

Dharmacon™

RNAi, Gene Expression & Gene Editing

Yeast DAmP Collection

Cat. #YSC5050, YSC5090, YSC5093, YSC5094

Product Description:

A library of *Saccharomyces cerevisiae* strains containing compromised alleles of essential genes that exhibit modest growth defects. These alleles are hypomorphic and cover > 950 of the essential yeast genes as diploid and > 800 genes as haploid. This enables quantitative genetic interaction measurements that allow in-depth functional dissection of pathways and complexes.

Current technologies allow for direct exploration of non-essential genes. Essential genes must be studied by inducible inactivation of the essential genes or by conditional protein destabilization. This resource is constructed a "library of hypomorphic alleles for ~ 82% of essential yeast genes utilizing the Decreased Abundance by mRNA Perturbation (DAmP) approach." Please note that the original library as documented in the accompanying reference paper contained more ORFs than available in the Dharmacon product line. Several ORFs were removed as the creators of the collection wanted to ensure that the ORFs represented were true, essential genes.

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 are shipped in media with 15% glycerol (added after growth of strains). All strains should be stored at -80 °C.

For collections: 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.

Background Information:

The strategy for this library is based on disruption of the 3'-UTR (untranslated region) with an antibiotic resistance cassette (kanamycin), which destabilizes the corresponding transcript and can reduce mRNA levels 4 to 10 fold. The library was constructed as heterozygous diploid using the BY4741/Y6683 strains (*MATa/a his3Δ1/his3Δ leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 met15Δ0/met15Δ0 CYH2+/cyh2*). Correct insertion of the kanamycin cassette was confirmed by PCR using gene-specific forward primers and a universal reverse primer complementary to the promoter region of the kanamycin cassette.

Correct diploids were transformed with URA3-marker plasmid pRS316-STE2pr-SpHIS5 encoding the *Schizosaccharomyces pombe* HIS5 gene driven by a *Mata*-specific promoter.

Table 1. Fold depletion of mRNA in selected DAmP strains relative to the corresponding heterozygous diploid strains. The RNA levels in each strain were normalized relative to the isogenic WT control.

Strain	Fold Reduction in mRNA Relative to Heterozygous Diploid
cdc21-DAmP	7.6+/-3.2
fol1-DAmP	3.6+/-1.8
fol3-DAmP	3.9+/-1.6
erg11-DAmP	3.8+/-0.8



GE Healthcare

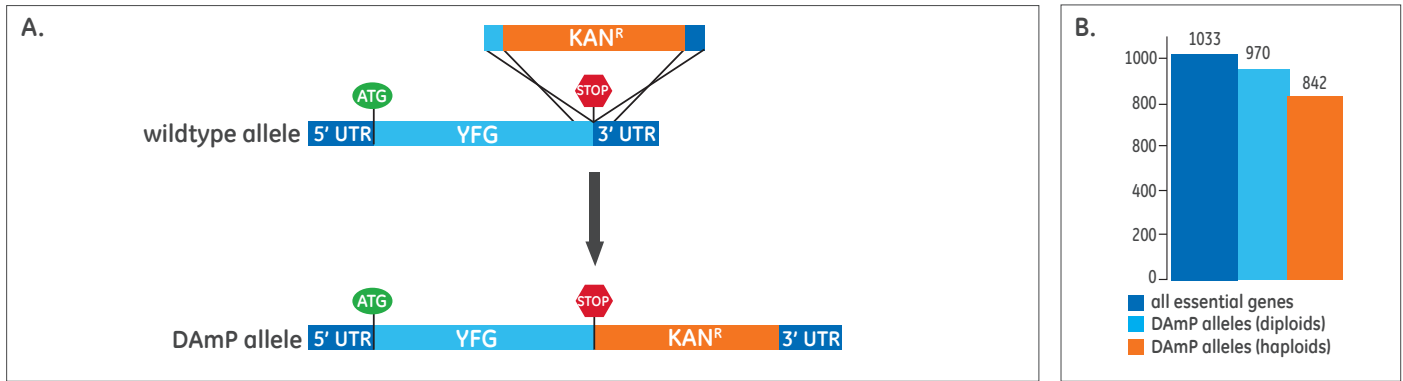


Figure 1. Construction of a library of hypomorphic alleles of essential yeast genes using the DAMP approach. **A.** Schematic of the strategy employed for constructing DAMP alleles. The KAN^R cassette was introduced immediately following the open reading frame of each gene by transformation with a PCR product encoding the KAN^R cassette flanked at each end with homology to the targeted locus to facilitate integration. **B.** Summary of the number of DAMP diploid and haploid strains obtained. Of all 1033 essential yeast genes, ~ 970 were obtained in diploid form and 842 were obtained as MATa haploids.

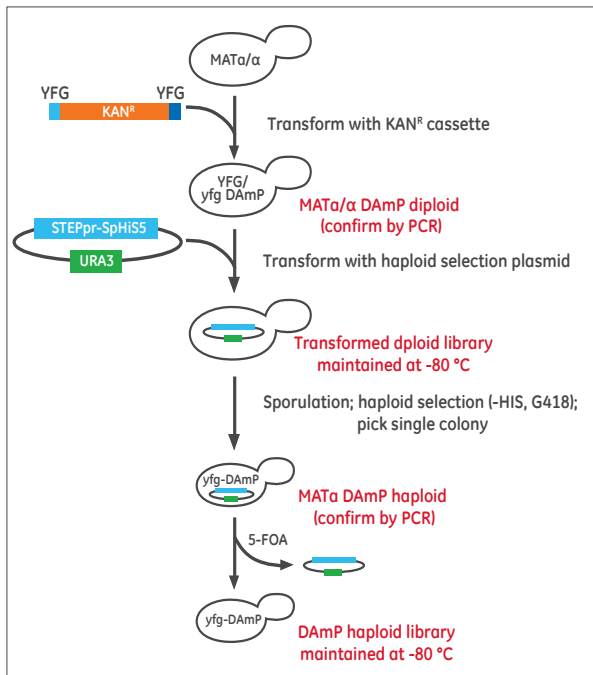


Figure 2. Schematic illustrating the strategy used for DAMP library construction. DAMP alleles were first constructed in diploid strains by transformation of a PCR product bearing 40 nt homology to the site of integration. DAMP diploids were confirmed by PCR then transformed with a plasmid to enable direct selection of MATa haploids following sporulation through the use of a *S. pombe* HIS5 marker driven by a MATa-specific promoter. Following sporulation, DAMP haploids were reconfirmed by PCR and passaged on 5-fluorouracil (5-FOA) to ensure loss of the haploid selection plasmid.

Protocols:

- The haploid DAMP library can simply be grown in YEPD + G418.
- The diploid library requires selection in media lacking URA to maintain the plasmid, so they should be grown in SD-URA + 418.
- Strains should be incubated at 30 °C for optimal growth.
- G418 in the growth media at a final concentration of 200 micrograms per mL. To get the 200 ug/mL final concentration, add 4 mLs of the 50 mg/mL solution of Geneticin prepared with the recipe below to every liter of medium. It is added after the media is made and autoclaved.
- These are sold as glycerol stocks. The final concentration of glycerol in our yeast sets is approximately 15%.

Protocol – Plate 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 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.

YEPD + G418 Medium 1 Liter:

YPD mix:

Yeast extract 10 g
 Peptone 20 g
 dH₂O 900 mL

Autoclave mixture for 20 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.

50 mg/mL Geneticin Solution (100 mL):

Geneticin/G418 5 g

dH₂O 100 mL

1. Measure dH₂O and pour into a clean bottle.
2. Add Geneticin(5 g) to the bottle.
3. Shake or vortex until completely dissolved.
4. Under a hood, sterile filter the solution with a 50 cc syringe and 0.2 µM syringe filter into a sterile bottle.
5. Aliquot 8 mL of the filtered solution into a 15 mL Falcon tube.
6. Repeat Step 5 until all of the solution is aliquotted. If the last tube filled contains < 8 mL, mark the lid with an "X."
7. Label the tubes (LVWIN60/samples/antibiotic labels/Geneticin).
8. Place a label in the designated area on this sheet.
9. Store tubes at -20° C until ready to use.

SD-URA-418 1 Liter:

CM Broth w/ Glucose – Uracil 23.5 g

dH₂O 980 mL

25% Ammonium Sulfate Solution 20 mL

1. Measure dH₂O and pour into 1 L bottle designated for SD-URA Medium.
2. Repeat step one until all bottles are filled.
3. Measure and add CM Broth w/ Glucose – Uracil (23.5 g) to each bottle.
4. Shake until in solution.
5. Autoclave bottles for 20 minutes at 121 °C.
6. In a sterile hood, add 20 mL of sterile 25% Ammonium Sulfate solution to each bottle.
7. Swirl to mix.
8. Place the bottle in the incubator to check for contamination.

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.

Other relevant information for any tables in manuals:

Deep well plates: Fisher Cat #07-200-700

Microporous film: Fisher Cat #50-820-083

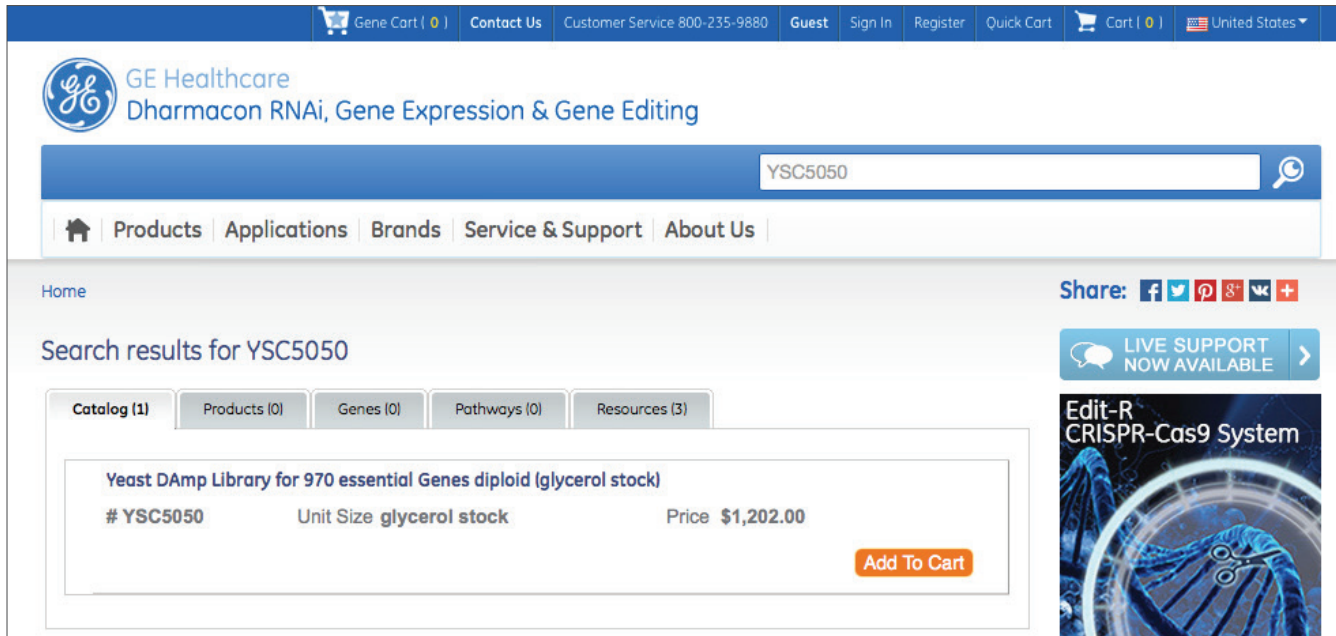
Heat seal: Fisher cat AB-3738

Making a Stock Culture:

We recommend making a stock of the pure culture. Inoculate the pure culture in either YPD + G418 or SD URA-418 (for haploid and diploid strains, respectively) 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.

Obtaining Clone Information:

The Dharmacon Product 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 3).



The screenshot shows the GE Healthcare Dharmacon website. At the top, there is a navigation bar with links for 'Gene Cart', 'Contact Us', 'Customer Service 800-235-9880', 'Guest', 'Sign In', 'Register', 'Quick Cart', 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 'YSC5050'. Below the search bar are navigation links: 'Products', 'Applications', 'Brands', 'Service & Support', and 'About Us'. The main content area shows 'Search results for YSC5050' with filters for 'Catalog (1)', 'Products (0)', 'Genes (0)', 'Pathways (0)', and 'Resources (3)'. The search results display a product card for 'Yeast DAmP Library for 970 essential Genes diploid (glycerol stock)' with item number '# YSC5050', unit size 'glycerol stock', and price '\$1,202.00'. An 'Add To Cart' button is present. To the right, there is a 'Share:' section with social media icons and a 'LIVE SUPPORT NOW AVAILABLE' banner. Below the banner is an advertisement for the 'Edit-R CRISPR-Cas9 System' featuring a DNA double helix.

Figure 3. Dharmacon Product Search.

Are the Constructs in this Collection MET-, or MET+? The Documentation for this Says that the Mat a Haploids were Constructed from a Double Met- diploid Strain (met15delta0/met15delta0):

Per the source lab, the diploid strain used to create this collection was heterozygous for MET, so half of the haploids are MET+ and half are MET-, therefore we cannot provide clone specific information regarding methionine selection.

Reference:

1. Breslow, D. K., D. M. Cameron, *et al.* (2008). A comprehensive strategy enabling high-resolution functional analysis of the yeast genome. *Nat Methods* 5(8): 711-8.

FAQS/Troubleshooting:

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

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