TECHNICAL MANUAL

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Yeast barcoders

Cat. #YSC5117

Product description

The Yeast Barcoders Collection was developed by Corey Nislow and Guri Giaever at the University of Toronto. The goal of this collection was to provide a simple and general method of barcoding yeast collections. The Yeast Barcoders Collection contains unique DNA sequence tags (barcodes) that can be systematically transferred to any *Saccharomyces cerevisiae* collection (Figure 1). The Yeast Barcoders Collection currently consists of approximately 1200 yeast strains that combines simple barcoding (Yan, Costanzo *et al.* 2008) with Synthetic Genetic Array (SGA sites.utoronto.ca/boonelab/sgatechnology/index.shtml;) (Tong and Boone 2006; Boone 2007).

Constructs are distributed as cultures of *S. cerevisiae* in YPD broth + G418 (300 µg/mL) + 15% glycerol.

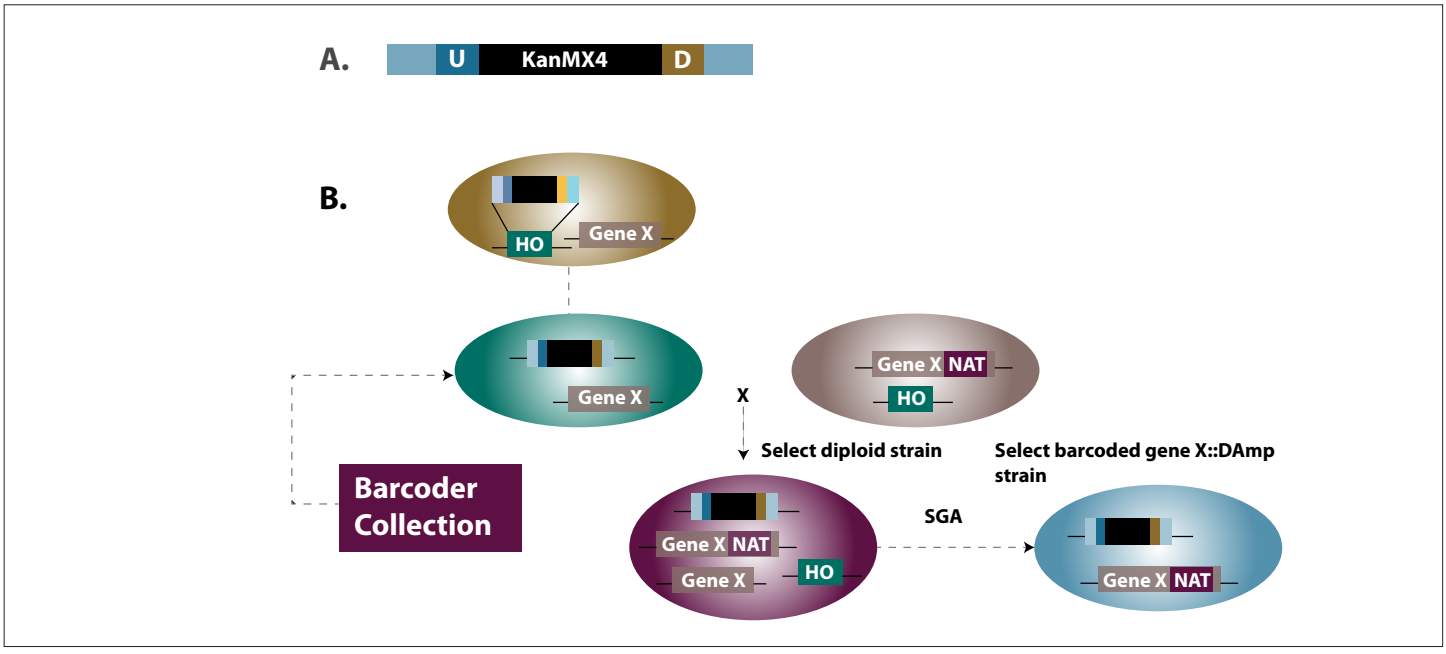

Figure 1. Strategy used to bar code DAmP strains. **A.** Two unique bar codes, upstream tag (U) and downstream tag (D), were linked to the KanMX4 cassette. **B.** The bar-coded KanMX4 cassette was used to replace HO in the MATa strain BY4741 by transformation and homologous recombination. A bar-coded BY4741 strain was then mated with a strain f MATa gene X::DAmP strain, and the diploid strains were selected on YPD medium containing G418 and nourseothricin. The diploid strains were then sporulated, and the SGA technology was used to select haploid double mutants that contain both the bar-coded *HO* and the gene X::DAmP allele. NAT represents the gene that encodes resistance to the antibiotic nourseothricin (Yan, Costanzo *et al.* 2008).

Strain information

Genotype of *S. Cerevisiae* **BY4741**
MATa his31 leu20 ura30 met15D0

Antibiotic resistance

The Yeast Barcoders contain the kanR gene which should be selected for with G418 (Table 1).

Table 1. Antibiotic resistance of *S. cerevisiae* yeast strain R1158.

Antibiotic	Concentration	Utility
G418	300 µg/mL	Eukaryotic selection marker

Validation

Construct validation

To verify correct integration of the barcode cassettes, genomic DNA was prepared from the resistant strains and used as template in PCR reactions using two primers common to the KanMX4 module (Yan, Costanzo *et al.* 2008).

Collection validation

To validate both the barcoders the technology was applied by generating a collection of barcoded 'decreased abundance by mRNA perturbation' (DAmP) loss-of function strains. (Yan, Costanzo *et al.* 2008).

Protocol I – replication

We recommend making a stock or working culture of the yeast strains. Grow the yeast strains in YPD broth with the appropriate antibiotic (300 µg/ml G418)*. 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.

Table 2. Materials for yeast 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 media for yeast clones, do not add the glycerol to the medium until after the clones have grown. Glycerol inhibits the growth of yeast**

YPD 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/dH2O 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/dH_2O mix.

Replication of 96-well plates

Prepare target plates

1. Prepare deep well 96-well target plates by dispensing 1.5mL 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 at 16-48 hours, based upon when growth is apparent.
8. When sufficient growth has been noted in the target plates, add 500 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.

Protocol II – strain verification

PCR can be used to verify the integration of the barcode cassette. Prepare genomic DNA from the resistant strains and use as template in PCR reactions using two primers common to the KanMX4 module. [KanB (5'-CTGCAGCGAGGAGCCGTAAT-3') and KanC (5'-TGATTTTGATGACGAGCGTAAT-3')] and two primers [A (5'-TATTAGGTGTGAAACCACGAAAAGT-3') and D (5'-CATGTCTTCTCGTTAAGACTGCAT-3')] flanking *HO* gene. Primer A is designed from region 244 base upstream of the start codon, whereas primer D is 356 bases downstream of the stop codon. For correct transformation strains, the A-KanB and the D-KanC PCR reactions should produce a 566 and 1964-bp band respectively when analyzed by gel electrophoresis. To further confirm correct integration of barcodes, two other primers [B (5'-ACTGTCATTGGGAATGTCTTATGAT-3') and C (5'-GAGTGGTAAAAATCGAGTATGTGCT-3')] were designed based on the sequence within the *HO* gene. The A-B and C-D PCR reactions should produce no band if integration is correct (Yan, Costanzo *et al.* 2008).

Related reagents

Table 3. Related Reagents.

Item	Vendor	Cat #
Yeast Barcoders Collection	Dharmacon	YSC5117
DAmp Library for 970 essential genes – diploid	Dharmacon	YSC5050
DAmP Library for 842 essential genes – haploid	Dharmacon	YSC5090
DAmP individual strain – diploid	Dharmacon	YSC5093
DAmP individual strain – haploid	Dharmacon	YSC5094

FAQS/troubleshooting

For answers to questions that are not addressed here, please email technical support ts.dharmacon@horizondiscovery.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

What clones are part of my collection?

A USB containing the data for this collection will be shipped with each collection.

What antibiotic should I use?

You should grow all Yeast Barcoders Clones in YPD broth + G418 (300 µg/mL). When preparing medium for yeast clones, do not add the glycerol to the medium until after the clones have grown if a freezer stock is needed. Glycerol inhibits the growth of yeast. We prepare a solution of 50% glycerol and 50% medium to add to the growth media after incubation.

Where can I find more information on the barcode primers—such as sequences?

The supplemental data included in the Yan 2008 reference contains primer sequences for the barcodes.

What Is SGA?

SGA stands for the Synthetic Genetic Array developed by the Boone lab at the Donnelly Centre for Cellular and Biochemical Research, University of Toronto (Tong and Boone 2006; Boone 2007).

References

1. Costanzo, M., G. Giaever, *et al.* (2006). Experimental approaches to identify genetic networks. *Curr Opin Biotechnol.* **17(5)**: 472-80.
2. Boone, A. T. a. C. (2007). High-throughput strain construction and systematic synthetic lethal screening in *Saccharomyces cerevisiae*. *Yeast Gene Analysis*. **36**: 369-386, 706-707.
3. Tong, A. H. and C. Boone (2006). Synthetic genetic array analysis in *Saccharomyces cerevisiae*. *Methods Mol Biol.* **313:** 171-92.
4. Yan, Z., M. Costanzo, *et al.* (2008). Yeast Barcoders: a chemogenomic application of a universal donor-strain collection carrying bar-code identifiers. *Nat Methods* **5(8)**, 719-725 (August 2008).

If you have any questions, contact

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