

# Dharmacon™

RNAi, Gene Expression & Gene Editing

## Yeast mTn Plasmid Clones and Collection

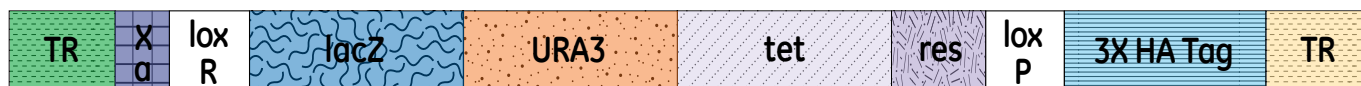
Cat #YSC1201, YSC1202

### Introduction:

The Yeast mTn (mini transposon) Plasmid Strains represent over 3,600 *E. coli* strains. Each plasmid contains yeast genomic DNA integrated with a 6 kb mini-transposon (mTn). This resource allows for transformation of the plasmid into the yeast strain(s) of interest, which can then provide a set of gene disruption mutants for phenotypic analysis\*.

In the original experiments, the mutagenized strains were developed via transposon insertional mutagenesis. The mini-transposons (mTn) were initially introduced into a plasmid library of yeast genomic DNA followed by transformation of the disruption alleles into a heterozygous diploid strain of yeast. Once introduced into the yeast the transposons integrate into the corresponding genomic locus by homologous recombination. The insertion of the transposon into the open reading frame (ORF) of the gene typically disrupts gene function, or insertion upstream or downstream of the ORF may result in mis-expression of the gene.

The 6 kb mTn contains the reporter gene *lacZ*, flanked by *lox* sites, and a 3xHA tag (Figure 1).



**Figure 1.** TR – Tn3 terminal repeats (with transposonase gene *tnpA* allows transposition to occur)

Xa – Xa protease site (can be used to remove HA tag– will remove everything downstream of the tag and possibly truncate the gene)

loxR/loxP – target sites for Cre recombinase

lacZ – for B-gal assays (no start codon or promoter)

URA3 – for negative selection. If media has substrate for URA3, cell dies.

tet – tetracycline resistance

res – aids in transposition with *tnpR* resolvase

3X HA Tag – 3 copies of epitope from hemagglutinin protein

The *lacZ* gene is lacking both its promoter and start codon, thus, the expression is dependent on the transposon being inserted in-frame into a gene and subsequently transcribed and translated. Using the site-specific DNA recombinase, Cre, the 6kb insert can be reduced to a 93-codon read-through element containing the 3X HA-tag (see HA-Tagged Yeast Collection, Cat #YSC1068). The mTn technology has been used for large-scale functional analysis of the yeast genome. Information on the insertion site, ORFs affected, and strains available from Dharmacon can be obtained by querying on our website by ORF ID.

\* Although disruption occurs in most cases, gene function may not always be disrupted by the insertion.

### Product Description:

#### Strain

*E. coli* DH10B in LB + tetracycline (3 µg/mL) + kanamycin (40 µg/mL) + 8% glycerol.

#### Library

Bacterial culture of *E. coli* in LB broth with an inert growth indicator + 8% glycerol + tetracycline (3 µg/mL) + kanamycin (40 µg/mL).

To allow any CO<sub>2</sub> that may have dissolved into the medium from the dry ice in shipping to dissipate, please store plates at -80 °C for at least 48 hours before thawing

### Storage:

4 °C for up to one week; -80 °C indefinitely.



GE Healthcare

## Plate Replication Protocol:

### 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 400 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.

Other relevant information for any tables in manuals:

Deep well plates: Fisher Cat #07-200-700

Microporous film: Fisher Cat #50-820-083

Heat seal: Fisher cat AB-3738

## Finding Additional Information on a Strain:

The Dharmacon™ Search provides a rapid means of locating relevant strain information. Simply enter the gene identifier into the query box and press the “+” sign to the left of the correct result to see further details (See Figure 2).

The screenshot shows the GE Healthcare Dharmacon website interface. At the top, there is a search bar containing 'YMR110C'. Below the search bar is a navigation menu with links for Home, Products, Applications, Brands, Resources, Service & Support, and About Us. The main content area displays 'Search results for YMR110C' with filters for Catalog (7), Products (0), Genes (1), Pathways (0), and Resources (13). Three search results are listed, each with a '+' icon on the left and an 'Add To Cart' button on the right:

Item Name	Item ID	Unit Size	Price
GST Tagged HFD1 (YMR110C) Strain	# YSC4515-202487787	glycerol stock	\$75.00
Yeast Mini-Transposon HFD1 (YMR110C) Strain	# YSC1073-213590553	glycerol stock	\$75.00
Plasmid Mini-Transposon HFD1 (YMR110C) Strain	# YSC1202-213592356	glycerol stock	\$66.00

A red arrow points to the '+' icon next to the 'Plasmid Mini-Transposon HFD1 (YMR110C) Strain' item. On the right side of the page, there is a promotional banner for the 'Edit-R CRISPR-Cas9 System' with the text 'We just changed the way you edit genes' and a 'LIVE SUPPORT NOW AVAILABLE' button.

Figure 2. Dharmacon Product Search.

## Strain Verification:

A PCR reaction using one gene specific and one mTn specific primer should yield a product with a known size, since the mTn insertion site is unique for each strain.

mTn sequence: NCBI GenBank accession U54828

[ncbi.nlm.nih.gov](http://ncbi.nlm.nih.gov)

## References:

1. P. Ross-Macdonald, *et al.*, *Nature* 402, 413 (1999).
2. Kumar, A. *et al.*, *Methods Enzymol.* 328. 550-574 (2000).
3. Kumar, A., Cheung, K.-H., Ross-Macdonald, P., Coelho, P.S.R., Miller, P., and Snyder, M. *Nucleic Acids Res.* 28, 81 (2000).
4. Siefert, *et al.*, *Proc. Natl. Acad. Sci. USA* 88, 5457-5461(1991)
5. Siefert, *et al.*, *Proc. Natl. Acad. Sci. USA* 83 (3), 735-739 (1986)

## FAQs/Troubleshooting:

For answers to questions that are not addressed here, please email technical support at [ts.dharmacon@ge.com](mailto:ts.dharmacon@ge.com).

GE, imagination at work and GE monogram are trademarks of General Electric Company. Dharmacon is a trademark of GE Healthcare companies. All other trademarks are the property of General Electric Company or one of its subsidiaries. ©2014 General Electric Company—All rights reserved. Version published August 2014. GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK



GE Healthcare

Orders can be placed at:  
[gelifesciences.com/dharmacon](http://gelifesciences.com/dharmacon)

Customer Support: [cs.dharmacon@ge.com](mailto:cs.dharmacon@ge.com)  
Technical Support: [ts.dharmacon@ge.com](mailto:ts.dharmacon@ge.com) or  
1.800.235.9880; 303.604.9499 if you have any questions.