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Arrays of DNA-binding sites

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Abstract

A DNA array has been used to measure the affinity of wild-type and mutant variants of a zinc-finger-protein DNA-binding domain for all combinations of its binding site on DNA.

Significance and context

It is clear that DNA arrays will eventually find more uses than simple gene expression profiling. Any work involving the study of DNA interactions can potentially benefit from the high-throughput power of DNA-array technology. Bulyk *et al*. are the first to describe a DNA-microarray-based method for characterizing sequence-specific DNA recognition by wild-type and mutant variations of a zinc-finger-protein (ZFP) DNA-binding domain.

Key results

DNA-array hybridization experiments proved highly sensitive in distinguishing binding domains with very similar DNA-sequence specificities. The arrays were also used to determine the binding-site preferences of zinc fingers with poorly characterized sequence specificity. One particular mutant could be isolated using more than a dozen variations of the 3 base-pair (bp) DNA-binding sequence, but was highly specific for only one of them. In contrast, another mutant was fairly nonspecific and showed relatively weak binding to a number of DNA sequences, consistent with a 3 bp consensus.

Methodological innovations

The authors demonstrate that DNA-protein hybridizations on glass arrays can be carried out with equal ease to the standard DNA-DNA hybridizations. To do this, a set of 64 double-stranded DNAs containing the wild-type binding sites for fingers 1 and 3 of the murine ZiF268 protein, and all possible 3 bp binding sites for finger 2, were arrayed on a glass slide. A bacteriophage display library containing randomized critical amino acids in the finger-2 DNA-binding site of Zif268 was then generated and used

to measure the binding affinity of wild-type and mutant finger-2 binding domains for the 3 bp wild-type and variant DNA-binding sequences on the array.

Developing this new twist on DNA arrays naturally required developing a new technique for measuring the amount of bound protein on each spot. The authors therefore modified a standard indirect fluorescent protein labeling technique involving the use of primary (anti-M13) and secondary antibodies, the secondary antibody being conjugated with R-phycoerythrin. They also used a clever, yet simple, approach for normalizing the amount of DNA on each spot. Separate microarrays (manufactured in the same print run as those used in the actual, phage display hybridizations) were stained with SybrGreen I. The average relative signal intensities from the zinc-finger binding to phage were then divided by each of the respective average relative signal intensities from SybrGreen I staining.

Finally, the relationship between relative fluorescence intensity derived from the array and DNA-binding affinity of the respective protein domains was calculated from a plot of apparent K_d versus average normalized relative fluorescence intensity. These plots were prepared (using zinc-finger phage enzyme-linked immunoassay, ELISA) by measuring the binding affinity of each variant Zif phage with a select few representative binding sequences (for finger 2) that spanned the dynamic range of relative fluorescence intensities.

Links

This article is available free from PNAS online at Exploring the DNA-binding specificities of zinc fingers with DNA microarrays.

Conclusions

One appealing feature of the technique is that it can be scaled up. Given the increasing density of DNA arrays, it should be possible to analyze even longer DNA-binding sites. For example, investigating all possible sequences of an 8 bp binding site would require approximately 65,000 spots, all of which can be printed onto a single slide. The authors suggest that this type of approach to measuring the affinity of protein binding domains for binding sites in DNA has a number of applications: these include deducing the optimum reaction conditions and cofactor requirements of DNA-binding proteins, predicting functions for uncharacterized transcription factors, and engineering DNA-binding domains for controlling gene expression in biotechnology applications. When more data accumulate from such experiments, it should also be possible to determine the rules that govern DNA recognition by sequence-specific transcription factors.

Reporter's comments

It is only a matter of time before innovation results in new and useful applications of DNA-array technology. Clearly, the type of experiment described is not limited to zinc-finger proteins and can be used to study any form of protein-DNA binding, such as that seen with steroid receptors, leucine zippers and helix-loop-helix domains. Indeed, we should soon expect to see more papers such as this, in which DNA arrays are used for studies involving not only protein-DNA binding, but any type of interaction involving DNA. It should also be no surprise to see approaches such as this reversed as proteomic technology evolves, so that arrays of immobilized proteins and their variants will be used to measure interactions with DNA, drugs, other proteins, and indeed any number of small molecules with a protein-binding capacity. One of the most useful applied applications of the approach described in this paper might be in pharmacogenomics, where it could be used to investigate the significance of single-nucleotide polymorphisms in gene promotor regions and thus help to determine the etiology and nature of individual drug responses.

Table of links

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Exploring the DNA-binding specificities of zinc fingers with DNA microarrays

References

1. Bulyk ML, Huang X, Choo Y, Church GM: Exploring the DNA-binding specificities of zinc fingers with DNAmicroarrays. Proc Natl Acad Sci U S A. 2001, 98: 7158-7163. 0027-8424