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Dharmacon™ Molecular Barcoded Yeast ORF (MoBY ORF Library)

Cat #YSC5432

Product description

The Charlie Boone lab at The University of Toronto has created the Molecular Barcoded Yeast ORF (MoBY-ORF) library. This is a collection in which each gene, controlled by its native promoter and terminator, has been cloned into a centromere-based vector along with two unique DNA barcodes. The MoBY-ORF resource has numerous genetic and chemical-genetic applications.

One example, from Ho et al. (2009), is the discovery of the genes responsible for spontaneous drug-resistance mutation. The MoBY-ORF library enables the simultaneous monitoring of the fitness level changes induced by each gene in the genome. This provides a comprehensive strategy for screening drug resistant or other mutations in yeast. Changes in fitness due to the expression of each MoBY-ORF can be measured in parallel in a heterogeneous populations. In addition to the ease of use, this greatly minimizes the amount of the drug required for the complementation assay.

Library construction

The MoBY-ORF library consists of plasmids that each carry a pair of oligonucleotide barcodes and a single yeast ORF that is flanked by its native upstream and downstream genomic sequences (Figure 1). The plasmid vector p5472 carries a URA3 selectable marker and a yeast centromere, which maintains one to three copies of the plasmid per cell. The vector was designed to be compatible with an *in vivo* bacterial cloning method, mating-assisted genetically integrated cloning (MAGIC), which facilitates the rapid construction of recombinant DNA molecules, enabling the barcoded clones to be transferred efficiently to other vector backbones, such as a high-copy vector (Li and Elledge, 2005). The barcodes were obtained from the yeast deletion mutant collection and comprise two unique 20-nucleotide DNA sequences (labeled the UPTAG and DNTAG) flanking a dominant selectable marker (KanMX) that confers resistance to the drug G418/kanamycin. The barcodes can be amplified with universal primers, enabling cells carrying a specific ORF to be quantitatively detected with a microarray having probes that hybridize to the barcode sequences. Each plasmid was constructed in a three-step process. First, each yeast ORF was PCR amplified from an average of ~ 900 bp upstream of the start codon to an average of ~ 250 bp downstream of the stop codon using a DNA template isolated from the sequenced S288C

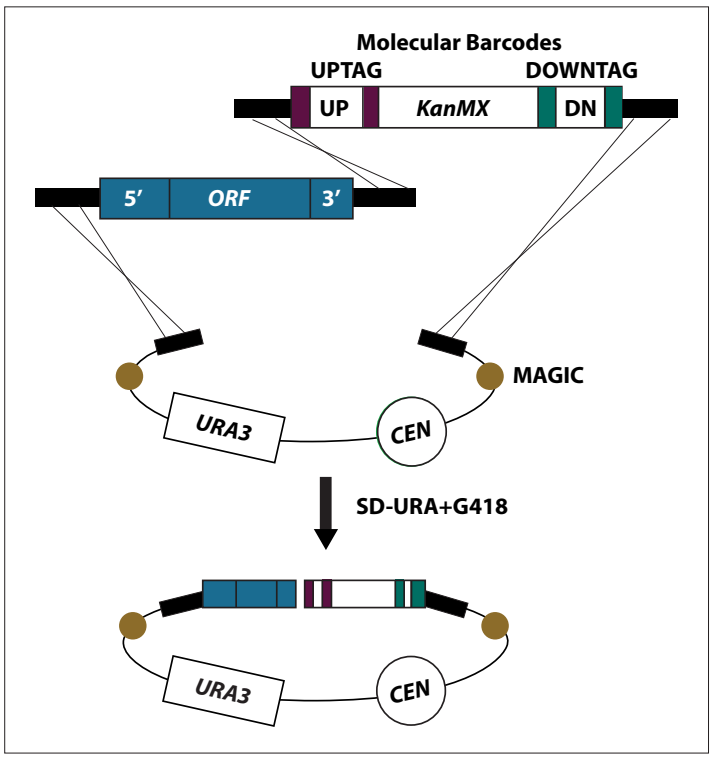

Figure 1. Construction of the MoBY-ORF library by homologous recombination in yeast. Yeast cells are co-transformed with a PCR product encoding an ORF, a KanMX cassette, which confers G418/kanamycin resistance, and an XhoI-linearized vector that carries a selectable marker (URA3) and a yeast centromere sequence (CEN). Transformants are grown on synthetic medium that selects for recombinant plasmids. The KanMX cassette is flanked by two different molecular barcodes, labeled UPTAG and DOWNTAG, each of which are flanked by common primer sites, indicated in purple and green. The KanMX barcode cassette for each gene was amplified from the corresponding deletion mutant for that gene. The vector backbone was designed to be compatible with the MAGIC system for manipulating plasmid inserts by homologous recombination in *Escherichia coli*. Filled yellow circles represent MAGIC recombination sites flanking the MoBY-ORF insert (Ho et al., 2009).

strain. In addition, the KanMX barcode sequences that uniquely identify the ORF were PCR amplified from the appropriate strain in the yeast deletion collection. Second, the plasmid was assembled by homologous recombination by transforming yeast with the ORF, the barcode PCR products and linearized p5472. Third, recombinant plasmids were recovered and used to transform bacteria to facilitate plasmid DNA isolation and subsequent diagnostic restriction digests to confirm the sizes of both fragments.

Each clone in the MoBY-ORF library was sequenced to confirm the 3′ portion of the gene and the barcodes. The authors identified 4,396 ORFs (88.7%) with two unique barcodes, but 560 with only one barcode (344 with only an UPTAG and 216 with a DNTAG), as the other barcode was either not unique within the collection (that is, multiple clones contained the same barcode sequence), or it had no corresponding sequence on the Affymetrix TAG4 microarray. In summary, the MoBY-ORF library version 1.0 contains 4,956 uniquely barcoded ORFs, representing ~ 90% of all non-dubious ORFs annotated in the *Saccharomyces* Genome Database (SGD). To assess the functionality of the clones, 254 different MoBY-ORF plasmids were introduced into a synthetic genetic array (SGA) query strain. The SGA method was then used to cross the plasmids into a set of corresponding temperature-sensitive mutants covering alleles of the same 254 essential genes, and the transformants tested for functional complementation at the restrictive temperatures. In total, 17 clones failed to rescue the temperature sensitivity of the corresponding mutant strain, suggesting that ~ 93% of the clones in the library should be functional.

Table 1. Antibiotic resistance of MoBY ORFs (Bacteria)

Antibiotic	Concentration	Utility
tetracycline	5 µg/ml	Bacterial selection marker-Magic plasmid
kanamycin	100 µg/ml	Bacterial selection marker-barcode
chloramphenicol	12.5 µg/ml	Bacterial selection marker-vector

Antibiotic resistance

The kanamycin and chloramphenicol select for the clone (chloramphenicol from the vector backbone and kanamycin from the barcoded insert). The tetracycline is to maintain a plasmid specific to the bacterial strain used for the MAGIC protocol (BUN20 [Δlac-169 rpoS(Am) robA1 creC510 hsdR514 ΔuidA(MluI):pir-116 endA(BT333) recA1 F′(lac+ pro+ ΔoriT:tet)] (Li & Elledge, 2005).

Verification

For each clone, two sequencing reactions were performed: one covering the UPTAG and 3′ ORF junction and the second covering the DNTAG (with sequencing primers 5′- TATACATGGGGATGTATGGGC-3′ and 5′-GGGCAACAACAGATGGCTG-3′ respectively). The sequencing reads were analyzed using computational scripts developed to identify the barcode position, based on the adjacent common primer sequences (5′-GACCTGCAGCGTACG-3′ for the UPTAG and 5′CGGTGTCGGTCTCGTAG-3′ for the DNTAG).

Protocol I – bacterial replication

We recommend making a stock or working culture of the bacterial strains. Grow the clones at 37 °C in LB-Lennox (low salt) medium plus tetracycline (5 µg/mL), kanamycin (100µg/mL) and chloramphenicol (12.5 µg/mL). Prepare media with 8% glycerol* and the appropriate antibiotics. After incubation, vortex the culture to evenly mix the bacteria throughout, and store at –80 °C. The culture can be stored indefinitely at –80 °C.

Replication of plates

Prepare target plates by dispensing ~160 µL of LB-Lennox (low salt) medium supplemented with 8% glycerol* and appropriate antibiotics.

Prepare source Plates

- Remove foil seals while the source plates are still frozen. This minimizes cross contamination.
- Thaw the source plates with the lid on. Wipe any condensation underneath the lid with a paper wipe soaked in ethanol.

Replicate

- Gently place a disposable replicator in the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the well.
- Gently remove the replicator from the source plate and gently place in the target plate and mix in the same manner to transfer cells.
- Place the lids back on the source plates and target plates.
- Repeat steps until all plates have been replicated.
- Return the source plates to the -80 °C freezer.
- Place the inoculated target plates in a 37 °C incubator without shaking for 18-19 hours.

Freeze at -80 °C for long term storage. Avoid long periods of storage at room temperature or higher. ***Glycerol can be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at -80 °C, 8% glycerol is required.**

References

Ho, CH. et al. A molecular barcoded yeast ORF library enables mode-of-action analysis of bioactive compounds. *Nature Biotechnology*. 27(4): 369-377 (2009).

Li, M.Z. & Elledge, S.J. MAGIC, an *in vivo* genetic method for the rapid construction of recombinant DNA molecules. *Nat. Genet.* 37(3), 311–319 (2005).

FAQs/troubleshooting

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