

Yeast Deletion Clones

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IMPORTANT!

Beginning in 2009,
all Invitrogen clone
manuals will only be
available online at
www.invitrogen.com

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General Description

Types of Products

This manual is supplied with the products described in the table below. Detailed information on the storage and contents of each product is described on the following pages.

Product	Quantity	Cat. no.
Yeast Deletion Clones		
Individual	1 stab culture	95400
Homozygous Diploid Complete Set	54, 96-well plates	95401.H1R3
Mat-A Complete Set	53, 96-well plates	95401.H2
Mat-Alpha Complete Set	52, 96-well plates	95401.H3
Heterozygous Diploid A Complete Set	67, 96-well plates	95401.H4R3
Yeast Deletion Pools		
Homozygous Diploid	5 × 0.2 ml cells	95401.H1Pool
Heterozygous Diploid	5 × 0.2 ml cells	95401.H4Pool

Description

The yeast deletion library was created by a collaboration of eight North American and eight European laboratories composing the *Saccharomyces* Genome Deletion (SGD) Project. The intent of this consortium was to produce a deletion clone for each gene in the yeast genome. The library consists of 4,828 Mat-alpha haploid clones, 4,848 Mat-A haploid clones, 5,996 heterozygous diploid clones, and 4,741 homozygous diploid clones.

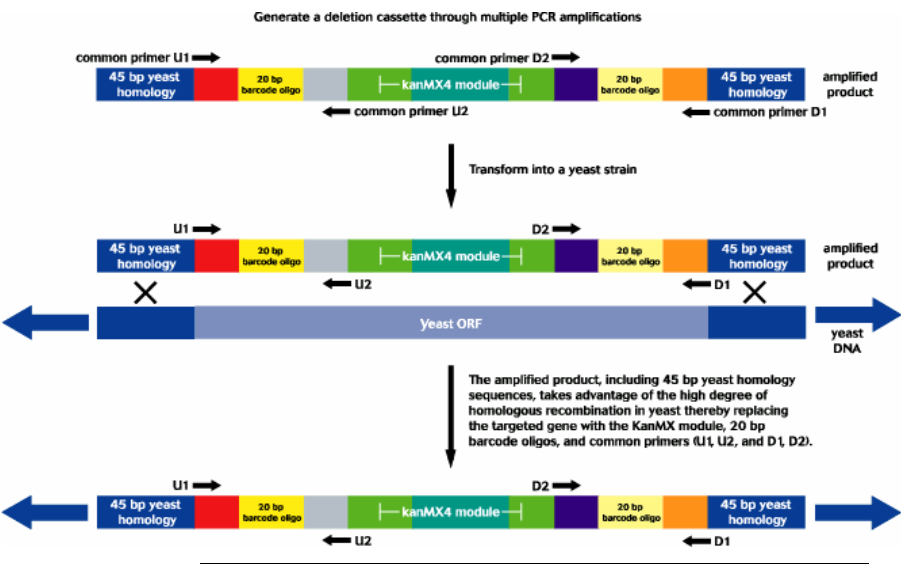
SGD Project

Additional information about the *Saccharomyces* Genome Deletion (SGD) Project, consortium member contacts, and protocols is available at:

http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html

General Description, Continued

Strategy The strategy used to generate the Yeast Deletion Clones is shown below.



Reference According to a July 2000 publication by T. R. Hughes *et al.* in *Nature Genetics* (25; 3: 333-337), approximately 8% of the screened heterozygous diploids exhibit aneuploidy for whole chromosomes or chromosomal segments.

Individual Yeast Deletion Clones

Contents and Storage

The individual Yeast Deletion Clones (Cat. no. 95400) generated by the *Saccharomyces* Genome Deletion (SGD) Project are supplied as stab cultures in yeast peptone dextrose (YPD) agar containing Geneticin® (G418) at a concentration of 200 mg/L YPD-Geneticin® (YPD-G). The parent strains are supplied in YPD agar without Geneticin®. **Make glycerol stock cultures of each mutant as described below on receipt.**

Store a stab culture of the clone at 4°C for up to two weeks.

Preparing Glycerol Stocks

Prepare a stock culture within 2 weeks of receiving the clone.

1. Use a sterile loop to inoculate a 50 ml tube containing 5 ml YPD-G medium with the clone stab. Use YPD media without Geneticin® when growing the parent strains.
 2. Incubate the cells at 30°C with shaking overnight or until the culture is turbid.
 3. Add 1 ml sterile 80% glycerol and mix thoroughly.
 4. Dispense the stock into ultra-low temperature vials and freeze at -80°C.
 5. Revive the yeast by transferring a small portion of the frozen sample onto an YPD-G agar plate. Use YPD agar without the Geneticin® when reviving the parent strains.
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Haploid Contamination

A protocol for testing strains for haploid contamination is available at:
<ftp://ftp.resgen.com/pub/deletions/haploidtest.txt>

Replacements

As a distributor of yeast deletions, Invitrogen Corporation cannot be held responsible for errors in deletion genotype and phenotype. The Yeast Deletion Consortium has performed a quality control on the deletions; therefore, replacements are issued at the discretion of Invitrogen.

Complete Set of Yeast Deletion Clones

Types of Sets	The following complete set of Yeast Deletion Clones are available from Invitrogen: • Homozygous Diploid Complete Set (Cat. no. 95401.H1R3) • Mat-A Complete Set (Cat. no. 95401.H2) • Mat-Alpha Complete Set (Cat. no. 95401.H3) • Heterozygous Diploid A Complete Set (Cat. no.95401.H4R3) For more details on each set, see below and the following pages.
Contents and Storage	The Yeast Deletion Clones, Complete Set generated by the <i>Saccharomyces</i> Genome Deletion Project are supplied in 96-well plates containing 200 µl YPD/15% glycerol media with Geneticin® (G418) at a concentration of 200 mg/L. Produce at least one working copy of the plates on receipt. Store the plates at -80°C.
Inoculation	Allow the plate to thaw completely and resuspend the cells before replication as yeast has a tendency to settle to the bottom (flocculate) of the well during growth. Avoid freeze-thaw as it decreases cell viability.
Avoiding Cross Contamination	• Wash the lids with ethanol before returning them to their 96-well plates. • When re-sealing 96-well plates, freeze the plates, remove a few plates at a time, and seal them before returning them to the freezer. • Remove the foil seal only when the plate is frozen and allow the plates to thaw with the lids removed under a hood.

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Complete Set of Yeast Deletion Clones, Continued

Homozygous Diploid Complete Set

The Homozygous Diploid Complete Set (Cat. no. 95401.H1.R3) contains 54 plates, including three plates (370 to 372) grown with 50 μ M riboflavin and two plates (380, 381) which contain quality control failures. Plates are "footprinted" by leaving two wells blank; the first sequentially identifies the plate number and the second (always in row H) indicates the strain background. For instance, plate 2 of the homozygous set has well A2 (indicating plate number) and well H1 (indicating strain) empty. More information about the deletions scoring key is available at:

http://clones.invitrogen.com/deletions/deletion_plate_key.txt

Plates 349, 371, 372, 380, and 381 are not full. Plate data including strain location, is available at:

<http://clones.invitrogen.com/deletions/>

Mat-A Complete Set

The Mat-A Complete Set (Cat. no. 95401.H2) consists of fifty-three plates. Plates are "footprinted" by leaving two wells blank; the first sequentially identifies the plate number, and the second (in row H) indicates the strain background. For instance, plate 2 of the Mat-A isolate set has well A2 (indicating plate number) and well H2 (indicating strain) empty. More information on the deletions scoring key is available at:

http://clones.invitrogen.com/deletions/deletion_plate_key.txt

Plates 51 and 71 are not full. Plate data including strain location is available at:

<http://clones.invitrogen.com/deletions/>

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Complete Set of Yeast Deletion Clones, Continued

Mat-Alpha Complete Set

The Mat-Alpha Complete Set (Cat. no. 95401.H3) contains fifty-two plates. Plates are "footprinted" by leaving two wells blank; the first sequentially identifies the plate number and the third (in row H) indicates the strain background. For instance, plate 2 of the Mat-Alpha isolate set has well A2 (indicating plate number) and well H3 (indicating strain) empty. More information on the deletions scoring key is available at:

http://clones.invitrogen.com/deletions/deletion_plate_key.txt

Plates 150 and 171 are not full. Plate data including strain location is available at:

<http://clones.invitrogen.com/deletions/>

Heterozygous Diploid A Complete Set

The Heterozygous Diploid A Complete Set (Cat. no. 95401.H4R3) consists of sixty-seven plates, including two plates (280, 281) that contain quality control failures and two plates (270, 271) that contain deletions with special media requirements. Plates are "footprinted" by leaving two wells blank; the first blank well identifies the plate number and the second (in row H) indicates the strain background. For instance, plate 2 of the heterozygous diploid A isolate set has A2 (indicating plate number) and well H4 (indicating strain) empty. More information about the deletions scoring key is available at:

http://clones.invitrogen.com/deletions/deletion_plate_key.txt

Plates 229, 263, 270, 271, 280, and 281 are not full. Plate data including strain location is available at:

<http://clones.invitrogen.com/deletions/>

Yeast Deletion Pools

Description

The Yeast Deletion Pools allow simultaneous analysis of large numbers of deletion strains through selective growth conditions.

Contents and Storage

The Yeast Deletion Pools are supplied in 500 µl tubes containing 200 µl yeast peptone dextrose (YPD)/15% glycerol media with Geneticin® (G418) at 200 mg/L. Each product contains five tubes with 200 l of cells per tube.

Store the tubes at -80°C.

Inoculation

Allow the plate to thaw completely and resuspend the cells before replication as yeast has a tendency to settle to the bottom (flocculate) of the well during growth. Avoid freeze-thaw as it decreases cell viability.

Homozygous Diploid Pools

The Yeast Deletion Homozygous Diploid Pools (Cat. no. 95401.H1Pool) contain 4,653 deletions and are produced by stamping the yeast deletion plates on YPD agar + Geneticin® (G418) and growing the cells to an equal size. The cells are washed with 30 ml of YPD/15% glycerol media with Geneticin® and pooled. Optical density readings of the plate pools are adjusted to a final concentration of 2×10^7 cells/ml. Pool record number, strain, and sequence information are available at <http://clones.invitrogen.com/deletions/>

Heterozygous Diploid Pools

The Yeast Deletion Heterozygous Diploid Pools (Cat. no. 95401.H4Pool) contain 5936 deletions and are produced by stamping the plates on YPD agar + Geneticin® (G418) and growing the cells to an equal size. The cells are washed with 30 ml of YPD/15% glycerol media with Geneticin® and pooled. Optical density readings of the plate pools are adjusted to a final concentration of 1.70×10^7 cells/ml. Pool data is available at <http://clones.invitrogen.com/deletions/>

Recipe

YPD Medium

Yeast Extract Peptone Dextrose Medium (1 liter)

1% yeast extract

2% peptone

2% dextrose (D-glucose)

1. Dissolve the following in 1000 ml of water:
 - 10 g yeast extract
 - 20 g peptone
 - 20 g dextrose (see note below if making plates)
2. **Optional:** Add 20 g agar, if making plates.
3. Autoclave for 20 minutes on liquid cycle.
4. Store medium at room temperature or cool the medium and pour plates. The shelf life is approximately 1-2 months.

Note: If making plates, omit dextrose from Step 1. Autoclaving agar and dextrose together will cause the dextrose to caramelize. Prepare a separate stock solution of 20% dextrose and autoclave or filter-sterilize. After the YPD broth (900 ml volume) has been autoclaved, add 100 ml of 20% dextrose to the medium.

Technical Support

World Wide Web



Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information
 - Access to the Invitrogen Online Catalog
 - Additional product information and special offers
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MSDS

Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis

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Purchaser Notification, Continued

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- Winzeler, E. A., *et al*. (1999). Functional Characterization of the *S. cerevisiae* Genome by Gene Deletion and Parallel Analysis. *Science* **285**: 901-906.
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Notes

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