

Dharmacon™

RNAi, Gene Expression & Gene Editing

Yeast GST Fusion Collection and Strains

Cat. #YSC4423, YSC4515

Product Description:

The Andrews lab at the University of Toronto has developed a collection of more than 5000 yeast strains that each overexpresses a different yeast open reading frame (ORF) when induced with galactose. More than 80 % of the *S. cerevisiae* genome is represented. ORFs are plasmid-encoded, tagged at the N-terminus with GST, and expressed under control of the GAL1/10 promoter². This collection is in a BY4741 (MATa) background and has been used for genome-scale overexpression screening¹.

Host strain: BY4741 (MATa his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 – s288c background). (Dharmacon Cat #YSC1048).

Yeast strains are shipped in CM broth with glucose-uracil (Teknova Cat #C7141) with 15% glycerol (added after growth of strains).

Open Labs Products:

We provide clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other vendors. In order to provide these as a public resource, we depend on the contributing laboratories for quality control. Therefore, these are distributed in the format provided by the contributing institution with no additional product validation or guarantee. Additional information can be found in the product manual as well as in associated published articles (if available).

Shipping and Storage:

Individual strains are shipped in 2 mL tubes at room temperature. The entire collection is shipped in 96-well plates on dry ice. All strains should be stored at -80 °C.

For collections: To allow any CO₂ that may have dissolved into the media from the dry ice in shipping to dissipate, please store plates at -80 °C for at least 48 hours before thawing.

Obtaining Clone Information:

The Dharmacon™ Search provides a rapid means of locating relevant strain information. Simply enter a record number into the query box and choose to search by "Yeast ORF ID". (See Figure 1)

The screenshot shows the Dharmacon website interface. At the top, there is a navigation bar with links for 'Gene Cart (0)', 'Contact Us', 'Customer Service 800-235-9880', 'Guest', 'Sign In', 'Register', 'Quick Cart', 'Cart (0)', and 'United States'. Below this is the GE Healthcare logo and the text 'Dharmacon RNAi, Gene Expression & Gene Editing'. A search bar contains the text 'YSC4423'. Below the search bar are navigation links: 'Home', 'Products', 'Applications', 'Brands', 'Service & Support', and 'About Us'. The main content area shows 'Search results for YSC4423'. There are filters for 'Catalog (1)', 'Products (0)', 'Genes (0)', 'Pathways (0)', and 'Resources (1)'. The search results list 'Yeast GST-Tagged Collection (glycerol stock)' with the identifier '# YSC4423', 'Unit Size glycerol stock', and 'Price \$3,979.00'. An 'Add To Cart' button is visible next to the product listing. On the right side, there are social media share icons and a 'LIVE SUPPORT NOW AVAILABLE' button. Below that is an advertisement for 'Edit-R CRISPR-Cas9 System'.

Figure 1. Dharmacon™ Product Search.

Vector Information:

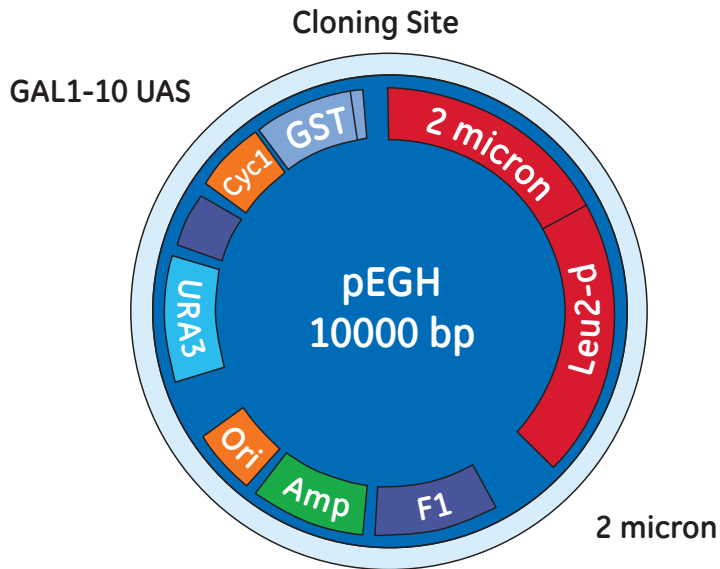


Figure 2. Yeast GST fusion plasmid map³. (Not to scale.) This vector was not sequenced by the source lab. The above graphic is the only information Dharmacon has on this vector.

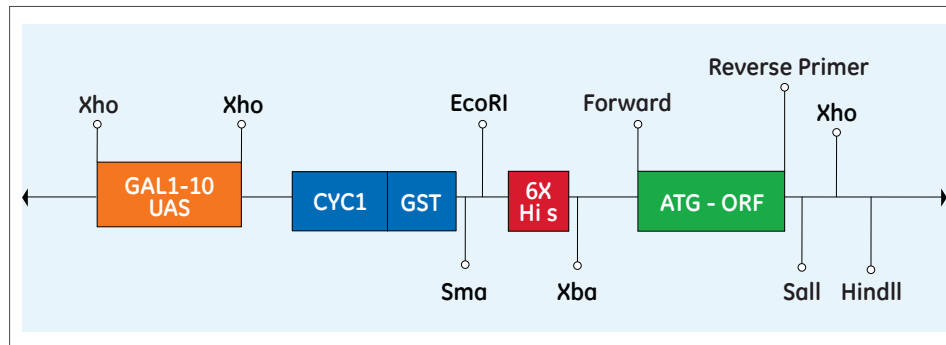


Figure 3. Schematic of the promoter/tag/ORF region of the Yeast GST-fusion plasmid. (Not to scale.)

Yeast Universal Primers:

Forward – 5'GGAATTCCAGCTGACCACC

Reverse – 5'GATCCCGGAATTGCCATG

Quality Control:

Individual plasmids were sequenced to ensure that the correct yeast ORF was fused in-frame to GST².

Confirmation of the size of the ORF can be accomplished by digesting with *Xho*I. If there is no internal *Xho*I site present, the excised band should be the ORF size + 1 kb.

Protocols:

Plate Replication Protocol

To allow any CO₂ that may have dissolved into the medium from the dry ice in shipping to dissipate, please store plates at -80 °C for at least 48 hours before thawing.

Table 1. Materials.

Item	Vendor	Cat #
CM broth with glucose – uracil	Teknova	C7141
Glycerol	Fisher Scientific	BP2291
Deep well plates	Fisher Scientific	07-200-700
Heat seal	Fisher Scientific	AB-3738
Disposable replicators	Genetix	X5054

Prepare Target Plates

1. Prepare deep well 96-well target plates by dispensing 1.5mL media with appropriate antibiotics.

Prepare Target Plates

1. Remove the lids and the aluminum seal from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
2. Allow the source plates to thaw completely with the lids on. Wipe any condensation that may appear under the lids with ethanol and an absorbent wipe.

Replicate

1. Gently place a sterile, disposable replicating tool into the source plate and lightly mix the yeast cells. Make sure to scrape the bottom of each well thoroughly ensuring maximum transfer of cells.
2. Gently remove the replicating tool from the source plate and gently insert the tool into the target plate. Mix the replicating tool around in the target plate.
3. Dispose of the plastic replicating tool.
4. Replace the lid of the target plate and the source plate.
5. Repeat steps 1-6 until all plates have been replicated.
6. Heat seal source plates and return to an ultralow freezer.
7. Cover with a microporous film and place the target plates on a 30 °C incubator with shaking for at 16-48 hours, based upon when growth is apparent.
8. When sufficient growth has been noted in the target plates, add 400 µL of 50% glycerol to each well for a final concentration of 12.5% glycerol.
9. Heat seal target plates and return to an ultralow freezer.

References:

1. Sopko, R., Huang, D., Preston, N., Chua, G., Papp, B., Kafadar, K., Snyder, M., Oliver, S.G., Cyert, M., Hughes, T.R., Boone, C. and Andrews, B. 2006. Mapping pathways and phenotypes by systematic gene overexpression. *Mol Cell* 21(3):319-330.
2. Zhu, H., Bilgin, M., Bangham, R., Hall, D., Casamayor, A., Bertone, P., Lan, N., Jansen, R., Bidlingmaier, S., Houfek, T., Mitchell, T., Miller, P., Dean, R.A., Gerstein, M. and Snyder, M. 2001. Global analysis of protein activities using proteome chips. *Science* 293: 2101-2105.
3. Mitchell, D.A., Marshall, T.K. and Deschenes, R.J. 1993. Vectors for the inducible overexpression of glutathione S-transferase fusion proteins in yeast. *Yeast* 9(7): 715-722.

FAQS/Troubleshooting:

For answers to questions that are not addressed here, please email ts.dharmacon@ge.com technical support with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

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