TECHNICAL MANUAL

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INSPIRED CELL SOLUTIONS

Yeast TAP-Fusion ORF clones and collection

Cat. #YSC1177, YSC1178, YSC1247

Product description

The Yeast TAP-Fusion Library (scTAP) is comprised of 4,247 Open Reading Frames (ORFs) tagged with a high-affinity epitope and expressed from their natural chromosomal locations. The tandem affinity purification (TAP) tag consists of a calmodulin binding peptide, a TEV cleavage site and two IgG binding domains of Staphylococcus aureus protein A. The scTAP Library is provided in 96-well microtiter plates containing frozen stock cultures in YPD plus 15% glycerol. While the scTAP strains are supplied in YPD media, SD complete or SD -HIS medium can also be used.

Strains are arranged in plates by protein expression levels (highest to lowest) and then by size (largest to smallest), with plate designation GS1 representing the "high expression" category and GS5 representing the "low expression" category. Membrane bound proteins are arrayed at the end of each category for convenience. Refer to USB shipped with the collection for expression category designation (datafile is also available online at dharmacon.horizondiscovery.com).

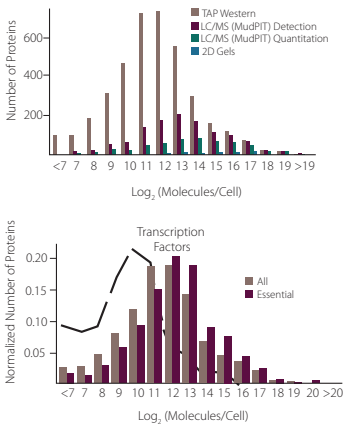


Figure 1. Protein detection1.

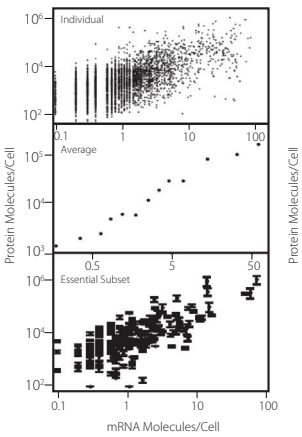


Figure 2. Protein detection per cell.

Overview

To facilitate global protein analyses, Dr. Erin O'Shea and Dr. Jonathan Weissman (UCSF) have created a Saccharomyces cerevisiae fusion library where each ORF is tagged with a high-affinity epitope and expressed from its natural chromosomal location. Through immunodetection of the common tag, a resource now exists that provides a census of proteins expressed during log-phase growth and quantifies their absolute levels. Characterization of this library revealed that ~ 80% of the proteome is expressed during normal growth conditions. The abundance of proteins ranged from fewer than 50 to more than 106 molecules/cell with many, including essential proteins and most transcription factors, having levels not readily detectable by other proteomic techniques, nor predictable by mRNA levels or codon bias measurements (Figures 1 and 2)[1].

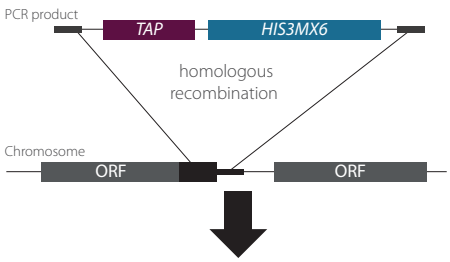
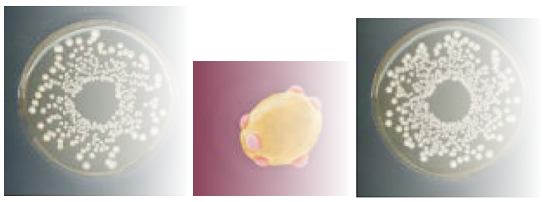


Figure 3. Insertion of C-terminal *TAP* cassette.



Useful websites	
O'Shea Lab	labs.mcb.harvard.edu/o%27shea
Weismann Lab	weissmanlab.ucsf.edu
Saccharomyces Genome Database	yeastgenome.org

The Yeast TAP-Fusion Library allows the purification and selection of the entire yeast proteome and associated components using two simple affinity selection steps in tandem, enabling the development of a range of high-throughput functional assays. A collection of ORF specific oligonucleotide primers was synthesized. Each primer pair possessed shared 3′ ends that allowed for PCR amplification of a common insertion cassette, as well as gene-specific 5′ ends that allowed for the precise introduction, through homologous recombination, of the amplified insertion cassettes as a perfect in-frame fusion at the C-terminal end of the coding region of each gene (Figure 3)[1].

The C-terminal TAP insertion cassette contains the coding region for a modified version of the TAP (Tandem Affinity Purification) tag, which consists of a calmodulin binding peptide, a TEV cleavage site and two IgG binding domains of Staphylococcus aureus protein A, as well as a selectable marker. In total, successful integrants were obtained for 98% of all ORFs annotated in the Saccharomyces Genome Database (as of April 2001), including 93% of all essential ORFs in haploid yeast.

Storage

The Yeast TAP-Fusion strains are shipped at ambient temperature. The strains and libraries should be stored at –80 °C.

To allow any CO_2 that may have dissolved into the medium from the dry ice during shipment to dissipate, please store plates at –80 °C for at least 48 hours before thawing.

Genotype of *Saccharomyces cerevisiae* used to construct scTAP strains

S288C: (ATCC 201388: MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0)

Strain verification

PCR can be used to verify that the TAP-tag has been integrated into the C-terminal coding region of each gene. The confirmation primers consist of a gene specific primer and a cassette specific primer that will produce a short band of approximately 500 base pairs in the mutant strains. Gene specific primer sequences used to verify the strains are found using the Dharmacon search bar. Simply search for the ORF and choose the "Non Mammalian" link from the proper result on the Gene tab. Then press the "+" next to the product of interest to obtain further details. The cassette specific primer (F2CHK) sequence is: AACCCGGGGATCCGTCGACC. A complete PCR protocol is attached in the Appendix.

Making a stock culture

Once the strain has been streak-isolated and the identity has been confirmed, we recommend making a stock of the pure culture. Inoculate the pure culture in YPD broth and incubate for 48 hours at 30 °C. Transfer 850 µL of culture into a polypropylene tube and add 150 µL sterile glycerol to make a 15% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at –80 °C.

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 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.

Reference

1. Ghaemmaghami, S. *et al.* Global analysis of protein expression in yeast. *Nature* **425**, 737–741 (16 October 2003).

Additional literature

1. Rigaut, G. *et al.* A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol* **17**, 1030–2 (1999).
2. Gavin, A. C. *et al.* Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**, 141–7 (2002).

Appendix

Liquid culture PCR

1. Grow cells to stationary phase.
2. Transfer 200 µL of culture to a PCR tube.
3. Spin down cells and discard supernatant by aspiration.
4. Suspend cells in 20 µL of 0.2% SDS solution.
5. Boil cell suspension at 99 °C for 10 minutes using PCR machine.
6. Add 1.5 (0.6) µL of boiled cell suspension to the PCR reaction mixture of final 50 (20) µL volume:

40.5 (16.2) µL	H_2O
5 (2) µL	10x *Taq* buffer
1.5 (0.6) µL	2 mM each dNTP
0.5 (0.2) µL	50 µM forward primer
0.5 (0.2) µL	50 µM reverse primer
0.5 (0.2) µL	5 U/µL *Taq* polymerase
0.1 (0.04) µL	10 mg/mL RNase

For library construction

(1) 0.6 µL boiled cells	
+	
(2) 2 µL 5 µM CHK primer	
+	(× 1300)
(3) 14.5 µL H2O	18.85 mL
2 µL 10x Taq buffer	2.6 mL
0.5 µL 2 mM each dNTP	0.65 mL
0.2 µL 50 µM F2CHK	260 µL
0.2 µL 5 U/µL Taq pol.	260 µL
0.04 µL 10 mg/mL RNase	50 µL

If you have any questions, contact

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