GROW'N'GLOW:
THE GFP TWO-HYBRID SYSTEM

Order #
GNGK01/GNGK02

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

The Grow'n'Glow system was developed by Dr. Robert S. Cormack and Dr. Imre E. Somssich at the Max-Planck-Institut für Züchtungsforschung, Cologne, Germany. The system is patented.

General knowledge: Users of the kit should be familiar with basic molecular biology and microbiological techniques!

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1. Introduction

1.1. General

The yeast two-hybrid system[1] or interaction trap[2] has rapidly become a widely used technique in molecular biology. It is now the method of choice to identify protein-protein interactions from either cDNA libraries or known gene sequences[3]. The method relies on the transactivation of reporter genes in *Saccharomyces cerevisiae* to identify positive interactions. Growth selection via activation of either a HIS3 or LEU2 reporter gene is used in conjunction with a second reporter gene, such as LacZ, which expresses β-galactosidase. The use of two reporter genes enables discrimination of false positives which activates only the auxotrophic marker. Yeast colonies that survive the growth selection scheme are chosen, re-streaked and tested for expression of the LacZ gene by means of a filter assay or growth on minimal medium plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). This latter step can often be time-consuming depending on the number of primary transformants obtained.

In order to simplify and accelerate the screening process of the two-hybrid systems, the new reporter plasmid pGNG1[4] was constructed that utilizes the green fluorescent protein (GFP) from the bioluminescent cnidarian Aequorea victoria[5].

1.2. Background

The Grow'n'Glow system is a LexA-based version of the yeast two-hybrid system originally developed by Fields and Song[1]. The yeast two-hybrid system has proven to be a powerful tool for identifying proteins from an expression library which can interact with one's protein of interest. The technology is based on the fact that many eukaryotic transcriptional activators, such as GAL4, consist of two physically separable modular domains, one DNA-binding domain and one transcriptional activation domain. In order to activate transcription, they have to be physically tethered to one another, and neither of them can activate transcription on its own. The same basic idea is followed in LexA/B42-based yeast two-hybrid systems. Here, the two proteins LexA and B42 (acid blob) are used as DNA-binding and transcriptional activation domain, respectively. The bacterial source of these proteins reduce the risk of false positives due to endogenous yeast protein binding to one of them. For this reason, LexA-based systems are often the method of choice. The conventional reporter gene generally used with yeast two-hybrid systems is β-galactosidase.

The Grow'n'Glow System was developed in co-operation with the Max-Planck Society as a more versatile and more accurate version of the yeast two-hybrid system, since it is using the reporter gene GFP. The practical advantages of this fluorescent protein over β-galactosidase are substantial. In a typical β-Gal screen, individual surviving yeast colonies are picked and then tested for β-galactosidase activity either by a filter assay or growth on minimal medium plates containing X-Gal. Both procedures are often labor-intensive, especially when hundreds of yeast colonies are obtained. In contrast, when pGNG1 is used as the reporter plasmid, the selection plates containing the yeast colonies are simply placed under a UV lamp (without the lid!) and positives become immediately identifiable by their green fluorescence. The Grow'n'Glow System is patented. In summary, pGNG1(see chapter 4.2.1.) is a valuable addition to the family of reporter plasmids for two-hybrid systems and makes a secondary screening of yeast colonies faster and more cost-effective than conventional LacZ assays. Detection of

protein-protein interactions via the green fluorescent protein provides global screening of colonies without bias and can be adapted to most yeast-based two-hybrid strategies.

1.3. GFP

The GFP of the jellyfish *A. victoria* is activated in vivo by an energy transfer via the Ca^{2+}-stimulation of the photoprotein aequorin[6]. The blue light generated by aequorin excites GFP and results in the emission of green light. GFP itself consists of 238 amino acids (Mr = 27 kDa) and is synthesized as an apoprotein in which post-translational formation of the chromophore occurs in an O2-dependent manner independent of any other gene products[7, 5]. It maximally absorbs light at 395 nm and has an emission peak of 509 nm. The nonsubstrate requirement for GFP activity makes this protein an attractive reporter for gene expression studies and this utility was initially demonstrated in both prokaryotes (*Escherichia coli*) and eukaryotes (*Caenorhabditis elegans*)[7]. It has subsequently been used to monitor gene expression in many organisms including mouse[8], Drosophila[9], zebrafish embryos[10], Arabidopsis[4] and yeast[11]. In addition to the non-invasiveness of GFP detection (long-wave UV light) the protein is very stable, non-toxic and resistant to photobleaching.

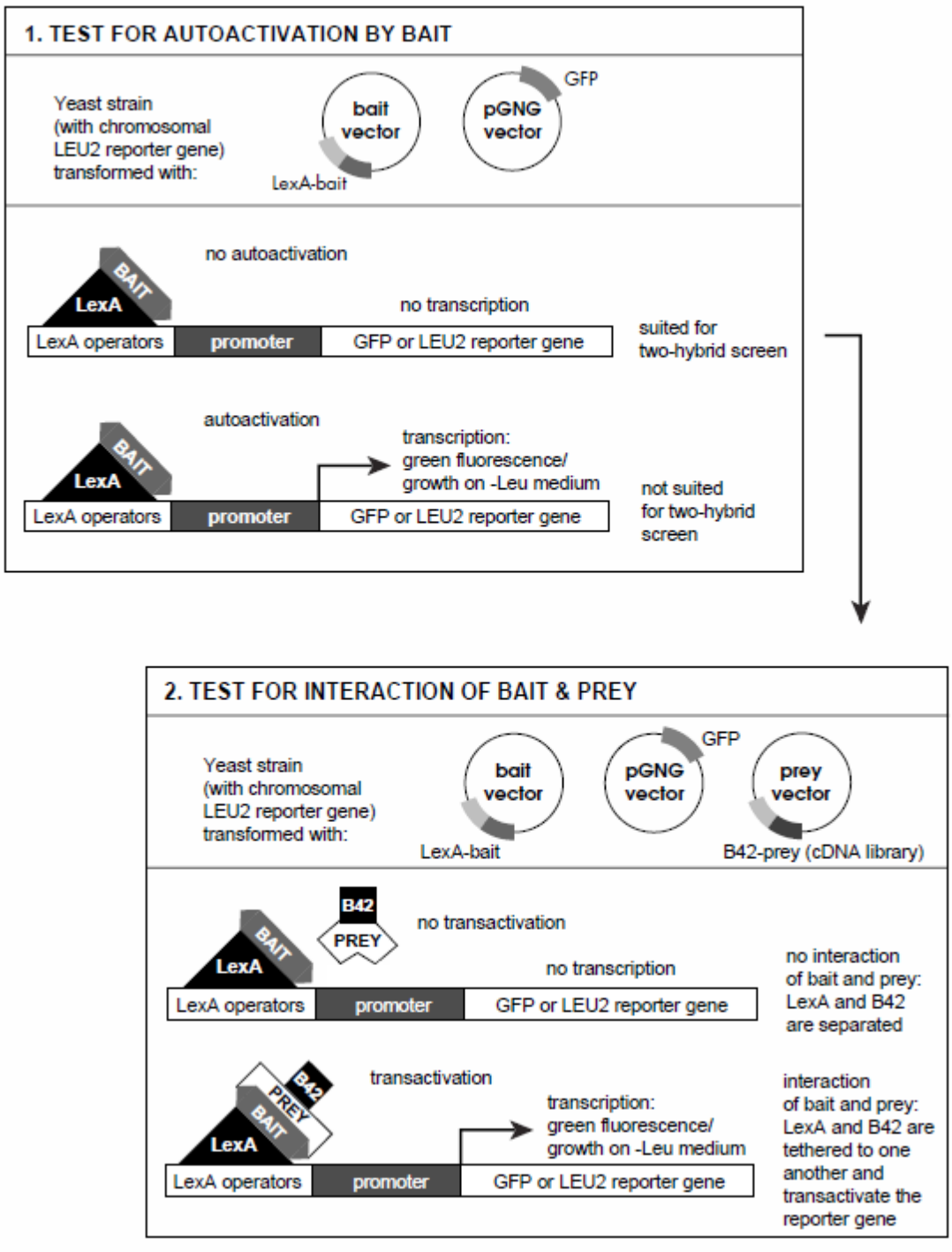
These properties make GFP a viable alternative to traditional reporter genes such as β-galactosidase (LacZ), β-glucuronidase (GUS), chloramphenicol acetyl transferase (CAT) or firefly luciferase which require substrate for their detection. Several modifications of the wild-type GFP cDNA have been engineered with optimized codon usage, improved fluorescence activity and red-shifted variants with altered excitation maxima intended for fluorescence microscopy[5]. The *A. victoria* GFP variant GFPuv is optimized for maximal fluorescence by UV-light excitation making it fluoresce 18 times brighter than wildtype GFP while retaining identical excitation and emission wavelength maxima.

2. Advantages of the Grow'n'Glow Two-Hybrid System

- protein-protein interaction is detected directly by visualisation under UV light
- greatly reduces amount of time & effort needed to screen a cDNA library
- allows immediate inspection of yeast colonies for gene activation
- non-invasive, chemical-free and cost-free assay
- GFP may be used as sole reporter gene
- higher threshold for detection of protein-protein interactions
- can be integrated simply into pre-existing LexA bait-based two-hybrid systems
- stable GFP expression
- using prokaryotic (LexA and B42) rather than eukaryotic (GAL4) proteins reduces number of false positives
- inducible expression of library fusion proteins (with galactose) reduces potential toxicity problems
- antibodies to HA-tag (fused downstream of B42) permit simple coimmunoprecipitation assay of bait and potential positive
- yeast strains with varying LEU2 sensitivities reduce problems associated with bait autoactivation

Legend to flow chart on page 7: Identification of molecular interactions with the *Grow'n'Glow GFP Two-Hybrid System*. To test whether the bait protein activates the reporter genes on its own ("autoactivation"), only the bait vector and the reporter vector are transformed into yeast. Clones growing on -LEU and/or fluorescing green under UV-light are not suitable for a two-hybrid screen. Clones which do not grow on -LEU medium and which do not fluoresce green, can be further tested for interaction of a bait and a prey protein in a two-hybrid screen. For this purpose, the bait vector, reporter vector and prey vector (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). Note: LEU2 can only be transcribed, if the B42-prey fusion protein is expressed. The expression of the latter is driven by the galactose-inducible GAL1 promoter. As a consequence, growth on galactose medium is essential for reporter gene transcription.

3. Schematic Overview of the Grow'n'Glow System



Legend see page 6

4. Kit Components: "Complete Kit" & "Basic Kit"

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.

Grow'n'Glow Two-Hybrid System **"Complete Kit":**

Vectors*		Primers*
pGNG1	5 µg	5'-BAITprimer 500 pmole
pEG202	5 µg	5'-PREYprimer 500 pmole
pJG4-5	5 µg	3'-PREYprimer 500 pmole
pEG202-p53	5 µg	
pJG4-5-LTA	5 µg	
pEG202-GAL4	8 µg	

Host Strain	
Yeast strain EGY48	1 ml
Yeast strain EGY194	1 ml
Yeast strain EGY188	1 ml

Grow'n'Glow Two-Hybrid System **"Basic Kit":**

Vectors	
pGNG1	5 µg
pEG202-p53	5 µg
pJG4-5-LTA	5 µg
pEG202-GAL4	5 µg

(Note: This kit comes without primers or host strains)

Before use, redissolve plasmid DNA in TE buffer (pH 8).

Order information see chapter 11.

NOTE: You may not require all of the components listed below. Read the manual carefully to determine which components will best suit your needs!

4.1. Yeast Strains

The provided yeast strains have the following genotypes:

High sensitivity strain:
S. cerevisiae EGY48[4]: MATα, trp1, his3, ura3, leu2::6 LexAop-LEU2

Medium sensitivity strain:
S. cerevisiae EGY194: MATα, trp1, his3, ura3, leu2::4 LexAop-LEU2

Low sensitivity strain:
S. cerevisiae EGY188: MATα, trp1, his3, ura3, leu2::2 LexAop-LEU2

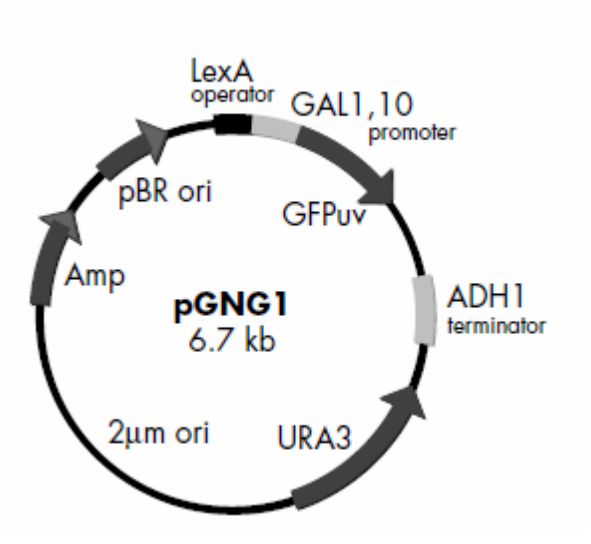
The strains differ in the genome-integrated LEU2 reporter gene which contains two to six copies of the LexA operator upstream the promoter, allowing growth selection via activation by LexA/B42 as well as a test for autoactivation of a reporter gene by the bait protein. The number of LexA copies is directly related to the sensitivity of the strain.

See chapter 7 for growth and maintenance of yeast.

4.2. DNA Vectors

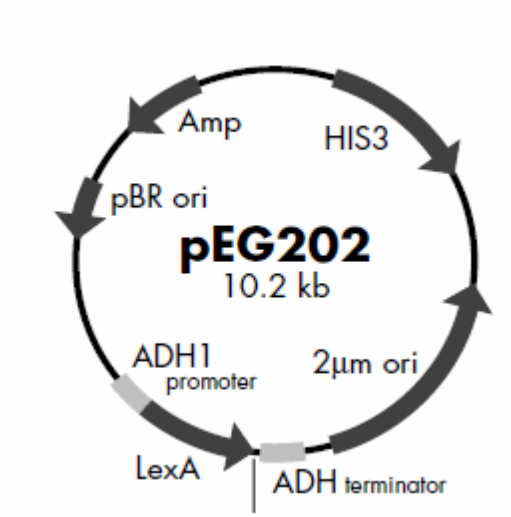
4.2.1. GFP Reporter Plasmid pGNG1[4]

The reporter plasmid is suited for twohybrid screens with the reporter gene GFP. This GFPuv gene is driven by a GAL1,10 minimal promoter containing eight binding sites for LexA (four LexA operators) upstream. Please note that this promoter is not galactose inducible. The 6.7 kb plasmid contains a URA3 selectable marker and a 2 µm origin to allow propagation in yeast, and the ampicillin resistance gene (Amp) and pBR origin of replication (ori) for propagation in *Escherichia coli.*



4.2.2. Bait Plasmid pEG202

The bait plasmid pEG20212 is used to generate fusions of LexA with a bait (target) protein of interest. Fusion protein expression is controlled by the strong, constitutive yeast ADH1 promoter. For selection in yeast, the vector contains the HIS3 (histidine) marker and the 2 µm origin of replication; for propagation in *E. coli* an ampicillin resistance (Amp) and pBR origin are present.



cloning site for bait:
EcoR I, BamH I, Sal I, Nco I, Not I, Xho I, Sal I
(with p53 or GAL4 in bait control vectors)

pEG202 polylinker:

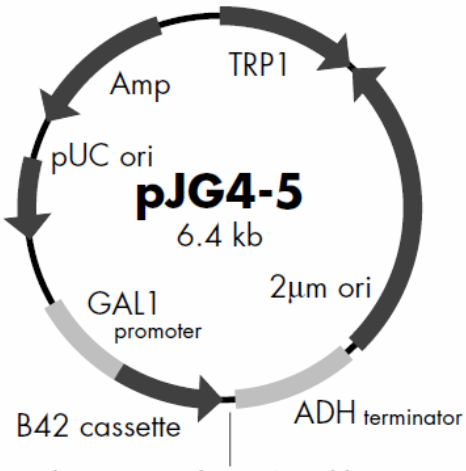
5'- -3'
GAA TTC CCG GGG ATC CGT CGA CCA TGG CGG CCG CTC GAG TCG ACC TGC AGC

EcoR I BamH I Sal I Nco I Not I Xho I Sal I

Polylinker of the vector indicating the open reading frame. The shown restriction endonucleases are only cutting the polylinker (single cutters).

4.2.3. Prey Plasmid pJG4-5

The prey plasmid pJG4-5[12] is used to express cDNAs or other coding sequences inserted into the EcoR I and Xho I sites as translational fusions to a cassette consisting of the SV40 nuclear localization sequence, the 88-residue acidic activator B42 (acid blob) and the HA (hemagglutinin) epitope tag. Fusion protein expression is controlled by the GAL1 inducible promoter, thus, transcription levels are very low in the presence of glucose and high with galactose. For selection in yeast, the vector contains the TRP1 (tryptophan) marker and the 2 µm origin of replication; for propagation in *E. coli* an ampicillin resistance (Amp) and pUC origin are present.



cloning site for cDNA library:
EcoR I, Xho I
(with LTA in prey control vector)

pJG4-5 polylinker:

5'-CCC GAA TTC GGC CGA CTC GAG AAG-3'

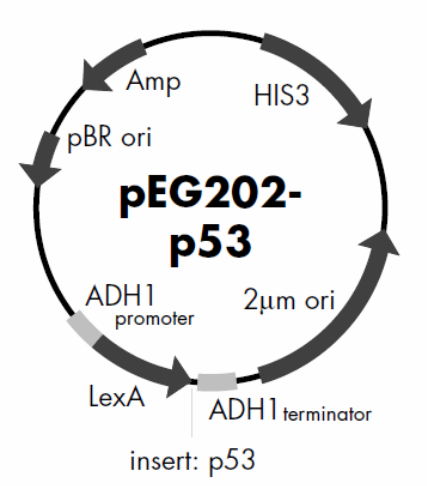
EcoR I Xho I

Polylinker of the vector indicating the open reading frame. The shown restriction endonucleases are only cutting the polylinker (single cutters).

4.2.4. Control Plasmids

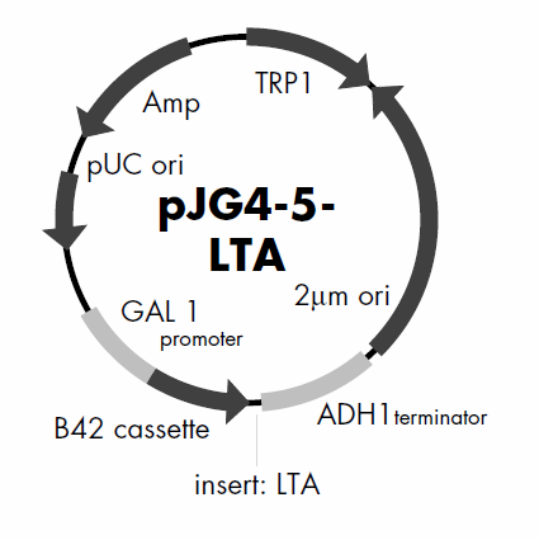
Bait control:

The bait control plasmid pEG202-p53 consists of pEG202 and the mouse p53 (amino acid # 72-390) sequence fused to the LexA DNA-binding domain. pEG202-p53 serves as positive control in combination with the prey control vector pJG4-5-LTA which carries the gene for LTA, a protein known to interact with p53.



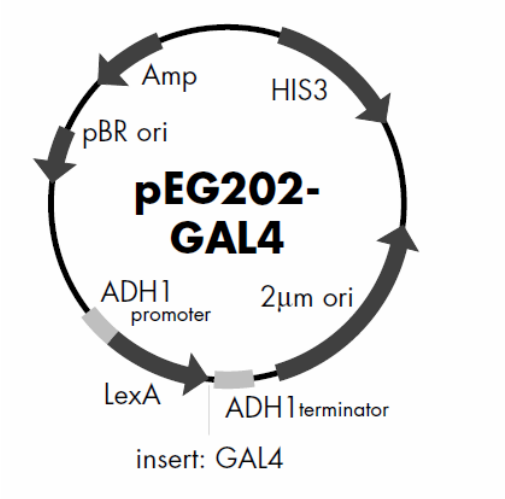
Prey control:

The control plasmid pJG4-5-LTA consists of pJG4-5 and the sequence of the large-T antigen (amino acid # 84-708) fused to the B42 activation domain.



Positive control:

pEG202-GAL4 consists of pEG202 and the GAL4 activation domain sequence fused to the LexA DNA-binding domain. This positive control is able to activate transcription of the GFP reporter gene by itself.



4.3. Primer

5'-BAITprimer:

5'-CGT CAG CAG AGC TTC ACC-3'

(5' forward primer for sequencing the junction between LexA and the bait gene of pEG202).

5'-PREYprimer:

5'-CTG AGT GGA GAT GCC TCC-3'

(5' forward primer used to determine the reading frame and identity of positive clones in pJG4-5; can be used with 3' PREYprimer to amplify clone by PCR).

3'-PREYprimer:

5'-GCC GAC AAC CTT GAT TG-3'

(3' primer used to determine the identity of positive clones in pJG4-5; can also be used with 5' PREYprimer to amplify a clone by PCR).

5. Materials Required, but not Supplied

Note: The specific materials listed below are the ones we have tested in the *Grow'n'Glow System*. For order information see chapter 11. Similar items from other sources may be interchangeable.

1. *Grow'n'Glow Yeast Growth Media* (see chapter 5.1.1.)
2. *Grow'n'Glow Bacterial Growth Media* (see chapter 5.1.2.)
3. Ampicillin, Roche Molecular Biochemicals order # 835269
4. For yeast transformations:
   Grow'n'Glow High Efficiency Yeast Transformation Kit (MoBiTec order # 2200-1) or, alternatively, *Grow'n'Glow Fast and Easy Yeast Transformation Kit* (MoBiTec order # 2100-1)
5. *Grow'n'Glow Yeast Plasmid Isolation Kit* (MoBiTec order # 2069-1)

5.1. Recipes for Media

For optimal results, we highly recommend to use the *Grow'n'Glow Yeast* and *Bacterial Growth Media* offered by MoBiTec (see chapter 5.1.1. and 5.1.2.) which are optimized for two hybrid systems. Rich medium YPD and the standard Wickerham yeast nitrogen base with carbon source optimized for *S. cerevisiae* can be added to water and autoclaved without the need to make concentrated solutions of vitamins, trace elements, salts or carbon sources. The powder dropout base formulation which is called DOB (Drop Out Base) medium or DOBA (Drop Out Base with Agar) is a complete standard Wickerham yeast nitrogen base with a carbon source. 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 dropout supplement for virtually all strains of *S. cerevisiae* containing different combinations of amino acids, adenine and uracil. Cells grow vigorously in DOB supplemented with CSM.

These media are very easy to handle and are delivered as powder. Some of our media are available in small bags with the appropriate amount of powder for 0.5 litre medium (10 bags per package are sufficient for 10 x 0.5 litre medium). Just add water and autoclave - ready!

For order information see chapter 11.

5.1.1. *Grow'n'Glow Yeast Growth Media*

Notes:
DOB = Drop Out Base
DOBA = Drop Out Base with Agar
"-URA" signifies: medium lacks uracil
"-TRP" signifies: medium lacks tryptophan
"-LEU" signifies: medium lacks leucine
"-HIS" signifies: medium lacks histidine

a)YPD and YPD agar (rich medium; 20 g peptone, 10 g yeast extract, 20 g glucose, pH 6.5, 17 g agar per litre):

YPD broth:
Pour the entire content of a YPD broth bag (MoBiTec order # 4001-1) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to at least 37 °C. Ready for use.

YPD agar:
Pour the entire content of a YPD agar bag (MoBiTec order # 4001-2) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates.
Ready for use.

b)YNB -URA -HIS -LEU -TRP (selective medium) with glucose or galactose/ raffinose:

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

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

DOBA:
DOB (glu or gal/raf) with 17 g agar per liter

CSM (Complete Supplement Mixture):
The formulation of CSM is a dropout 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.

DOB -URA -HIS -LEU -TRP (glucose):
Pour the entire content of a DOB glucose bag and 0.3 g CSM -HIS -LEU -TRP -URA (MoBiTec order # 4540-0) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to at least 37°C. Ready for use.

DOBA -URA -HIS -LEU -TRP (glucose):
Pour the entire content of a DOBA glucose bag and 0.3 g CSM -HIS -LEU -TRP -URA (MoBiTec order # 4540-0) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOB -URA -HIS -LEU-TRP (galactose/raffinose):
Pour 18.4 g of DOB gal/raf (MoBiTec order # 4025-2) and 0.3 g CSM –HIS -LEU -TRP -URA (MoBiTec order # 4540-0) into a 0.5 l flask, add 500 ml H_2O.
Autoclave. Cool to at least 37 °C. Ready for use.

DOBA -URA -HIS -LEU -TRP (galactose/raffinose):
Pour 26.9 g DOBA gal/raf (MoBiTec order # 4026-2) and 0.3 g CSM -HIS -LEU -TRP-URA (MoBiTec order # 4540-0) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOB -URA -HIS -TRP (glucose):
Pour the entire content of a DOB glucose bag (MoBiTec order # 4025-1) and 0.35 g CSM -URA -HIS -TRP (MoBiTec order # 4530-8) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to at least 37 °C. Ready for use.

DOBA -HIS -LEU (galactose/raffinose):
Pour 26.9 g DOBA gal/raf (MoBiTec order # 4026-2) and 0.335 g CSM -HIS -LEU (MoBiTec order # 4520-4) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOBA -URA -HIS (galactose/raffinose):
Pour 26.9 g DOBA gal/raf (MoBiTec order # 4026-2) and 0.375 g CSM -URA -HIS (MoBiTec order # 4520-3) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOBA -HIS (galactose/raffinose):
Pour 26.9 g DOBA gal/raf (MoBiTec order # 4026-2) and 0.385 g CSM-HIS (MoBiTec order # 4510-3) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOBA -HIS (glucose):
Pour 21.85 g DOBA glu and 0.385 g CSM-HIS (MoBiTec order # 4510-3) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

DOBA -URA (glucose):
Pour 21.85 g DOBA glu and 0.385 g CSM-URA (MoBiTec order # 4511-2) into a 0.5 l flask, add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

5.1.2. Grow'n'Glow Bacterial Growth Media

a) LB Medium:
Pour the entire content of an LB medium bag (MoBiTec order # 3002-1) into a 0.5 l flask add 500 ml H_2O. Autoclave. Cool to at least 37 °C. Ready for use.

b) LB Agar Medium:
Pour the entire content of an LB agar medium bag (MoBiTec order #3002-2) into a 0.5 l flask add 500 ml H_2O. Autoclave. Cool to 55 °C. Pour into plates. Ready for use.

c) LB Amp Medium (ampicillin selection):
Cool the LB medium (a, above) to 37 °C and add 4 ml of 25 mg/ml ampicillin (in distilled water, filter-sterilized) per litre of medium. Mix.

d) LB Amp Agar Medium (ampicillin selection):
Cool the LB agar medium above to 55 °C and add 4 ml of 25 mg/ml ampicillin (in distilled water, filter-sterilized) per litre of medium. Mix. Pour into plates.

Recipes for preparing *E. coli* growth media are described in Sambrook *et al*. (1989)[13] and Ausubel *et al*. (1997)[14], recipes for the preparation of yeast growth media in Ausubel *et al*. (1997)[14] and Guthrie and Fink (1991)[15].

6. Grow'n'Glow cDNA Libraries

For your convenience, several pre-made *Grow'n'Glow* cDNA Libraries from different sources (e.g. human, mouse, rat, *S. cerevisiae, C. elegans, D. melanogaster*) which have been constructed in pJG4-5 (for vector details see chapter 4.2.3) are provided by OriGene. A complete list of the libraries presently available can be found on their web site (www.origene.com). The cDNA they used for the libraries was made by oligo d(T) priming. It was cloned unidirectionally between the EcoR I and Xho I sites of pJG4-5. These libraries are provided as 100 µg ready-to-use plasmid DNA and, in addition, as frozen *E. coli* glycerol stocks fo rther propagation of the library.

7. Growth and Maintenance of Yeast

The three yeast strains (*Saccharomyces cerevisiae*; see chapter 4.1.) in our "Complete Kit" are provided in YPD medium with 20% glycerol and can be maintained indefinitely at -80 °C. Streak the yeast strains (do not thaw the strains ever) on YPD plates. Start cultures from single colonies and grow in YPD medium at 30 °C for 2-3 days. General remarks: Yeast can be grown on plates or in liquid culture, like *E. coli*. However, antibiotics, which work on *E. coli*, do not work on yeast, making good sterile technique mandatory when working with yeast. The optimum growth temperature for yeast is 28 – 32 °C. The growth rate is relatively rapid, with a doubling time of 90 - 120 minutes. Budding yeast is very amenable to genetic and molecular biological methods due to its ability to be transformed by foreign DNA and its highly efficient system of homologous recombination.

8. *Grow'n'Glow Two-Hybrid System* Protocol

8.1. Constructing the Hybrid Gene LexA-Bait

Using standard recombinant DNA techniques, subclone your bait protein gene in the correct orientation into the polylinker of pEG202 (see chapter 4.2.2.). Design the subcloning of the bait gene such that it fuses in-frame with LexA. We strongly recommend verifying the sequence of the LexA-bait junction with the sequencing primer (5'BAITprimer) provided in the "Complete Kit" to make sure that a LexA-bait fusion protein is going to be expressed correctly.

Note: We highly recommend testing your bait fusion protein in the assays below before performing a full-scale library screen.

8.2. Autoactivation of LEU2

A two-hybrid system library screen does not have to be performed, if the bait protein activates the reporter genes on its own ("autoactivation"). Since LEU2 is the reporter used in the initial screen, it is important not to have a high background of colonies arising due to autoactivation of the LEU2 gene. Also, for some baits, the LEU2 reporter in EGY48 is more sensitive than the GFPuv reporter on pGNG1. Therefore, the ability of the bait to autoactivate the LEU2 reporter should be tested before performing a large screen. To test for autoactivation by your bait fusion protein, transform yeast strain EGY48 with the bait vector containing your bait gene in the correct reading frame.

8.2.1. Yeast Transformation Protocol

We recommend the *Grow'n'Glow High Efficiency Yeast Transformation Kit* (order # 2200-1). However, for the above described testing of autoactivation other transformation procedures can be used as well (see Appendix I).

a) Use the protocol supplied with the *Grow'n'Glow High Efficiency Yeast Transformation Kit* using 200 ng of plasmid DNA for transformation or the procedure described in Appendix I (up to step j).

b) Spread 50-100 µl of each transformation onto separate DOBA (glu) –HIS plates.

c) Incubate at 30 °C for 2-3 days.

d) Streak 4 colonies from each plate onto another DOBA (glu) -HIS plate.

e) Incubate at 30 °C 1-2 days.

f) Perform a LEU2 autoactivation assay as follows (see 8.2.2.):

8.2.2. Testing Procedure for LEU2 Autoactivation

a) Transfer a colony of EGY48 containing the bait plasmid into 0.5 ml of sterile distilled water.

b) Vortex.

c) Dilute 100 µl into 1 ml of sterile distilled water.

d) Vortex: this is "Dilution 1".

e) Do three more serial 1:10 dilutions ("Dilutions 2-4") such that:

if "Dilution 1" is considered "undiluted",
"Dilution 2" = 1:10 diluted,
"Dilution 3" = 1:100 diluted, and
"Dilution 4" = 1:1000 diluted.

f) Plate 100 µl of each of "Dilutions 1-4" onto DOBA (gal/raf) -HIS plates and onto DOBA (gal/raf) -HIS -LEU plates.

g) Incubate at 30 °C for 1-2 days. You should see colonies on the -HIS plates, but not on the -HIS -LEU plates.

Note: Galactose plates are used in this experiment since that is the carbon source that will be used during the LEU2 selection step of the large-scale screen. If you do obtain many colonies on the -HIS -LEU plates, then your bait is autoactivating and you should perform the assays again using your bait in strains EGY194 (medium sensitivity) and EGY188 (low sensitivity). If you do not obtain the expected results with one of these strains, you can try to subclone parts of your bait gene in order to delete those sections responsible for the autoactivation activity. If you are failing again to obtain the expected results with this control assay, you should not attempt a two-hybrid screening with the bait anymore.

Test	Plasmids	Growth
Autoactivation Test	Bait plasmid pEG202 with insert	?
Positive control	Control plasmid pEG202-Gal4	+
Negative control	Bait plasmid without insert	-

Table: Expected results of LEU autoactivation by the bait protein cloned into pEG202. Yeast is grown on DOBA (gal/raf) -HIS -LEU plates.

8.3. Autoactivation of GFP

A two-hybrid system library screen does not have to be performed, if the bait protein activates the reporter genes on its own. To test for autoactivation by your bait fusion protein, transform yeast strain EGY48 with the following combinations of vectors:

Plasmids	Plates	Expected Results
pEG202 (with insert) + pGNG1 (Test for GFP autoactivation)	DOBA (glu) -URA -HIS	No Fluorescence
pEG202 (without insert) + pGNG1 (Negative control)	DOBA (glu) -URA -HIS	No Fluorescence
pEG202 (with insert) + pGNG1 pJG4-5 (without insert) (Test for GFP autoactivation)	DOBA (glu) -URA -HIS -TRP	No Fluorescence
pEG202-Gal4 + pGNG1 (Positive control)	DOBA (glu) -URA -HIS	Fluorescence
pEG202-p53 + pGNG1 + pJG4-5-LTA (Positive control)	DOBA (gal/raf) -URA -HIS -TRP -LEU	Fluorescence
pEG202-p53 + pGNG1 + pJG4-5-LTA (Induction control)	DOBA (glu) -URA -HIS -TRP	No Fluorescence

Table: Expected results of testing for GFP autoactivation by the bait protein cloned into pEG202. * pJG4-5 is only induced on galactose/raffinose medium.

Expected results:

The colonies containing pEG202-GAL4 + pGNG1 or pEG202-p53 + pGNG1 + pJG4-5-LTA should emit green light, the colonies containing bait plasmid pEG202 (without insert) + pGNG1 (+ pJG4-5 without insert) should not fluoresce green, and the colonies containing bait plasmid pEG202 (with insert) + pGNG1 may or may not emit green light. If yeast colonies with your bait protein do not fluoresce green, then the bait does not autoactivate reporter gene expression and can be used for screening. If the clones containing your bait protein and the pGNG1 plasmid (and the "empty" pJG4-5) do emit green light in the above assay, then you must subclone only parts of the gene encoding your protein into bait plasmid pEG202 and test for a part that does not autoactivate. Once you are convinced that your bait fusion can bind to LexA operators without autoactivating either of the two reporter genes, then you are ready to perform a large-scale library screen. It is thought that LexA or a fusion of LexA and another protein can enter the nucleus passively by diffusion through nuclear pores (Brent and Ptashne, 1984; 1985)[16,17].

Note: For an unknown reason, some baits can autoactivate the reporter genes in a large-scale screen even when they did not autoactivate in small-scale tests.

8.4. Large-Scale Library Screen Protocol

This step is very critical. The number of recombinants transformed with the prey clones should be as high as possible. We therefore recommend the use of the *Grow'n'Glow High Efficiency Yeast Transformation Kit* (MoBiTec order # 2200-1) for obtaining the best results, since it contains an optimized library transformation procedure. Otherwise, you can try an up-scaling of the procedure (as described in Appendix II) with the following information:

To screen up to 10^6 independent clones you should use 100 µg library plasmid DNA for transformation of one litre yeast culture. When screening a prey library to find new proteins that interact with the bait protein, the bait and prey plasmids (see chapter 4.2.) can either be transformed into the yeast strain simultaneously or sequentially. We recommend the following strategy:

a) First introduce the pGNG1 and bait simultaneously and plate onto selective DOBA (glu•) -URA -HIS plates.

b) One yeast colony from the DOBA (glu•) -URA -HIS plates is re-streaked onto a DOBA -URA -HIS plate. One colony is then selected and propagated for transformation with the prey library (or one prey plasmid with your insert of interest).

c) Dilute 100 µl transformation suspension (see Appendix II j) in 900 µl sterile distilled water and spread the diluted suspension onto 10 DOBA (gal/raf) -HIS -URA -TRP -LEU plates (150 mm). Spread serial dilutions of the transformation suspension onto a DOBA (glu) -HIS - URA - TRP plate to determine the transformation efficiencies (see Appendix III, k-l).

Note: The transformation of the yeast strain with pEG202 and pGNG1 is performed as small-scale transformation and the library transformation as a large-scale transformation.

If you do not obtain colonies after transforming yeast cells simultaneously with bait and pGNG1 we recommend a sequential transformation with the bait plasmid first (selection on DOBA (glu) - HIS plates) and thereafter with pGNG1 (selection on DOBA (glu) - HIS -URA plates):

- Transform yeast cells with bait plasmid and select on DOBA (glu) -HIS plates.
- Transform cells containing bait plasmid in a second step with pGNG1 and select double-transformed cells on DOBA (glu) -HIS -URA plates.

8.5. Green Fluorescent Protein (GFP) Assay Protocol

The DOBA (gal/raf) -HIS -LEU -URA -TRP plates containing the putative positive colonies are exposed to standard UV-light in a darkroom. The colonies showing green fluorescence are putative (most probably) positive. These colonies are isolated by streaking them onto new DOBA (gal/raf) -HIS -LEU -URA -TRP plates (master plate) and by incubating them for 24-72 hours at 30 °C until colonies appear, which are then checked again for GFPuv expression under a UV-lamp. The colonies that appear positive at this point are tested for dependence of the prey protein on growth and GFPuv expression of these potential positive transformants (see 8.6.).

Note: Check colonies on plates (without lid!) for fluorescence using an UV handlamp.

Caution

When using ultraviolet (UV) radiation, it is important to protect yourself with a full-length UV-blocking face shield and other appropriate skin-covering garments. In order to avoid damage to the DNA, it is best to minimize the exposure time of the yeast to the UV light. The shortest duration possible is the best. Exposing any plate of yeast to UV for more than 2 minutes total does not provide reliable results. As much as possible, try to maintain sterile technique when working with yeast.

Disclaimer: MoBiTec is not responsible or liable for any harm or damage to any person or to any DNA as a result of exposure to UV radiation.

8.6. Elimination of False Positives

Since the expression of the target protein is dependent on galactose, any colonies which can activate LEU2 gene expression and grow in the presence of glucose on DOBA (glu) -HIS -LEU -URA -TRP medium are false positives and should not be further characterized. Colonies which can grow on DOBA (gal/raf) -HIS -LEU -URA -TRP medium (containing galactose), but that cannot grow on DOBA (glu) -HIS -LEU -URA -TRP medium (containing glucose) are potentially true positives and should be tested for GFPuv expression. Re-streak colonies from the master plates on the following types of plates:

• DOBA (glu) -HIS -URA -TRP -LEU (colonies on this plate are false positives)
• DOBA (gal/raf) -HIS -URA -TRP -LEU (colonies on this plate are putative positives)

Incubate at 30 °C for 1-3 days, until growth occurs.

Potential positive transformants will grow on the galactose/raffinose plates, but not on the glucose plates, and will emit green light under a UV-lamp.

Note: The chromosomal reporter gene LEU2 will only be transcribed upon binding of the B42/LexA complex. B42 is only expressed, if the GAL1,10 promoter on pJG4-5 is induced by galactose (see also chapter 4.2.3.).

Due to catabolite repression, the galactose medium has to be glucose•-free. Raffinose supports the switch from glucose to galactose metabolism.

8.7. Further Characterization of Putative Positives

Putative positive colonies are picked from the master plate and are inoculated in 3 ml DOB (glu) -HIS -TRP -URA medium and are grown overnight. Freeze 1 ml of every culture in 20% glycerol at -80 °C. If the number of potential positives is small (<50), then all should be recovered and further characterized. If >50 potential positives are obtained, then you should characterize the first 50 that arise and freeze the rest in 1 ml aliquots at -80 °C in 20% glycerol.

8.7.1. Plasmid Isolation from Yeast

To isolate DNA from the potential positives we recommend the *Grow'n'Glow Yeast Plasmid Isolation K*it to obtain best results (MoBiTec order # 2069-1).
Alternatively, you can use the procedure in Appendix III.

8.7.2. Transformation of *E. coli* with Plasmids Isolated from Yeast

Use a standard transformation procedure ($CaCl_2$ or electroporation procedures; Sambrook *et al*., 1989[13]; Ausubel *et al*., 1997[14]) or the protocol listed in Appendix IV:

Use 5 µl plasmid DNA (for *Grow'n'Glow Yeast Plasmid Isolation Kit* users) to transform *E. coli*, spread the transformation solution onto LB-Amp agar plates and incubate at 37 °C overnight. Colonies arising at this stage contain either the bait-, prey- or pGNG1 reporter plasmid.

8.7.3. Prey Plasmid Identification by PCR

Follow the protocol a) to f):

a) Inoculate 6 colonies from each plate in 2 ml LB-Amp medium. Grow overnight at 37 °C.
b) 5 µl culture are transferred to a PCR tube and centrifuged for 5 sec at maximal speed. Discard supernatant. Add 30 µl PCR-mix and mix.

PCR-mix:

20 pmole	3'-PREYprimer
20 pmole	5'-PREYprimer
3 µl	10 X dNTP (2.5 mM dNTP)
3 µl	10 X Taq DNA polymerase buffer
	Taq DNA polymerase (use appropriate amount as suggested by supplier)

H_2O up to 30 µl

c) Run the reaction in a thermal-cycler as follows:

1 cycle	2 min, 95 °C		
20 cycles	30 sec, 95 °C	30 sec, 60 °C	120 sec, 72 °C
1 cycle	5 min, 72 °C		

d) Load the PCR reactions on an agarose gel.Clones with an amplified fragment contain a prey plasmid with an insert of interest*.

e) Go back to the cultures (see point 8.7.3.a). Isolate plasmids from the cultures using the Grow'n'Glow Yeast Plasmid Isolation Kit (MoBiTec order #2069-1), which can also be used for bacterial plasmid purification. Alternatively, use another appropriate method for plasmid purification.

f) Sequence the prey plasmid insert with the 5'PREYprimer. Use the DNA sequence to search against a database of choice.

* Performing a PCR with the 5'-PREYprimer and 3'-PREYprimer using the prey plasmid (carrying no insert) as template results in a 125 bp DNA fragment.

8.8. Other Verification Methods of Protein-Protein Interaction

The protein-protein interaction should always be confirmed by an independent method. The *Grow'n'Glow GFP Two-Hybrid Kit* offers the possibility to use coimmunoprecipitation analysis as a verification method using the antibodies to the HA-tag (downstream of B42 in the prey vector pJG4-5) which are commercially available (MBL). If available, you could also use antibodies against your bait protein and then verify with the HA-tag antibody.

Moreover, you can also verify your results by switching cloning vectors (i.e. moving the library insert from the AD to the DNA-BD-vector and vice versa, and then repeat the two-hybrid assay) or using a different two-hybrid system (e.g. MoBiTec's ACE1-based Grow'n'Glow Two-Hybrid System, # ACE01).

Another possibility is generating mutations of your prey (by deletions, substitutions or frame-shift mutations of the gene) and assaying the relative strength of the interactions using the GFP reporter assay and/or growth selection on medium containing different concentrations of copper ions.

9. Literature

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10. Appendix

Appendix I: Small Scale Yeast Transformation Procedure

a) Grow a 5 ml culture of yeast in YPD medium at 30 °C with shaking (for 24 hours). Inoculate by picking a colony from a streaked EGY48 plate.

b) Measure the OD_{600} of a 1:10 dilution of the overnight culture. Calculate the OD_{600} of the 5 ml culture and use that to inoculate a 60 ml YPD culture to an OD_{600} = 0.1. Grow at 30 °C with vigorous shaking.

c) Once the OD_{600} = 0.5-0.7 (approximately 4-6 hours after inoculation), pellet the cells by spinning the culture at 1500 x g for 5 minutes. Resuspend in 20 ml of sterile distilled water, spin again at 1500 g, and resuspend the pellet in 5 ml of 1 X TE/LiOAc.

d) Spin again at 1500 g, and resuspend the pellet in 300 µl of 1x TE/LiOAc.

e) Boil the carrier DNA 3 times 5 minutes and quickly chill on ice. This is essential for obtaining a maximum efficiency of transformation.

f) Add 250 ng of each plasmid DNA (e.g. pEG202 + insert and pGNG1) to 25 µg of denatured carrier DNA (10 µl) and mix.

g) Add 50 µl resuspended cells from step d) to the DNA (10 µl) from step f).

h) Add 340 µl of 1x TE/LiOAc/PEG, mix by inversion, and put the tubes at 30 °C (with or without shaking) for 30 minutes.

i) Incubate at 42 °C (without shaking) for 15 minutes.

j) Add 600 µl sterile distilled water, spin at 1500 g in a microcentrifuge for 5 minutes, pour off the supernatant, and resuspend each pellet in 100 µl of sterile distilled water.

k) Dilute 10 µl of each transformation suspension in 990 µl sterile distilled water and spread 100 µl of this dilution onto separate appropriate selective DOBA plates (e.g. -HIS or -URA). Incubate at 30 °C for 2-3 days. Calculate the number of transformants obtained by counting the number of colonies on the plate. 100 colonies on the plate corresponds to an efficiency of 2 x 10^4/µg plasmid DNA (when transforming with e.g. 250 µg of bait and 250 µg of reporter plasmid).

l) Streak 4 colonies from each plate onto another DOBA (glu) -HIS -URA plate. Incubate at 30 °C 2-3 days. Perform a GFP assay with a UV-lamp in a darkroom.

Appendix II: Large-Scale Yeast Transformation Procedure

a) Grow a 30 ml culture of yeast in YPD medium at 30 °C with shaking (for 24 hours). Inoculate by picking a colony from a streaked EGY48 plate.

b) Measure the OD_{600} of a 1:10 dilution of the overnight culture. Calculate the OD_{600} of the 30 ml culture and use that to inoculate 2 x 500 ml YPD culture to an OD_{600} = 0.1. Grow at 30 °C with vigorous shaking.

c) Once the OD_{600} = 0.5-0.7 (approximately 4-6 hours after inoculation), pellet the cells by spinning the culture at 1500 x g for 5 minutes. Resuspend in 2 x 50 ml of sterile distilled water, transfer to two 50 ml sterile conical tubes, spin again, and resuspend the two combined pellets in 20 ml of 1 x TE/LiOAc.

d) Spin again at 1500 g for 5 minutes, and resuspend the pellet in 5 ml of 1 x TE/LiOAc.

e) Boil the carrier DNA 3 times for 5 minutes and quickly chill on ice. This is essential for obtaining a maximum efficiency of transformation.

f) Add 500 ng of each plasmid DNA to 25 µg of denatured carrier DNA (10 µl) and mix. (For large-scale transformation of yeast cells containing already bait plasmid DNA [pEG202 + insert] with library plasmid DNA use 1 µg of pJG4-5 based plasmid library DNA and 50 µg of carrier DNA. Do not use more than 1 µg of library DNA per tube since multiple plasmids can enter the same yeast cell and give confusing results in later analyses).

g) Add 50 µl resuspended cells from step d) to the DNA (10 µl) from step f).

h) Add 340 µl of 1x TE/LiOAc/PEG, mix by inversion, and put the tubes at 30 °C (with gentle or no agitation) for 30 minutes.

i) Incubate at 42 °C (without shaking) for 15 minutes.

j) Add 600 µl water to each tube, spin at 1500 g in a microcentrifuge for 5 minutes, pour off the supernatant, and resuspend each pellet in 100 µl of sterile distilled water.

k) Dilute 10 µl of each transformation suspension in 990 µl sterile distilled water and spread 100 µl of this dilution onto separate appropriate selective DOBA plates (e.g. -HIS -URA -TRP). Incubate at 30 °C for 2-3 days until colonies appear.

l) Calculate the number of transformants obtained by counting the number of colonies on the plate. 200 colonies on the plate corresponds to an efficiency of 2 $x10^4$/µg library DNA. Thus, 100 µg library DNA will yield 100 x 2 x 10^4 = 2 x 10^6 transformants). A saturating screen of a mammalian library requires at least 2 x 10^6 transformants.

m) Streak 4 colonies from each plate onto another DOBA (Glu) -HIS -URA plate. Incubate at 30 °C 2-3 days. Perform a GFP assay with a UV-lamp in a darkroom.

Note: For yeast transformation procedures especially electrotransformation (electroporation) see Ausubel et al. (1997)[14].

Materials:

10 X TE:	100 mM Tris-HCl (pH 7.5) 10 mM EDTA
10 X LiOAc:	1 M lithium acetate

Polyethylene Glycol: 50 % (w/v) Polyethylene Glycol-3350 (in H_20)

1 X TE/LiOAc:	Right before use, mix 1 part 10 X TE 1 part 10 X LiOAc 8 parts sterile distilled water
1 X TE/LiOAc/PEG:	Right before use, mix 1 part 10 X TE 1 part 10 X LiOAc 8 parts 50 % PEG-3350
Carrier DNA:	Sonicated salmon or herring sperm DNA (Sigma) 20 mg/ml (size < 10 kb)

Appendix III: Isolation of Plasmids from Yeast for *E. coli* Transformation

a) Grow a yeast colony in 2 ml DOBA (glu) -HIS -URA -TRP overnight or until culture shows abundant yeast growth.

b) Spin down 1.5 ml at 14,000 g for 1 minute.

c) Discard supernatant.

d) Resuspend in 200 µl lysis-buffer.

e) Add 100 µl of phenol and 100 µl of chloroform/isoamylalcohol (see below).

f) Add 0.3 g (~ 200 µl) glass beads (see below).

g) Vortex at maximal speed for at least 5 minutes.

h) Centrifuge at 14,000 g for 10 minutes.

i) Transfer supernatant carefully to fresh tube.

j) Add 8 µl 10 M ammoniumacetate. Mix.

k) Add 500 µl 96% ethanol.

l) Place at -20 °C for at least 30 minutes or 10 minutes at -70 °C.

m) Centrifuge at 14,000 g for 10 minutes.

n) Discard supernatant and dry pellet.

o) Resuspend in 20 µl sterile TE buffer.

Materials:

Lysis-buffer: 2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris-HCl, pH 8.0
1 mM EDTA

TE-buffer: 10 mM Tris-HCl, pH 7.5
1 mM EDTA

Phenol: Tris-saturated (pH 8.0)
Chloroform/Isoamylalcohol: 24:1 (v/v)
Glass-beads: acid washed 400 - 600 nm glass-beads; Sigma

Appendix IV: Competent E. coli Cells and Transformation Procedures

Competent cells:

a) Inoculate one colony of the *E. coli* strain in 2.5 ml SOB-medium (see below) and incubate over night at 37 °C.

Note: Use a freshly streaked plate with your *E. coli* strain of choice.

b) Incubate 250 ml SOB with 2.5 ml overnight culture. Incubate at 37 °C and 200 rpm.

c) Once an OD_{550} of about 0.4 to 0.5 is reached (after about 2 to 2.5 hours), place cells on ice for 15 minutes. It is very important to keep the temperature at 0 °C unless otherwise stated.

d) Pellet cells by centrifugation. Spin 10 minutes at 6,000 rpm (3,000 g) at 4 °C.

e) Discard the supernatant.

f) Resuspend in 80 µl ice-cold RF I-buffer (see below).

g) Place on ice for 20 minutes.

h) Spin 6,000 rpm for 10 minutes at 4 °C.

i) Discard the supernatant. Resuspend the pellet in 20 ml ice-cold RF IIbuffer (see below).

j) Aliquote the cells in 200 µl aliquots (Eppendorf tubes) on ice. Quick-freeze immediately in liquid nitrogen.

k) Place at -80 °C until use.

Materials:

SOB: 2% (w/v) Bacto Tryptone
0.5% (w/v) yeast extract
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$

RF I-buffer: 100 mM RbCl (rubidiumchloride)
30 mM KAc, pH 7.5
10 mM $CaCl_2$
15% (w/v) glycerol (87%)
Adjust to pH 5.8 with 0.2 M acetic-acid.
Add 50 mM $MnCl_2$.

RF II-buffer: 10 mM MOPS
10 mM RbCl
75 mM $CaCl_2$
15% (w/v) glycerol (87%)
Adjust to pH 6.8 with 1 M NaOH.

Transformation Procedure:

a) Thaw the frozen, competent cells at 4 °C and store on ice.
b) Add plasmid DNA.
c) Incubate on ice for 30-60 minutes.
d) Incubate in a water bath preheated to 42 °C for exactly 2 minutes.
f) Add 1 ml LB medium.
g) Incubate at 37 °C for one hour.
h) Plate the transformation solution onto selective media (LB medium supple mented with 100 µg/ml ampicillin).
i) Incubate overnight (or 8-16 hours) at 37 °C.

Note: For *E. coli* transformation procedures especially electrotransformation (electroporation) see Ausubel *et al*. (1997)[14] or Sambrook *et al*. (1989)[13]. Protocols for the isolation of plasmid DNA from *E. coli* can also be found in both manuals.

11. Order Information, Shipping and Storage

Order#	Product	Quantity
GNGK01	Grow 'n' Glow Two-Hybrid System 'Complete Kit'	
	pGNG1, lyophilized DNA	5 µg
	pEG202, lyophilized DNA	5 µg
	pJG4-5, lyophilized DNA	5 µg
	pEG202-p53, lyophilized DNA	5 µg
	pJG4-5-LTA, lyophilized DNA	5 µg
	pEG202-GAL4, lyophilized DNA	5 µg
	5'-BAITprimer	500 pmole
	5'-PREYprimer	500 pmole
	3'-PREYprimer	500 pmole
	Yeast strain EGY48, glycerol stock	1 ml
	Yeast strain EGY194, glycerol stock	1 ml
	Yeast strainEGY188, glycerol stock	1 ml
shipped on dry ice; vectors & primers stored at 4 °C, yeast strains stored at -20 °C		

Order#	Product	Quantity
GNGK02	Grow 'n' Glow Two-Hybrid System 'Basic Kit'	
	pGNG1, lyophilized DNA	5 µg
	pEG202-p53, lyophilized DNA	5 µg
	pJG4-5-LTA, lyophilized DNA	5 µg
	pEG202-GAL4, lyophilized DNA	5 µg
shipped at RT; stored at 4 °C		

Related Products

Order#	Product	Quantity
GNGK03	Grow 'n' Glow One-Hybrid System	Kit
2100-1	Grow 'n' Glow Yeast Fast & Easy Transformation Kit	200 transf.
2200-1	Grow 'n' Glow High Efficiency Yeast Transformation Kit	250 transf.
2069-2	Grow 'n' Glow Yeast Plasmid Isolation Kit	100 preps
ACE01	Grow'n'Glow ACE1 Two Hybrid System 'Complete Kit'	Kit

Grow'n'Glow Yeast and Bacterial Growth Media:

order #	description	amount
Bags for 0.5 litre medium each:		
4001-1	YPD broth bags (with 25 g for 0.5 litre medium each)	10 bags
4001-6	YPD broth bags	10 x 10 bags
4001-2	YPD agar bags (with 33.5 g for 0.5 litre medium each)	10 bags
4001-7	YPD agar bags	10 x 10 bags
3002-1	LB medium bags (with 12.5 g for 0.5 litre medium each)	10 bags
3002-6	LB medium bags	10 x 10 bags
3002-2	LB agar bags (with 20 g for 0.5 litre medium each)	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)
4510-3	CSM -HIS supplement	10 g
4511-2	CSM -URA supplement	10 g
4520-3	CSM -HIS -URA supplement	10 g
4520-4	CSM -HIS -LEU supplement	10 g
4520-5	CSM -TRP -URA supplement	10 g
4530-8	CSM -HIS -TRP -URA supplement	10 g
4540-0	CSM -HIS -LEU -TRP -URA supplement	10 g

Order numbers for Grow'n'Glow cDNA Libraries see Appendix V.

* DOB = Drop Out Base **DOBA = Drop Out Base with Agar

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