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Bacterial two-hybrid screening

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Abstract

A new bacterial alternative to the yeast two-hybrid system and phage display for detecting protein-protein and protein-DNA interactions is described.

Significance and context

One way of finding protein or DNA sequences that bind a target molecule is to screen large libraries. Each DNA sequence in the library, or its expressed protein, is offered to the target, and those that bind tightly are selected and identified. Protein designers search libraries synthesized from random DNA sequences for new, non-biological binders; biochemists search libraries of genomic sequences for biological binders. Two standard library screening methods are the yeast two-hybrid and phage display techniques. Joung *et al*. describe a new screening method which is related to the two-hybrid scheme, but uses bacteria as the host. The method may prove a useful alternative to traditional screening approaches.

Key results

Joung *et al.* test their technique by screening a library of three-finger zinc-finger proteins (derived from Zif268) which are randomized at six positions on one finger. The targets used to screen this library are sequences from the nuclear receptor element (NRE), TATA box or p53 promoters. The authors report the sequences of zinc-finger variants that bind the targets. Some are specific: they bind only one target. As results are available from a comparable phage display experiment which used the same targets, Joung *et al.* can compare their binders to those identified from phage display. Many of the zinc finger variants that bind the TATA and p53 sequences appear to be similar in the two cases, but those that bind the NRE are different. As a control, Joung *et al.* used the NRE binders identified from phage display as binders in the bacterial two-hybrid protocol. Results indicated that the phage display sequences did not bind their targets in bacteria, suggesting that the bacterial screen may be more stringent.

Methodological innovations

The technique is an extension of a bacterial two-hybrid scheme previously reported by the same authors in 1997. In this implementation, targets are DNA sequences and test candidates from the library are proteins. A test candidate from the library is identified as a strong binder to the target if it can promote transcription of a reporter gene *in vivo* by causing interactions between a pair of mediator proteins. Joung *et al*. use sequences from the NRE, TATA box or p53 promoters as target DNA. The library contains variants of zinc-finger proteins and the reporter gene is the bacterial gene *hisB*, which encodes an enzyme of the histidine biosynthetic pathway. Their mediator proteins are Gal4 and Gal11P from yeast. It all comes together as follows. Joung *et al*. make one set of plasmids containing the *hisB* gene under the control of a promoter containing one of the targets (NRE, TATA or p53). The authors make another set of plasmids containing *GAL4* fused to the gene for the RNA polymerase alpha subunit from *Escherichia coli*. They transfect both plasmids into a strain of *E. coli* that lacks *hisB*. Next they make oligonucleotides of the zinc-finger test sequences, ligate each to the gene for the mediator protein Gal11P, and introduce them into phage to form the library. Finally, the authors transfect the library phage into the deficient *E. coli* and grow them in medium lacking histidine. A successful 'hit' happens in the following sequence. *E. coli* expresses the zinc-finger test protein linked to Gal11P, and Gal4 linked to the polymerase subunit. If the test protein binds to the target promoter, Gal11P recruits Gal4 to the promoter. The polymerase linked to Gal4 causes transcription of *hisB* and the enzyme is synthesized. *E. coli* is then able to synthesize histidine from the medium and grow stably.

Conclusions

Joung *et al.* conclude that their method can pick out DNA-binding proteins from a randomized library. These proteins may bind more strongly and more specifically than those identified by phage display, and the procedure to detect them may be faster than in a yeast two-hybrid screen because *E. coli* has a higher transformation efficiency and growth rate.

Reporter's comments

This paper describes a complex and creative new technique for library screening. What it does not provide is a quantitative comparison against previous methods. For that, Joung *et al.* will need to measure their identified proteins' binding affinities to the target. The authors also do not provide evidence for the 'coverage' of their library - how many unique sequences they screened in *E. coli*.

Table of links

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References

1. Joung JK, Ramm EI, Pabo CO: A bacterial two-hybrid selection system for studying protein-DNA and protein-protein interactions. Proc Natl Acad Sci U S A. 2000, 97: 7382-7387. 0027-8424