TECHNICAL MANUAL

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# Histone H3 and H4 Mutant Collection

Cat #: YSC5105, YSC5106

## Product description

The Yeast Histone H3 and H4 Mutant Collection was developed by Dr. Jef Boeke at Johns Hopkins University. The goal of this collection is to probe the importance of every histone residue, ultimately elucidating contributions of all residues to nucleosome function. The Yeast Histone H3 and H4 Mutant Collection is a systematic library of histone H3 and H4 mutants consisting of 486 constructs. Each amino acid residue has been systematically substituted with alanine (naturally existing alanine residues were changed to serine) (Figure 1. Additionally, unique molecular barcodes were integrated into each of the histone mutants allowing for identification of histone mutant pools.

The Yeast Histone H3 and H4 Mutant Collection is available in both yeast and bacteria. The bacterial collection contains all 486 mutants in *Escherichia coli*. In the yeast format, there is a non-essential and essential collection in a MATa yeast strain. The yeast clones containing lethal mutations were transfected with an additional plasmid pJP11 (CEN LYS HHF1-HHT1) to compensate for the lethality and rearrayed into a separate collection, the Essential Histone H3 and H4 Mutant collection.[1]

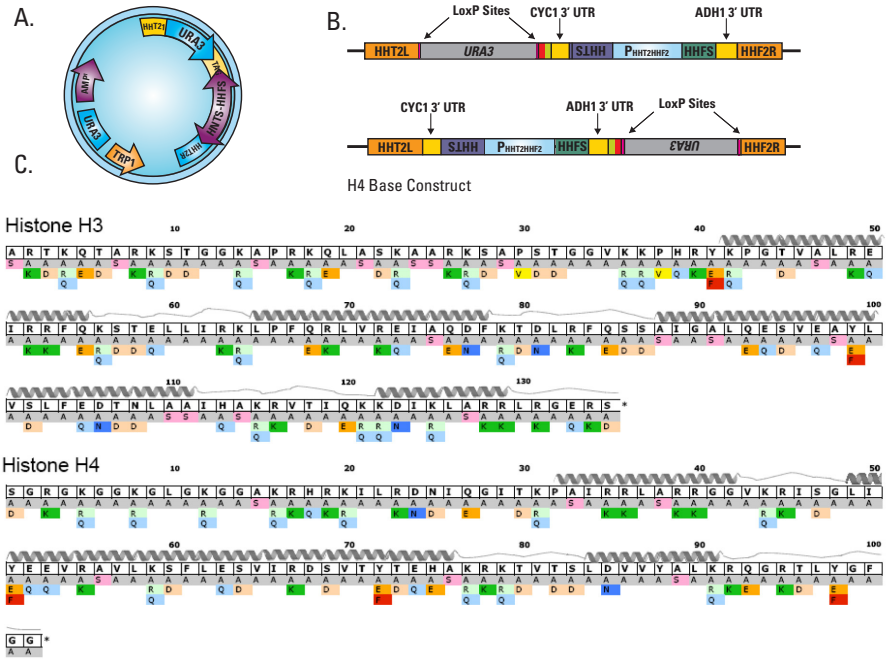


**Figure 1.** Features of synthetic histone cassette. **(A.)** Schematic representation of histone H3/H4 cassette in pRS414. The two selectable markers, *TRP*1 and *URA*3, can be ued to select an episomal copy or an integrated cassette respectively. **(B.)** Cassettes contain synthetic H3 and H4 genes (*HHTS* and *HHFS*) flanking a central native *HHT2*/*HHF*2 promoter region (PHHT2-HHF2). Mutations are engineered into either HHTS or HHFS and tagged with molecular barcodes. Upper cassette indicated is used as base construct for *HHTS* (H3) mutants; lower one is used as base construct for *HHFS* (H4) mutagenesis. **(C.)** The mutant library consists of an alanine scan with other systematic residue swaps and systematic tail deletions, totaling 486 mutants. (Figure[1])

**Table 1.** Available Yeast Histone H3 and H4 Mutants.

| Product Description | Cat # |
|---|---|
| Non Essential Histone H3 and H4 Mutant Individual Strain (Yeast) | YSC5105 |
| Non Essential Histone H3 and H4 Mutant Collection (Yeast) | YSC5106 |

## Strain information

**Non essential histone H3 and H4 Mutants (yeast):** Genotype of *Saccharomyces cerevisiae MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]*-URA3*

**Essential histone H3 and H4 mutants (yeast):** Genotype of *S. cerevisiae MATa his3Δ200 leu2Δ0 lys2Δ0 trp1Δ63 ura3Δ0 met15Δ0 can1::MFA1pr-HIS3 hht1-hhf1::NatMX4 hht2-hhf2::[HHTS-HHFS]*-URA3 Plus pJP11 plasmid* (CEN LYS HHF1-HHT1)

**[HHTS-HHFS]* denotes the synthetic histone gene cassette bearing the mutation of interest (which may be either in H3 (HHTS) or H4 (HHFS) respectively).**

**Histone H3 and H4 mutants (bacteria):**
The source lab did not track the host strain of the bacterial constructs. The clones are in either XL10gold or DH5alpha.

XL10gold (Stratagene) genotype: *TetrD (mcrA)183 D(mcrCB-hsdSMR-mrr) 173 endA1 supE44 thi-1recA1 gyrA96 relA1 lac Hte [F' proABlaclqZDM15 Tn10 (Tetr) Amy Cam']*

*DH5alpha genotype: F- φ80dlacZM15 (lacZYA-argF)U169 deoR recA1 endA1 hsdR17(r k-, m k+) phoA supE44 thi-1 gyrA96 relA1 λ-*

## Antibiotic resistance

**Table 2.** Antibiotic resistance of histone H3 and H4 mutants (bacteria).

| Antibiotic | Concentration | Utility |
|---|---|---|
| carbenicillin (ampicillin) | 100 µg/mL | Bacterial selection marker |

**Table 3.** Antibiotic resistance of histone H3 and H4 mutants (yeast)

| Antibiotic | Concentration | Utility |
|---|---|---|
| nourseothricin | 100 µg/mL | Eukaryotic selection marker |

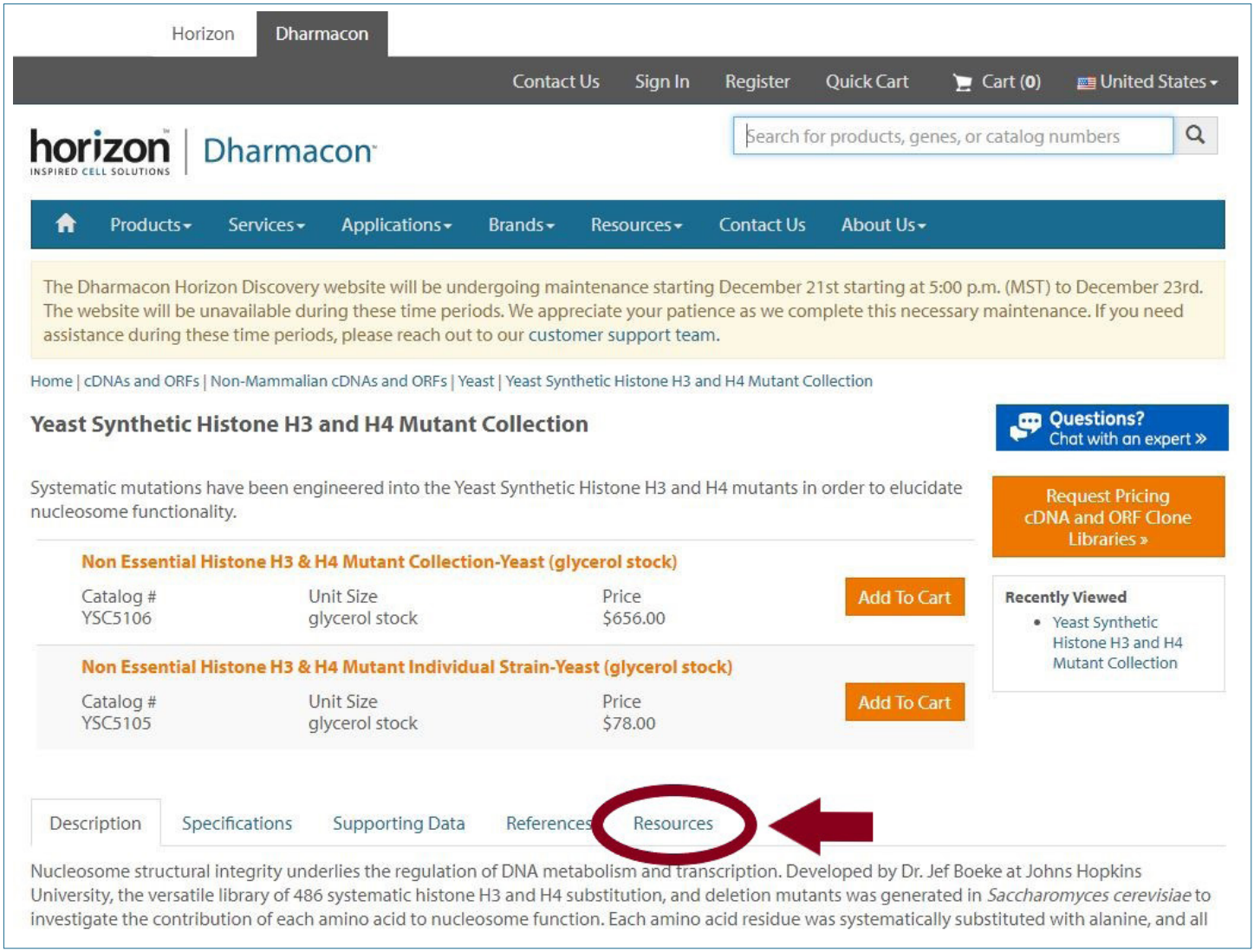


**Figure 2.** How to find platemap and clone information for yeast clones.

## Obtaining clone information

To find clone information, simply go to the Yeast Synthetic Histone H3 and H4 Mutant Collection and click on the Resources tab. There you will find platemaps with clone information and plate coordinates

## Protocol I – yeast replication

We recommend making a stock or working culture of the yeast strains. Grow the yeast strains for 24-48 hours at 30 °C in YPD broth or SD-Ura broth with appropriate antibiotic. Transfer 850 µL of culture into a polypropylene tube and add 150 µL sterile glycerol to make a 15% glycerol freezing solution. Vortex the culture to evenly mix the glycerol throughout the culture. The culture can be stored indefinitely at -80 °C.

**Table 4.** Materials for yeast replication.

| Item | Vendor | Cat # |
|---|---|---|
| Yeast Extract, 500 g, granulated | Fisher Scientific | BP1422-500 |
| Peptone, granulated, 2 kg – Difco | Fisher Scientific | BP9725-2 |
| Glucose (D(+)-glucose monohydrate) | EMD Millipore | 1.08342.2500 |
| Glycerol | Fisher Scientific | BP2291 |
| G418 | Calbiochem | 345810 |
| 96-well microplates | Nunc | 260860 |
| Aluminum seals | Nunc | 276014 |
| Disposable replicators | Genetix | X5054 |

***When preparing medium for yeast clones, do not add the glycerol to the medium until after the clones have grown. Glycerol inhibits the growth of yeast. We prepare a solution of 50% glycerol and 50% medium to add to the growth medium after incubation.**

## YPD medium 1 liter

**YPD mix:**

| | |
|---|---|
| Yeast extract | 10 g |
| Peptone | 20 g |
| $dH_2O$ | 900 mL |

Autoclave mixture for 20 minutes at 121 °C

## SD-ura medium 1 liter

| | |
|---|---|
| CM Broth w/ Glucose – Uracil | 23.5 g |
| $dH_2O$ | 980 mL |

Autoclave mixture for 20 minutes at 121 °C

Add 25% Ammonium Sulfate Solution 20 mL

## Glucose/$dH_2O$ mix

| | |
|---|---|
| Dextrose | 20 g |
| $dH_2O$ | 100 mL |

Shake until in solution and autoclave for 20 minutes at 121 °C. Under hood, add YPD mix to 100 mL of sterile glucose/$dH_2O$ mix.

## Yeast 96-well plate replication

**Prepare target plates**

1. Prepare 96-well target plates by dispensing 150 µL of YPD into each well.

**Pepare 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 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 65 µL of 50% glycerol to each well for a final concentration of 15% glycerol.
9. Heat seal target plates and return to an ultralow freezer.

Freeze at -80 °C for long term storage. Avoid long periods of storage at room temperature or higher.

***Glycerol can be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at -80 °C, and glycerol is required.**

## Protocol II – bacterial replication

We recommend making a stock or working culture of the bacterial strains. Grow the clones at 37 °C in LB-Lennox (low salt) medium plus 100 µg/mL carbenicillin. Prepare medium with 8% glycerol* and the appropriate antibiotics if an archive stock is required. After incubation, vortex the culture to evenly mix the bacteria throughout, and store at -80 °C. The culture can be stored indefinitely at -80 °C.

**Table 5.** Materials for bacterial replication.

| Item | Vendor | Cat # |
|---|---|---|
| LB-Lennox broth (low salt) | Fisher Scientific | BP1427500 |
| Peptone, granulated, 2kg – Difco | Fisher Scientific | BP9725-2 |
| Yeast Extract, 500g, granulated | Fisher Scientific | BP1422-500 |
| Glycerol | Fisher Scientific | BP2291 |
| Carbenicillin | Fisher Scientific | BP2648-250 |
| 96-well microplates | Nunc | 260860 |
| Aluminum seals | Nunc | 276014 |
| Disposable replicators | Genetix | X5054 |
| Disposable replicators | Scinomix | SCI-5010-OS |

## 2X-LB broth (low salt) medium preparation

| | |
|---|---|
| LB-Broth-Lennox | 20 g/L |
| Peptone | 10 g/L |
| Yeast Extract °C | 5 g/L |

Appropriate antibiotic(s) at recommended concentration(s)

## Bacterial 96-well plate replication

**Prepare target plates**

1. Prepare 96-well target plates by dispensing 150 µL of media with appropriate antibiotics into each well.

**Pepare 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 ultralow freezer.
7. Cover with a microporous film and place the target plates on a 37 °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 65 µL of a sterile 50/50 mixture of glycerol and YPD to each well and mix. This will bring the total glycerol percentage in each well to 15%.
9. Heat seal target plates and return to an ultralow freezer.

Freeze at -80 °C for long term storage. Avoid long periods of storage at room temperature or higher.

***Glycerol can be omitted from the medium if you are culturing for plasmid preparation. If making copies of the constructs for long term storage at -80 °C, and glycerol is required.**

**Frequently asked questions**

| Questions | Answers |
|---|---|
| What does the pJP11 plasmid do? | In the parental strain, the hht1-hhf1 is knocked out by NatMX4 and hht2-hhf2 is knocked out by HygMX4. Since the strain can't survive when both copies of H3/H4 are knocked out, it is covered by a plasmid (pJP11) before the second copy is knocked out. The parental strain used contains pJP11 which was knocked in with the mutant constructs, marked by URA3. PCR was performed to confirm the correct integration of the synthetic construct at the right locus. If the histone mutant is viable, the pJP11 can be lost. In the essential mutants, the pJP11 can not be lost and is retained in the cell.<br><br>The addition of the pJP11 changes the genotype from lys2delete to LYS2+ and from hht1delete hhf1delete to HHT+ HHF+, thus changing the phenotype. |
| Where can I find more information on the Barcode Primers – such as sequences? | The supplemental data included in the Yan 2008 reference contains primer sequences for the barcodes. |

## Reference


1. Dai, J, J.D. Boeke, *et al.* Probing nucleosome function: A highly versatile library of synthetic histone H3 and H4 mutants. *Cell.* Volume 134, Issue 6, 19 September 2008, Pages 1066-1078.


## FAQS/troubleshooting

We provide certain clone resources developed by leading academic laboratories. Many of these resources address the needs of specialized research communities not served by other commercial entities. In order to provide these as a public resource, we depend on the contributing academic laboratories for quality control.

Therefore, these are distributed in the format provided by the contributing institution "as is" with no additional product validation or guarantee. We are not responsible for any errors or performance issues. Additional information can be found in the product manual as well as in associated published articles (if available). Alternatively, the source academic institution can be contacted directly for troubleshooting.


**If you have any questions, contact**

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