

Yeast Knockout Collections and deletion strains

TKY1000, TKY1001,TKY1002,TKY1003,TKY3500,TKY3501,TKY3502,TKY3503,TKY3504

Overview

A collection of barcoded yeast deletion strains was created as part of the Saccharomyces Genome Deletion Project. This collection includes over 20,000 knockout strains corresponding to 5,916 genes (including 1,159 essential genes). Each strain has a complete start-codon to stop-codon deletion of an open reading frame (ORF) that is flanked by two molecular barcodes. Strains can be used individually or pooled for parallel gene analysis.

Homozygous diploid, heterozygous diploid, MAT-a haploid and MAT-alpha haploid genome collections as well as an essential gene set are available. Product formats include individual strains, custom sets and collections.

Receipt and storage:

Strains are shipped either in individual tubes or arrayed into micro-titer plates. Yeast stocks are provided in YPD (define?) broth with 15% glycerol. Individual tubes will be shipped at room temperature and should be stored at -80°C long-term. Arrayed plates will be shipped frozen (on dry ice) and need to be placed directly at -80°C. CO₂ will have dissolved into the media from the dry ice so please store plates at -80°C for at least 48 hours before thawing.

Catalog numbers:

Yeast Knock Out Homozygous Diploid Strain	TKY1000
Yeast Knock Out Heterozygous Diploid Strain	TKY1001
Yeast Knock Out MATa Haploid Strain	TKY1002
Yeast Knock Out MAT α Haploid Strain	TKY1003
Yeast Knock Out Homozygous Diploid Collection	TKY3500
Yeast Knock Out Heterozygous Diploid Collection	TKY3501
Yeast Knock Out MATa Haploid Collection	TKY3502
Yeast Knock Out MAT α Haploid Collection	TKY3503
Yeast Knock Out Essential Gene Collection	TKY3504

Parent strain, genotype and deletion genotype:

Strain	Background	Genotype
MATa	BY4741	his3 Δ 1 leu2 Δ 0 met15 Δ 0 ura3 Δ 0
MAT α	BY4742	his3 Δ 1 leu2 Δ 0 lys2 Δ 0 ura3 Δ 0
MATa/ α	BY4743	his3 Δ 1/his3 Δ 1 leu2 Δ 0 /leu2 Δ 0 lys2 Δ 0/LYS2 MET15/met15 Δ 0 ura3 Δ 0 /ura3 Δ 0 (4741/4742)

Homozygous diploids are in the BY4743 background unless 4730/4739 is indicated

Gene Deletion and Barcodes:

A PCR based gene deletion strategy was used to generate deletions of each ORF in the yeast genome. The targeted gene was replaced with a KanMX cassette and tagged with one or two unique 20mer barcode sequences (Figure 1). This allows the yeast XX? in this collection to be selected on G418. The barcodes can be amplified using primer sites which are common to every deletion (U1/U2 and D1/D2 shown in Fig. 1) These can be detected via microarray hybridization or next generation sequencing.

To date, the *Saccharomyces* Genome Deletion Project consortium disrupted 90% of the yeast genome and at least 10% of the yeast genome is redundant. Due to primer-design issues, some highly similar ORFs are not available as deletions.

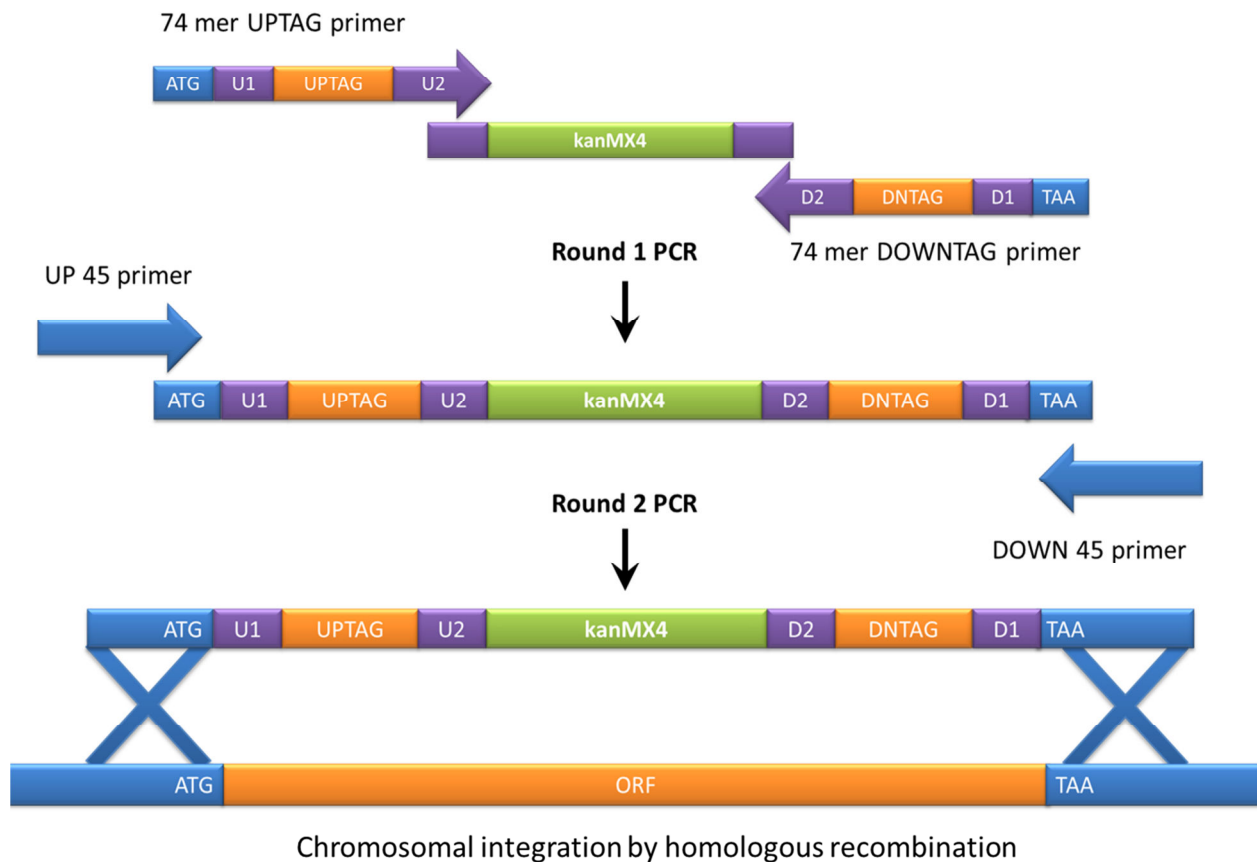


Figure 1: Chromosomal integration by homologous recombination. A two-step PCR reaction was used to construct each deletion cassette. Round 1 amplification includes the KanMX gene (G418 resistance) and the unique barcode sequences. In the second round of PCR ORF homology is extended to 45 bp to increase homologous recombination targeting specificity. PCR priming sites (U1/U2 or D1/D2) flank the barcodes. These are common to every strain and are used for barcode amplification for genome-wide pooled screening.

Obtaining Strain Information:

Strain information is available via gene search using FETCH my gene search at www.transomic.com. Valid search terms include gene ID, SGD ID, systematic name, gene symbol and Refseq accession. Click on the clone ID for details on each strain. Gene information, strain background, strain confirmation primers as well as expected PCR product sizes are provided.

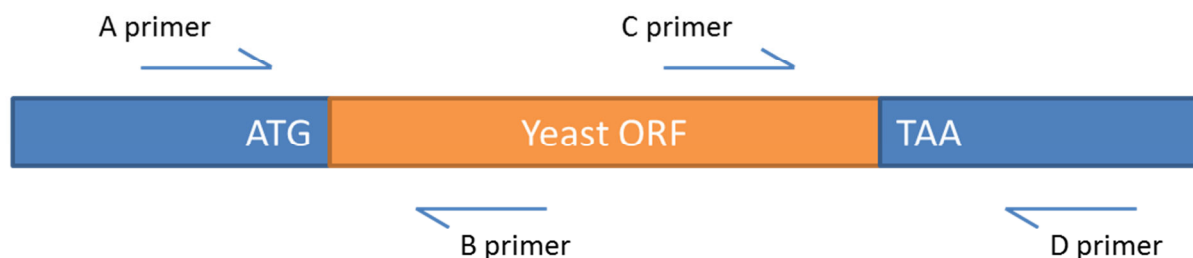
Strain Confirmation

Strain identity can be confirmed by PCR using primers specific to the gene and the selection marker. Knockout alleles require primers specific to the knockout cassette for PCR amplification (Fig. 2B) while wild type alleles are PCR amplified by gene specific primers (Fig. 2A).

Haploid and homozygous deletion strains are positive for knock out allele PCR products and negative for wild type allele PCR products. Heterozygous strains should be positive for all bands. Amplification using the A and D primers will produce PCR product from both alleles.

Primer sequences and expected PCR amplicon sizes are available on the clone details page for each strain.

A. Wild Type Allele



B. Knock Out Allele

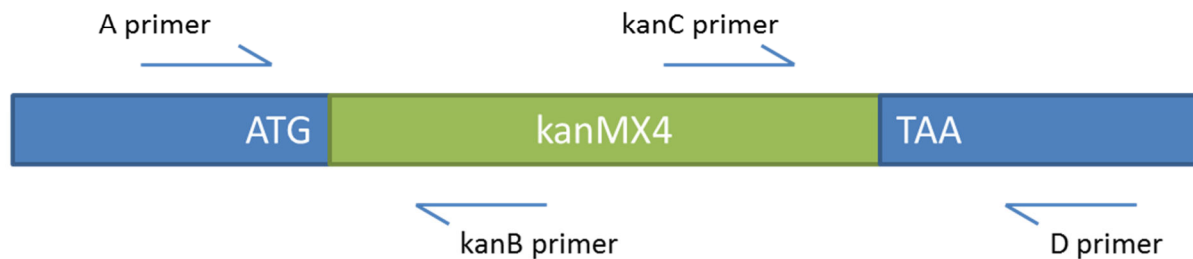


Figure 2: Schematic of wild type allele (A) and Knock out allele (B) showing primer pairs used to detect each.

Making stock cultures from yeast strains

Individual yeast clones

Culture yeast strains in YPD broth with the 200µg/ml G418 (Geneticin Disulfate Sale, G418 - Teknova G5005), at 30°C for 48 hours. Place 850µl of culture into a polypropylene tube and add 150µl sterile glycerol (15% glycerol). Mix well and store at –80°C.

Plate replication

1. Dispense sterile YPD broth with 200µg/ml G418 into 96-well microtiter plates.
2. Remove the foil seals (VWR - 73520-056) from the source plates.
NOTE: Removing the seals while the source plates are frozen will minimize cross-contamination.
3. Place a sterile/disposable replicator (Genetix - X5054) into the thawed plate and gently rotate replicator in the wells to mix the culture. Make sure to scrape the bottom of the plate.
4. Place the replicator into the target plate and rotate again to transfer the cells.
5. Reseal the source plates and return to the –80°C freezer.
6. Place the inoculated target plates in a 30°C incubator for 48 hours. Once the cultures have grown, supplement the wells with 15% glycerol, prior to storage at -80°C.
NOTE: the addition of glycerol prior to incubation impedes yeast growth.

Further information and protocols:

The *Saccharomyces* Genome Deletion Project web page contains a wealth of information on the collection and its creation.

Saccharomyces Genome Deletion Project

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

Detailed description of the deletion strategy

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

KanMX4 cassette sequence and primers

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

Additional protocols and technical information

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