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MOLECULAR SCREENING

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Superior Screening of Protein-Protein and DNA-Protein Interaction

Understanding the interactions between biomolecules, predominantly proteins, is key to the complexity of biological functions and the disorders associated with them. There are a multitude of methods to detect protein-protein interactions. Each of these approaches has its own strengths and weaknesses, especially with regard to sensitivity and specificity. MoBiTec went along with many of these methods from the early days on and offers today well-selected tools to detect and quantify molecular interactions.

This brochure focuses on several yeast one- and two-hybrid systems and a phagemid system, that are useful for high-throughput molecular screening of protein-protein and DNA-protein interactions. Further tools, such as biochemical assays, immunological and fluorescence-based methods, are available from MoBiTec as well.

The yeast two-hybrid system has rapidly become one of the most widely used techniques in molecular biology. It still is the method of choice to identify protein-protein interactions from either cDNA libraries or known gene sequences *in vivo*. The method relies on the transactivation of reporter genes in *Saccharomyces cerevisiae* identifying positive interactions.

Grow'n'Glow GFP Reporter Systems

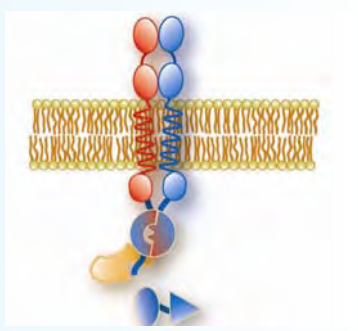
MoBiTec's Grow'n'Glow one- and two- hybrid systems are powerful tools for gene discovery and functional analysis. A GFP reporter allows one-step selection of positive clones under UV light.

Grow'n'Glow GFP Two-Hybrid System: Time-saving identification of positive clones with the well-established GFP reporter system, suitable for high-throughput screening assays. See page 2-3.

Grow'n'Glow GFP One-Hybrid System: Ideal to isolate genes of novel DNA-binding proteins or to map residues/motifs responsible for DNA-binding. See page 4.

Grow'n'Glow ACE1 Two-Hybrid System: Stringent selection and more efficient detection of protein interactions using the copper-inducible CUP1/ACE1 system. (Page 5).

Y2H Membrane Systems



MoBiTec's split-ubiquitin based systems circumvent the restrictions that are usually associated with traditional Y2H systems, now allowing to detect interactors with integral membrane proteins, self-activating and transcriptionally active proteins.

Y2H Membrane System: Versatile, reliable detection and analysis of specific binding partners of full-length integral membrane and membrane-associated proteins. See page 6-8.

Y2H Transactivating Protein System: Screens transcriptionally active and self-activating proteins that cannot be screened using classical Y2H systems. See page 7-8.

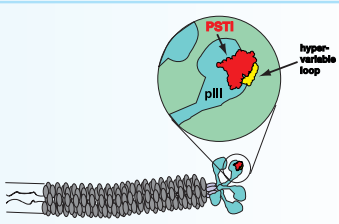
Tools and Media

MoBiTec offers many tools optimized for working with Y2H and yeast: Kits to transform yeast cells, kits to purify plasmids from yeast, media, supplements and fermentation technology.

Yeast Transformation & Yeast Plasmid Purification Kits: See page 9.

Yeast Media: See page 10.

Phagemid System



Phagemid Display System: Easy-to-handle, fast and low-cost approach for high-throughput and high-content screening of protein-protein interactions. See page 11-12.

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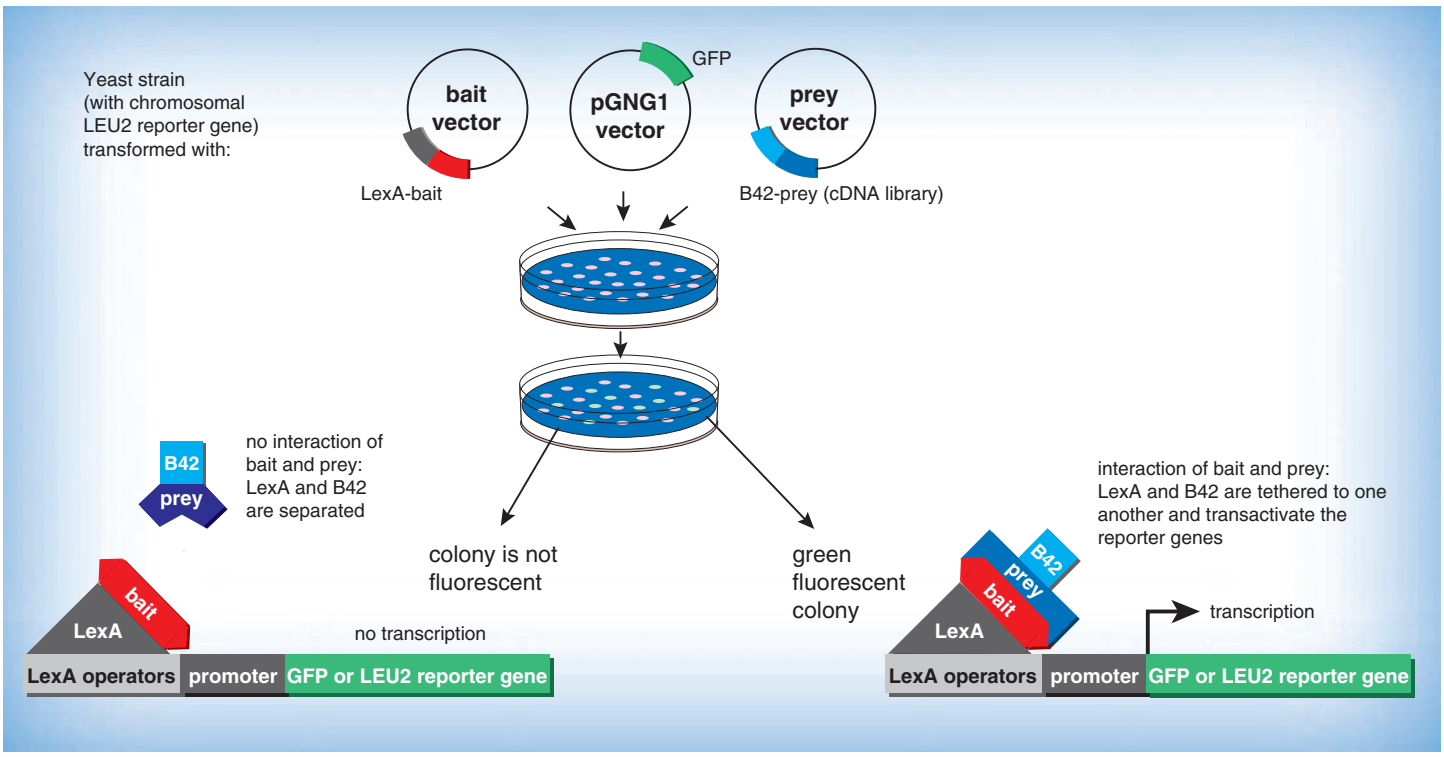
Powerful Tool for Gene Discovery and Functional Analysis

Grow'n'Glow is a patented system, which simplifies and accelerates the screening process of two-hybrid systems tremendously. The method combines the advantages of the well known gene expression reporter protein GFP (green-fluorescent protein) with the LexA-based yeast two-hybrid technology[1,2,3]. As a result, protein-protein interactions can be detected *in vivo* in a one-step selection under UV light.

Features

- One-step selection of potential protein-protein interactions under UV light
- No time-consuming β-galactosidase lift assays
- Due to galactose-inducible expression, also toxic interacting proteins can be screened for



Identification of molecular interactions with the Grow'n'Glow GFP Two-Hybrid System. Bait, reporter and prey vector (with e.g. a cDNA library) are transformed into yeast. If the expressed bait and prey proteins are interacting, B42 and LexA are tethered to one another and transactivate the reporter genes LEU2 (integrated in the yeast chromosome) and GFP (located on pGNG1).

Selection of Interacting Proteins

Interacting proteins result in expression of two reporter genes - the LEU2 nutritional selection gene and the GFP gene from *Aequorea victoria*[4]. Expression of LEU2 allows yeast with interacting proteins to grow on medium lacking leucine, and expression of the GFP gene is readily detected by visualizing colonies under ultraviolet (UV) light. Use of the tightly controlled LEU2 and the easily detectable GFP reporters reduces background and offers more clear-cut results, which expedite the entire screening process.

Fewer False Positives

Our two-hybrid system uses the *E. coli* LexA protein as a DNA-binding protein to provide promoter specificity. Since LexA is a prokaryotic protein, there is little chance of getting non-specific activation, as it is the case when the GAL4 DNA-binding domain is used. As an endogenous protein, GAL4 may interact with a variety of proteins in a yeast cell. These spurious interactions might affect yeast growth creating false positives. Using the prokaryotic LexA DNA-binding protein minimizes these effects.

No β-Gal Lift Assays

Using the Green Fluorescent Protein (GFP) reporter makes the time-consuming β-galactosidase lift assay unnecessary. The particular GFP variant expressed by the pGNG1 plasmid[8], GFPuv, has the same excitation and emission maxima as wild-type GFP but is 18 times brighter than the wild-type variant[5]. Placing the plate with the yeast colonies on a standard 300 nm UV table or under a handlamp in a darkroom, colonies with interacting proteins are glowing brightly green.

Inducible System Allows Screening for Potentially Toxic Interactions

Expression of fusion proteins by the prey plasmid is controlled by the GAL1 upstream-activation sequence. In yeast with an intact galactose regulatory system, the GAL1 activation sequence is induced by galactose and repressed by glucose. This regulation delays the expression of library/activation domain fusions to the actual screening process. Using such an inducible system, also toxic proteins can be selected for without encountering the problem that a potentially toxic library protein may affect growth of the yeast.

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Order Information

The Grow'n'Glow GFP Two-Hybrid System is offered as a "Complete Kit" for scientists starting to establish the two-hybrid technology, or as a "Basic Kit" for researchers already working with a LexA-based two-hybrid system. Both kits include the GFP reporter vector pGNG1[6].

"The Complete Kit" contains the LexA-bait vector[7] for cloning the gene coding for the protein of interest as well as the B42-prey vector, which is used to clone a cDNA library or the gene coding for the target protein of interest.

In addition, several control plasmids are provided: a bait control, a prey control and a "positive control" that can activate GFP by itself. The three sequencing primers 5'-BAITprimer, 5'-PREYprimer and 3'-PREYprimer are designed for determining the sequence of the genes cloned into the bait and prey vectors, respectively. 5'-PREYprimer and 3'-PREYprimer can additionally serve as primers for amplifying the cloned gene by PCR.

For your convenience, we provide three yeast strains carrying the second reporter gene LEU2 in the chromosome. They have varying sensitivities towards transcriptional activation of LEU2, allowing to use a less sensitive strain, if a bait protein of interest autoactivates this reporter gene.

Our "Basic Kit" for inclusion into pre-existing LexA two-hybrid systems contains only the bait, prey and positive control plasmids next to the reporter vector pGNG1.

Please also note, that we offer easy-to-handle yeast transformation and plasmid purification kits (see page 9) as well as yeast and bacterial growth media which have been optimized for two-hybrid applications (see page 10).

Order No.	Description	Amount
GNGK01	**Grow'n'Glow GFP**	
	Two-Hybrid System "Complete Kit"	**1 kit**
	GFP reporter vector pGNG1, lyophilized DNA	5 µg
	bait plasmid, lyophilized DNA	5 µg
	prey plasmid, lyophilized DNA	5 µg
	bait control plasmid, lyophilized DNA	5 µg
	prey control plasmid, lyophilized DNA	5 µg
	positive control plasmid, lyophilized DNA	5 µg
	5'-BAITprimer (0.1 nmole/µl)	500 pmole
	5'-PREYprimer (0.1 nmole/µl)	500 pmole
	3'-PREYprimer (0.1 nmole/µl)	500 pmole
	yeast strain "high sensitivity", glycerol stock	1 mL
	yeast strain "medium sensitivity", glycerol stock	1 mL
	yeast strain "low sensitivity", glycerol stock	1 mL
GNGK02	**Grow'n'Glow GFP**	
	Two-Hybrid System "Basic Kit"	**1 kit**
	GFP reporter vector pGNG1, lyophilized DNA	5 µg
	bait control plasmid, lyophilized DNA	5 µg
	prey control plasmid, lyophilized DNA	5 µg
	positive control plasmid, lyophilized DNA	5 µg
GNGV01	**Grow'n'Glow GFP**	
	Two-Hybrid reporter vector pGNG1	5 µg

References

1. Fields, S.; Song, O., Nature 340 (1989) 245-246
2. Bartel, P. L.; Fields, S., Methods Enzymol. 254 (1995) 241-63
3. Wilson, T. E. *et al.*, Science 252 (1991) 1296-1300
4. Cubitt, A. B. *et al.*, Trends Biochem. Sci. 20 (1995) 448-455
5. Crameri, A. *et al.* (1996): Nature Biotechnol. 14: 315-319
6. Cormack, R. S. et al., The Plant Journal 14 (6) (1998) 685-692
7. Gyuris, J. *et al.*, Cell 75 (1993) 791-803

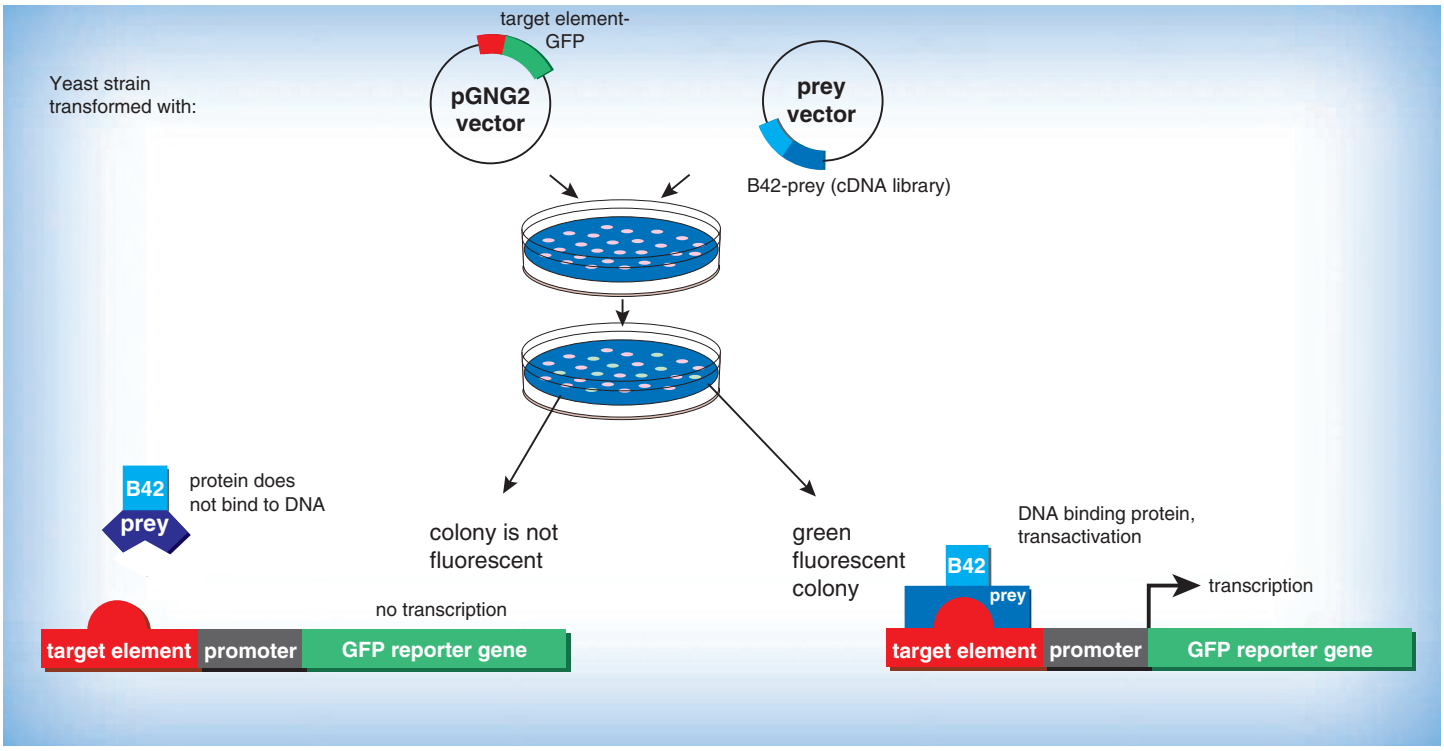
Faster and more cost-effective than conventional assays

The Grow'n'Glow GFP One-Hybrid Kit isolates genes of proteins that bind a specific DNA element of interest[1,2,3]. In addition to finding novel DNA-binding proteins, the one-hybrid system can be used to identify the bases and amino acids involved in specific DNA-protein interactions.

Proteins can be found that bind to any short DNA element of interest. The Grow'n'Glow system offers maximal sensitivity[4] because detection of the DNA-protein interaction occurs *in vivo*, where proteins are more likely to be in their native conformation.

Features

- Isolates genes of novel DNA-binding proteins
- Maps residues and regions responsible for DNA-binding
- One-step selection of potential positive colonies under UV light
- No time-consuming β-galactosidase lift assays
- Finds potentially toxic proteins by screening with galactose-inducible expression libraries



Identification of molecular interactions with the Grow'n'Glow GFP One-Hybrid System. Reporter and prey vector (with e.g. a cDNA library) are transformed into yeast. If an expressed prey protein is interacting with the DNA target element, the B42 activator peptide is brought into a position that initiates transcription of the reporter gene GFP (located on pGNG2).

Order Information

Order No.	Description	Amount
GNGK03	**Grow'n'Glow GFP One-Hybrid System**	**1 kit**
	pGNG2, lyophilized DNA	5 µg
	pJG4-5, lyophilized DNA	5 µg
	pGNG2-p53, lyophilized DNA	5 µg
	pJG4-5-p53, lyophilized DNA	5 µg
	GNGprimer	500 pmole
	5'-PREYprimer (0.1 nmole/µl)	500 pmole
	3'-PREYprimer (0.1 nmole/µl)	500 pmole
	yeast strain EGY48, glycerol stock	1 mL
GNGV02	**Grow'n'Glow GFP One-Hybrid reporter vector pGNG2, lyophilized DNA**	5 µg

References

1. Wang, M. M. & Reed, R. R., Nature 364 (1993) 121-126
2. Strubin, M., Newell, J. W. and Matthias, P. (1995) Cell 80:497-506
3. Luo, Y., Vijaychander, S., Stile, J. and Zhu, L. (1996) Biotechniques 20: 564-568
4. Crameri, A., *et al*. (1996) Nature Biotechnol. 14:315-319

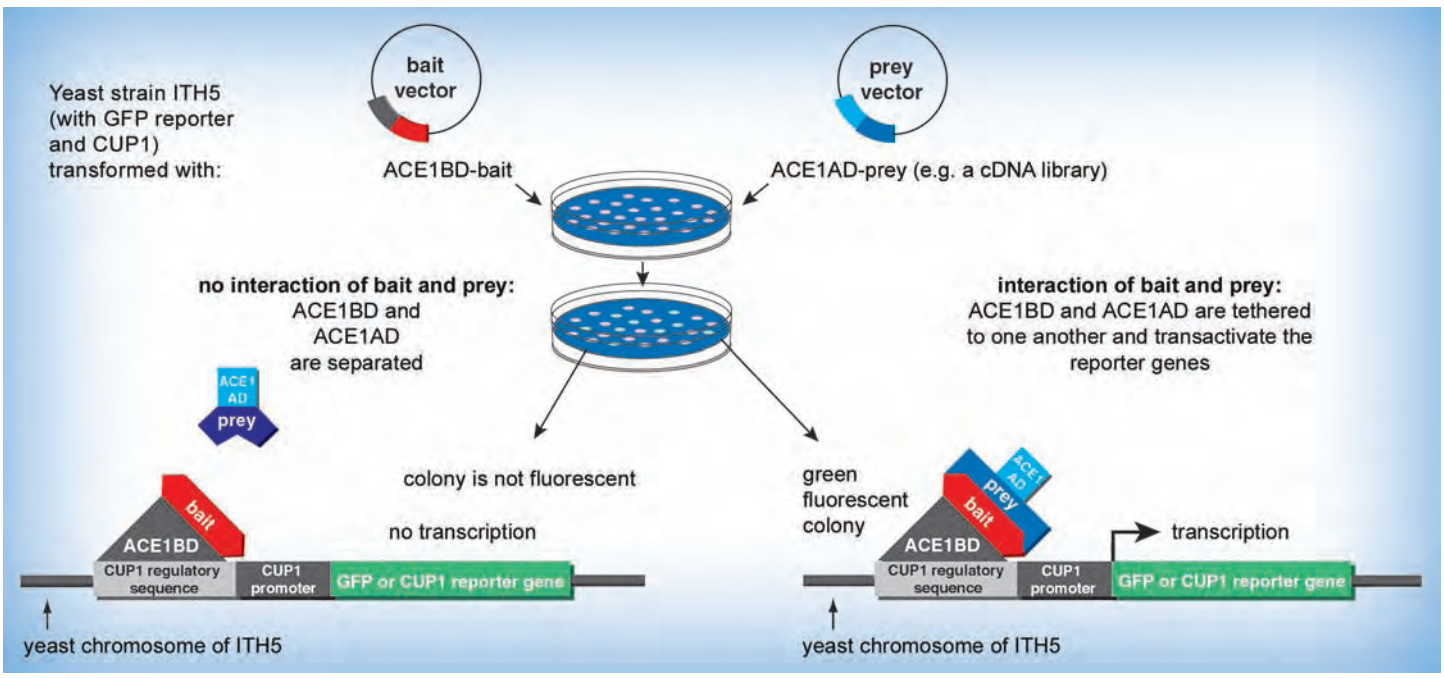
The ACE of the Two-Hybrid Systems

MoBiTec's ACE1 Two-Hybrid System is a powerful tool for gene discovery and functional analysis, and very useful for efficiently identifying protein-protein interactions *in vivo*. It combines the advantages of the copper-inducible transcription factor ACE1 and the copper resistance-mediating CUP1 gene with the green fluorescence protein (GFP) as reporter. Interaction of two proteins fused to the DNA-binding and transactivation domains of ACE1, respectively, can be consequently detected by 1) growth of the cells on copper-containing media and 2) the copper-dependent expression of the strongly fluorescent GFPuv which renders those colonies to fluoresce brightly green under UV light.

Thus, this innovative technique greatly facilitates an efficient one-step selection of potential positives expressing interacting proteins in copper-containing media. It allows a fast, highly sensitive and more reliable monitoring of protein interactions in living cells because interacting clones are identified by a growth selection as well as a strong fluorescence signal. Changing the copper concentration allows to select for different protein-protein interaction strengths. Thus, this tool is a very useful alternative to LexA-based two-hybrid technology.

Features

- More efficient detection of protein interactions *in vivo* by the copper-inducible CUP1/ACE1 system
- Fewer false-positives by stringent selection for copper resistance
- Allows to select for different protein interaction strengths
- Improved time-saving selection of positives with the well-established advantageous GFP reporter
- No requirement for external substrates when screening for positives
- Suited for the development of HTS assays



Detection of protein-protein interactions using the Grow'n'Glow ACE1 Two-Hybrid System. The coding sequences of two potentially interacting proteins ("bait" and "prey") are ligated into the ACE1-bait vector pTY137 (carrying the ORF coding for the ACE1 binding domain and the HIS3 gene) and the ACE1-prey vector pTM114 (carrying the ORF coding for the ACE1 activation domain and the URA3 gene), respectively. Both plasmids are transformed simultaneously in the yeast strain ITH5 which carries several tandemly amplified copies of the CUP1 gene and a CUP1 promoter-GFP reporter fusion. Double-transformed cells are spread directly onto agar plates lacking histidine and uracil but supplemented with 30 – 100 µM $CuSO_4$. In the presence of copper ions, the ACE1BD-bait hybrid binds to regulatory sequences within the CUP1 promoter. Interaction of the prey and bait hybrid proteins allows a re-assembly of the ACE1 DNA binding and transactivation domains *in vivo*. This reconstitution of a functional ACE1 transcription factor causes an activation of both, the CUP1 genes and the GFP reporter (which are stably integrated in the yeast chromosome) resulting in copper-resistant green-fluorescent colonies. Thus, only those clones containing both plasmids and expressing interacting proteins will grow on the selection plates as fluorescent colonies.

Order Information

The Grow'n'Glow ACE1 Two-Hybrid System is offered as a "Complete Kit" for scientists starting to establish the two-hybrid technology or searching for an intelligent alternative to already existing two-hybrid systems (e.g. LexA- or GAL4-based systems). The kit includes the bait and prey vectors, as well as several controls. As a host strain for transformation, the *Saccharomyces cerevisiae* strain ITH5 is provided having several copies of the CUP1 gene and a CUP1 promoter-GFP reporter fusion stably integrated in its genome.

Order No.	Description	Amount
ACE01	Grow'n'Glow ACE1 Two-Hybrid System	1 kit
	bait plasmid pTY137, lyophilized DNA	5 µg
	prey plasmid pTM114, lyophilized DNA	5 µg
	bait control plasmid pTY143, lyophil. DNA	5 µg
	prey control plasmid pTM125, lyophil. DNA	5 µg
	positive control plasmid pTY139, lyophil. DNA	5 µg
	5'-BAITprimer (0.1 nmole/µl)	500 pmole
	5'-PREYprimer (0.1 nmole/µl)	500 pmole
	3'-PREYprimer (0.1 nmole/µl)	500 pmole
	yeast strain ITH5, glycerol stock	1 mL

Discover Novel Interaction Partners of Integral Membrane Proteins

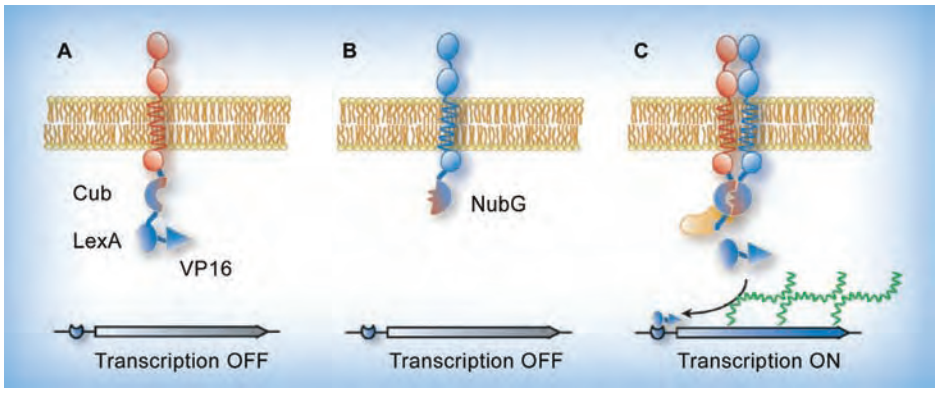
The Y2H Membrane System is a genetic assay based on the split-ubiquitin system originally developed by N. Johnsson and A. Varshavsky in 1994[1,2,3].

It is designed to detect interactions between an integral membrane protein and:

- other integral membrane proteins (e.g. receptor subunits or co-receptors)
- membrane-associated proteins (e.g. signalling components or members of the sorting machinery)
- soluble proteins (e.g. adaptors, kinases and other signalling components)

The Y2H Membrane System can be used for the following applications:

- Detection of pairwise protein interactions
- Identification of novel protein interactions by cDNA library screenings
- Investigation of ternary complexes
- Interaction domain mapping



Detection of protein interactions with an integral membrane protein using the Y2H Membrane System. Specific bait and prey vectors are transformed into yeast, leading to expression of a bait protein and a prey protein in the yeast cell. (A) The bait is a fusion of the integral membrane protein under investigation (e.g. GPCR, receptor tyrosine kinase, ion channel) and the C-terminal half of ubiquitin followed by a transcription factor (LexA-VP16). Since the transcription factor is immobilized at the membrane, it is unable to reach the nucleus. (B) The prey is a fusion of a second integral membrane protein or a soluble protein fused to the N-terminal half of ubiquitin bearing a point mutation (NubG). (C) If bait and prey interact as a consequence of protein-protein interaction, Cub and NubG are forced into close proximity and associate to form split-ubiquitin. The formation of split-ubiquitin activates proteases, which cleave the transcription factor from the bait. The free transcription factor then relocates to the nucleus and activates its cognate reporter genes integrated into the yeast genome. The protein-protein interaction at the membrane is thus detected by measuring the output of a set of reporter genes. To screen for novel protein interactions, the prey is replaced by a cDNA library encoding millions of different proteins or protein fragments fused to NubG.

Features

- Interactions of full-length integral membrane proteins are detected under physiological conditions at the membrane
- Novel interactors are identified through screening of cDNA libraries; no need to express and purify proteins
- Easy subcloning of full-length cDNA inserts using *Sfi* I technology
- NMY51 reporter strain with improved stringency, resulting in fewer false positives

References

1. Johnsson and Varshavsky (1994) Proc. Natl. Acad. Sci. USA.91, 10340-4.
2. Stagliar *et al.* (1998) Proc. Natl. Acad. Sci. USA. 95, 5187-5192.
3. Suter *et al.* (2006) Biotechniques 40, 625-644

Order Information

Order No.	Description	Amount
P09901DS	Y2H Membrane Starter Package (incl. P01001, P01002, P01003)	1 kit
P01001DS	Y2H Membrane Kit 3:	1 kit
P03230DS	Type II bait vector pBT3-N (N-terminal fusion)	5 µg
P03231DS	Type I bait vector pBT3-C (C-terminal fusion)	5 µg
P03232DS	Type I signal sequence bait vector pBT3-SUC (C-terminal fusion)	5 µg
P03233DS	Type I leader sequence bait vector pBT3-STE (C-terminal fusion)	5 µg
P03234DS	Prey vector pPR3-N (NubG-cDNA orientation)	5 µg
P03235DS	Prey vector pPR3-C (cDNA-NubG orientation)	5 µg
P03236DS	Signal sequence prey vector pPR3-SUC (cDNA-NubG orientation)	5 µg
P03237DS	Leader sequence prey vector pPR3-STE (cDNA-NubG orientation)	5 µg
	Control bait vector pCCW-Alg5	5 µg
	Control prey vector pAI-Alg5	5 µg
	Control prey vector pDL2-Alg5	5 µg
	Yeast reporter strain NMY51	lyoph. yeast
P01002DS	HTX β-galactosidase assay kit	4 x 96 assays
P01003DS	Y2H Yeast transformation kit	1 kit
P06001DS	Single-stranded DNA	3 x 1 mL
P06004DS	Mouse anti-*E. coli* LexA monoclonal antibody	40 µL

Order information

The Y2H Membrane Kit contains all vectors, strains and controls necessary to use the system. Single components can be ordered separately as well. To get started quickly, take advantage of the Y2H Membrane Starter Package that additionally includes:

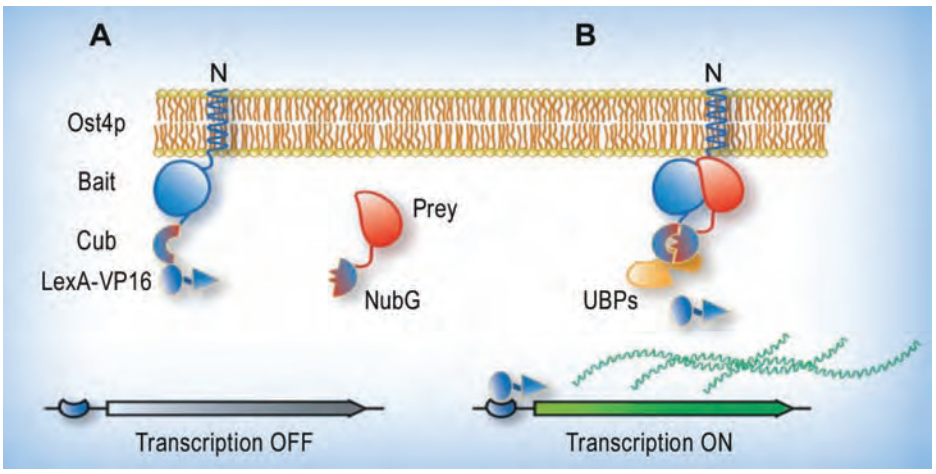
- HTX β-galactosidase assay kit (4x 96 reactions) for easy determination of β-galactosidase levels
- Yeast transformation kit sufficient for 7 library scale yeast transformations

Designed to Circumvent the Pitfalls of Transactivating Proteins

The Y2H Transactivating System[1,2] has been designed as a solution for those proteins which present problems in classical protein interaction assays, such as transcriptionally active proteins or proteins containing clusters of highly acidic proteins. Using the Transactivating Protein kit, a protein of interest or a protein fragment or domain is immobilized at the membrane by means of a small membrane anchor. At its C-terminus, the protein is fused with a reporter cassette, containing the C-terminal half of ubiquitin (Cub) and the artificial transcription factor LexA-VP16.
The Y2H Transactivating Protein System is designed to identify novel protein-protein interactions by cDNA library screening. All cDNA libraries of the Y2H Membrane System can also be used with the Y2H Transactivating Protein System.

Features

- Screens transcriptionally active and self-activating proteins that cannot be screened using classical Y2H systems
- No need to truncate your protein of interest or to select subdomains
- NMY51 reporter strain with improved stringency, resulting in fewer false positives



Detection of protein interaction with transactivating proteins. (A) A protein of interest (the bait) is inserted between the membrane protein Ost4p and the C-terminal half of ubiquitin (Cub), followed by the artificial transcription factor LexA-VP16. A second protein (the prey) is fused to the mutated N-terminal half of ubiquitin (NubG). (B) If bait and prey interact, Cub and NubG complement to form split-ubiquitin, followed by cleavage and translocation of LexA-VP16 to the nucleus and transcriptional activation of endogenous reporter genes. The protein interaction between bait and prey is detected using the output of the reporter genes, either via a growth selection on minimal medium, or via the color marker lacZ. Replacing the plasmid encoding the prey by a cDNA library expressing millions of different proteins or protein fragments allows to detect novel protein interactions.

References

1. Johnsson and Varshavsky (1994) Proc. Natl. Acad. Sci. USA. 91, 10340-4.
2. Möckli *et al.* (2007) BioTechniques 42(6), 725-730.

Order Information

Order No.	Description	Amount
P01005DS	Y2H Transactivating Protein Kit:	1 kit
P03501DS	Bait vector pDHB1	5 µg
	Prey vector pPR3-N	5 µg
P03503DS	Control bait vector pDHB1-largeT	5 µg
P03504DS	Control prey vector pDSL-p53	5 µg
	Control prey vector pAI-Alg5	5 µg
	Control prey vector pDL2-Alg5	5 µg
	Yeast reporter strain NMY51	lyoph. yeast
P01002DS	HTX β-galactosidase assay kit	4 x 96 assays
P01003DS	Y2H Yeast transformation kit	1 kit
P06001DS	Single-stranded DNA	3 x 1 mL
P06004DS	Mouse anti-*E. coli* LexA monoclonal antibody	40 µL

There are two types of cDNA libraries for the Y2H Membrane Protein and Transactivating Protein Systems:
NubG-x libraries (expressing NubG-prey fusion proteins) and x-NubG libraries (expressing prey-NubG fusion proteins).

For the Y2H Membrane System, we recommend NubG-x libraries as a starting point, in particular when screening for cytosolic interactors and interactors of type II integral membrane proteins (e.g. ion channels, transporters). We recommend x-NubG libraries when screening for interactors of type I integral membrane proteins (e.g. receptor tyrosine kinases, cell adhesion molecules, GPCRs).

For use with the Y2H Transactivating Protein System, we recommend NubG-x libraries.

Order Information
Libraries are supplied as 200 µg purified plasmid DNA, ready for screening.
The quantity supplied is sufficient for seven library scale screens.

Oder No.	Description	Amount
	cDNA library:	
P02201DS	Mouse adult whole brain / NubG-x	200 µg
P02205DS	Jurkat T cells, unstimulated / NubG-x	200 µg
P02206DS	Mouse adult heart / x-NubG	200 µg
P02207DS	Mouse adult heart / NubG-x	200 µg
P02208DS	Human adult kidney / x-NubG	200 µg
P02209DS	Human adult liver / x-NubG	200 µg
P02210DS	Arabidopsis thaliana / NubG-x	200 µg
P02212DS	HeLa cell line / NubG-x	200 µg
P02211DS	Saccharomyces cerevisiae / x-NubG	200 µg
P02213DS	Human embryonal brain / NubG-x	200 µg
P02215DS	Human adult colon / NubG-x	200 µg
P02218DS	Caenorhabditis elegans adult / NubG-x	200 µg
P02220DS	Human adult liver / NubG-x	200 µg
P02221DS	Human adult brain / NubG-x	200 µg
P02222DS	Caenorhabditis elegans eggs / NubG-x	200 µg
P02223DS	Drosophila melanogaster whole embryo (16 hours) / NubG-x	200 µg
P02224DS	Mouse adult spleen / x-NubG	200 µg
P02226DS	Human adult kidney / NubG-x	200 µg
P02227DS	Human adult brain / x-NubG	200 µg
P02228DS	LNCaP cell line / NubG-x	200 µg
P02229DS	Drosophila melanogaster / NubG-x	200 µg
P02230DS	Human adult lung / NubG-x	200 µg
P02231DS	Mouse adult kidney / NubG-x	200 µg
P02232DS	Mammary epithelial cells / NubG-x	200 µg
P02233DS	Rat neonatal cardiomyoctes / NubG-x	200 µg
P02234DS	Mouse whole embryo, 11 days / NubG-x	200 µg

Grow'n'Glow High Efficiency Yeast Transformation Kit

The High Efficiency Yeast Transformation Kit provides all reagents required for 25 preparations of competent yeast cells and 5 - 10 transformations per prep. The entire procedure can be completed in less than 90 minutes and routinely provides a transformation efficiency of greater than 10^4 transformants per µg of DNA, using YEP24 plasmid DNA.

Features

- High efficiency (≥ 10^4 transformants per µg DNA)
- Applicable for linear or plasmid DNA

Order Information

Order No.	Description	Amount
2200-1	**Grow'n'Glow High Efficiency Yeast Transformation Kit**	250 transformations
	Kit Components:	
	TE, pH 7.5, sterile solution	275 mL
	Lithium/cesium acetate	125 mL
	Carrier DNA, 10 mg/mL, sterile	1.25 mL
	Histamine, sterile solution	1.25 mL
	PEG	200 mL
	TE/cation salt solution	50 mL
	rich medium, sterile solution	40 mL

Grow'n'Glow Fast and Easy Yeast Transformation Kit

The Fast and Easy Yeast Transformation Kit is designed for high-throughput transformations. It is ideal for cases where large numbers of transformations are required but only a few transformants are needed (i.e. transformation with a single homogenous vector). Thus, this kit is ideal for analyzing „putative positives" obtained from two-hybrid screens. The Fast and Easy Yeast Transformation Kit does not require competent cells prior to transformation. A simple, five-minute protocol is followed by overnight incubation at room temperature and plating on selective media.

Features

- Fast and easy procedure with minimal hands-on time
- No need for fully competent cells
- Moderate transformation efficiency (approximately 1 x 10^3 per µg DNA)
- Ideal for high-throughput analysis of clones in two-hybrid screens

Order Information

Order No.	Description	Amount
2100-1	**Grow'n'Glow Fast and Easy Yeast Transformation Kit**	200 transformations
	Kit Components:	
	Transformation solution	110 mL
	Carrier DNA, 10 mg/mL, sterile	1.1 mL

Grow'n'Glow Yeast Plasmid Isolation Kit

The Grow'n'Glow Yeast Plasmid Isolation Kit is designed for the isolation of plasmid DNA from yeast or bacterial cells for use in any downstream application (PCR, sequencing, restriction enzyme analysis, labeling, transformation etc.). It uses DNA purification with porous glass in conjunction with spin filter technology to increase throughput and achieve optimal DNA quality. This dual purpose kit is ideal for screening putative positive yeast transformants obtained from two-hybrid screens.

Features

- Fast and easy purification of pure plasmid DNA from yeast (or bacteria), e.g. for PCR and transformation
- Porous glass & spin filter technology; no phenol/chloroform extraction; no alcohol precipitation

Order Information

Order No.	Description	Amount
2269-2	**Grow'n'Glow Yeast Plasmid Isolation Kit**	100 preps
	Kit Components:	
	Yeast lysis matrix	0.4 g x 100 tubes
	Alkaline lysis solution	32 mL
	Neutralizing solution	32 mL
	Spin buffer with porous glass	32 mL
	Wash solution concentrate	56 mL
	Spin filters	100
	Catch tubes	100

Ready-to-use Media for Two- and One-Hybrid Screening

Our media formulations are supplied as ready-to-use powder mixtures. They come in small bags with the appropriate amount of powder for 0.5 liter medium. You only add water and autoclave for use. Grow'n'Glow yeast media are optimized for two- and one-hybrid studies and strongly recommended for our Grow'n'Glow Systems to achieve best results.

Features

- Ready-to-use media, simply add water and autoclave
- Optimized for two- and one-hybrid screenings

Grow'n'Glow Yeast Media

We offer a complete standard Wickerham yeast nitrogen base with carbon source optimized for *S. cerevisiae* that can be added to water and autoclaved without the need to make concentrated solutions of vitamins, trace elements, salts, or carbon sources. This powder drop-out base formulation is called DOB Medium, or DOBA (with agar). A complete supplemented synthetic defined medium is easily made by mixing two powders, DOB (or DOBA) and CSM (Complete Supplement Mixture). The formulation of CSM is a drop-out supplement for virtually all strains of *S. cerevisiae* containing different combinations of amino acids, adenine and uracil. Cells grow vigorously in DOB or DOBA supplemented with CSM.

YPD: 20 g peptone, 10 g yeast extract, 20 g glucose per liter, pH 6.5 at 30 °C (rich medium)

YPD Agar: YPD with 17 g agar per liter

DOB (glu): 26.7 g/liter (1.7 g YNB, 5 g ammonium sulphate, 20 g glucose)

DOBA (glu): DOB (glu) with 17 g agar per liter

DOB (2% gal/1% raf): 36.7 g/liter (1.7 g YNB, 5 g ammonium sulphate, 20 g galactose, 10 g raffinose)

DOBA (2% gal/1% raf): DOB (gal/raf) with 17 g agar per liter

Grow'n'Glow Bacterial Media

LB Medium: 10 g tryptone, 5 g yeast extract, 10 g NaCl per liter

LB Agar: LB medium with 15 g agar per liter

Find out more about MoBiTec's lab-scale 2 L and 100 mL fermenters in our Lab Supplies Catalog!

Order Information

Order No.	Description	Amount
Bags for 0.5 liter medium each:		
4001-1	YPD broth bags	10 bags
4001-6	YPD broth bags	10 x 10 bags
4001-2	YPD agar bags	10 bags
4001-7	YPD agar bags	10 x 10 bags
3002-1	LB medium bags	10 bags
3002-6	LB medium bags	10 x 10 bags
3002-2	LB agar bags	10 bags
3002-7	LB agar bags	10 x 10 bags
Powder:		
4025-2	DOB 2% galactose/1% raffinose	0.5 lb (227 g)
4025-7	DOB 2% galactose/1% raffinose	2.2 lb (1 kg)
4026-2	DOBA 2% galactose/1% raffinose	0.5 lb (227 g)
4026-7	DOBA 2% galactose/1% raffinose	2.2 lb (1 kg)
4540-0	CSM -HIS -LEU -TRP -URA suppl.	10 g
4530-8	CSM -HIS -TRP -URA supplement	10 g
4520-3	CSM -HIS -URA supplement	10 g
4520-4	CSM -HIS -LEU supplement	10 g
4510-3	CSM -HIS supplement	10 g
4520-5	CSM -TRP -URA supplement	10 g
4511-2	CSM -URA supplement	10 g

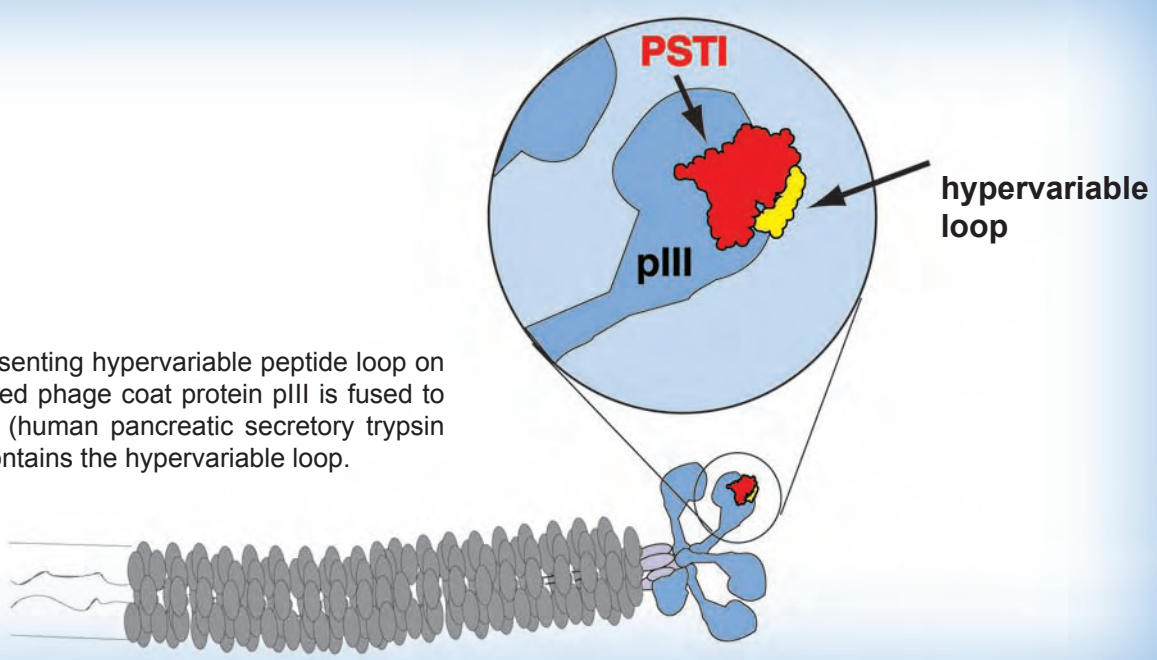
Molecular Evolution Inside your Lab

The pSKAN Phagemid Display System is a highly sophisticated screening tool that allows selection and production of novel peptides with the desired affinities to a given target molecule[1,2,3,4]. It is also a fast and easy-to-handle alternative to antibodies or immunoglobulin domain presenting phagemids. As the pSKAN Phagemid Display Libraries present a very small hypervariable amino acid loop, they can probe difficult to access areas on a protein. This is an advantage over many other types of display libraries for probing tight regions that matters, since many drugs recognize their targets, and enzymes their substrates, through binding interactions in deep pockets in the protein surface.

Features

- Easy-to-handle, fast and low-cost approach for high-throughput and high-content screening of protein-protein interactions
- Direct physical link between genotype and phenotype
- Monovalent display of specific epitopes
- No need for subcloning, no tedious cell culture or animal experiments
- Multiple applications in research, diagnostics and drug discovery, e.g. screening for high-affinity binders, ligands, inhibitors[5], structure-based protein design

Patented!



pSKAN phage presenting hypervariable peptide loop on its surface. Enlarged phage coat protein pIII is fused to the protein hPSTI (human pancreatic secretory trypsin inhibitor), which contains the hypervariable loop.

The pSKAN phagemid vectors combine genetic elements from bacterial plasmid and bacteriophage, uses the ColE1 plasmid origin for replication in *E. coli* and the phage fd origin for production of single stranded DNA upon superinfection with the helper phage M13. The phagemid DNA is then packaged into the phage coat, creating a new phagemid generation with pSKAN-DNA. A hybrid protein consisting of a fusion between one of the M13 phage coat proteins (pIII) and the human pancreatic secretory trypsin inhibitor (hPSTI) is expressed from the phagemid DNA. Statistically, only one hybrid protein is integrated in every second phagemid. Thus, a population of phages is produced, which monovalently displays the hybrid protein with the hypervariable loop and which contains pSKAN phagemid DNA.

Based on three sets of combinatorial phagemid libraries expressed in *E. coli*, the phage coat protein pIII is fused to human pancreatic secretory trypsin inhibitor protein (hPSTI), which contains a short stretch of hypervariable amino acids. This loop is exposed and displays about 3 x 10^7 randomly recombined amino acid variants, an least two orders of magnitude larger number of variants than immunoglobulin phagemids.

In repeated cycles of selection and amplification, a so-called ”panning assay”, the phagemid system produces a combinatorial library containing millions of site-specific mutant proteins that are screened for specific binding to a target molecule of interest. Variants with optimal affinity are selected and propagated in *E. coli*. The sequence of the binding domain can be determined easily by DNA sequencing. In comparison to antibodies, the pSKAN System is able to target very small epitopes, since it searches for binding sites with „only a fingertip“ instead of „two whole hands“ like antibodies. Within a period of only three weeks, the scientist is able to get the target molecule of interest.

References

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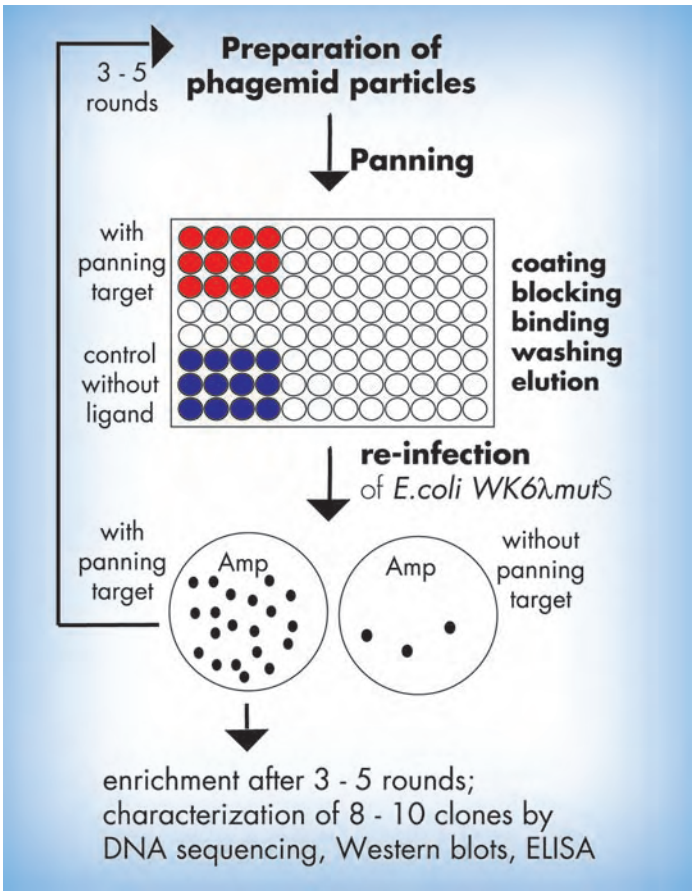

Panning with microplates. One well per library (HyA, HyB, HyC) for each reaction condition (# 1 - 4) is coated with the ligand of interest. After 3 - 5 rounds an increase in the pSKAN phagemid titer is observed. The control without panning target shows the background of unspecifically binding phagemids. (Amp, ampicillin.) Note: Since M13 derived phagemids do not lyse the cells, colonies and not plaques are counted!

Order Information

MoBiTec provides three pSKAN libraries which differ in the extent of their hypervariable loop: HyA, HyB and HyC with 8, 7 and 6 variable amino acids, respectively. To determine the sequence of a selected hypervariable loop, two sequencing primers are available. Antibodies against pIII and hPSTI can be obtained for Western blots. For the production of soluble hPSTI protein with the desired binding properties, the vector pMAMPF3-PSTI4 can be used. To construct your own peptide library, pSKAN8 vector DNA is available.

Order No.	Description	Amount
PSKAN	pSKAN Phagemid Display System:	
	HyA, HyB, HyC pSKAN libraries	3 x 1 mL
	host strain	1 mL
PS1255	18mer-sequencing primer 1255	500 pmole
PS2897	19mer-sequencing primer 2897	500 pmole
PSKAN3	Anti-pIII antibody (monoclonal mouse IgG1)	100 µL
PSKAN2	Anti-hPSTI antibody (monoclonal mouse IgG1)	100 µL
PSKAN8	pSKAN8 vector, lyophilized DNA	5 µg
PSKAN4	pMAMPF3-PSTI4 vector, lyophilized DNA	5 µg

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The pSKAN Phagemid Display System has been developed by Prof. Dr. J. Collins and Dr. P. Röttgen at the GBF Braunschweig, Germany (US patent no. 5,925,559). Use of the pSKAN Phagemid Display System is covered by U.S. Patents 5,223,409, 5,403,484, 5,571,698 & 5,837,500; and EPO Patent 436,597. Any commercial use of the pSKAN Phagemid Display System, including the discovery or development of commercial products, requires a license from Dyax Corp.

PCR is a process covered by patents owned by Hoffmann-La Roche. Use of this process requires a licence.

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MOLECULAR SCREENING

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