designed to perform the PCR in a standard block cycler. This enabled us to copy the DNA from a self-filled 454 chip onto a microarray (fig.3).

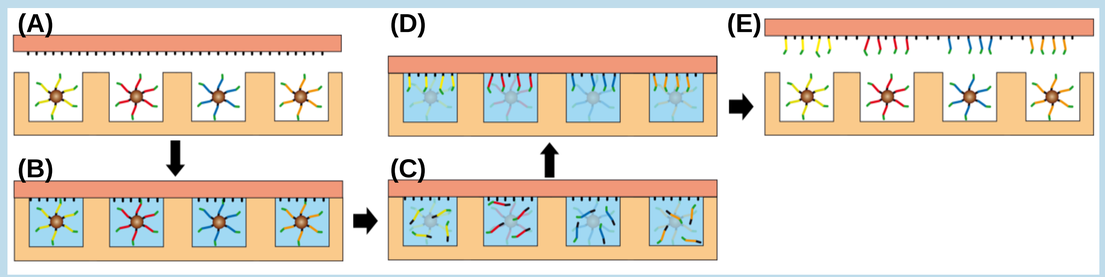


Fig.2: NGS microarray copying of DNA: A sequencing chip (A) is filled with PCR mix (B) and a PCR is performed. First the DNA is copied from bead into solution (C) and then onto the primer bearing surface (D). After 20 to 25 cycles a DNA microarray (E) is generated via this solid-phase PCR as identical copy of the NGS chips DNA pattern.

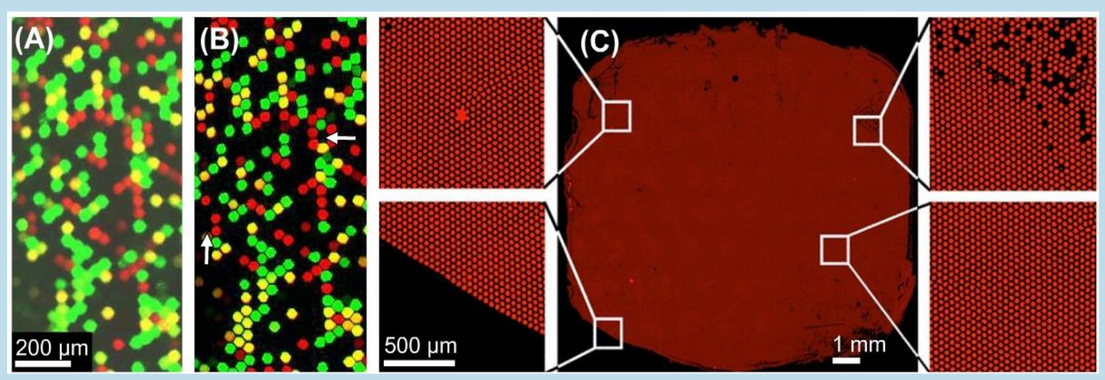
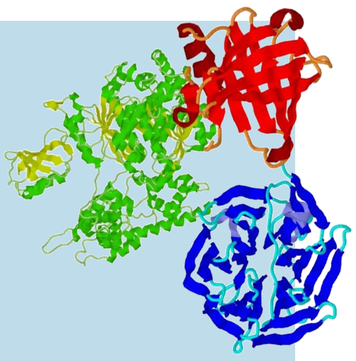


Fig.3: The self-filled NGS chip (A) contained two different DNAs stainable by red/green (yellow means both DNA on same poistion). The according copy (B) is quite identical except of some missing „pixels“ (white arrows). In a proof of concept experiment over 110,000 cavities could be copied (C).

DNA-to-protein copying [6]



Cell-free expression mixes enable to produce in-vitro directly from DNA the according encoded protein. With this cell-free mix a protein microarray can be made as copy from a DNA microarray (fig.4). The protein copying device is very simplistic design with a flow cell clamped by a magnet holder (fig.4 A&B), but it enables protein microarray copies at room temperature in less then an hour (fig.4B).

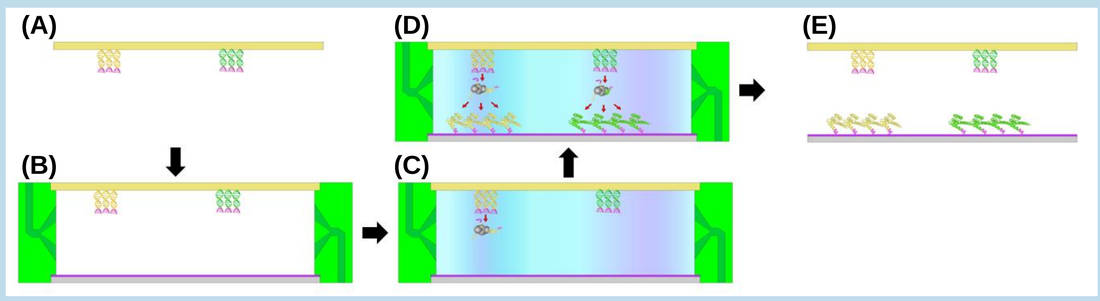


Fig.4: Protein microarray copying generated from a DNA microarray: A DNA mciroarray (A) is put into a microfluidic device (fig.5 B) in close proximity of a NTA glass slide (B). Cell free expression mix is filled in to generate RNA and the according protein (C). As the proteins diffuse to the NTA surface they attach there (D), generating a slightly blurred protein image of the original DNA array. After the process a protein microarray is generated (E).

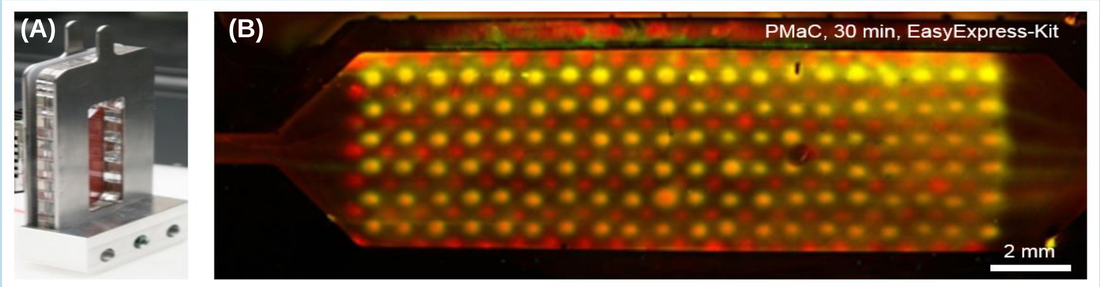


Fig.5: The Protein Microarray Copier (PMaC) is a magnetic holder (A) clamping together the DNA microarray close to a protein catcher surface. In cooperation with Lab Taussig, Cambridge, UK we made a first protein copy of two different DNA templates spotted in a square pattern. The proteins have been stained specifically with antibodies labeled with different proteins. As such the first copies ield several hundred protein dots (B) and are 3-times faster than the DAPA system of Taussig [7] .

Conclusion

We showed how to copy the DNA out of a NGS chip to generate a DNA microarray and how to copy a DNA microarray to generate a protein microarray. Taken both steps together, this enables to generate protein microarrays from a NGS chip. And as RNA is the intermediate to proteins, RNA microarray copies will become also possible.

We have taken the very first steps into microarray copying and we like to realize a microarray copier device – capable to ‘copy’ Next Generation Sequencing chips DNA to synthesize DNA, RNA or protein Next Generation Microarrays.