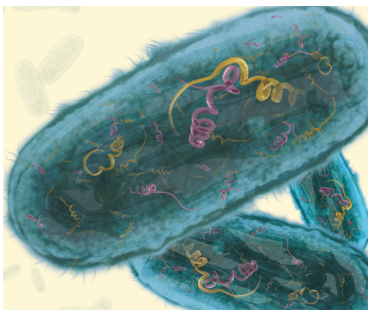
TECH TALK

Q&A QUESTIONS & ANSWERS

FROM STRATAGENE'S TECHNICAL SERVICES DEPARTMENT



Quickly Detect Protein-Protein Interactions

The **BacterioMatch™ two-hybrid system*** is a simple alternative or complement to yeast two-hybrid systems for in vivo detection of protein-protein interactions. Because the two-hybrid assay is performed in bacteria, results are obtained more easily and quickly than in yeast. The system is based on transcriptional activation of a primary ampicillin-resistant reporter and a secondary β-galactosidase reporter for validation. The BacterioMatch two-hybrid system is based on a methodology developed by Dove, Joung, and Hochschild of Harvard Medical School.[1,2]

Q What are the advantages of the BacterioMatch™ two-hybrid system over traditional yeast two-hybrid systems?

A The BacterioMatch™ two-hybrid system offers the ability to screen larger libraries for more difficult-to-find binding partners, due to the 1000-fold higher *E. coli* transformation efficiency. All screening is done in bacteria, transformation and plating is easy and colonies grow by the next day. Two-hybrid screening in bacteria also reduces the chance that the host harbors an eukaryotic homolog of one of the interacting protein partners, which may help to reduce false positives or potentially toxic effects.

Q What are some of the capabilities of the BacterioMatch two-hybrid system?

A The system identifies novel target proteins from a cDNA library that interact with a known bait protein. It also verifies protein-protein interactions between a known or potential interacting protein pair. In addition, it can characterize interactions between a known protein pair as weak, medium, or strong affinity.

Q How does the BacterioMatch two-hybrid system work?

A The BacterioMatch two-hybrid system is based on transcriptional activation (Figure 1). A protein of interest— the bait— is fused to the full-length bacteriophage repressor protein (λcI). The corresponding target protein is fused to the amino-terminal domain of the α-subunit of RNA polymerase (RNAPα). The bait is tethered to the λ operator sequence upstream of the reporter promoter through the DNA-binding domain of λcI.

If the bait and target interact, they recruit and stabilize the binding of RNA polymerase close to the promoter and activate the transcription of the ampicillin-resistant reporter gene in the BacterioMatch two-hybrid reporter strain. The β-galactosidase reporter gene provides an additional mechanism to validate putative protein-protein interactions.

Q Are BacterioMatch two-hybrid system premade libraries available?

A Stratagene offers many human, mouse, and rat premade cDNA libraries in the BacterioMatch two-hybrid system. For current BacterioMatch system premade library information, please visit www.stratagene.com.

Q What is the sensitivity of the BacterioMatch system?

A The system can detect interactions between pairs of protein domains with equilibrium dissociation constants in the high nanomolar range. To date, a positive interaction has been detected using the dimerization domain of the yeast transcriptional activator Gal4 and a domain derived from the mutant form of the Gal11 protein, Gal11^{P}. This protein pair has an equilibrium dissociation constant of approximately 10^{-7} M.

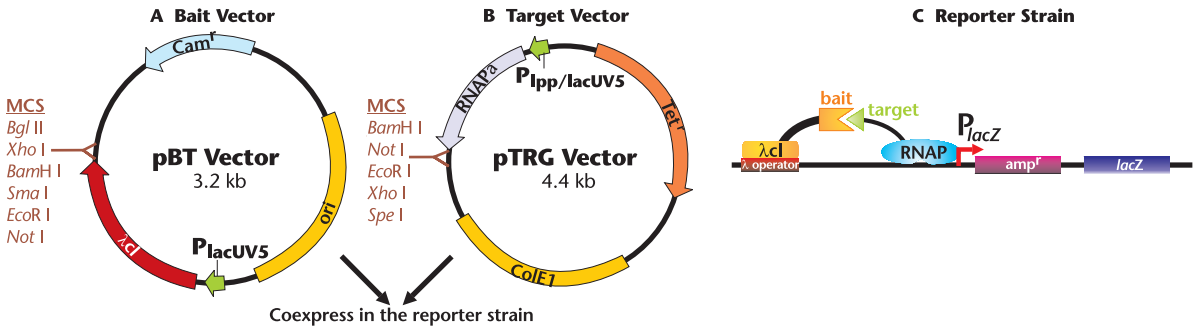


Figure 1

The BacterioMatch™ Two-Hybrid System
A. **Bait Vector**: The bait vector, pBT encodes the full-length bacterial phage cI protein under the control of the strong *lac*UV5 promoter. A protein of interest is fused to the bacterial phage λcI protein by inserting its gene into the multiple cloning site at the 3′ end of the λcI gene. A multiple cloning site present makes it convenient to subclone a bait gene that is already present in many yeast two-hybrid bait plasmids.
B. **Target Vector**: The target plasmid, pTRG is compatible with Stratagene's cDNA library construction kit. The target plasmid directs transcription of the amino-terminal domain of RNA polymerase α-subunit and linker region under the control of tandem promoters, lpp and *lac*UV5. The target gene is fused in-frame to the α-subunit NTD through a multiple cloning site at the 3′ end of the α-subunit gene.
C. **Reporter Strain**: The reporter strain is derived from XL1-Blue MRF′. The strain lacks all restriction systems in order to be compatible with current cDNA library construction methods. The *lac* I^q gene located on the F′ episome represses synthesis of the bait and target until induction. The reporter cassette is also located on the F′ episome in the cell. The *lac*Z gene serves as a secondary reporter to provide a visible phenotype for identifying positive protein-protein interactions.

Q What types of interactions have been performed using this system?

A Stratagene has demonstrated interaction with the following protein pairs:

Insert Cloned Into pTRG	Target Insert Description	Insert Cloned Into pBT	Bait Insert Description	Interaction Observed
Gal11^P	mutant form of the yeast Gal 11 protein	Gal 4	yeast transcriptional activator (dimerization domain)	yes
p53	murine tumor suppressor protein	p53	murine tumor suppressor protein	yes
p53	murine tumor suppressor protein	SV40-T	SV40 large T antigen	yes
Max	human transcription factor	Maxi	human transcription factor	yes
CD40	human TNF cell surface receptor (cytoplasmic tail)	TRAF2	human TNF receptor-associated factor 2	yes

Q Which antibiotic is used to select for protein-protein interactions using the primary reporter gene?

A Carbenicillin is used to screen for protein-protein interactions. Compared to ampicillin, carbenicillin is more stable and less sensitive to destruction by the ampicillin-resistance gene (β-lactamase), reducing satellite colony growth and the appearance of false positives.

Q Can the system be used to study proteins with both strong and weak affinities?

A Yes, expression from the test promoter of the reporter cassette is proportional to the strength of the protein-protein interaction between bait and target. The carbenicillin concentration can be adjusted to screen for protein-protein interactions of varying affinities.

Q Does the system require a special *E. coli* strain for screening?

A Yes, screening is performed in the BacterioMatch system two-hybrid reporter strain, which contains the appropriate reporter cassette. This reporter strain has been engineered to decrease basal transcription from the promoter. To achieve this, a minimal *lac* promoter has replaced the original *lac* promoter upstream of the reporter genes and the CRP binding site at -62 has been deleted.

REFERENCES
1. Dove, S.L., Joung, K., and Hochschild, A. (1997) *Nature* 386: 627-630.
2. Dove, S.L. and Hochschild, A. (1998) *Genes & Development* 12: 745-754.

* See license reference 1, on page 124.

For more information on the BacterioMatch two-hybrid system, please contact technical services or your local sales representative or visit www.stratagene.com

If you have a question for Tech Talk please send it to techservices@stratagene.com. If your question is chosen, we'll send you a free gift.