TECHNICAL MANUAL

horizon™
INSPIRED CELL SOLUTIONS

Yeast knockout clones and collections

Cat #	Description
YSC1021	Yeast: yeast knock out strain
YSC1053	Yeast mata collection
YSC4298	Yeast mata (plates 72–73)
YSC4341	Yeast mata (plate 73)
YSC4506	Yeast mata (plates 74–75)
YSC1054	Yeast matalpha collection
YSC4299	Yeast matalpha (plates 172–173)
YSC4507	Yeast matalpha (plates 174–175)
YSC1055	Yeast heterozygous collection
YSC4300	Yeast heterozygous (plates 281–282)
YSC4343	Yeast heterozygous plate 282
YSC4508	Yeast heterozygous (plates 283–284)
YSC4619	Yeast heterozygous (plates 285–286)
YSC1056	Yeast homozygous diploid collection
YSC4301	Yeast homozygous (plates 69–70)
YSC4344	Yeast homozygous plate 70
YSC4509	Yeast homozygous (plates 71–72)
YSC1057	Yeast essential collection

Product description

A nearly complete set of yeast open reading frame (ORF) knock-outs has been produced by the *Saccharomyces* Genome Deletion Project (SGDP). A PCR based strategy was used to replace each ORF with a KanMX cassette containing unique tags or barcodes for each deletion. Four different mutant collections have been generated: haploids of mating types MATa and MATalpha, homozygous diploids for non-essential genes, and heterozygous diploids, which contain the essential and non-essential ORFs.

Strain	Background	Genotype
Mat A	BY4730	MAT a leu2Δ0 met15Δ0 ura3Δ0
Mat Alpha	BY4739	MAT alpha leu2Δ0 lys2Δ0 ura3Δ0
Mat A	BY4741	MAThis3Δ1 leu2Δ0 met15Δ0 ura3Δ0
Mat Alpha	BY4742	MAT alpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0
Het/Hom Dip	BY4743	4741/4742
Homozygous diploids are in the BY4743 background unless 4730/4739 is indicated		

Background strains and genotypes of knockout strains generated by SGDP, including mutant collections.

Shipping and storage

Individual clones are shipped at room temperature and may be stored for up to one week at + 4 °C. They may be stored indefinitely at –80 °C. Plates are shipped on dry ice and should be stored at –80 °C. 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.

Obtaining clone information

Clone details such as vector information, antibiotic resistance and sequence of insert are available at dharmacon.horizondiscovery.com. Search by clone ID and press the "+" to the left of the correct result to see further clone details including antibiotic resistance, vector map and sequence files.

An Excel file denoting the plate locations of each particular knock-out strain in the collection can be found at dharmacon.horizondiscovery.com.

Glycerol stock replication

Culture *S. cerevisiae* in YPD broth + G418 (200 µg/mL). Add 12.5% glycerol prior to freezing.

Replication of individual clones

Inoculate the yeast culture into the appropriate liquid media plus antibiotics or supplements (no glycerol) and incubate for at least 48 hours at 30 °C with shaking. After incubation add enough sterile 50/50 glycerol/YPD mixture to bring the total glycerol percentage to 12.5%. The culture can then be stored indefinitely at –80 °C.

Replication of 96-well plates

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 50% glycerol to each well for a final concentration of 12.5% glycerol.
9. Heat seal target plates and return to an ultralow freezer.

References

1. Winzeler, E.A., *et al.*, Functional characterization of the *Saccharomyces cerevisiae* genome by gene deletion and parallel analysis, *Science*, **285** 901–906, 1999.
2. Giaever, G., *et al.*, Functional profiling of the *Saccharomyces cerevisiae* genome, *Nature* **418** 387-391, 2002.
3. Wach, A., Brachat, A., Poehlmann, R. & Philippsen, P., New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae, Yeast* **10** 1793-1808, 1994.

Yeast knockout useful information

Strain verification

For each ORF, two independent deletion mutants must be produced. This can be done in one of three ways:

1. Transformation of haploids. The diploids are then obtained by mating.
2. Transformation of diploids. The haploids and homozygous diploids are obtained by sporulation and mating
3. Transformation of one of the haploid strains and the diploid strain. The other haploid strain is obtained from the diploid by sporulation and the homozygous diploid is made by mating of the two haploids. The genotypes of the a and alpha segregants have to match those of BY4741 and BY4742, respectively.

Haploid transformants

A–KanB and KanC–D tests must be positive with the deletion mutant and negative with wild-type. If one of these reactions doesn't work, the A-D* test can substitute.

A–B and C–D tests: Both must be negative with the deletion strain and positive with wild-type.

Heterozygotes

A–B, C–D, A-KanB and KanC-D PCR products must be present. An A-D* test can substitute if either the A-KanB or KanC-D PCR doesn't work.* Because the sizes of the A–D bands can be similar in wild-type and mutant strains, it can be helpful to digest part of the A-D reaction with HindIII to test for the HindIII site in the KanMX4 cassette

Haploid segregants

A–B and C–D products must be present in wild-type but not in the mutant. For at least one deletion-specific primer pair, either an A-KanB, KanC–D, or an A–D band of the expected size must be present.

Homozygous diploids

Must be met+lys+, streaked to single colonies, and tested for both mating and sporulation.

Helpful links

Deletion strategy

www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html

Deletion primers

www-sequence.stanford.edu/group/yeast_deletion_project/Deletion_primers_PCR_sizes.txt

Genotypes

www-sequence.stanford.edu/group/yeast_deletion_project/faqs.html#strainbkgrds

Deletion strain confirmation

www-sequence.stanford.edu/group/yeast_deletion_project/project_desc.html#delconfirm

Strain verification

www-sequence.stanford.edu/group/yeast_deletion_project/verification.html

Strain confirmation

www-sequence.stanford.edu/group/yeast_deletion_project/project_desc.html#delconfirm

Kan cassette sequence

www-sequence.stanford.edu/group/yeast_deletion_project/KanMX4.html

kanB and kanC primers

www-sequence.stanford.edu/group/yeast_deletion_project/kanB_kanC_seq.html

KO specific primers

www-sequence.stanford.edu/group/yeast_deletion_project/downloads.html

FAQS/troubleshooting

We provide certain clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other commercial entities. In order to provide these as a public resource, we depend on the contributing academic laboratories for quality control.

Therefore, these are distributed in the format provided by the contributing institution "as is" with no additional product validation or guarantee. We are not responsible for any errors or performance issues. Additional information can be found in the product manual as well as in associated published articles (if available). Alternatively, the source academic institution can be contacted directly for troubleshooting.

If you have any questions, contact

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