

SKY48/pLacGUS

Catalog no. C832-00

Technical Information

Introduction

SKY48/pLacGUS is a yeast host strain designed for use with the Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System (Catalog no. K5200-01) available from Invitrogen. The yeast strain may be transformed with LexA and/or lambda cI-containing bait plasmids to generate a bait strain suitable for use in the following applications:

- Conducting an interactor hunt to simultaneously identify proteins that interact with LexA and cI-containing baits.
- Testing for an interaction with a known protein.

The SKY48 yeast strain (Serebriiskii *et al.*, 1999), derived from the EGY48 strain has been transformed with the pLacGUS reporter plasmid (see next page). See below for the genotype of SKY48/pLacGUS.

For more information about construction of bait plasmids, yeast transformation, and how to perform an interactor hunt or test for protein-protein interactions, refer to the Dual Bait Hybrid Hunter™ System manual available on our Web site at www.invitrogen.com.

Shipping/Storage

The yeast strain is supplied as 20% glycerol stock in a total volume of 0.5 ml and is shipped on dry ice. Upon receipt, store the glycerol stock at -80°C.

Genotype/ Phenotype

The genotype and phenotype of SKY48/pLacGUS yeast strain is described below. The SKY48/pLacGUS strain has been transformed with the pLacGUS reporter plasmid. For more information on pLacGUS, see the next page.

Genotype

MAT α *ura3 trp1 his3 6lexAop-LEU2 3cIop-LYS2*
transformed with pLacGUS (*URA3*)

Phenotype

Ura⁺, Trp⁻, His⁻, Leu⁻, Lys⁻

Product Qualification

SKY48/pLacGUS is tested for growth on YPD and YC-uracil.

SKY48/pLacGUS is also qualified in a functional assay. The strain is transformed with the following vectors as listed below, plated on selective medium, and assayed for growth or no growth as expected. A β -glucuronidase overlay assay is performed with colonies on the YC-UHWK Z200 Gal/Raff plates. Color development must be visible within one hour after addition of the X-Gluc staining solution.

Transformation	Selective Medium	Growth
pHybLex/Zeo-Fos2	YC-UHWL Z200 Gal/Raff or YC-UHWK Z200 Gal/Raff	Yes
pYESTrp-Jun		
pHybcI/HK-Krev		
pYESTrp2-RalGDS		
pYESTrp2		No

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This product is distributed for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen TECH-LINESM 800 955 6288

Technical Information, continued

Features of SKY48/pLacGUS

The SKY48/pLacGUS yeast strain exhibits the following features:

- The strain contains the pLacGUS reporter plasmid already transformed. Note that the pLacGUS plasmid contains a *URA3* gene for selection in yeast, therefore, the SKY48/pLacGUS strain is no longer auxotrophic for uracil.
 - *GAL1* promoters are inducible by galactose and repressed by glucose. This helps to eliminate false positives and allows detection of potentially toxic interactors.
 - The strain is wild-type for *GAL4*, a regulator of *GAL1* expression.
 - The strain will express cDNA libraries that have been cloned into pYESTrp, pYESTrp2, and pJG4-5.
 - The strain contains two auxotrophic markers - *LEU2* and *LYS2*, whose expression is controlled by 6 LexA operators and 3 cI operators, respectively.
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pLacGUS

pLacGUS (Catalog no. V616-20) is a *lacZ* and *gusA* reporter plasmid in which expression of the *lacZ* gene is controlled by 8 LexA operator sites and expression of the *gusA* gene is controlled by 3 cI operator sites. The plasmid contains the *URA3* selectable marker, the 2 μ origin to allow propagation in yeast, the kanamycin resistance gene, and the pUC origin to allow replication in *E. coli*. The complete sequence of pLacGUS is available for downloading from our Web site (www.invitrogen.com) or contact Technical Service (see page 4).



To propagate and use this yeast strain, the user should be familiar with basic yeast molecular biology and microbiological techniques including preparation of YC minimal medium and YPD medium. Refer to *Current Protocols in Molecular Biology*, Unit 13 (Ausubel *et al.*, 1994) for information on preparing yeast media and handling yeast. Recipes for preparation of YC and YPD media are also provided in the Dual Bait Hybrid Hunter™ System manual.

Initiating Cultures

To initiate cultures from frozen stocks, streak a small amount of frozen stock on a YPD plate. Once growth is established, check the phenotype of each strain by streaking the strain on a minimal plate supplemented with the appropriate amino acids. Be sure to make a glycerol stock of each strain. If you plan to use the strains directly from plates, be sure the streaked plates are less than 4 days old.

Yeast Transformation

You may use any yeast transformation protocol of choice to transform a bait plasmid (or any other plasmid) into SKY48/pLacGUS. Invitrogen offers the *S. c.* EasyComp™ Kit (Catalog no. K5050-01) for quick and easy preparation of competent yeast cells that can be used immediately or stored frozen for future use. Transformation efficiency is guaranteed at >10³ transformants per μ g DNA. Contact Technical Service for more information (see page 4).

References

The SKY48 yeast strain was isolated and constructed as described in Serebriiskii *et al.*, 1999. For more information about the dual bait two-hybrid system and interaction trap systems in general, refer to the following references:

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). *Current Protocols in Molecular Biology* (New York: Greene Publishing Associates and Wiley-Interscience).

Serebriiskii, I., Khazak, V., and Golemis, E. A. (1999). A Two-Hybrid Dual Bait System to Discriminate Specificity of Protein Interactions. *J. Biol. Chem.* 274, 17080-17087.

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**Information for
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Customers**

The SKY48/pLacGUS yeast strain is genetically modified and contains the plasmids, p3LexLEU2 and pcIL-2 stably integrated into the genome (Serebriiskii et al., 1999). This yeast strain also contains the pUC-derived plasmid, pLacGUS. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Technical Service

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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 2. Follow instructions on the page and fill out all the required fields.
 3. To request additional MSDSs, click the 'Add Another' button.
 4. All requests will be faxed unless another method is selected.
 5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.
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