Technical Manual

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Yeast Protein Interactome

Cat. #YSC5097, YSC5098, YSC5849

Product Description:

This collection, developed at the University of Montreal, has provided an 8 nm resolution map of the yeast protein interactome which illuminates the protein architecture of fundamental cellular processes (Tarassov *et al.*, 2008). This technology represents a systematic binary screen for protein-protein interactions (PPIs) at a genome wide scale in *Saccharomyces cerevisiae* using a protein-fragment complementation assay (PCA) based on the murine dihydrofolate reductase (mDHFR) assay adapted to yeast (Figure 1). The DHFR PCA is a survival selection assay based on a mutant of DHFR that is insensitive to the DHFR inhibitor methotrexate but retains full catalytic activity and allows detection of PPIs that occur in any subcellular location.

Shipping and Storage:

Individual strains are shipped in YPD supplemented with appropriate antibiotic (100 ug/mL nourseothricin for MATa [bait] transformed strains; 250 ug/mL hygromycin B for MATα [prey] transformed strains). The clones are shipped in 2 mL tubes at room temperature; the entire collection is shipped in 96-well plates on dry ice. All strains are shipped in media supplemented with 15% glycerol (added after growth of strains). All strains should be stored at -80 °C. For collections: To allow any CO_2 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.

Background Information:

Current PPI detection technologies have many limitations which are addressed by this collection:

The Yeast Two Hybrid System (Y2H)
- High false positives rate
- Interaction must occur in the nucleus

Affinity Tags (such as TAP-tag and FLAG-tag)
- High false negative rate

Yeast Cross Capture System
- Multiple steps involved

This Yeast Protein Interactome technology includes a DHFR protein-fragment complementation assay for studies in yeast. The mDHFR PCA was previously developed for *Escherichia coli*, plant protoplasts and mammalian cell lines. To adapt the DHFR PCA for high-throughput screening in *Saccharomyces cerevisiae* the authors created a double mutant (L22F and F31S) that is 10,000 times less sensitive to methotrexate than wildtype *Saccharomyces cerevisiae* Dihydrofolate reductase (scDHFR), while retaining full catalytic activity. The mutant was designed so that methotrexate inhibition of the wildtype scDHFR activity would be complemented by the activity of the reconstituted DHFR PCA reporter. Simply, yeast cells are naturally sensitive to methotrexate which will kill them. The methotrexate binds very well to DHFR. There are, however, mutants available that the DHFR does not bind methotrexate and therefore are much less sensitive to it. By creating two types of these mutants, the authors have essentially allowed the selection of methotrexate resistant colonies.

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The two DHFR mutant proteins together render the yeast colonies highly resistant to methotrexate as shown in Figure 1. This is a variation of a complementation assay.

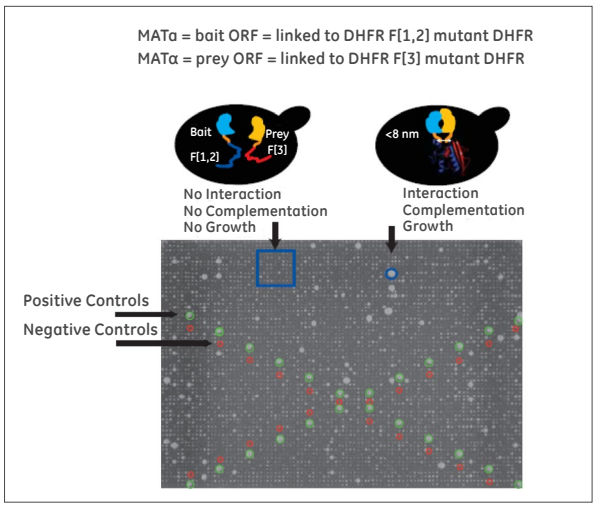

Figure 1. *In vivo* PCA screen of the yeast protein interaction network. Strategy high density array screening of the yeast PIN (protein interaction network) by DHFR PCA. Both positive (green circles (MATa/α, CDC19-F[1,2], MCK1-F[3]) and negative (red circles (MATa/α, CDC19-F[1,2], CLN3-F[3]) controls are included on each plate to ensure that each transfer and selection step has occurred correctly.

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When two proteins interact in the mated yeast, the two mutant DHFR proteins are brought together and the resulting yeast is able to survive treatment with methotrexate.

MATa = bait ORF = linked to DHFR F[1,2] mutant DHFR
MATα = prey ORF = linked to DHFR F[3] mutant DHFR

Below is a schematic for the large scale screening of yeast protein interactions (Interactome) using this complementation assay.

Protocols:

The transformed strains were grown on YPD agar plates plus appropriate antibiotic agar plates (100 mg/mL nourseothricin for MATa transformed strains or 250 mg/mL hygromycin B for MATα transformed strains).

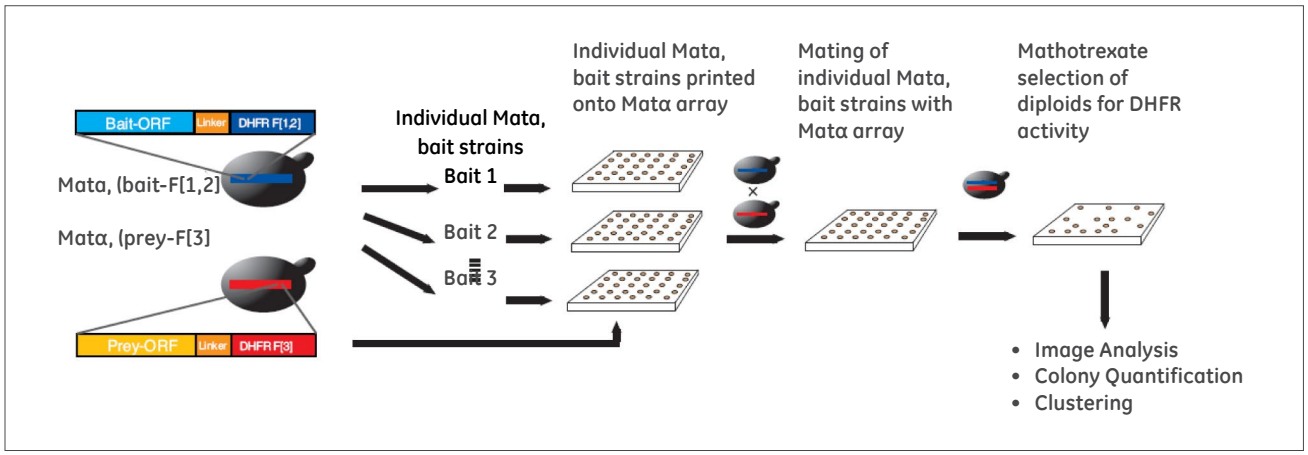

Figure 2. *In vivo* PCA screen of the yeast protein interaction network. Strategy for single bait versus prey array screening of the yeast PIN by DHFR PCA.

Protocol I – Replication:

Table 1. Materials for replication.

Item	Vendor	Cat #
Yeast Extract, 500 g, granulated	Fisher Scientific	BP1422-500
Peptone, granulated, 2k g – Difco	Fisher Scientific	BP9725-2
Glycerol	Fisher Scientific	BP2291
Nourseothricin	—	—
Hygromycin	—	—
96-well microplates	Nunc	260860
Aluminum seals	Nunc	276014
Disposable replicators	Fisher Scientific	NC9584102

***When preparing medium 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 medium after incubation.**

YPD Mix:

Yeast extract 10 g
Peptone 20 g
dH_2O 900 mL
Autoclave mixture for 20 minutes at 121 °C

Plate Replication:

Prepare Target Plates

1. Prepare deep well 96-well target plates by dispensing 1.5mL 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.

Making A Stock Culture Of Individual Clones
We recommend making a stock of the pure culture. Inoculate the pure culture in either YPD + appropriate antibiotic 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.

Protocol II – Interactome Assay:
Please refer to the Tarassov et al. (2008) Supplemental Online Material for protocols.

FAQs/Troubleshooting:
What Are The Background Strains Used And Their Genotypes?
The strains BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) and BY4742 (MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) were used.
For answers to questions that are not addressed here, please email Technical Support at ts.dharmacon@ge.com

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Dharmacon provides 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).

Reference:
Tarassov, K., *et al.* (2008). An *in vivo* map of the yeast protein interactome. *Science* 320(5882): 1465-70.

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Orders can be placed at:
gelifesciences.com/dharmacon

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