TECHNICAL MANUAL

horizon™
INSPIRED CELL SOLUTIONS

Yeast mTn plasmid clones and collections

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).

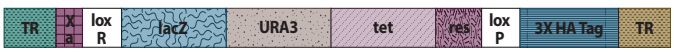

Figure 1. TR – Tn3 terminal repeats (with transposonase gene tnpA allows transposition to occur)
Xa—Xa protease site (can be used to remove HA tag— will remove everything downstream of the tag and possibly truncate the gene)
loxR/loxP—target sites for Cre recombinase
lacZ—for B-gal assays (no start codon or promoter)
URA3—for negative selection. If media has substrate for URA3, cell dies.
tet—tetracylcine 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_2 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.

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 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.

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)

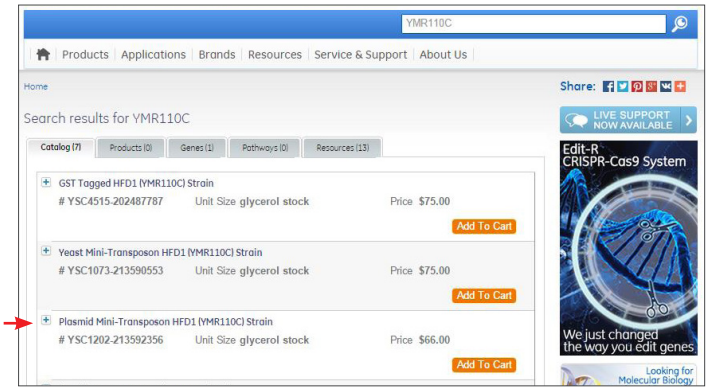

Figure 2. Dharmacon Product Search

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)

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@horizondiscovery.com.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

horizon™
INSPIRED CELL SOLUTIONS

V31118