Yeast Knockout Collections and Deletion Strains

Format: YPD Culture

Section 1: Introduction

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, arrayed collections, pooled collections, and custom arrays.

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 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 Figure 1). These can be detected in high-throughput 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.

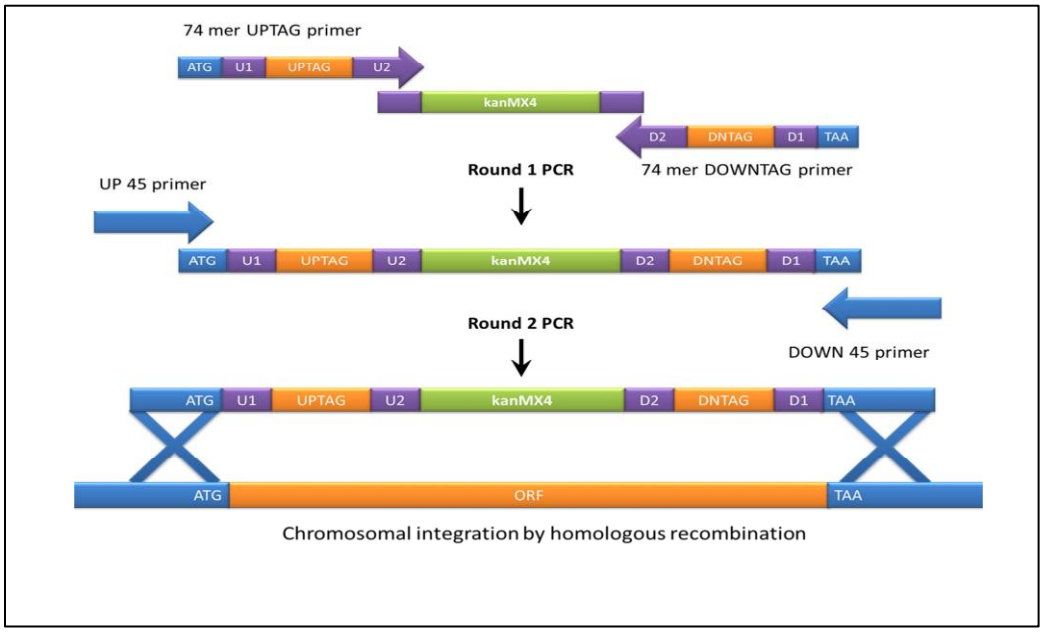

Figure 1: Chromosomal integration by homologous recombination. A two-step PCR reaction was used to construct each deletion cassette. Round 1 amplification includes the KanMX gene (G418 resistance) and the unique barcode sequences. In the second round of PCR ORF homology is extended to 45 base pairs to 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.

Section 2: Product information

Receipt and storage

Strains are shipped in individual tubes or arrayed into microtiter plates. Yeast stocks are provided in YPD (TEKNOVA, Catalogue # Y5311) broth with 15% glycerol. Individual tubes are shipped at room temperature and should be stored at -80°C long-term. Arrayed plates will be shipped on dry ice and should be stored at -80°C.

NOTE: CO_2 dissolves into the media from dry ice during shipment so please store plates at -80°C for at least 48 hours before thawing.

Obtaining Strain Information

Strain information is available via gene search using the 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 for each strain. Gene information, strain background, strain confirmation primers as well as expected PCR product sizes are provided.

Strain	Background	Genotype
MATa	BY4741	his3Δ1 leu2Δ0 met15Δ0 ura3Δ0
MATα	BY4742	his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0
MATa/α	BY4743	his3Δ1/his3Δ1 leu2Δ0 /leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0 /ura3Δ0 (4741/4742)
Het/Hom Diploid	BY4743	4741/4742

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

Section 3: Propagation Protocols

Individual yeast clones

Culture yeast strains in YPD broth with the 200µg/ml G418 (Geneticin Disulfate Salt, 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.

Section 4: 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 (**Figure 2B**) while wild type alleles are PCR amplified by gene specific primers (**Figure 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.

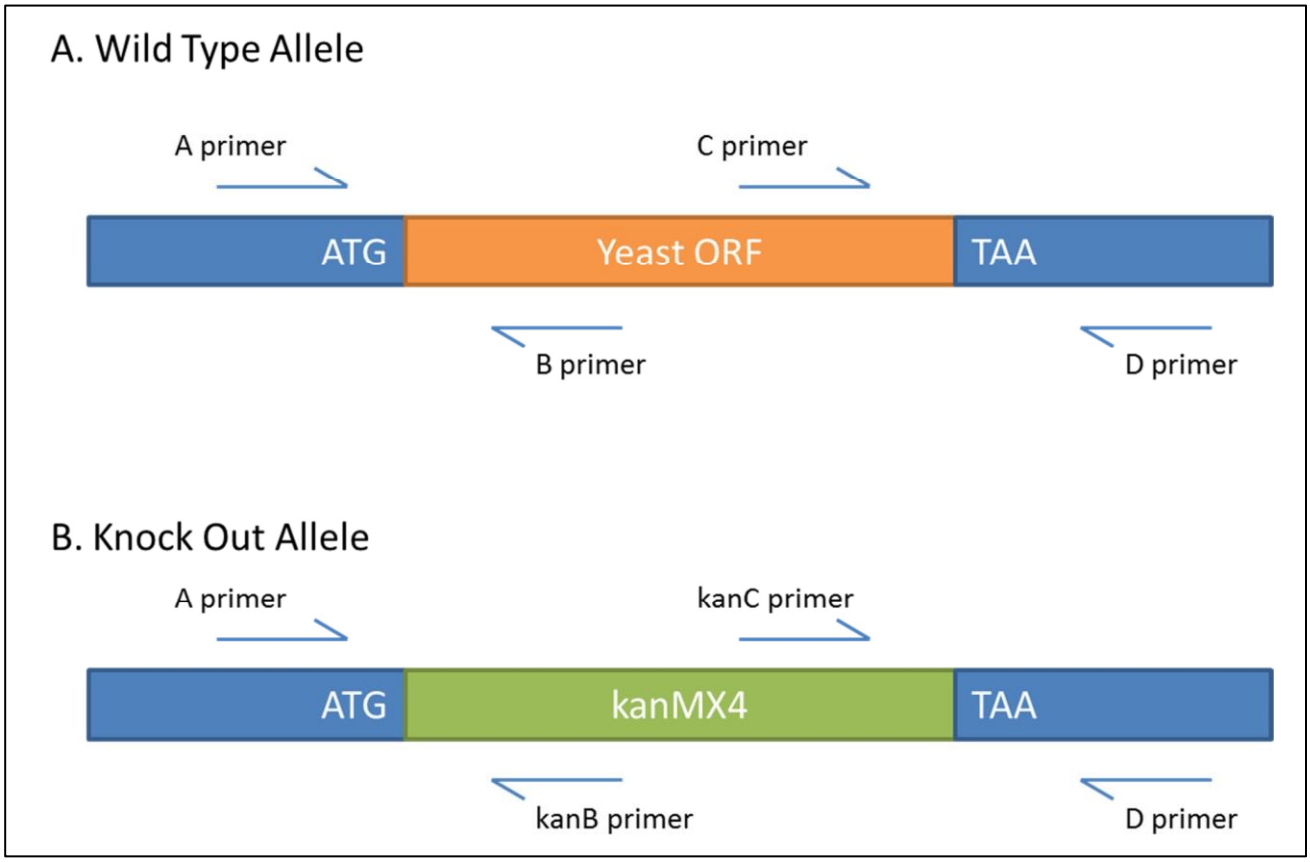

Figure 2: Schematic of wild type allele (A) and Knock out allele (B) showing primer pairs used to detect each.

Appendix: 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/project_desc.html

Additional protocols and technical information
http://www-sequence.stanford.edu/group/yeast_deletion_project/protocols.html

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Contact Information

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Corporate Headquarters
Transomic Technologies Inc.
601 Genome Way, Suite 2021
Huntsville, AL 35806 USA
Phone: 866-833-0712 Fax: 256-327-9515
E-mail: support@transomic.com