DupLEX-A Libraries

APPLICATION GUIDE

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PACKAGE CONTENTS AND STORAGE CONDITIONS

List of DupLEX-A™ cDNA Libraries

DupLEX-A™ cDNA Libraries	Insert	Cat. #	DNA (ug)	E. coli (mL) Glycerol
MDBK Cell (bovine kidney)	cDNA	DLBK100	100	1
C. elegans (adult)	cDNA	DLCE100	100	1
D. melanogaster (adult)	cDNA	DLDM100	100	1
Human Liver	cDNA	DLH100	100	1
Human Fetal Brain	cDNA	DLH101	100	1
WI-38 Cell Line (lung fibroblast cell line)	cDNA	DLH102	100	1
HeLa Cell Line	cDNA	DLH103	100	1
Human PBL (peripheral blood leukocytes)	cDNA	DLH104	100	1
Human Fetal Liver	cDNA	DLH105	100	1
Human Fetal Kidney	cDNA	DLH106	100	1
Human Prostate, Normal (pooled / 8 adults)	cDNA	DLH107	100	1
Human Prostate, Tumor (pooled / 8 adults)	cDNA	DLH108	100	1
LNCaP Cell (untreated)	cDNA	DLH109	100	1
LNCaP Cell (treated)	cDNA	DLH110	100	1
SKOV3 (ovarian cancer cell line)	cDNA	DLH111	100	1
MCF7 Cell (estrogen depleted)	cDNA	DLH112	100	1
MCF7 Cell (estrogen treated)	cDNA	DLH113	100	1
Human Adult Ovary	cDNA	DLH114	100	1
Jurkat T-cell Line	cDNA	DLH115	100	1
SKBR3 Cell (estrogen receptor negative)	cDNA	DLH116	100	1
MCF7 Cell (serum grown)	cDNA	DLH117	100	1
MG63 Cell Line (osteosarcoma cell line)	cDNA	DLH118	100	1
Mouse Brain	cDNA	DLM100	100	1
Mouse Spleen	cDNA	DLM101	100	1
Mouse Liver	cDNA	DLM102	100	1
Mouse Ovary	cDNA	DLM103	100	1
Mouse Prostate, Normal	cDNA	DLM104	100	1
Mouse Breast, Normal (lactating)	cDNA	DLM105	100	1
Mouse Breast, Normal (involuting)	cDNA	DLM106	100	1
Mouse Breast, Normal (Virgin)	cDNA	DLM107	100	1
Mouse Breast, Normal (pregnant, 12-day)	cDNA	DLM108	100	1
Mouse Embryo (whole, 19-day)	cDNA	DLM110	100	1
Mouse Skeletal Muscle	cDNA	DLM111	100	1
Rat Thymus	cDNA	DLR100	100	1
Rat Testis	cDNA	DLR101	100	1
Rat Brain	cDNA	DLR102	100	1
Rat Adipocyte (9 week old Zucker rat)	cDNA	DLR103	100	1
S. cerevisiae	genomic DNA	DLY100	100	1

Each DupLEX-A™ cDNA Library package contains:

1 tube of plasmid DNA (100 ug/ 100 ul) in TE buffer (10 mM Tris HCl, pH8.0, 1mM EDTA)
1 tube of transformed bacterial cells (1 ml) in 15% Glycerol

The above components are shipped with dry ice and should be kept at -80°C for storage. If properly stored, they will maintain activity for several years.

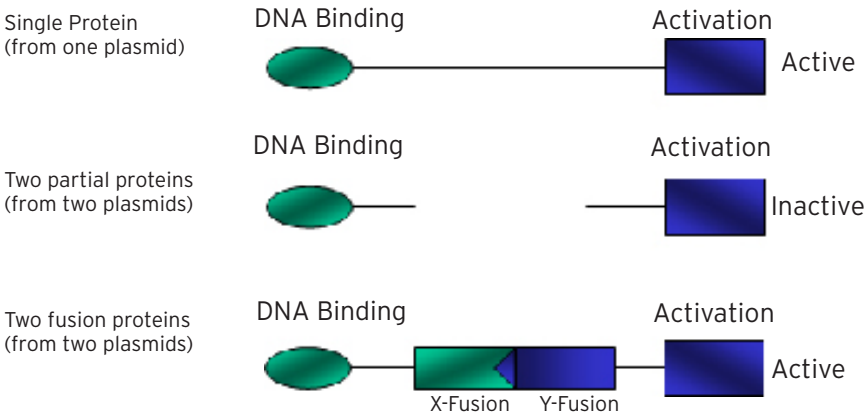
NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

Related Products
TrueClone cDNA clones http://www.origene.com/cdna/
HuSHTM shRNA Plasmids http://www.origene.com/rna/
Validated Antibodies http://www.origene.com/antibody/
Functional Proteins http://www.origene.com/protein/
Northern Blots http://www.origene.com/geneexpression/rna_blots/

Introduction

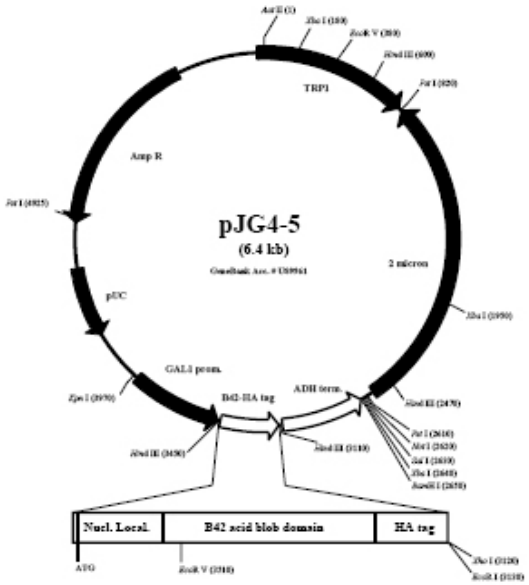
Yeast two-hybrid systems were designed for identifying and validating protein-protein interactions. Some transcriptional factors, such as Gal4, have two specific domains: a DNA binding domain and a transcriptional activation domain, which are flanked by a less specific region.

Figure 1: Schematic Diagram of the Yeast Two-Hybrid System



Fields and Song constructed two plasmids, one containing the Gal4 DNA-binding domain and the other the Gal4 activation domain. Yeast cells expressing the two truncated proteins lack Gal4 activation. Subsequently they made two more plasmids. One plasmid expresses a fusion protein containing the Gal4 DNA binding domain and a protein X, and the other expresses a fusion protein containing the Gal4 activation domain and a protein Y. Protein X and Protein Y are known to interact with each other. Yeast cells expressing both fusions now generate the Gal4 activity. Presumably the protein complex containing the two fusion proteins linked together through the X-Y interaction restored the Gal4 function. This finding established the base for testing whether two proteins interact using the system. To do so, one just needs to fuse one protein to the DNA binding domain and the other to the activation domain, and examine whether the cells with the two constructs can activate a reporter gene: in most case, beta-galactosidase. This system has been successfully used not only for testing protein-protein interaction in laboratories, but it has been also proven to be efficient in identifying novel proteins that interact with a known protein, by screening a cDNA library constructed using the binding domain-containing vector.

Vector information and cloning strategy



OriGene's yeast two-hybrid libraries are designed for researchers to identify proteins interacting with their proteins of interest. The libraries are made from mRNAs isolated from a broad range of tissues in the normal or diseased stage to increase the chances of a successful screen. The libraries are all constructed in the pJG4-5

vector (accession #: U89961). pJG4-5 is a standard target (library) plasmid used for inducible expression of B42-HA tag-target fusion protein in yeast. It contains the yeast selectable marker TRPI. The 2 um origin of replication produces a high copy number of plasmids in yeast. The B42-HA sequence encodes a peptide containing three major elements. A stretch of positive amino acids at the N-terminus serves as the nuclear localization signal for targeting the fusion protein to the nucleus. The B42 domain, when complexed with the DNA binding domain, activates transcription of a reporter gene. The HA tag is used for convenient detection of the fusion protein. EcoR I and Xho I sites near the c-terminus of B42-HA are used for subcloning a target gene. The entire coding sequence is directed under the GAL1 promoter and terminated by the ADH terminator. Gal1 promoter is a strong promoter in the presence of galactose but shows weak or no activity in the presence of glucose. This character allows the expression of some toxic proteins. There is a termination codon in all three reading frames to insure a proper translational termination. The pUC origin of replication is to maintain the plasmid in E. coli, and the ampicillin resistance gene is for subcloning selection in E. coli.

The B42 domain and included elements in pJG4-5:

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ATG GGT GCT CCT CCA AAA AAG AAG AGA AAG GTA GCT GGT ATC AAT AAA GAT ATC   54
 M   G   A   P   P   K   K   K   R   K   V   A   G   I   N   K   D   I    18
                         NLS
GAG GAG TGC AAT GCC ATC ATT GAG CAG TTT ATC GAC TAC CTG CGC ACC GGA CAG  108
 E   E   C   N   A   I   I   E   Q   F   I   D   Y   L   R   T   G   Q    36

GAG ATG CCG ATG GAA ATG GCG GAT CAG GCG ATT AAC GTG GTG CCG GGC ATG ACG  162
 E   M   P   M   E   M   A   D   Q   A   I   N   V   V   P   G   M   T    54

CCG AAA ACC ATT CTT CAC GCC GGG CCG CCG ATC CAG CCT GAC TGG CTG AAA TCG  216
 P   K   T   I   L   H   A   G   P   P   I   Q   P   D   W   L   K   S    72

AAT GGT TTT CAT GAA ATT GAA GCG GAT GTT AAC GAT ACC AGC CTC TTG CTG AGT  270
 N   G   F   H   E   I   E   A   D   V   N   D   T   S   L   L   L   S    90

GGA GAT GCC TCC TAC CCT TAT GAT GTG CCA GAT TAT GCC TCT CCC GAA TTC GGC  324
 G   D   A   S   Y   P   Y   D   V   P   D   Y   A   S   P   E   F   G   108
                     HA tag                              EcoR I
CGA CTC GAG AAG CTT TGG ACT TCT TCG CCA GAG GTT TGG TCA AGT CTC CAA ---
 R   L   E
     Xho I
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NLS: Nuclear localization signal
B42 fusion moiety molecular weight size:

- No insert: 163 amino acids (18,398 Dalton)
- With insert: 107 amino acids (11,835 Dalton) + insert

Compatibility

There are two major yeast two-hybrid systems frequently used in laboratories. One is the yeast Gal4-based, and the other is E.coli LexA protein-based. It has been demonstrated that the activation domains of the two proteins are interchangeable in terms of activating transcription of a reporter gene. Therefore the yeast two-hybrid libraries constructed in pJG4-5 can be used in both Gal4 and LexA systems as long as the yeast host can accommodate the Trp selectable marker in pJG4-5.

cDNA library construction

For each cDNA library construct, the starting materials are polyA+ RNA. First strand cDNAs were synthesized using a reverse transcriptase and oligo dT-Xho I linked primer. After synthesis of the second strand cDNAs, a EcoR I linker were ligated to the 5′ prime of the DNAs. The cDNA were digested with EcoR I and Xho I, purified and then ligated to the EcoR I and Xho I sites of the pJG4-5 vector. The mixed construct library is transformed in E. coli for library preparation and plasmid DNA purification.

5′ *EcoR* I linker sequence: AATTCGGCACGAGGCG-3′
GCCGTGCTCCGC-5′

Quality Control

Each DupLEX-A™ cDNA Library has been tested in a stringent quality control process. First a cDNA library must have an adequate number of independent clones. OriGene's cDNA libraries have 3.5 x 10^6 to 10^7 independent clones. Second, the percentage of the vectors with an insert must be above 95%, and large size inserts must be presented in the cDNA libraries. The 100 ug DNA included in the package is transformation-ready and is sufficient for one round of screening. The E. coli glycerol stock has been amplified once and can be used for a large scale DNA plasmid preparation.

Methods
Protocol for Amplifying DupLEX-A cDNA Libraries

1. Titer frozen bacteria by doing serial dilution plating; the provided bacterial cell stock should have ~10,000,000 pfu/ul glycerol medium. When handling frozen bacteria, gouge a chunk of cells with a sterile spatula or toothpick and keep the rest frozen.
2. Plate bacteria on LB plates containing ampicillin at 100ug/ml, at a density of about a half-million colonies per 150mm petri dish. A total of five million colonies (on ten 150mm petri dishes) are usually enough to represent the whole library.
3. Grow bacteria for about 10 hours at 37 °C or until the colonies are 1mm in diameter. Lower incubation temperatures (e.g. 32-35 °C) are also suggested. Note: DO NOT LET THE COLONIES OVERGROW.

4. Flood each plate with LB medium (~5 ml/150mm plate). Using a sterile cell scraper, rub colonies off from the plates and make a bacterial suspension.
5. Pool the suspensions from different plates and purify DNA from the pool using a commercially available plasmid DNA purification kit, or the method described below.

Modified alkaline lyses method (Current Protocols in Molecular Biology, p1.6.7):

As observed by researchers who work on yeast transformation, in many cases, some 'crude' plasmid DNA preparations have much higher transformation efficiencies than commercial-kit purified plasmid DNAs. The following DNA preparation method generally yields DNAs with satisfactory transformation efficiencies:

Solutions:

Sol I: Glucose /Tris/EDTA
50 mM glucose
25 mM Tris.Cl, pH 8.0
10 mM EDTA
Autoclave and store at RT

Sol II: NaOH/SDS solution
0.2 N NaOH
1% (w/vol) sodium of dodecyl sulfate

Sol III: 5 M potassium acetate, pH 4.8
29.5 ml glacial acetic acid
KOH pellets to pH 4.8
H_2O to 100 ml

Phenol/chloroform (1 :1 vol/vol)
95% Ethanol
70% Ethanol

NOTE: No RNase A should be used
1. Collect cells by centrifuging 10 min at 4,000 x g at 4 oC.
2. Discard the supernatant and re-suspend the cells in 4 ml of Sol I.
3. Add 8 ml of Sol II and mix it by inverting the tube several times.
4. Add 6 ml of Sol III and mix it by gentle vortexing.
5. Put the tube on ice for 10 min
6. Centrifuge the tube at 12,000 x g for 30 min.
7. Transfer the supernatant to a fresh polyethylene tube.
8. Add equal volume of cold phenol/chloroform and mix.
9. Centrifuge the tube at 12,000 x g for 10 min.
10. Transfer the upper layer to a new centrifuge tube.
11. Add 2X volume of 100% ethanol (room temperature) and mix.

12.Centrifuge the tube at 12,000 x g for 30 min
13.Discard the supernatant without disturbing the pellet.
14.Add 20 ml of 70% cold ethanol to the pellet.
15.Centrifuge the tube at 12,000 x g for 15 min.
16.Discard the supernatant and dry the tube by inverting it on a piece of paper towel.
17.Re-suspend the pellet in 1 ml of TE buffer (pH 8.0).
18.Estimate the DNA concentration by running a sample on an agarose gel (a spectrophotometer cannot be used for measuring the quantity because of the presence of a large amount of RNA in the sample).
19.Aliquot the DNA sample to several tubes and store at –20 oC.
20Use a small amount for yeast transformation to test the yeast transformation efficiency.
21.Calculate the amount of plasmid DNAs needed for a library-scale screening, or follow recommendations from your yeast two-hybrid system user manual.

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