

Dharmacon™

RNAi, Gene Expression & Gene Editing

Yeast Tet-promoters Hughes (yTHC)

Cat #YSC1180, YSC1182, YSC1210

Product Description:

The creation of a genome-wide yeast deletion collection ([sequence-www.stanford.edu/group/yeast deletion project deletions3.html](http://sequence-www.stanford.edu/group/yeast%20deletion%20project/deletions3.html)) revealed that there are over 1000 protein-coding genes that are essential for viability. The function of many of these essential genes has yet to be defined. In part this is due to the difficulty of utilizing essential genes in functional assays. The Tet-promoters yeast collection (yTHC) from the Hughes Laboratory, University of Toronto, helps to overcome this barrier.

The Yeast Tet-promoters Hughes (yTHC) Collection contains over 800 essential yeast genes for which expression is regulated by doxycycline. The endogenous promoter has been replaced with a TET-titratable promoter in the genome. Thus, the expression of the gene can be switched off by the addition of doxycycline to the yeast's growth medium.

Constructs are distributed as cultures of *S. cerevisiae* in YPD broth + G418 (300 µg/mL) + 15 % glycerol.

Strain Information:

The yTHC mutant strains are provided in the haploid MATa strain R1158. This strain was created from the background strain BY4741 by a one-step integration of the tTA transactivator, under the control of the CMV promoter, at the URA3 locus (Hughes, Marton et al. 2000). A plasmid carrying a kanR-tetO7-TATA cassette was then integrated into the genome replacing the endogenous promoter. The correct integration was verified by PCR across the junctions and again using gene specific primers.

Additionally, each strain was qualified by its growth phenotype on YPD with 10 µg/mL doxycycline. Without doxycycline in the media the Tet-promoter is fully activated. The addition of doxycycline in a titratable manner allows for down regulation of the promoter until the gene of interest is no longer expressed at detectable levels. The optimal condition for each strain needs to be determined empirically.

Genotype of *S. cerevisiae* R1158:

URA::CMV-tTA MATa his3-1 leu2-0met15-0

Antibiotic Resistance:

The yeast strain R1158 has the kanR gene which should be selected for with G418 (Table 1).

Table 1. Antibiotic resistance of *S. cerevisiae* yeast strain R1158

| Antibiotic | Concentration | Utility |
|------------|---------------|-----------------------------|
| G418 | 300 µg/mL | Eukaryotic selection marker |



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Obtaining Clone Information:

The Dharmacon Search provides a rapid means of locating relevant strain information. Simply enter a clone ID into the query box and choose to search. (See Figure 1). Press the "+" next to the proper search result to display details.

The screenshot shows the GE Healthcare Dharmacon website interface. At the top left is the GE Healthcare logo and the text "Dharmacon RNAi, Gene Expression & Gene Editing". A search bar at the top right contains the text "TH_2788". Below the search bar is a navigation menu with links for "Products", "Applications", "Brands", "Service & Support", and "About Us". The main content area displays "Search results for TH_2788" with filters for "Catalog (1)", "Products (0)", "Genes (0)", "Pathways (0)", and "Resources (1)". A single search result is shown: "Yeast Tet-promoters Hughes (yTHC) Strain YLR229C (CloneID:TH_2788)" with a plus sign icon on the left, "# YSC1180-202218878", "Unit Size glycerol stock", and "Price \$66.00". An "Add To Cart" button is located to the right of the result. On the right side of the page, there are social media share icons, a "LIVE SUPPORT NOW AVAILABLE" button, and a promotional banner for the "Edit-R CRISPR-Cas9 System" featuring a DNA double helix.

Figure 1. Dharmacon Search.

Protocol I – Replication:

Table 2. Materials for replication.

| Item | Vendor | Cat # |
|-----------------------------------|-------------------|--------------|
| Yeast Extract, 500 g, granulated | Fisher Scientific | BP1422-500 |
| Peptone, granulated, 2 kg – Difco | Fisher Scientific | BP9725-2 |
| Glucose (D+)-Glucose Monohydrate) | EMD Millipore | 1.08342.2500 |
| Glycerol | Fisher Scientific | BP2291 |
| G418 | Calbiochem | 345810 |
| 96-well microplates | Nunc | 260860 |
| Aluminum seals | Nunc | 276014 |
| Disposable replicators | Genetix | X5054 |

*When preparing media for yeast clones, do not add the glycerol to the media until after the clones have grown. Glycerol inhibits the growth of yeast. We prepare a solution of 50% glycerol and 50% media to add to the growth media after incubation.

YEPD Medium 1 Liter:

YPD mix:

Yeast extract 10 g
Peptone 20 g
dH₂O 900 mL

Autoclave mixture for 180 minutes at 121 °C.

Glucose/dH₂O Mix

Dextrose 20 g
dH₂O 100 mL

Shake until in solution and autoclave for 20 minutes at 121 °C.

Under hood, add YPD mix to 100 mL of sterile Glucose/dH₂O mix.

Plate Replication Protocol:

Prepare Target Plates

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

Prepare Source 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 ultra-low 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 ultra-low freezer.

Protocol II – Strain Verification:

PCR can be used to verify that the KanR-tet07-TATA-cassette has been integrated into the promoter region of each gene. The confirmation primers will produce a short band of 200-500 bases in a wild type strain while the mutant strain will have a longer band (>2 Kb). Gene specific primer sequences used to verify the strains are found in the Dharmacon Search (See Obtaining Clone Information, Figures 1-3). Optimum temperature for most PCR reactions using the recommended primers is 58 °C. However, some primer pairs will require temperature optimization.

What Clones Are Part Of My Collection?

A USB containing the data for this collection will be shipped with each collection. This file contains the location and ORF information for each construct in the collection. This data file can be downloaded from the Yeast Tet-promoters Hughes (yTHC) product page at dharmacon.gelifesciences.com.

Table 3. Related Reagents

| Reagents | Vendor | Cat # |
|--|-----------|---------|
| Yeast Tet-promoters Hughes (yTHC) Collection | Dharmacon | YSC1182 |
| Yeast Tet-promoters Hughes (yTHC) Strain | Dharmacon | YSC1180 |
| Yeast Tet-promoters Hughes Parental Strain - R1158 | Dharmacon | YSC1210 |

What Antibiotic Should I Use?

yTHC clones are shipped in YPD broth + G418 (300µg/mL) + 15% glycerol. When preparing media for yeast clones, do not add the glycerol to the medium until after the clones have grown. Glycerol inhibits the growth of yeast. We prepare a solution of 50% glycerol and 50% medium to add to the growth media after incubation.

Where Can I Get The Doxycycline For Use With The yTHC Collection?

We order our doxycycline from VWR. The brand is EMD Biosciences (CalbioChem), catalog number 80056-372.

How Much Doxycycline Should I Use To Induce These Clones?

For this collection, each strain was qualified by its growth phenotype on YPD with 10 µg/mL doxycycline. The data file contains the details of these verification studies under the column 'Growth defect (10 ug/mL Doxycycline)'.

This file is also downloadable from our website at dharmacon.gelifesciences.com.

Without doxycycline in the medium the Tet-promoter is fully activated. Addition of doxycycline in a titratable manner allows for down regulation of the promoter until the gene of interest is no longer expressed at detectable levels. The optimal condition for each strain needs to be determined empirically. It is very important to perform serial dilutions of both the yeast and the doxycycline. If you have too heavy an inoculum of yeast, the expression of the gene or protein may be too great to allow for any reduction to be detectable.

Why Are My yTHC Strains Growing In The Absence Of Uracil In The Medium?

The strains are supposed to be URA3 + because this is the selectable marker included in the CMV-tTA cassette. This information is listed in the Hughes 2000 Cell paper.

Where Can I Find An Explanation Of Various Primers Listed For yTHC Collection?

hugheslab.ccb.utoronto.ca/supplementary-data/Mnaimneh

"List of primers" has all primers listed by gene name.

"Hughes Lab TET collection strains", lists which were confirmed with "inside" or "outside" primers.

-UP_Primer, DOWN_primer (amplifies the kan cassette)

-UP_Extenders, DOWN_Extenders (amplifies the amplified cassette, in order to get longer homology arms)

-Confirm-UP, Confirm-DOWN (flanking primers used for confirmation)

"Inside" means confirmation with one of the flanking primers vs. an internal primer in the kan cassette. "Outside" means PCR was performed using the two confirmation primers to verify that the original allele was replaced with the tet allele. It has been reported that using the "outside" strategy for confirmation may give null results, even when the "inside" PCR data and strain's growth defect indicates the wild type allele is gone.

References:

1. Hughes, T. R., M. J. Marton, *et al.* (2000). Functional discovery via a compendium of expression profiles. *Cell* **102**(1): 109-26.
2. Mnaimneh, S., A. P. Davierwala, *et al.* (2004). Exploration of essential gene functions via titratable promoter alleles. *Cell* **118**(1): 31-44.
3. Winzler, E. A., D. D. Shoemaker, *et al.* (1999). Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**(5429): 901-6.

FAQS/Troubleshooting:

For answers to questions that are not addressed here, please email Technical Support at ts.dharmacon@ge.com.

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