- BACTERIAL TRANSFORMATION—NO NEW TECHNIQUES TO LEARN
- NEW REPORTER STRAIN REDUCES BACKGROUND 100-FOLD
- PREMADE LIBRARIES AND LIBRARY CONSTRUCTION KITS AVAILABLE

NEW

Two-Hybrid System Reduces Background 100-Fold

*The new **BacterioMatch® II two-hybrid system** makes library screening faster and easier than in yeast. A new reporter strain reduces background by 100-fold compared to the original BacterioMatch system, so you analyze fewer colonies to obtain your positive protein-protein interactions.*

Yeast two-hybrid screening is traditionally used to detect protein-protein interactions, but it is limited by the basic biology of yeast; colonies take several days to grow, transformation efficiencies are low, and DNA manipulation requires special techniques.

Fast and Efficient

The BacterioMatch® two-hybrid system* is easier and 75% faster than yeast two-hybrid systems. All screening and validation steps are performed in bacteria, so there are no new techniques to learn. Additionally, bacteria have higher transformation efficiencies than yeast, making it easier to screen large libraries and detect rare interacting moieties. Protein-protein interactions are detected by an initial selection for histidine prototrophy and a secondary screen for streptomycin resistance.

Background Reduced by More Than 100-Fold

We have improved the original BacterioMatch two-hybrid system by replacing the reporter strain with a new strain that exhibits a significant reduction in background. Whereas the original reporter strain (BacterioMatch I strain) used ampicillin resistance and β-galactosidase expression selection markers, the new reporter strain (BacterioMatch II strain, a histidine auxotroph) uses the yeast His3 gene and aadA, a gene which confers streptomycin resistance, to select for interacting pairs (Figure 1). These new selection markers give the BacterioMatch® II system* much lower background, significantly reducing the number of false-positive colonies. To measure background, we took complex cDNA libraries and separately co-transformed them with empty bait vector into the improved BacterioMatch II strain and plated equal volumes on both selective

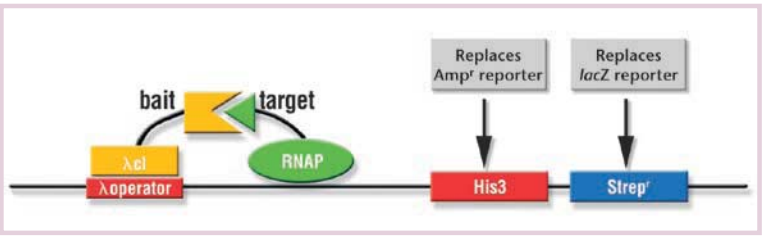

Figure 1
The BacterioMatch® II Reporter Competent Cell
The BacterioMatch® II reporter competent cell replaces the ampicillin resistance gene and *LacZ* reporter gene from the original strain with the His3 gene and a gene that confers streptomycin resistance.

Co-transformation	# CFUs following primary screen
Empty pBT and human ovary cDNA library pTRG	0 cfu/µg vector
Empty pBT and mouse embryo cDNA library pTRG	0 cfu/µg vector
pBT-LGF2 + pTRG-Gal11 (positive control)	4.1 x 10^5 cfu/µg vector

Table 1
BacterioMatch® II Two-Hybrid System Exhibits Exceptionally Low Background

and non-selective plates. No colonies were detected on selective plates, indicating extremely low breakthrough of background colonies. On non-selective plates, we observed 2.5 x 10^5 cfu/µg of DNA. We repeated the experiment with a second library, and no background was observed. To ensure that interacting partners could be detected, we co-transformed pBT-LGF2 and pTRG-Gal11 into the strain and plated onto the selective plates (Table 1).

Uncompromised Sensitivity

To demonstrate the BacterioMatch II system's ability to detect low abundance interacting partners, we simulated library screening conditions by diluting the Max human transcription factor target protein (pTRG-Max) 20,000-fold into a complex cDNA library and co-transforming into the BacterioMatch II strain with the known interacting partner Mxi (pBT-Mxi). 38 colonies were isolated after the primary screen on 5 mM 3-AT. Plasmids were isolated from the colonies, and the inserts were PCR-amplified to ascertain identity of the inserts. Of the 38 colonies, 28 contained the Max sequence.

Two Grades of Reporter Competent Cells

The BacterioMatch II two-hybrid system has two different grades of reporter strains for use at various steps throughout experiments. For library screening and other experiments demanding higher transformation efficiency, we offer the BacterioMatch II screening reporter competent cells. For validation and testing of known protein-protein interactions, we offer the BacterioMatch II validation reporter competent cells, a lower-efficiency, economical alternative to the screening competent cells.

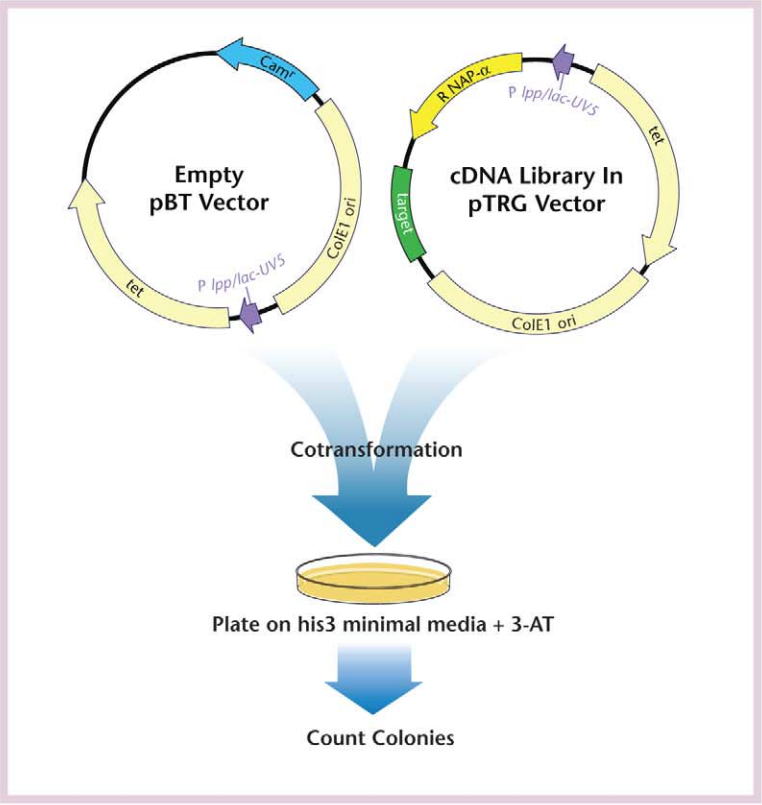

Figure 2
Measuring Background in the BacterioMatch® II Reporter Competent Cells

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* U.S. Patent No. 5,925,523
U.S. Patent No. 5,925,523, covering the BacterioMatch® two-hybrid system, is licensed exclusively by Stratagene. Research use of theBacterioMatch two-hybrid system by commercial entities requires a license from Stratagene. For license information, please contact: Vice President of Business Development at (858) 535-5400.

BacterioMatch® Two-Hybrid System	Catalog
BacterioMatch® II Screening Reporter Competent Cells	#200190
BacterioMatch® II Validation Reporter Competent Cells	#200192
BacterioMatch® II Two-Hybrid System Vector Kit	#240065
BacterioMatch® II Two-Hybrid System cDNA Library Construction Kit	#200412
See *www.stratagene.com/Cloning* for a complete list of BacterioMatch® premade libraries	

BacterioMatch® II Two-Hybrid System cDNA Libraries

Source and type	Description	Primary Library Size (x 10^6)	Avg. Insert Size	Catalog #
Yeast cDNA				
S. cerevisiae	DBY 746 strain	2.7	1.8 kb	982050
Human cDNA				
Brain, cerebellum	24 pooled, normal cerebellum, male/female, 16-70 years	3.2	1.4 kb	982207
Breast	10 pooled samples, 33-80 years	2.5	1.0 kb	982215
Cervix	Pooled squamous cell carcinoma samples, 42-81 years	2.4	1.3 kb	982214
Colon	Normal female (59 years)	1.7	1.6 kb	982261
Fetal Brain	Pool of 10 male and female, 21 to 30 weeks old	3.4	1.5 kb	982260
HeLa Cell	Hela S-3 cells	4.3	1.3 kb	982208
K-562 Cell	Cells grown to semiconfluency	2.8	1.8 kb	982218
Kidney	Male, 82 years	3.8	1.4 kb	982205
Lung	Pooled donors, normal, whole lung, male/female 30 & 40 years	2.5	1.3 kb	982201
Melanoma	G361 malignant cell line	2.3	1.7 kb	982256
Pancreas	Female, 21 years, Black	1.8	1.4 kb	982213
Uterus	8 pooled donors, normal, whole uteri, female 21-60 years	2.1	1.5 kb	982202
T-cell, PMA stimulated	Semi-confluent Jurkat cells, PMA stimulated	3.3	1.7 kb	982212
Mouse cDNA				
Heart	Pooled BALB/c, male and female 8-12 weeks	3.4	2.0 kb	982303
Kidney	Pooled kidney tissue, BALB/c, male, 7-10 weeks	4.8	1.9 kb	982304
Liver	7 pooled livers, BALB/c, male, 7-10 weeks	4.8	1.6 kb	982307
Lung	Pool of 200 normal, whole lungs, BALB/c, male, 8-12 weeks old	1.5	1.5 kb	982305
Spleen	Pooled, whole spleen specimen, BALB/c, male, 8-12 weeks old	6.9	1.1 kb	982306
Testis	100 pooled testes, BALB/c, 7-10 weeks	2.9	1.8 kb	982308
Rat cDNA				
Brain	Pooled brain tissues, Sprague-Dawley, male, 10 weeks	2.1	1.8 kb	982504
Heart	Pool of 100, whole hearts, Sprague-Dawley, male, 10-12 weeks	2.5	1.3 kb	982501
Liver	Pooled specimens, Sprague-Dawley, male, 10 weeks	2.0	1.5 kb	982502
Skeletal Muscle	Pooled muscle tissues, Sprague-Dawley, male, 10 weeks	2.8	1.4 kb	982505
Smooth Muscle	100 pooled muscle tissues, Sprague-Dawley, male, 10-12 weeks	2.3	1.2 kb	982506
Insect cDNA				
Drosophila Adult	*Drosophila melanogaster* adult	2.0	2.1 kb	982602
Drosophila Embryo	Pooled normal whole embryos	1.8	1.6 kb	982600
Drosophila Larva	Pooled from *Canton S* Larvae	2.5	1.3 kb	982605
Drosophila S2 Cells	S2 cell line	4.6	1.1 kb	682604
***Xenopus* cDNA**				
Xenopus Embryo	Pooled normal whole embryos	3.0	1.8 kb	982650

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