TECHNICAL MANUAL

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HA-tagged yeast clones and collection

Cat. #YSC1070, YSC1068

The Dharmacon™ HA-Tagged Yeast Clones and Collection contains over 2,400 yeast mutagenized strains. Minitransposon (mTn) constructs and shuttle mutagenesis were utilized to develop a collection of mutagenized yeast genes for analysis of expression patterns, localization, and phenotypic analysis[1,2]. The constructs originally contained a 6-kb multipurpose transposon containing a reporter sequence, selection markers for both *E. coli* and *Saccharomyces*, flanked by lox sites and the required transposon elements. Following reduction by Cre-lox recombination, a 93-codon region, consisting primarily of the read through triple hemagglutinin epitope tag (3xHA tag), remains. The mTn technology has proven useful for large-scale functional analysis of the yeast genome. The HA tags permit a variety of functional studies including immunolocalization, immunoprecipitation and analysis of binding sites using immuno detection. Insertion via mini-transposon (mTn) mutagenesis allows for tagging within the coding region of the protein in a nonbiased fashion, resulting in the tagging of both annotated and previously unidentified ORFs[1].

The HA insertion provides a means to study the function of yeast genes in a large-scale fashion by analysis of conditional phenotypes. The generation of conditional alleles and hypomorphic mutants that exhibit partial gene function is especially important for the analysis of essential genes[2,3]. It has also been shown that full-length HA tagged proteins can effectively localize to the appropriate cellular region where they can be detected using an antibody to the HA epitope[4]. Additionally, native amounts of protein can easily be purified from individual yeast strains using immunoprecipitation with a commercially available HA antibody.

Product description

Culture of *S. cerevisiae* in YPD broth + 12.5% glycerol.

Shipping and storage

Individual clones are shipped at room temperature and may be stored for up to one week at +4 °C. Clones may stored indefinitely at –80 °C. Plates are shipped on dry ice and should be stored at –80 °C. To allow any CO_2 that may have dissolved into the media from the dry ice in shipping to dissipate, please store plates at –80 °C for at least 48 hours before thawing.

Making a stock culture

Inoculate the yeast culture into the appropriate liquid medium plus antibiotics or supplements (no glycerol) and incubate for at least 48 hours at 30 °C with shaking. After incubation add enough sterile 50/50 glycerol/YPD mixture to bring the total glycerol percentage to 15%. The culture can then be stored indefinitely at –80 °C.

Replication of plates

Prepare target plates

1. Prepare deep well 96-well target plates by dispensing 1.5 mL 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 1–4 until all plates have been replicated.
6. Heat seal source plates and return to an ultra low 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 400µL 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 3xHA-tag specific primer should yield a product with a known size, since the 3xHA-tag insertion site is unique for each strain.

3xHA Tag sequence
GCGGCCGTTTACCCATACGATGTTCCTGAC
TATGCGGGCTATCCCTATGACGTCCCGGAC
TATGCAGGATCCTATCCATATGACGTTCCAG ATTACGCTCCGGCCGCC

FAQs/troubleshooting

What is the size of HA-tag in kDa?
The size of HA-tag (3xHA in our HA-tagged strains) is approximately 3kDa.

Are the HA-tagged yeast diploid?
Yes, the HA-tagged yeast are diploid (BY4741 strain), but the tag may only be on one allele. The yeast Y800 genotype is as follows: MATa leu2-D98cry1R/ MATalpha leu2-D98CRY1 ade2-101 HIS3/ade2-101 his3-D200 ura3-52 caniR/ ura3-52CAN1 lys2-801/lys2-801 CYH2/cyh2R trp1-1/TRP1 Cir0] carrying pGAL-cre (amp,ori, CEN, LEU2)[5].

How can I find the HA-tagged insertion site?
The insertion site can be found in the data file. The number given is the amino acid location where the tag was inserted.

For answers to questions that are not addressed here, please email technical support ts.dharmacon@horizondiscovery.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

References

1. P. Ross-Macdonald, *et.al.*, *Nature* **402**, 413 (1999).
2. P. Ross-Macdonald, A. Sheehan, G.S. Roeder, and M. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 190 (1997).
3. Kumar, A., des Etages, S.A., Coelho, P.S.R., Roeder, G.S., and Snyder, *M. Methods Enzymol* **328**, 550 (2000).
4. A. Kumar, *et.al.*, *Genes & Development* **16**, 707 (2002).
5. Burns *et al.*, *Genes & Dev.* **8**:1087-1105 (1994).

If you have any questions, contact

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