TECHNICAL MANUAL

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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 a constructed "library of hypomorphic alleles for ~ 82% of essential yeast genes utilizing the Decreased Abundance by mRNA Perturbation (DAmP) approach."[1] 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 CO_2 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.

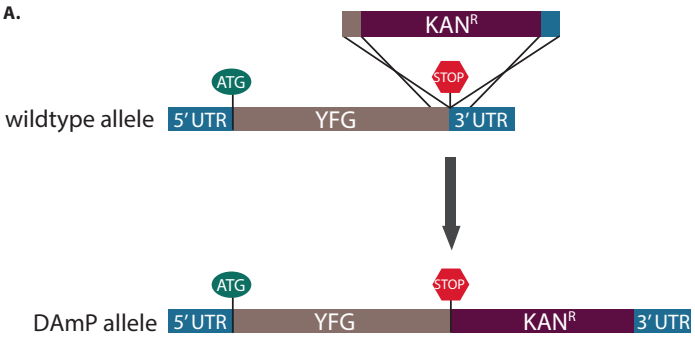
Background information

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/α his3Δ1/his3Δ1leu2Δ0/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



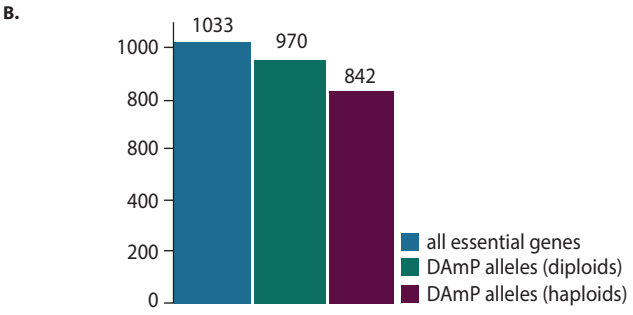


Figure 1. Construction of a library of hypomorphic alleles of essential yeast genes using the DAmP approach. A. Schematic of the strategy employed for constructing DAmP alleles. The KAN^R cassette was introduced immediately following the open reading frame of each gene by transformation with a PCR product 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.

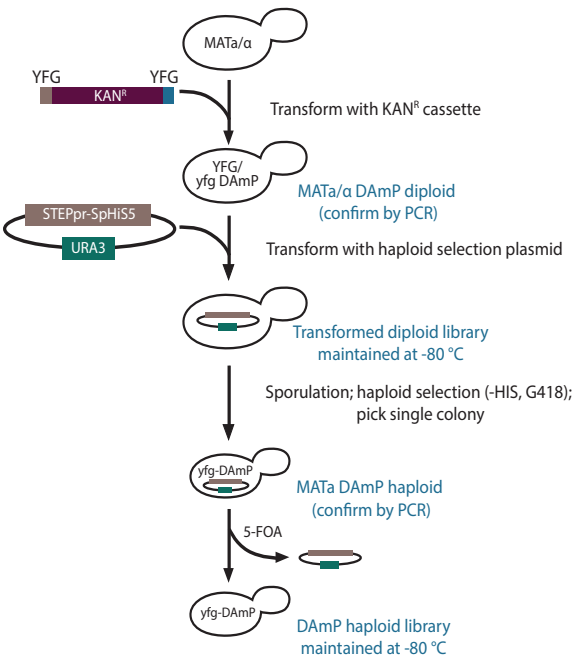


Figure 2. Schematic illustrating the strategy used for DAmP library construction. DAmP alleles were first constructed in diploid strains by transformation of a PCR product bearing 40 nt homology to the site of integration. DAmP diploids were confirmed by PCR then transformed with a plasmid to enable 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 + G418.
- 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
dH_2O	900 mL

Autoclave mixture for 20 minutes at 121 °C

Glucose/dH_2O mix

Dextrose	20 g
dH_2O	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
dH_2O	100 mL

1. Measure dH_2O 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
dH_2O	980 mL
25% ammonium sulfate solution	20 mL

1. Measure dH_2O 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 1.5 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 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

Our search provides a rapid means of locating relevant strain information. Simply enter the gene identifier into the query box and press the "+" sign to the left of the correct result to see further details (See Figure 3).

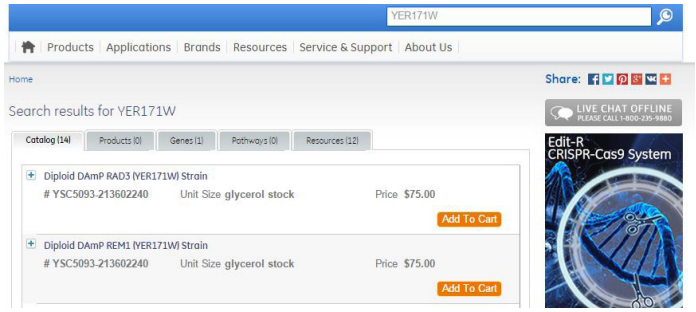


Figure 3. Dharmacon product search.

Reference

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

FAQs/troubleshooting

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.

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

If you have any questions, contact

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