

Yeast insertional mutant clones and collection

Cat #YSC1073, YSC1074

Introduction

The Yeast Insertional Mutant Strains represent over 3,600 mutagenized yeast strains providing a set of gene disruption mutants for phenotypic analysis*. The mutagenized strains were developed via transposon insertional mutagenesis. The minitransposons (mTn) are 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).

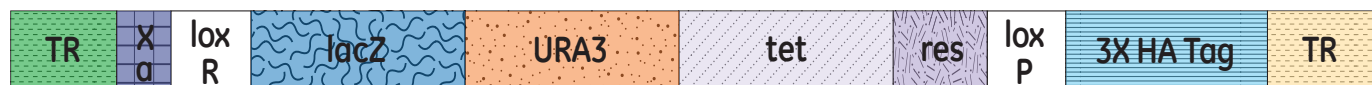


Figure 1. TR – Tn3 terminal repeats (with transposonase gene *tnpA* allows transposition to occur)

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

Product description

Culture of *S. cerevisiae* in synthetic defined (SD) dropout broth (-leu, -ura) + 15% glycerol.

Storage

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

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 6 kb insert can be reduced to a 93-codon read-through element containing the 3X HA-tag (The Yeast HA-Tagged 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. Additionally, phenotypes observed should be confirmed to be linked to the indicated insertion.**

Making a stock culture

Once the strain has been streak isolated and the identity of the strain has been confirmed, we recommend making a stock of the pure culture. Inoculate the pure culture in SD dropout broth (-leu, -ura) and incubate for 48 hours at 30 °C. Transfer 850 µL of culture into a polypropylene tube and add 150 µL sterile glycerol to make a 15% glycerol freezing solution. Vortex to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at -80 °C.

Replication of 96-well plates

Strain verification

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.

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](https://www.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).

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

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