

sciFLEXARRAYER Application Note  
No. 08007

HTS of protein-protein interactions in mammalian cells using transfected cell arrays

Investigation of protein-protein interactions (PPI) within cells is essential for the elucidation of biological processes and cellular networks. The two-hybrid system is the most commonly used method for PPI analysis. A high-throughput, cost-effective method for analysing PPI directly in mammalian cells was established: The cell array-based protein-protein interaction assay (CAPPIA).

Materials and methods

The DNA samples were spotted and immobilized on glass slides in array formats. Each DNA spot contained bait and prey expression plasmids in addition to a reporter plasmid coding for an autofluorescent protein. After spotting the vector constructs with the sciFLEXARRAYER, adherent human cells were added, creating a monolayer on the surface of the slides. Only cells growing on top of the DNA spots became transfected. In case of chimeric bait and prey protein interaction, the reporter gene was expressed, resulting in formation of fluorescent reporter protein. Signals resulting from PPI were analysed directly by fluorescent detection (Fig. 1).

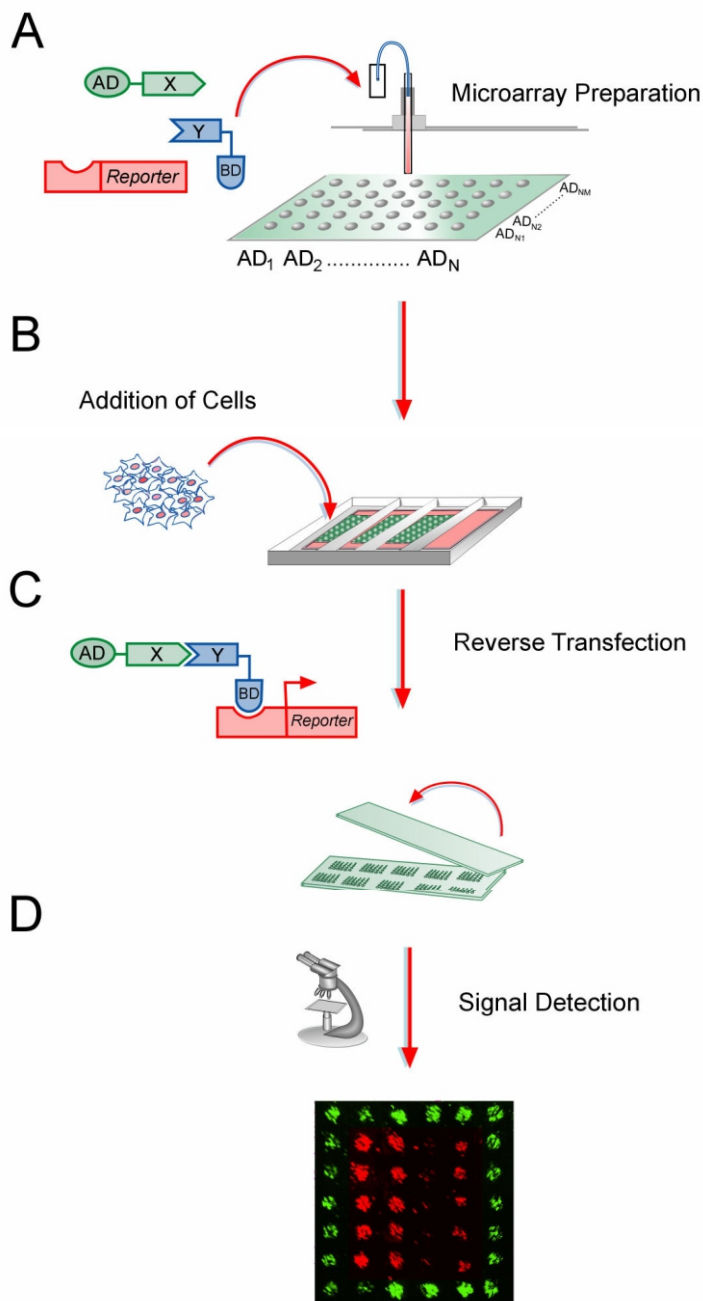


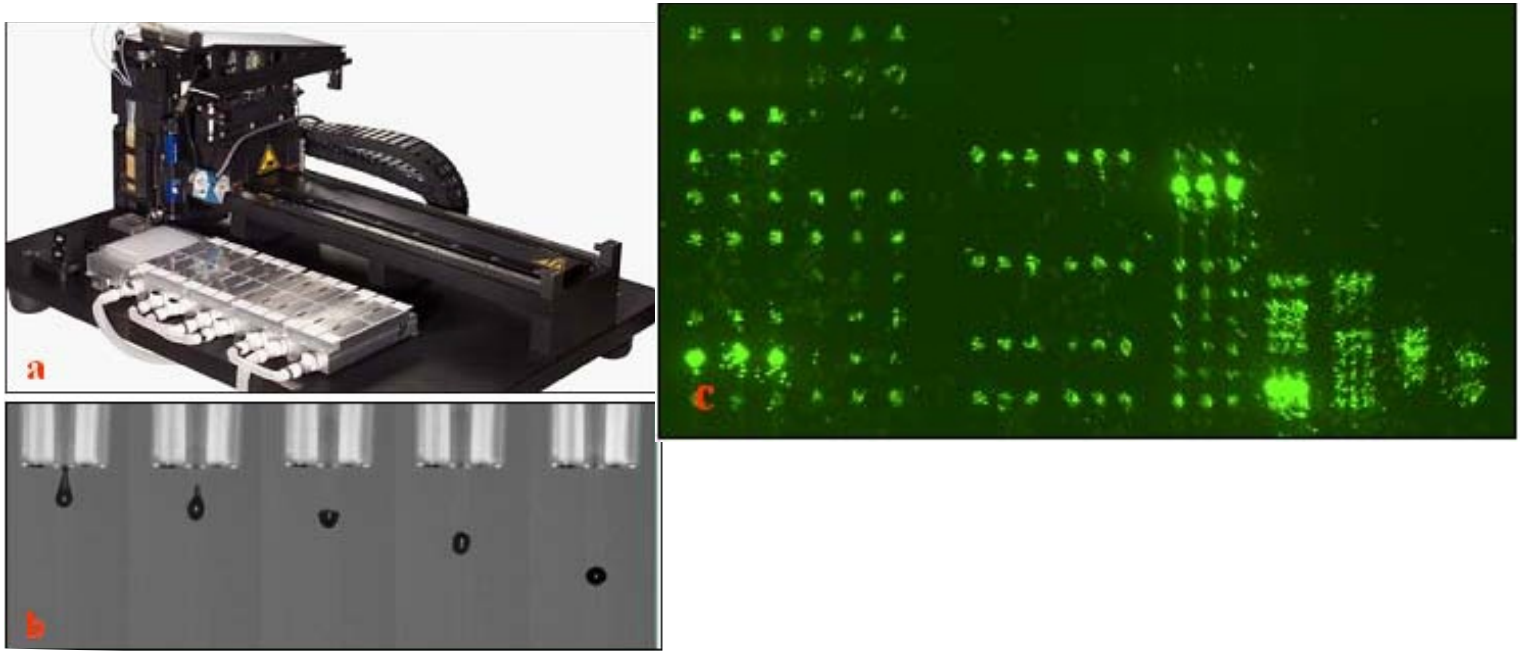
Fig. 1 The CAPPIA process. For details, see reference on reverse site.

## Results and discussion

At first, production of the cell array and transfection conditions were optimised. Out of the two microarray spotting systems tested, the sciFLEXARRAYER was chosen for automated spotting (Fig. 2). Starting with the gelatine method for reverse transfection where the DNA is diluted with 0.2% gelatine, and comparing it to the lipid-DNA-method using Effectene (QIAGEN), the latter proved to be better suited for automated spotting. Subsequently CAPPIA was shown to specifically and quantitatively detect PPI in various mammalian cell lines.

Moreover, screening of a small prey library against the human androgen receptor demonstrated that CAPPIA is well suited for the detection of hormone-dependent PPI. Finally, it was shown that the possible combinatorial screens could be increased by application of slides without bait.

One of the advantages of the mammalian compared to the yeast two-hybrid system is that the expressed proteins maintain their native conformation, and additional factors necessary for the interaction of both proteins are available. But currently mammalian two-hybrid systems involve high reagent consumption and are therefore not practical for screening PPI in mammalian cells. The high sample capacity of the cell arrays and the low reagent consumption make CAPPIA currently one of the most economical high-throughput detection assays for PPI in mammalian cells and by that an excellent tool for functional genomics.



**Fig. 2** Automated spotting was done using the sciFLEXARRAYER S5 depicted in a with formation of droplets of ca. 400 pl size from a 70  $\mu$ m nozzle used for CAPPIA shown in b, repetitive dispensing of 20 drops with 500 Hz for one spot of 8 nl were found to give the best results in CAPPIA. A slide with transfected cell arrays is shown in c (image acquired with BIOccd camera), spotted with the sciFLEXARRAYER for testing different parameters. Distance between dots for CAPPIA experiments was chosen to be 1 mm to be sure of separate transfection spots.

Courtesy of Andrea Fiebitz. An article on this topic has been published by Fiebitz, A. et al.: High-throughput mammalian two-hybrid screening for protein-protein interactions using transfected cell arrays; BMC Genomics 2008, 9:68 (06 Feb 2008) Fig 1 and 2 were taken from Andrea Fiebitz' PhD thesis: High throughput screening of protein-protein interactions in mammalian cells using transfected cell arrays. Online available at [www.diss.fu-berlin.de/2007/59/index.html](http://www.diss.fu-berlin.de/2007/59/index.html)

This work was performed at the Max Planck Institute for Molecular Genetics, Berlin.

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