TECHNICAL MANUAL

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Dharmacon™ Molecular Barcoded Yeast ORF 2.0 (MoBY ORF 2.0 Library)

Cat #YSC11751

Product description

The Charlie Boone lab at The University of Toronto has created the Molecular Barcoded Yeast ORF 2.0 (MoBY-ORF 2.0) library for drug-target screening applications in *S. cerevisiae*. Each gene from the MoBY-ORF 1.0 collection was transferred using MAGIC (Mating-Assisted Genetically Integrated Cloning; Li, 2005) from the original centromere-based vector into a high-copy plasmid backbone (p5476), and transformed into the BY4741 yeast strain. Each gene was moved in its entirety and is controlled by its native promoter and terminator and has two unique DNA barcodes. The MoBY-ORF 2.0 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 population. In addition to the ease of use, this greatly minimizes the amount of the drug required for the complementation assay.

Library construction

MoBY-ORF, v1.0 bacterial strains were inoculated from frozen stocks in 96-well plates into a shallow 96-well plate in which each well had 100 µL 2X YT containing tetracycline at 5 µg/mL, kanamycin at 50 µg/ml, and chloramphenicol at 12.5 µg/mL. Cultures were grown for ~ 16 hours at 37 °C. P5530 (genotype: *lacI^{Q}rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 galU95 ΔendA9:FRT ΔrecA635:FRT umuC:ParaBAD-I-SceI-FRT)*, the MAGIC recipient strain carrying plasmid p5476, was inoculated into 5 mL of YE+Gluc, containing 0.2% glucose, spectinomycin at 10 µg/mL, and carbenicillin at 200 µg/mL. Cultures were grown for ~ 22 hours at 30 °C.

The following day, the OD_{600} values of the recipient strain and of 3 bacterial (donor) strains from the MoBY-ORF 1.0 96-well plate were taken; the average of the 3 wells was used as the average OD_{600} for the entire plate. Cultures were diluted to OD_{600} ~ 0.10 and mixed together for mating in a 1:1 ratio in a total volume of 100 µL in a fresh 96-well plate. Cells were shaken at 30 °C for 2 hours, at which time L-arabinose was added to a final concentration of 0.2% to each well. Cells were incubated without shaking at 37 °C for 2 hours, and then transferred to a shaking incubator at 37 °C for 2 hours. 2 µL of the 100 µL mating reaction were plated onto YE+Glyc containing 0.2% glycerol, 0.2% DL-chlorophenylalanine, carbenicillin at 200 µg/mL, and kanamycin at 50 µg/mL, and incubated at 41 °C overnight.

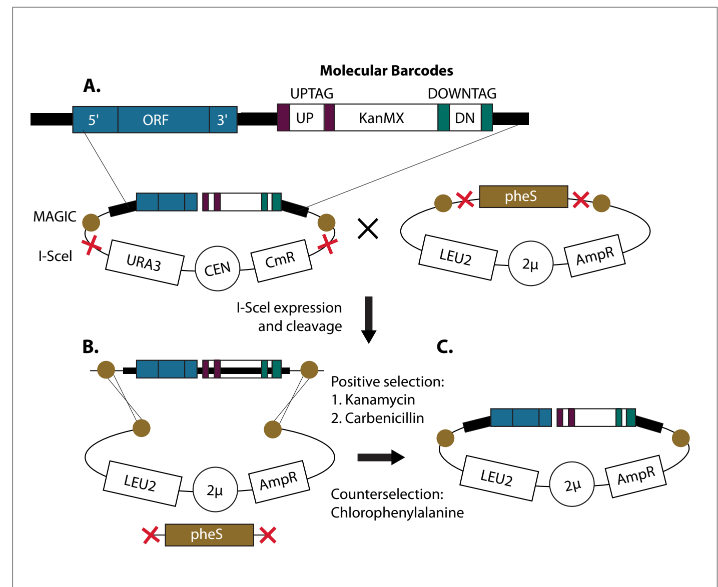

Figure 1. Construction of MoBY-ORF library 2.0 using MAGIC

A. The donor bacterial strain carrying the CEN-based MoBY-ORF 1.0 plasmid is mated to a recipient bacterial strain carrying a 2µ-based vector.

B. The barcoded ORF is released and the recipient vector linearized. Subsequent expression of a recombinase induces homologous recombination between the barcoded ORF and linearized vector using the MAGIC sequences (filled gold circles).

C. The recipient strain was grown on medium containing ampicillin, kanamycin, and DL-chlorophenylalanine that selects against any non-recombined vector and for the recombinant plasmid (Ho, 2011).

Mating products were streaked out for individual colonies onto 2X YT containing 0.2% glucose, carbenicillin at 200 µg/mL, and kanamycin at 50 µg/mL and incubated at 37 °C overnight. Miniprep DNA was prepared from a single bacterial colony, doubly digested using *Xho*I and *Eco*RI (Fermentas), and resolved on a 0.8% agarose gel to confirm vector and insert fragment sizes. Two individuals performed gel analysis independently, and the results were compared to determine clone validity. If the primary *Xho*I/*Eco*RI digest was ambiguous, a secondary digest using *Bam*HI/*Hind*III was performed. ORF fragment sizes for both double digests, along with complete ORF sequence and barcodes, can be obtained from the MoBY-ORF database: (http://moby.ccbr.utoronto.ca).

Antibiotic resistance

The kanamycin and chloramphenicol select for the clone (chloramphenicol from the vector backbone and kanamycin from the barcoded insert). The chlorophenylalanine is to act as a counter selection agent against any non-recombined vectors.

Protocol – yeast replication

We recommend making a stock or working culture of the yeast strains. Grow the clones at 30 °C in SD(MSG)-LEU containing 200 µg/mL G418. Prepare medium with 20% glycerol* and the appropriate antibiotics. After incubation, vortex the culture to evenly mix the yeast 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 SD(MSG)-LEU+G418 plus 20% glycerol medium.

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 30 °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, 20% glycerol is required.**

References

Ho, CH. Molecular Barcoded Plasmid Yeast ORF Library: Linking Bioactive Compounds to their Cellular Targets and Mapping Dosage Suppressor Networks. http://hdl.handle.net/1807/29745 (2011)

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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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