GE Healthcare

Dharmacon™ Yeast Genomic Tiling Collection

Cat #YSC4613

Product Description

The Yeast Genomic Tiling Collection consists of over 1,500 glycerol stock cultures each containing a unique clone of a segment of the yeast *S. cerevisiae* genome in an *E. coli*-yeast shuttle vector, designed by Dr. Greg Prelich of Albert Einstein College of Medicine. These plasmid clones represent a unique and virtually complete overlapping clone collection of the entire *S. cervisiae* genome. This clone collection constitutes a "minimal functional pathway" through the yeast genome and represents ~ 97% of the length of the yeast genome at the physical level, and ~ 95% of the genome at the functional level. The genomic location represented by each insert was identified in collaboration with the Sanger Institute by sequencing the insert-vector junctions and comparing the obtained sequences to the complete yeast genome sequence in the public database. Analysis revealed only ~ 284 kb of the genome missing in 92 gaps (for a 3 kb average gap size). The genome coordinates (such as the known yeast genes) and well position on the microtiter plate of each clone is precisely known and supplied to the customer. This collection was designed specifically for systematic overexpression screens.

The library was constructed by partially digesting prototrophic yeast genomic DNA with *Mbo*I and subcloning into the *Bam*HI sites of the *E. coli*-yeast shuttle vector, pGP564. Since the library was not made by PCR, it contains no PCR-induced mutations. The proteins are untagged and expressed from their endogenous wild-type promoter. The inserts are flanked by attL sites, allowing transfer of inserts to appropriate destination vectors with attR sites by the Gateway reaction.

The pGP564 shuttle vector contains the *LEU2* selectable marker and 2-micron plasmid sequences necessary for maintenance of a high copy number in yeast. The average insert size in this library is approximately 10 kb, with each insert containing an average of 4-5 genes.

The collection is provided as bacterial cultures of *E. coli* (DH10B) in LB broth with an inert growth indicator + 8% glycerol + kanamycin at a concentration of 50 µg/mL.

Open Labs Products

We provide clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other vendors. In order to provide these as a public resource, we depend on the contributing laboratories for quality control. Therefore, these are distributed in the format provided by the contributing institution with no additional product validation or guarantee. Additional information can be found in the product manual as well as in associated published articles (if available).

Shipping and Storage

Plates are shipped on dry ice and should be stored at -80 °C.

Source Of Yeast Genomic DNA

The parental yeast strain for isolation of genomic DNA was FY4, a MAT*a* prototroph, originated from Dr. Fred Winston's lab (Harvard Medical School Department of Genetics).

Plate Replication Protocol

To allow any CO_2 that may have dissolved into the medium from the dry ice in shipping to dissipate, store plates at –80 °C for at least 48 hours before thawing.

Procedure

Replication of Plates

1. Dispense ~ 160 µL of sterile (low salt) LB medium into 96-well microtiter plates. The LB should be supplemented with 8% glycerol and the appropriate antibiotic.

Prepare Source Plates

1. Remove the foil seals from the source plates. Removing the seals while the source plates are frozen will minimize cross-contamination.
2. Thaw the source plates with the lids on. Wipe away any condensation under the lid.

Replicate

1. Gently place a disposable replicator into 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 plate of the well.
2. Carefully remove the replicator from the source plate and gently place the replicator into the target plate. Lightly move the replicator back and forth in the target plate to transfer cells.
3. Discard the replicator.
4. Place the lids back on the source plates and target plates.
5. Seal the source plates, being mindful to avoid cross contamination.
6. Repeat this process until all plates have been replicated.
7. Return the source plates to the –80 ºC freezer.
8. Place the inoculated target plates in a 37 ºC incubator. Incubate the plates for 12-24 hours.

Obtaining Clone Information

Individual clone information and plate locations can be found on the USB drive that accompanies the collection.
Alternatively, the datasheet can be downloaded from our website. (gelifesciences.com/dharmacon)

Reference

Jones, G. M., J. Stalker, *et al.* (2008). A systematic library for comprehensive overexpression screens in *Saccharomyces cerevisiae. Nat Methods* **5**(3): 239-41.
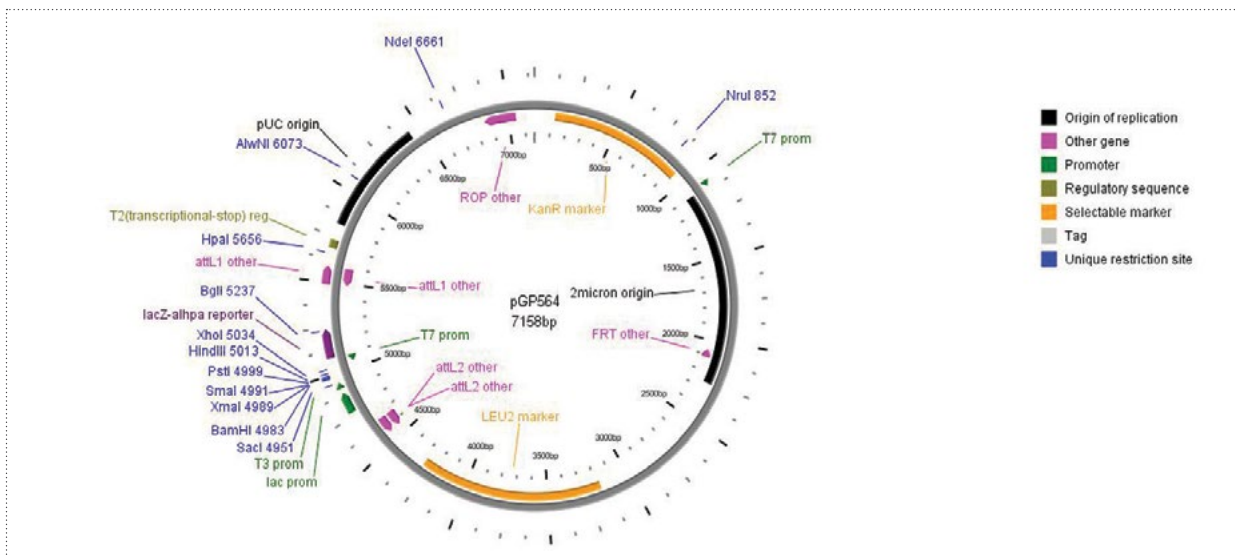

Figure 1. pGP564 Map. The vector is pBR322-based (not pUC-based), so the DNA yield may be a bit lower than a pUC-based vector yield.

Appendix I
Vector Sequence pGP564

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Other Gene	6853..7044
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FAQS/Troubleshooting

For answers to questions that are not addressed here, please email technical support ts.dharmacon@ge.com

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