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Combinatorial effect of histone H3 and H4 mutations with deletion of YEATS domain protein Sas5 in *Saccharomyces cerevisiae*

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Combinatorial effect of histone H3 and H4 mutations with deletion of YEATS domain protein Sas5 in Saccharomyces cerevisiae

Abstract

The single cell eukaryote, *Saccharomyces cerevisiae* or budding yeast, synthesizes three YEATS domain containing proteins associated with chromatin remodelling and modifying complexes. Previous experiments have shown that deletion of either of the YEATS domain containing proteins, Taf14 or Yaf9, in combination with mutations in the N-terminal tails of histones H3 and H4 is lethal for the cells. In this thesis the aim was to assess the effects of deletion of SAS5, the third YEATS-domain containing protein, in combination with mutations in histones. A decrease in the viability for cells carrying *sas5Δ* and H3 acetylation and methylation mutations was detected.

Keywords

Sas5 protein, YEATS domain, *Saccharomyces cerevisiae*, histone acetyltransferase, mutant histones

CERCS: B220- Genetics, cytogenetics, B230 - Microbiology, bacteriology, virology, mycology

Histoonide H3 ja H4 mutatsioonide ning YEATS domeeniga valgu Sas5 deletsiooni mõju pagaripärmi (*Saccharomyces cerevisiae*) kasvule

Lühikokkuvõte

Üherakuline eukarüoot Saccharomyces cerevisiae ehk pagaripärm toodab kolme YEATS-domeeniga valku, mis on seotud kromatiini struktuuri mõjutavate kompleksidega. Varasemad uuringud on näidanud, et YEATS-domeeniga valkude Taf14 või Yaf9 eemaldamine koos mutatsioonidega histoonide H3 ja H4 N-terminaalsetes sabades on rakkudele letaalne. Käesoleva töö eesmärgiks oli uurida kolmanda YEATS-domeeniga valgu SAS5 eemaldamise mõju kombinatsioonis histoonimutatsioonidega. Katsetulemused näitasid, et *sas5Δ* ja H3-mutantseid histoone kandvate rakkude eluvõime oli vähenenud.

Võtmesõnad:

Sas 5 valk, YEATS domeen, *Saccharomyces cerevisiae*,, histooni atsetüültransferaas, mutantsed H3 histoonid.

CERCS: B220 - Geneetika, tsütogeneetika, B230 - Mikrobioloogia, bakterioloogia, viroloogia, mükoloogia

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TERMS, ABBREVIATIONS AND NOTATIONS

5-FOA..... 5-Fluoroorotic acid

C-terminal..... Carboxyl-terminal

HDAC..... Histone deacetylases

HGB..... Hygromycin

HIS ..... Histidine

HML ..... locus homothallic mating left

HMR .....locus homothallic mating right

KAT ..... lysine acetyltransferase

N-terminal..... amino-terminal

NuA3 ..... nucleosome acetyltransferase of histone 3 PTMs post-transcriptional modifications

PMT..... post-translational modification

RNAPII .....RNA polymerase II

Rpb9..... RNA Polymerase II Subunit I

RSC.....Chromatin Structure Remodeling Complex

SAS complex.....Something About Silencing

SWI/SNF..... switching defective/sucrose nonfermenting Taf14 transcription initiation factor

TFIID subunit 14..... transcription factor II D

Taf14.....TBP associated factor 14

TFIIF ..... transcription factor II F

TRP .....tryptophan

WT .....wild-type

Yaf9 ..... Yeast Associated Factor 9

YCP ..... YEATS domain-containing protein

YEATS ..... **Y**af9, **E**NL, **A**F9, **T**af14, **S**as5

YPD ..... yeast extract/peptone/dextrose

INTRODUCTION

In eukaryotic cells, histones play a role in DNA packaging and its organization into chromatin, and are instrumental in the expression and dynamics of chromatin. Histone tail regions can undergo post-translational modifications, which influence chromatin remodelling. These post-translational modifications of histones, such as acetylation, methylation, phosphorylation, and crotonylation hold an essential role in epigenetic regulations by influencing transcription and replication in all organisms with similar chromatin structure to *Saccharomyces cerevisiae*.

Sas5 is a subprotein of the SAS protein complex, an acetyltransferase complex involved in transcriptional silencing at telomeres and at HML locus, as well as rDNA silencing in *S. cerevisiae*. The YEATS domain contained in Sas5 proteins, as well as Yaf9 and Taf14 proteins in *S. cerevisiae*, is a recognition domain that binds to histone lysine acylation. For Sas5 proteins, it is shown to have interaction also with unmodified H3 and H4's histone tails as it is involved in the regulation of histone modifications (Shanle et al., 2015).

The purpose of the experiments with this thesis was to analyze the viability of yeast cells with modified histones, lacking Sas5 protein. In previous experiments involving YEATS domain containing proteins done by our research group, the deletion of YAF9 or TAF14 from budding yeast cells became lethal when in combination with methylation or acetylation mutation on histone N-terminal tails. The results analyzed from the previous experiment lead to the hypothesis that Sas5 deletion in *S. cerevisiae* would induce a visible impact in cell viability when experiencing a lack of specific histone modifications, such as acetylation and methylation, at targeted sites known for YEATS domain activity.

1 LITERATURE REVIEW

1.1 Chromatin

Eukaryotic cells' chromosomal DNA is considerable in length, making compiling and organizing DNA a key requirement for DNA replication, management of gene expression, regulation and preventing DNA damage. This is done through compacting the long DNA molecules into a denser structure that fits within the nucleus of the cell. Chromatin, a nucleoprotein complex composed of DNA, and proteins, is designed to package DNA efficiently (Luger et al., 2012). Nucleosomes, the segmented units of chromatin, consist of DNA wound around histone octamer complexes, comprising two copies of H2A-H2B and H3-H4 dimers, forming a beads-and-string resembling structure, which allows for the compaction level of DNA from 10,000 to 20,000 times (Nothof et al. 2022) (Figure 1)

Nucleosomes fold into 30-nanometer chromatin fibers, consisting of nucleosome arrays in their most compact form (Nothof et al. 2022). The compaction of chromatin serves several critical functions in cellular processes. It provides protection for the DNA molecule, shielding it from environmental factors such as chemical agents and radiation, which can induce mutations or breaks in the DNA. Additionally, chromatin compaction regulates gene expression by controlling the accessibility of DNA to transcription factors and other regulatory proteins. Regions of tightly compacted chromatin, such as heterochromatin, typically repress gene expression, while regions with a more relaxed chromatin structure, such as euchromatin, allow for greater access to the DNA, facilitating gene transcription (Talbert and Henikoff, 2010).

Proper chromatin compaction is essential for DNA replication, as the compacted chromatin structure must be temporarily relaxed to allow the replication machinery access to the DNA strands. Errors in chromatin compaction can lead to chromosomal abnormalities which can cause physiological changes in the cell or stagnate, if not completely inhibit cellular replication, highlighting the importance of chromatin structure in maintaining genome stability and proper cellular function (Nothof et al. 2022).

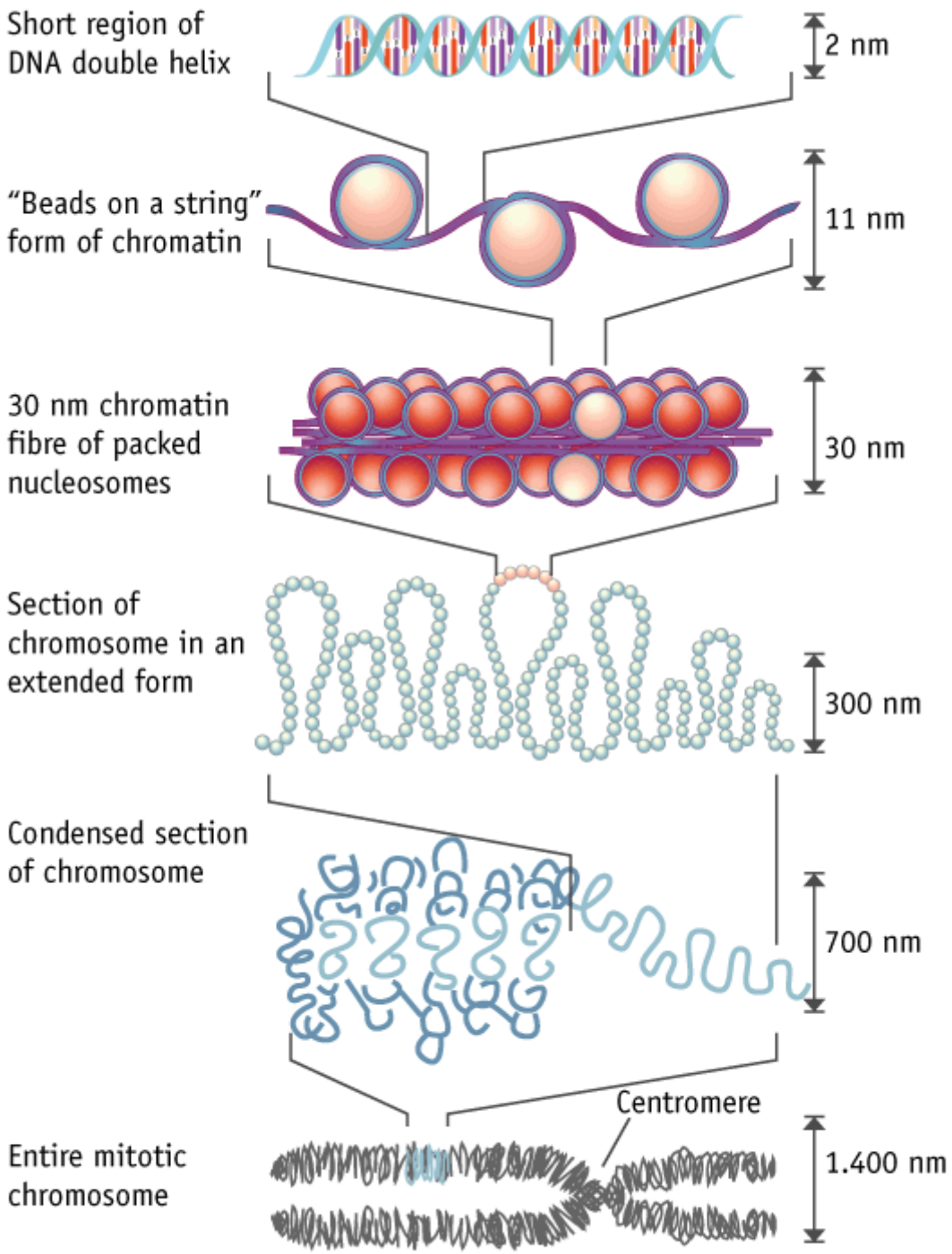

Figure 1. Organization of chromosomal DNA. DNA is winded by two superhelical turns and wound around a histone octamer, creating a nucleosome. The nucleosome resembles a basic unit of a repeating, bead-on-a-string structure which folds into a fiber about 30 nm in diameter. Further folding is condensed into fiber called chromatin fibers. These compact fibers make up for discrete sections of the chromosome which folds and loops in order to condense, tightly packaging together in a resemblance of a chromosome's arm. (Felsenfeld and Groudine, 2003).

1.1.1 Histones

Histones within nucleosomes are crucial for maintaining the structural integrity of chromosomal DNA and play significant roles in replication and gene expression. Histones are positively charged nuclear proteins that act as spools around which DNA coils to form nucleosomes preventing DNA from becoming damaged or tangled. There are 4 main types of histone proteins in yeast: H2A, H2B, H3, and H4; each having variants to fulfil important functions respectively between different species. (Luger et al., 2012) (Figure 2). Each histone protein possesses a structurally conserved motif called a histone fold, which facilitates histone-histone and histone-DNA interactions, contributing to nucleosome stability (Figure 2). Additionally, histones have unstructured regions known as histone tails, which extend from the nucleosome core and can undergo various post-translational modifications.. These modifications can alter chromatin structure and dynamics, influencing processes. (Luger et al., 2012).

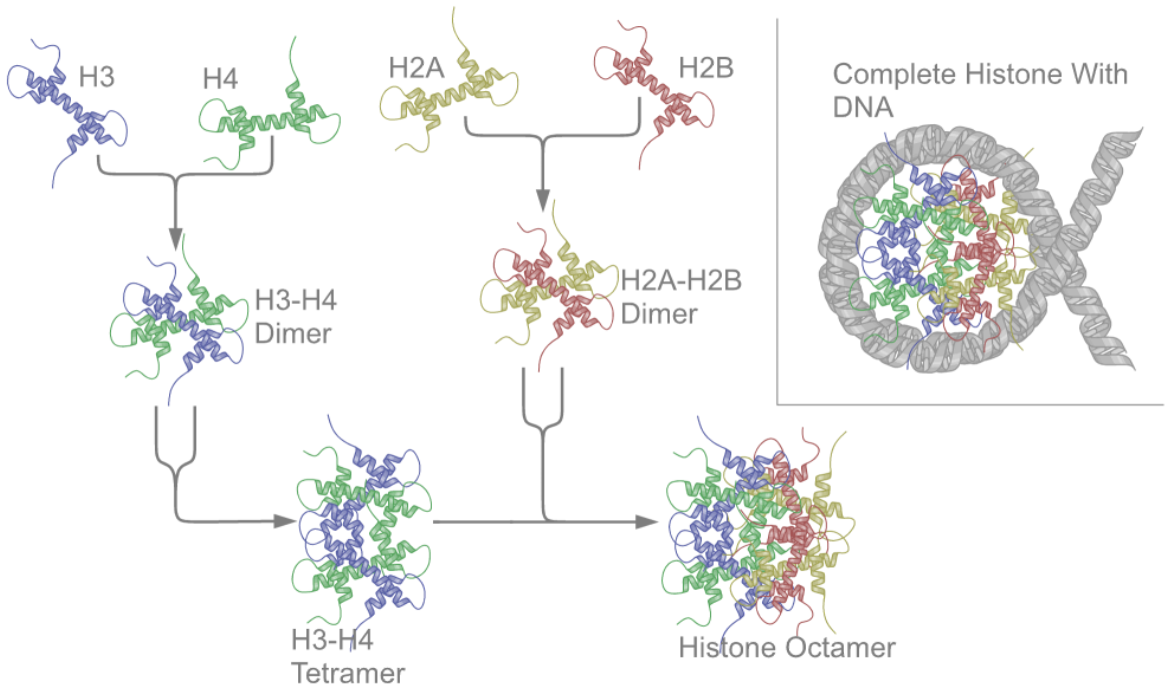

Figure 2. Histone and nucleosome structure. Nucleosomes are made of 4 different core histone proteins. These proteins have a fold, composed of 3 alpha helices, connecting 2 loops in order to create a globular structure. Each globular domain extends a tail-like protrusion (N-terminal and C-terminal tails) which is involved in histone-histone and histone to DNA interactions. Each core histone protein (H2A (yellow), H2B (red), H3(blue), and H4(green) links to their complementary histone protein, creating a dimer. The H3-H4 dimers are doubled into tetramer which serves as a central core. This core is flanked by 2 H2A-H2B dimers, forming a histone octamer protein complex which becomes the central structural unit of the nucleosome. The nucleosome is formed from the wrapping of DNA around the histone octamer creating a bead-and-string-like structure. (Shuaib, 2012)

1.1.2 Histone post-translational modifications

Each histone in a nucleosome has an unstructured, flexible region of amino acid residues that protrudes from the core, known as a histone tail, which are enriched with lysine and arginine amino acid. These tails extend outwards from the nucleosome core for proteins and enzymes that facilitate in gene regulation and chromatin structure remodeling through post-translational modifications (PTMs) of the amino acids of the tail and domains that recognize these modifications. PTMs, such as acetylation, methylation, phosphorylation, and ubiquitination, modify histone tails and influence chromatin characteristics and, in turn, cellular processes in synergistic or antagonistic manners. (Nothof et al. 2022).

Different chromosomal proteins contain recognition domains that interpret histone modifications and recruit effector proteins to specific chromatin regions. Examples of recognition domains include bromodomain (recognizes acetyl-lysine), chromodomain (recognizes methylated lysine with histones), double PHD (Plant Homeodomain, recognizes unmodified and methylated amino-terminal tails in histone H3), and YEATs (will be discussed later in thesis). These recognition domains facilitate the binding of proteins involved in chromatin remodeling, transcriptional regulation, DNA repair, and other cellular processes. (Nothof et al. 2022)

1.1.3 Histone acetylation

Histone acetylation is a prominent histone modification involving the addition of acetyl groups to lysine residues (Kac) by the use of lysine K-acetyltransferases (KATs) (Figure 3). Acetylation neutralizes the positive charge of lysine residues, loosen DNA histone interaction to facilitate transcription factor binding and promote nucleosome remodeling via recruitment of chromatin remodelers and DNA-binding factors in transcription and repair. (Nothof et al. 2022). The removal of acetyl groups by Histone deacetylases (HDACs) restores the positive charge, repressing transcription and stabilizing nucleosomes. This “acetylation–deacetylation” cycle provides a reversible switch for regulating chromatin accessibility (Nothof et al. 2022).

Proteins containing Kac readers domains play a role in the recognition and binding of proteins or protein domains to acetylated lysine residues. The most well-studied are bromodomains-containing factors which play a role in transcriptional regulation.

There are several KATs that have been identified in yeasts, each that exhibits substrate preferences and have distinct roles in chromatin regulation. In H3 and H4 histones, lysine

acetylation affects chromatin regulation directly and indirectly by assisting and regulating gene expression, either by removing positive charges of lysine side chains by acetylation or recruiting a 'reader' domain'. Single acetylation of H4 at K16 in nucleosomes reduces the inter-nucleosome interaction, while hyperacetylation of H4 at K5, K8, K12, and K16 causes internucleosomal decompaction in reconstituted systems (Kikuchi et al., 2022)

For YEATS domain containing proteins in *S. cerevisiae*, the binding to acetylated histones has been analyzed mostly using acetylated histone peptides. Taf14 showed a strong preference for to binding of H3K9ac, acting as selective reader of histone for the site, and weak interaction toward H3K4ac, H4K14ac, H4K18ac, H4K27ac, and the single-site H4 acetylations at K5, K8, K12, and K16. Likewise prefers H3K9ac and displays modest affinity for H3K27ac. Other YEATS domain containing proteins with similar activity also show similar binding behavior for those acetylated histone peptides sites such as binding of AF9-YEATS preferencing H3K9ac peptide and ENL, YEATS2, and GAS41 preferencing H3K9ac and H3K27ac peptides (Kikuchi et al., 2022).

1.1.4 Histone methylation

Histone methylation is a complex modification that occurs on arginine and lysine residues of histones. Up to two methyl groups can be added to an arginine residue, while lysine can accept up to three. Unlike acetylation, methylation does not alter the charge of lysine or arginine side chains. Methylated lysine and arginine residues act as binding sites for various protein domains, including PHD and chromodomains (Kouzarides, 2007). The methylation is set by methyltransferases and removed by demethylase enzymes, which control histone lysine methylation. (Separovich and Wilkins, 2021)

In *S. cerevisiae*, the H3K4, H3K36, and H3K79 sites are histone lysine methylation marks associated with transcriptional activation and are involved in key cellular processes, including the regulation of the DNA damage response, genomic stability, and cell death. Each site has multiple methylation states, each with distinct functions that can either activate or repress gene expression, as well as regulate other processes across the genome. The three states, mono-, di-, and trimethylation each have a feature, for example, for H3K4, its monomethylation state is usually at enhancer, while its trimethylation marks transcription start sites. (Separovich and Wilkins, 2021)

1.1.5 Other post-translational modifications on histones

Other histone post-translational modifications are observed in histone tails such as phosphorylation, the modification of serine, threonine, and lysine with a hydroxyl group. This promotes the agglutination of chromatin into chromosomes, and creates interactions between other histone PTMs (Lui et al, 2023). Ubiquitination, a covalent attachment of ubiquitin moieties to histone lysine residues, orchestrates histone turnover, DNA repair pathways, and chromatin compaction through a coordinated enzymatic cascade involving E1, E2, and E3 ubiquitin ligases. (Figure 3). Hydroxylation, catalyzed by hydroxylases, emerges as a novel modification implicated in gene regulation and chromatin organization, contributing to transcriptional activation and DNA repair processes (Zurlo et al., 2016).

While less prevalent, N-linked glycosylation, involving oligosaccharide attachment to asparagine residues, intricately regulates chromatin remodeling and gene expression modulation (Hirabayashi and Suzuki, 2012).

Lastly, crotonylation, one of the more recently discovered PTMs, is where a crotonyl group is added to a lysine residue on a histone tail. These crotonylation marks on histones impact gene expression through several key protein factors with different functions; writers (proteins that exhibits detectable histone crotonyltransferase (HCT) activity), erasers (histone decrotonylases that binds to the crotonylated histone peptide via a π–π interaction and removing the Kcr marker), readers (recognition of histone Kcr marker that play a role in epigenetic regulation. YEATS domain was the first discovered reader) , or regulators (histones proteins that act as transcriptional promoters or repressors). (Li and Wang, 2021)

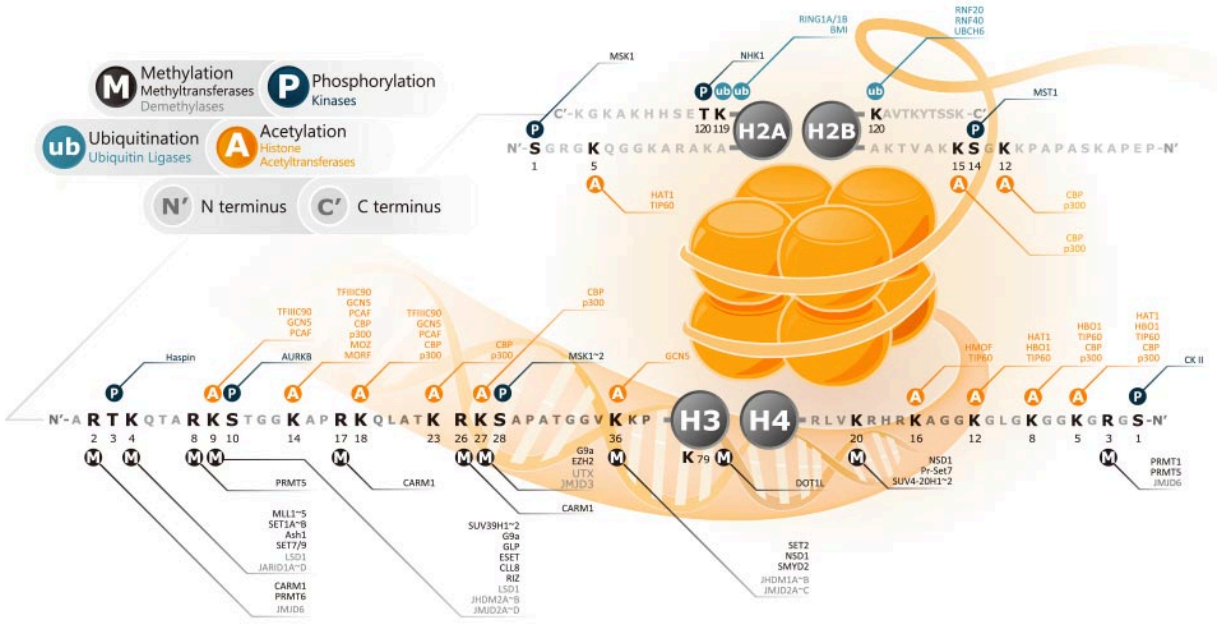

Figure 3. Histone post-translational modifications (GeneTex, n.d.). In histones, H2A, H2B, H3, and H4 are subunits of the histone complex of a nucleosome. Each subunit has terminal tails with H2A and H2B containing prominent C-terminal and N-terminal tails, and H3 and H4 containing extensive N-terminal tails. Each tail extends out from the nucleosome core. Each tail is composed of amino acid sequence, that vary in length, that can accept post-translational modifications. (Luger et al., 2012)

1.2 YEATS - Domain Containing Proteins

YEATS domain is a recognizing domain for histone lysine acylation, similar to bromodomain, and is a component of various chromatin-modifying and transcription-regulating complexes. Capable of reading bulkier lysine acylation marks than bromodomains, this domain has a high affinity for lysine crotonylation and exhibits physiologically relevant crotonyllysine-binding activity (Andrews et al, 2016). It is found in primarily chromatin-associated proteins such as Yaf9, ENL, AF9, Taf14, and Sas5, with each initial letter forming the acronym YEATS. In total, 3,741 YEATS domain-containing proteins (YCPs) have been identified across 1,442 eukaryotic species, including human proteins ENL, AF9, YEATS2, and GAS41, as well as yeast proteins Yaf9, Taf14, and Sas5 (Li et al., 2022). This family of proteins are usually implicated in chromatin remodeling and transcriptional regulation. A single copy of the YEATS-domain is located at the N-terminus of proteins of all YEATS-domain-containing proteins. The domain consists of approximately 130 residues and contains an antiparallel β-sheet structure that functions to recognize and bind to Kac and other acylated lysines. In yeast, double deletions of YEATS domain-containing genes lead to reduced growth ability. (Kikuchi et al, 2022)

YEATS domains-containing proteins can recognize a repertoire of short-chain histone acylations, preferring for lysine crotonylation (Li et al., 2017). This preferential binding is facilitated by "aromatic-π-aromatic" stacking interactions between the crotonyl amide group and specific aromatic residues for Taf14 and aromatic-amide–aliphatic-aromatic π–π–π-stacking mechanism facilitates the specific recognition of the crotonyl moiety (Andrews et al, 2016). For AF9's YEATS domain, a similar binding preference as Taf14 is observed for H3K9cr, as well as recognizes histone crotonylation at H3K9, K18, and K27. Contrastingly, YEATS2 and ENL perferes a selective binding to H3K27cr.

1.3 Proteins containing YEATS domain in budding yeast

There are 3 YEATS domain-containing proteins in *S. cerevisiae*: Taf14, Sas5, and Yaf9. These proteins are present in eight different complexes that collectively play crucial roles in chromatin remodeling, histone modification, and transcriptional regulation.

1.3.1 Yaf9

Yaf9 is a YEATS domain-containing protein in *S. cerevisiae* and holds an important part as a subunit of NuA4 acetyltransferase and the SWR1 chromatin remodeling complexes. NuA4 acetyltransferase complexes is responsible for acetylating histones H4, H2A and histone variant, H2A.Z, and regulates transcriptional and DNA repair programs, while SWR1 chromatin remodeling complexes is ATP-dependent and is responsible for incorporation of H2A.Z into chromatin at gene promoters, as well as is necessary for genome stability (Klein et al., 2017).

The YEATS domain in Yaf9 is located in the N-terminus of the protein and is theorized to bind acetylated H3 and H4 histones to recruit SWR1 or NuA4 complexes to specific promoters, similar to the activity of YEATS domain in human ENL proteins (Zeisig et al. 2005). It acts as a preferential reader of H3K27ac and associates with succinylated H3K14, H3K56, H3K79, H3K122, H4K12, and H4K31 peptides *in vitro* (Wang et al., 2018). When inactivated by mutation of Yaf9 YEATS domain, a decrease deposition of H2A.Z into the PHO5 promoter region due to impaired function of the SWR1 complex, which Yaf9 is a subprotein of. (Klein et al., 2018)

1.3.2 Taf14

Taf14 is another YEATS domain-containing protein in S. cerevisiae, which is found in chromatin-remodeling complexes, such as TFIIF, TFIID, INO80, SWI/SNF and RSC complexes, and the histone acetyltransferase complex NuA3 (Andrews et al., 2016). Taf14 is a selective reader of histone H3 Lys9 acetylation (H3K9ac) as well as preference for crotonylated H3K9 (H3K9cr) due to its YEATS domain within the protein. In addition, the YEATS domain for Taf14 also reads acylation and binds for DNA damage response and gene transcription (Shanle et al, 2015).

The absence of Taf14 protein in yeast cells is observed to cause reduced growth rate, sensitivity to DNA damage, and elevated temperatures. However, these phenotypes can be rescued when truncated Taf14 that lacks its YEATS domain is expressed. This confirms that Taf14, while critical for RNA polymerase 2, is not essential for yeast viability. (Peil et al., 2020)

1.3.3 Sas5

Sas5 is a subunit of the KAT complex known as SAS protein complex (something about silencing). SAS protein complex is a trimeric acetyltransferase composed of Sas2, Sas4, and Sas5 and has a primary function to acetylate free histones and nucleosomes, despite weak nucleosomal KAT activity in comparison to NuA4 complex. The SAS complex acetylates both free histones and nucleosomes and is required for the acetylation of histone H4K16 and H3K14. The 3 proteins of the protein complex were identified in a screen for genes regulating the silencing of the heterochromatin-like mating-type locus in yeast called HMR (Xu et al. 1999a). Sas5, which forms a complex with Sas2 and Sas4, was found to be required to affect telomere position in the telomere silencing assay (Xu et al. 1999b). This was noted after deletion of *SAS5* gene disrupted the complex's structure which affects silencing at the HML locus while at the same time suppressing silencing defects at the HMR locus (Xu et al. 1999b). It is suggested that Sas5 may be needed to stabilize SAS complex or help in substrate recognition, due to its requirement in maximal histone acetyltransferase activity of the complex.

The YEATS domain in the N-terminus of Sas5 shares 46% of its identity with Taf14's YEATS domain and interacts with H3 and H4 peptides (Shanle et al., 2015) . It is found to be essential for nuclear localization and is imported into the nucleus by karyopherins/importins Kap123 and Pse1/Kap121. In addition, Gel‑filtration of yeast cells suggest that Sas5 has a higher-molecular-mass fractions, indicating Sas5 may play a part in other protein complexes

(Meijsing and Ehrenhofer-Murray 2001). However, further research regarding the influence onto other proteins and functions is needed.

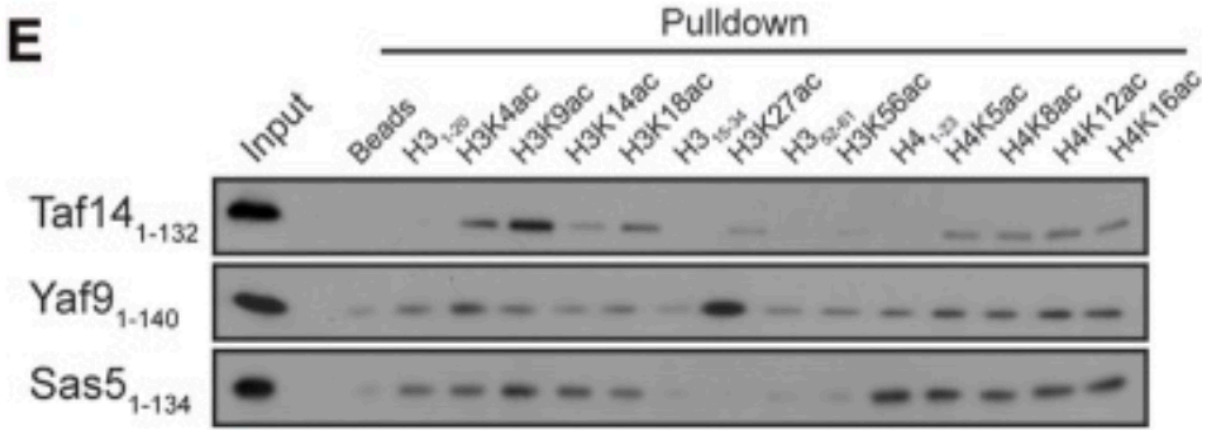

Figure 4. Western blot analyses of peptide pull-down assays with the corresponding GST-tagged YEATS domains and histone peptides. This figure presents the results of Western blot analyses for Taf14, Yaf9, and Sas5 proteins, each containing a YEATS domain, as they interact with acetylated lysine residues on histone H3 and H4 peptides. Sas5 showed affinity for unmodified H3 peptide 1-20 and unmodified H4 1-23 and for H3 acetylation sites; K4ac, K9ac, K14ac, and K18ac, and H4 acetylation sites; K5ac, K8ac, K12ac, and K16ac. (Shanle et al. 2015.)

2 THE AIMS OF THE THESIS

The aim of the thesis is to evaluate the viability of yeast cells with different histone H3 or H4 lysine to arginine mutations in the *sas5Δ* background.

- Construction of yeast strain with SAS5 deletion into histone plasmid exchange strain background
- Transformation of constructed strain with plasmids expressing mutated histones H3 or H4 in SAS5 deletion background.
- Analyzing the growth rate of cells harboring SAS5 deletion in combination with histone mutations using yeast spot test assay

3 EXPERIMENTAL PART

3.1 MATERIALS

Yeast strains: All *Saccharomyces cerevisiae* strains were derived from the W303 background. All the used yeast strains are described in supplementary table S1.

Plasmids: Plasmids with HIS3 marker gene: pRS413 - plasmid lacking gene for H3/H histone production and is a negative control, pRS413_wtH3/wtH4 - contains H3/H4 histone genes and acts as a positive control, pRS413_wtH3/H4K16R - expresses histone H4 with lysine 16 mutated to arginine abolishing the possibility of acetylation of the residue. pRS413_wtH3/H4K5,8,12R - expresses histone H4 with lysines 5, 8, 12 mutated to arginine. pRS413_H3K9,14,18,23,27R/wtH4 - expresses histone H3 with lysines 9, 14, 18, 23, 27, mutated to arginine.pRS413_H3K4,36,79R/wtH4 - expresses histone H3 with lysines 4, 36, and 79 mutated to arginine, abolishing possible methylation at these sites. Plasmid for NatMX6: p3x1E2-NatMX6 - Plasmid containing nourseothricin resistance gene, used to amplify the marker gene with SAS5 3'and 5`homology regions to knockout the SAS5 gene.

Plasmid for HGBMX6: p3xMYC-HphMX6 - Plasmid containing hygromycin resistance gene, used to amplify the marker gene with SAS5 3'and 5`homology regions to knockout the SAS5 gene.

Plasmid for TRP1: - p3x1E2-TRP1 - Plasmid containing TRP1 gene needed for tryptophan biosynthesis, used to amplify the marker gene with SAS5 3'and 5`homology regions to knockout the SAS5 gene.

Primers: For amplifying marker genes that contain SAS5's 5′ and 3′ homology regions. Sequence of each primer in supplementary table S3: SAS5KO_3-1570, SAS5KO_5-1569

Control primer for yeast cells with knockout: SAS5_ctrl-1571, FBA1_CTRL_2

Growth Media: YPD plates [agar 20 g/l, yeast extract 10 g/l, mycological peptone 20 g/l, glucose 20 g/l]

YPD media [yeast extract 10 g/l, mycological peptone 20 g/l, glucose 20 g/l]

SC (Synthetic complete) plates (excluding histidine) for spot test assay [agar 20 g/l, YNB (Yeast nitrogen base, Applichem) 6,7 g/l, glucose 20 g/l, Drop-out mix 2 g/l].

SC 5-FOA plates for the selection of the strains of *Saccharomyces cerevisiae* that do not contain the *URA3* marker gene [5-FOA 1 mg/ml, YNB 6,7 g/l, agar 2%, glucose 2%, nitrogen bases and amino acids – adenine 20 μg/ml, uracil 20 μg/ml, histidine 40 μg/ml, tryptophan 40 μg/ml, leucine 80 μg/ml, lysine 80 μg/ml]

Nourseothricin (NAT) containing plates for the selection of *Saccharomyces cerevisiae* [agar 20 g/l, YNB 6.7 g/l, glucose 20 g/l, Nourseothricin (NAT) 0.1 g/l]

Tryptophan (-TRP) Plate [agar 20 g/l, YNB 6.7 g/l, glucose 20 g/l, Drop-out mix 2 g/l]

3.2 METHODS

3.2.1 Polymerase chain reaction (PCR)

Two types of PCR were used during this experiment. The first was to amplify *NatMX6, TRP1*, and *HGB* genes, along with the SAS5's 5' and 3' homology regions, from their respective plasmid with the aim of replacing the SAS5 gene in the yeast genome with a marker gene. The second PCR uses DNA extracted from colonies transformed with PCR products from the aforementioned reaction to verify successful deletion of the SAS5 gene from chromosomal DNA using control primers - one at the promoter region of the SAS5 gene and the other on the transformed foreign DNA.

3.2.1.1 PCR for amplification of DNA fragment from plasmid

A 50 µl PCR reaction mixture consisting of 1 µl purified plasmid DNA (100 ng/µl), 10 µl 5x Phusion green HF buffer, 5 µl Nucleoside triphosphate (dNTP) (5mM), 2 µl each of forward (SAS5KO_5-1569) and reverse primers (SAS5KO_3-1570) designed for SAS5's 5' and 3' homology regions (10 µM), 1 µl Phusion polymerase (2u/µl), and 29 µl double distilled water (ddH2O), is made. Mix was made 3 times for each plasmid with target gene (*NatMX6*, *HGBMX6*, and *TRP1*. After preparing the mix, thermal cycling conditions involving an initial temperature of 96°C suitable for denaturation is set for 2 min, followed by 30 cycles of denaturation for 15 sec (96°C), annealing for 20 sec (60°C), and extension for 1 min 20 sec (72°C) with an extended extension for 5 min and resting for 12°C for the targeted sequences programmed on a thermocycler.

3.2.1.2 Colony PCR

This is a PCR to verify SAS5 deletion in chromosomal DNA. A 10 µl PCR reaction mixture consisting of 1 µl extracted and purified chromosomal (procedure 3.1.4) DNA, 2 µl 5x FIREPol Master Mix Ready to Load (12,5 mM MgCl2; *Solis Biodyne*), 0,25 µl each of the control (SAS5_ctrl-1571) and reverse primer (FBA1_CTRL_2) (10 µM) and 6,5 µl double distilled water (ddH2O), is made. 3 samples of each DNA template are made. After preparing the mix, thermal cycling conditions involving an initial temperature of 96°C suitable for denaturation is set for 5 min, followed by 33 cycles of denaturation for 25 sec (96°C), annealing for 20 sec (55°C), and extension for 40 sec (72°C) with an extended extension for 5 min and resting for 12°C for the targeted sequences programmed on a thermocycler.

3.2.2 Chromosomal DNA extraction procedure

After growing transformed yeast colonies on solid selective media, the cells are suspended in 100 µl of 200 mM LiOAc, 1% SDS solution to lysis the cells. It is then

incubated at 70°C for 5 min. 300 µl of 96% ethanol is then added to precipitate the DNA. The solution is then vortexed and spun down at 15,000 Ref for 3 min. The supernatant is then removed and the remaining pellet washed with 400 µl of 70% ethanol. The pellet is then dissolved with 100 µl of ddH_2O, and then spun down using 15,000 Ref for 15 sec. From then 1 µl of the supernatant for PCR.

3.2.3 Agarose gel electrophoresis

1% Agarose was prepared in 1x TAE buffer (40 mM Tris-acetate; 1 mM EDTA) and ethidium bromide (EtBr) (0,5µg/ml) was added for analysis of PCR.

3.2.4 Transformation of *S. Cerevisiae* using LiOAc

The transformation of *S. cerevisiae* using the LiOAc method was conducted as follows. 20 ml of liquid YP-glucose media was inoculated with the desired yeast strain and incubated overnight at 30 °C with agitation at 200 rpm. The yeast culture is measured at $4x10^7$ cells/ml and diluted to a concentration of approximately 10^7 cells/ml. 1.5 ml of the cell culture was transferred to 1.5 ml tubes and centrifuged at 5000 rpm for 2 minutes to collect the cells. The cell pellet was resuspended in 0.5 ml of 0.1 M LiOAc + TE solution and centrifuged at 5000 rpm for 2 minutes. Carrier DNA was denatured at 95 °C for 6 minutes and placed on ice. The cell pellet was resuspended in 40 ul of LiOAc + TE solution, and 7 µl of denatured carrier DNA-d was added. DNA of interest (4 µl used for plasmid transformation - 7 µl used for amplified PCR product transformation) was added to the cell-carrier mixture, followed by incubation at room temperature for 5 minutes. 0.3 ml of 0.1 M LiOAc + PEG4000 solution was added, and the mixture was vortexed before incubating at room temperature for 15 minutes. 30 µl of DMSO was added and vortexed, followed by heat shocking the cells at 42 ℃ for 10 minutes and keeping them on ice for 1 minute. The cells were collected by centrifugation at 3000 rpm for 3 minutes, and the supernatant was removed. The cells were resuspended in 150 µl of sterile water and plated on growth media. The media was determined on the transformation, where cells transformed with *NAT* or *HGB* marker genes are plated on YPD and, after one day, were replica plated to their respective *NAT* or *HGB* selective media, cells transformed with TRP1 marker gene are plated directly on minimal selective media lacking tryptophan, and cells transformed with modified histone plasmids are plated on plates lacking histidine. The plates were incubated at 30 ℃ for 3-4 days to allow for colony formation.

3.2.5 Plasmid shuffling system

In our study, we employed a plasmid shuffling system to analyze the effect of mutant histones on the growth of sas5Δ strain. In strain AKY202, previously created in our work

group, both chromosomal copies of the histones H3 and H4 genes were deleted from the genome, and wild-type H3 and H4 histones were expressed from a plasmid carrying a URA3 marker gene, which encodes Orotidine 5'-phosphate decarboxylase (ODCase). For the expression of mutant histones, cells were transformed with plasmids expressing various mutant histones and containing a HIS3 marker gene. After colony formation on selective plates void of histidine, cells were plated onto 5-FOA selection plates, causing the enzyme Orotidine-5'-phosphate (OMP) decarboxylase to conversion of 5-FOA into the toxic metabolite 5-fluorouracil, leading to the survival of only those cells that had lost the initial plasmid expressing wild-type histones.

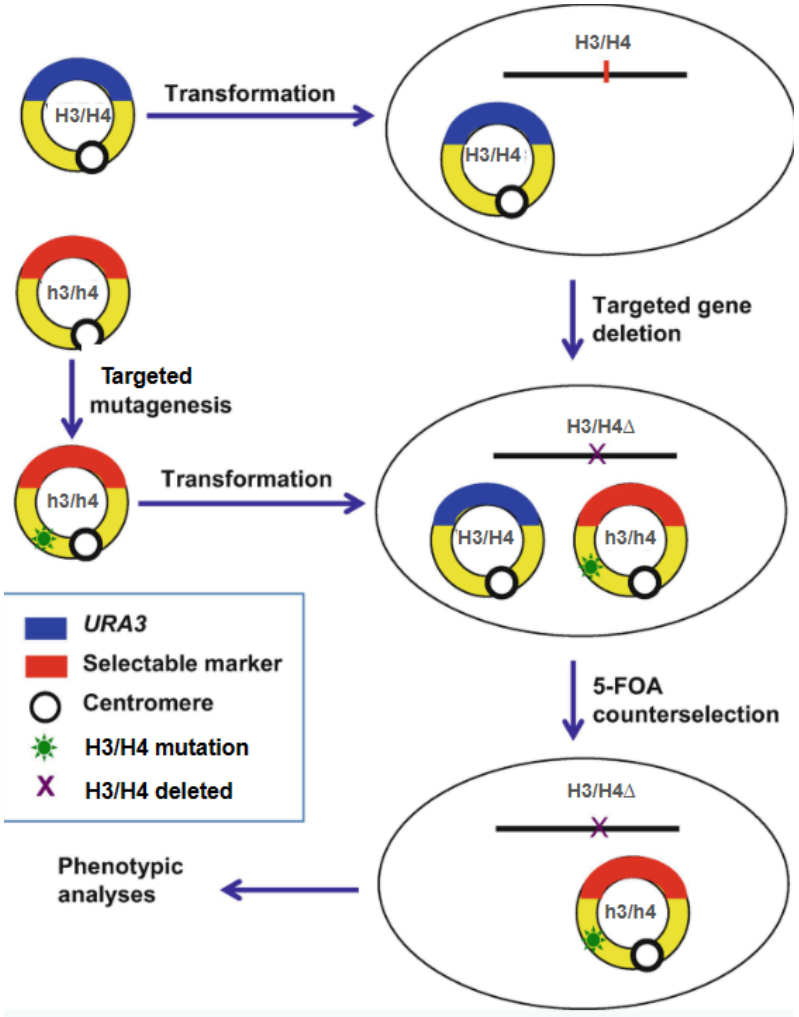

Figure 5. Plasmid Shuffling. Yeast cells are transformed with a histone coding plasmid containing H3/H4 genes with URA3 marker. The H3/H4 genes are then deleted from the chromosomal DNA allowing only H3/H4 plasmid DNA to produce histones as replacement. The cell is then transformed with mutated histone coding plasmid containing h3/h4 genes. After transformation, the cells are grown on a 5-FOA plate, which produces 5-fluorouracil when interacting with Orotidine-5'-phosphate (OMP) decarboxylase, which is toxic to the cells. This forces the yeasts containing the URA3 plasmids to discard it for survival, allowing only newly added plasmids lacking URA3 marker to substitute for the production of histones. (Adapted from Li Fan & Wei Xiao, 2020)

3.2.6 Yeast spot assay

Yeast Spot assay is an imaging-based quantitative analyzing spotting assay used to evaluate the growth and survivability of yeast cells, through comparing and evaluating the cell colonies density or number of visible colonies, size of individual colonies, and intensity or concentration of each colony within each spot in a series of dilutions. Transformed yeast with histone modified plasmids are suspended in 400 µl of sterile water within 1.5 ml tubes for each sample and mixed. The optical density of this initial suspension is measured at 600 nm. According to the OD value, the number of cells in 1 ml is calculated. 10 000 cells/µl cell suspension is created. 10-fold dilution series is made from the initial suspension. Each dilution of 200 µl achieved from 20 µl from initial suspension added to 180 µl of ddH_2O, vortexed, and again 20 µl taken from the dilution to another 180 µl of ddH_2O. This is performed 5 times and plated as spot dilution series, 5 µl for each spot for each dilution, on both -HIS plate and 5-FOA containing plate and incubated at 30°C for 3 days. Plates are scanned with EPSON Perfection 1200PHOTO with EpsonScan software .

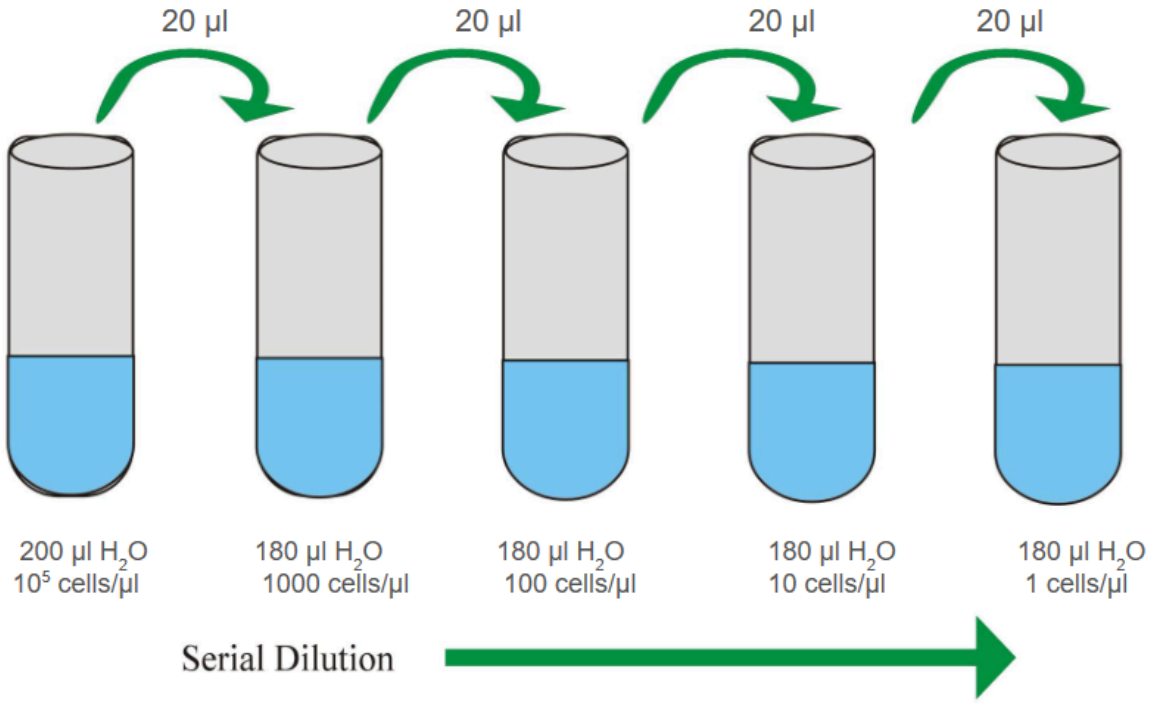

Figure 6. Yeast spot assay series dilution. The histone coding plasmid transformed yeast cells are collected, suspended, and diluted in ddH_2O for a concentration of 10000 cells/µl in 200 µl. A 10x series dilution is then made 4 more times, where 20 µl is taken from the previous dilution and added to a new 1.5 ml tube and vortexed afterwards. This divides the initial concentration by 10 by each step. The dilutions are then used for yeast spot assay.

3.3 RESULTS

3.3.1 Construction of yeast cells with SAS5 gene deletion

To analyze the effects of the depletion of Sas5 protein in yeast containing mutated histone H3 or H4, the SAS5 gene was deleted in AKY202 yeast strain. The strains' H3 and H4 histone coding regions were deleted from their genome and replaced with plasmid DNA that contains genes for expression of wild-type H3 and H4 histones along with a URA3 marker gene. To knockout SAS5 from the strain, the gene was targeted and cells transformed with PCR-amplified marker genes that flanked the promoter and terminator regions of the SAS5 gene (Figure 7). By homologous recombination, the SAS5 gene was replaced by the chosen marker genes in the chromosomal DNA, creating yeast strains with *sas5Δ* background (*sas5Δ)*. Three new yeast strains were made, each with different markers that knocked out SAS5: NATMX, which grants yeast resistance to nourseothricin; TRP1, which grants biosynthesis of tryptophan; and HGBMX gene, which grants hygromycin resistance (Figure 7). The 3 markers were amplified in DNA fragments using PCR from plasmids and transformed into the cells. The cells were grown in YPD plates after transformation and transferred to respective selective minimal media suited to the markers.

Successful transformation and recombination was confirmed through colony-PCR. For that, genomic DNA was extracted from 3 colonies from each transformed yeast strain, preceding with PCR of the colony-extracted DNA using primers, one complementary to the regions in the genome before the SAS5 gene and other targeted to the terminator region of the inserted marker gene construct in order to confirm the insertion of the marker gene and SAS5 knockout. The PCR products were then analyzed using agarose gel-electrophoresis confirming creation of new *S. cerevisiae* strains (Figure 7). Out of 9 colonies, 3 showed positive PCR signals with expected size of 524 bp (lane 1, 4 and 8). The positive colonies with different marker genes and *SAS5* gene deletion were propagated and made into stock. The newly *sasΔ* strains containing TRP1 and HGBMX genes, were designated strain database names AKY2671 and AKY2673, and strain containing *NATMX* gene, designated AKY2672. This strain was used for further experiments (Figure 8).

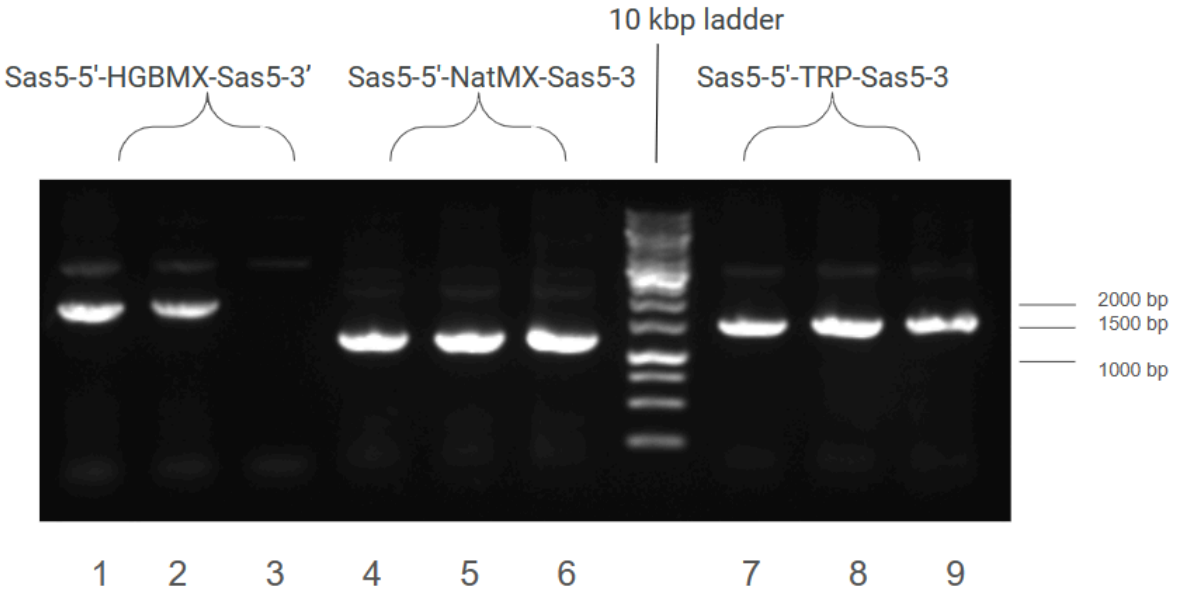

Figure 7. Gel-electrophoresis of SAS5KO maker genes PCR. 3 PCR samples were prepared for each marker gene. 5 ul of PCR were loaded on gel. Well 1 to 3 contains Sas5-5'-HBGMX-Sas5-3´, well 4 to 6 contains Sas5-5'-NATMX-Sas5-3´, and well 7 to 9 contains Sas5-5'-TRP-Sas5-3´, Expected sizes of the PCR products: TRP1 - 1,5 kb; NatMX - 1,2 kbp; HGBMX - 1,8 kbp. 1 kbp DNA ladder was used for size comparison.

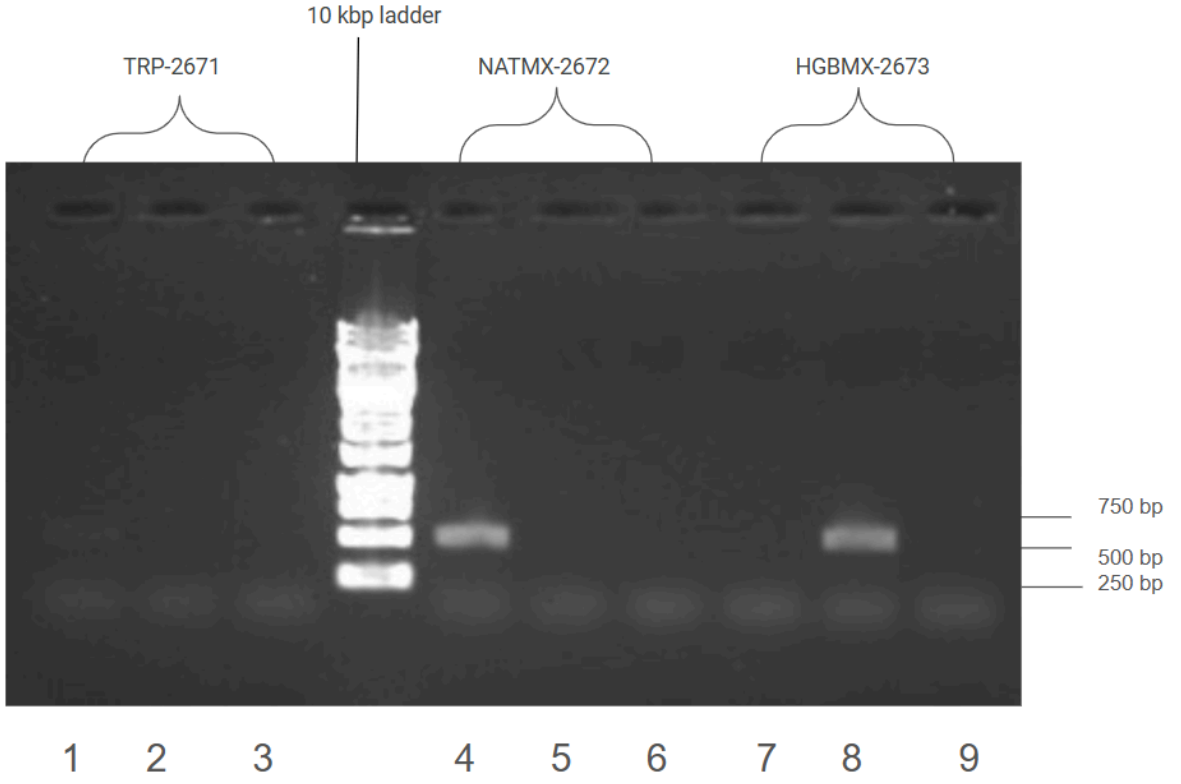

Figure 8. Agarose gel-electrophoresis of colony-PCR products. Colonies carrying NATMX marker gene (lanes 4-6, strain AKY2672); HGBMX marker gene (lanes 7-9; strain AKY2672); TRP1 marker gene (lanes 1-3, strain AKY2761). 1 kb DNA ladder was used for size comparison. Given the results, the colony used for PCR product on lane 4, AKY2672, was used for further experimentation.

3.2.2 Growth analysis of *sas5Δ* yeast strain in combination with histone mutants

To analyze the influence of Sas5 protein for the survivability of *S. cerevisiae* cells with specific histone modifications, cells from parental strain AKY202 and newly created *sas5Δ* strain AKY2672 were transformed with plasmids expressing either wild type or mutant histones. Transformants were firstly plated on -HIS selection media. (Figure 9). 3 colonies from *sas5Δ* strain were picked from -HIS selective media plates for each plasmid transformed, pRS413_**H3K9,14,18,23,27R**/wtH4, pRS413_**H3K4,36,79R**/wtH4; and modified H4 histone genes, pRS413_wtH3/**H4K16R** or pRS413_wtH3/**H4K5,8,12R**, and spread onto SC-5-FOA selective media plates and left to grow in 30◦C over 2-3 days. This will cause plasmid shuffling in the cells as 5-Fluoroorotic Acid reacts with the orotidine-5'-phosphate decarboxylase, produced from *URA3* gene, to create 5-fluoro-uracil, which is toxic to cells, causing the cells to remove the plasmid containing *URA3* to survive. Only cells that can survive *sas5Δ* and the unique histone modification will show growth in the media.

Compared to WT H3 and H4, all mutations had visibly slower growth in AKY2672. No growth in cells with empty plasmid was seen. Biggest growth defects were detected in AKY2672 containing H4K5,8,12R modified histones. Differences in viability and intensity of growth are also visible in cells of colony 1 and 3 of H3K9,14,18,23,27R, and in colony 2 of H3K4,36,79R.

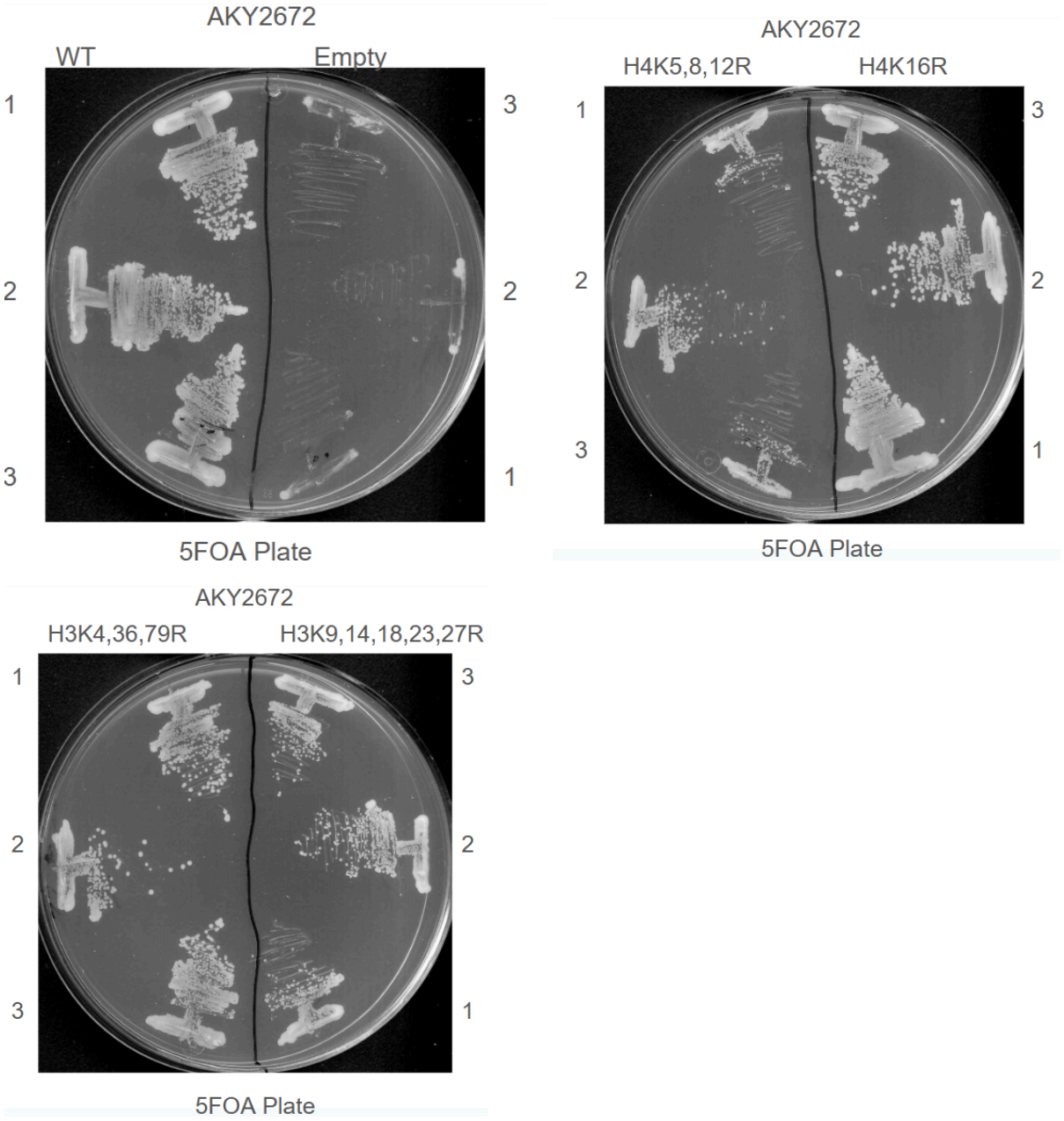

Figure 9. Growth analysis of *sas5Δ* yeast cells in combination with wt or mutated histones. After transformation of AKY2672 with histone plasmids, 3 colonies (1, 2, 3) of each transformed cell strain were taken from -HIS media and spread onto 5-FOA plates in order for plasmid shuffling to occur. Transformed plasmids are indicated on top of the plates, 2 plasmids per plate separated by a black line. Results were recorded two days after plating to 5-FOA containing plates.

3.3.3 Spot dilution assays of *sas5Δ* yeast strain with plasmids expressing mutant histones

Although the general growth test on 5-FOA is a good indicator of the effects of histone mutations on cells with *sasΔ* background, the results can vary depending on the amount of cells plated in each case. To acquire semi-quantitative results using a known number of cells for comparison a yeast spot assay was performed. The plasmids with

modified H3 histones genes with HIS marker, pRS413_**H3K9,14,18,23,27R**/wtH4, pRS413_**H3K4,36,79R**/wtH4; and modified H4 histone genes, pRS413_wtH3/**H4K16R** or pRS413_wtH3/**H4K5,8,12R**, were transformed into the *sas5Δ* AKY2672 cells, as well as in AKY202 yeast cell strain in order to compare the growth of the cells in parallel, using LiOAc method and plated on -HIS plates. Using spot dilution assay on selective media plates, -HIS and 5-FOA, the fitness and survival of the yeast can be examined in a 10x series of dilutions. The 2 strains, AKY202 and AKY2672, are compared for each mutant histone. pRS413_wtH3/wtH4 plasmid strain is used as positive control while nonhistone expressing plasmid, pRS413, as a negative control on both -HIS and 5-FOA plates. The -HIS media plate shows the baseline of cell growth with wild type histones as URA3 plasmids are still present, while 5-FOA plate results display the growth of cells with modified histones. The wtH3/wtH4 positive control plasmid shows the growth of *sasΔ* cells providing a baseline, while pRS413 shows no growth of cells, ensuring detection of lack of growth when expected in the 5-FOA (Figure 10). The experiment was performed 3 times for each colony (additional figures available in addendum no. 1)

For the WT strain (AKY202), on -HIS plates, a stable growth for all mutant strains can be seen as plasmids expressing wt histones and carrying a URA3 marker are still present in cells. On 5-FOA plates, no visible growth is observed for pRS413 and similar colony growth results for wtH3/wtH4 as on -HIS plates. For **H4K5,8,12R** modifications, there is a significant reduction in the number of colonies for 1000 cell/µl, 100 cell/µl, with no colony growth in 10 cell/µl and 1 cell/µl spots. For **H3K9,14,18,23,27R**, there is a reduction in colony density for 1000 cell/µl, 100 cell/µl, and 10 cell/µl, with no growth in 1 cell/µl. In **H3K4,36,79R**, there is no notable visible effect on colonies on 5-FOA plates (Figure 9).

For *sas5Δ* (AKY2672), on -HIS plates, no notable difference is observed in growth when compared with WT. On 5-FOA plating, no growth is observed for pRS413, while for wtH3/wtH4, a reduction is observed in colony size and slight decrease in density. For **H4K5,8,12R** and **H3K9,14,18,23,27R**, there is a significant decrease in colony size, density, and intensity with no colony growth in 10 cell/µl and 1 cell/µl spots. For **H3K4,36,79R**, there is also a reduction in colony size and density 10^4 cell/µl, 1000 cell/µl, 100 cell/µl and no growth in 1 cell/µl (Figure 9).

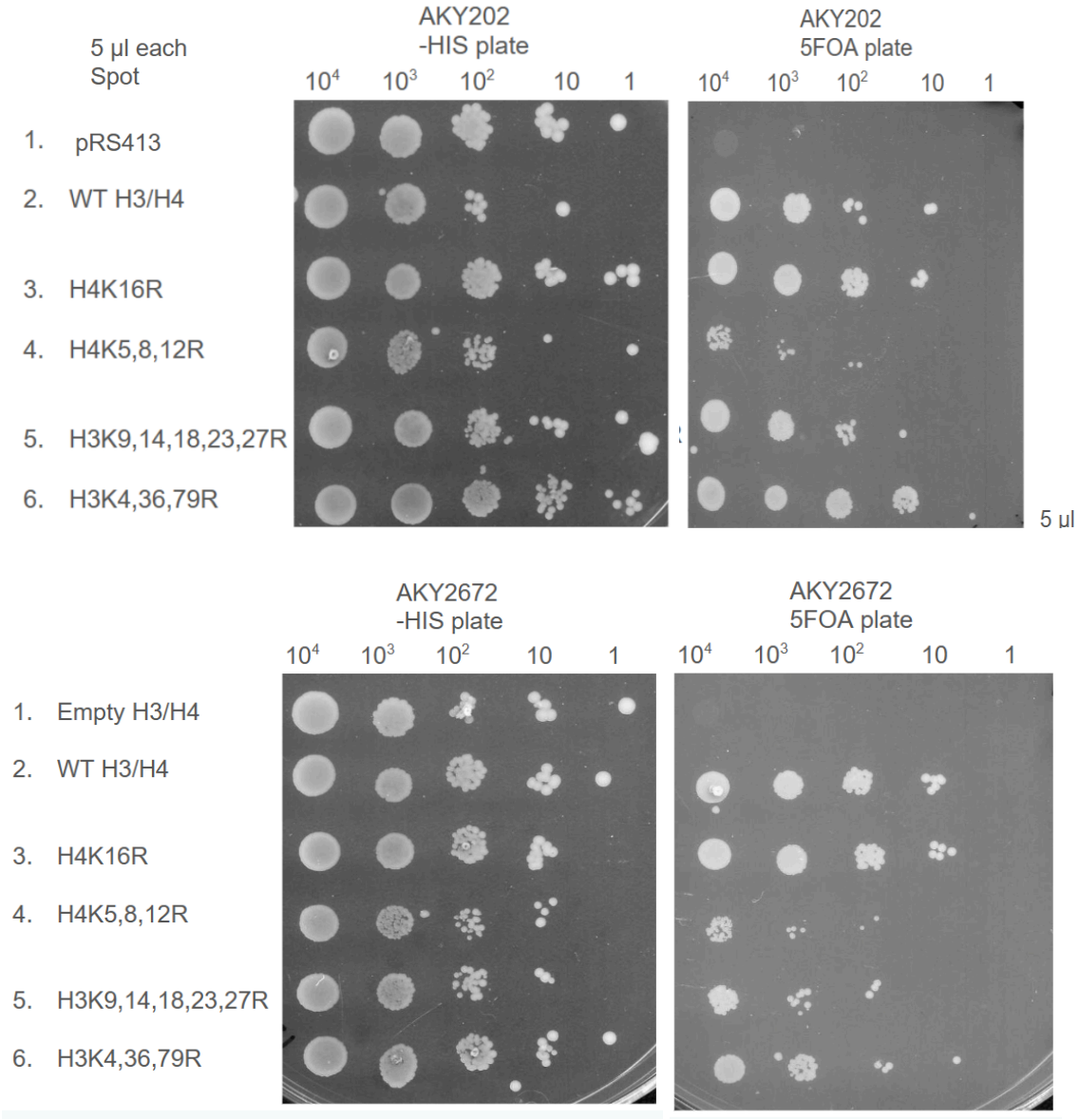

Figure 10. Spot assay analysis of parental, AKY202, and *sas5Δ,* AKY2672, yeast cells. Using a dilution series, both AKY202 and AKY2672 cell strains with wt and mutated histone plasmids were plated on both a -HIS and 5-FOA plates. Each row for each histone modified plasmid and each column for each dilution concentration. 5 µl is pipetted for strain for each dilution and left to grow in parallel for 2 (on the figure) to 3 days. Plates were scanned using EPSON Perfection 1200PHOTO with EpsonScan software .

3.4 DISCUSSION

As chromatin is composed of DNA wrapped around histone proteins, it serves not only as a structural scaffold but also as a responsive medium that undergoes conformational changes to control the accessibility of genetic material. These changes are orchestrated by PTMs on histones and other chromatin-associated proteins. PTMs, which include modifications such as methylation, acetylation, phosphorylation, and ubiquitination, acts as chemical signals that influences chromatin dynamics, impacting transcription, replication, and repair processes in a context-dependent manner. Crucial to this regulatory mechanism is the role of proteins that recognize these PTMs, often termed "reader" proteins. These specialized proteins interpret PTMs by binding to modified residues on histones, serving as molecular translators that recruit additional factors required for downstream cellular processes. By recognizing specific modifications, these proteins initiate a cascade of events that can either promote an open chromatin structure conducive to transcription or enforce a closed configuration that can silence gene expression.

YEATS-domain is an example of a domain in "reader" protein that assists in recognizing PTMs. YEATS domain recognizes histone lysine acylations and, for *S. cerevisiae,* found in 3 chromatin-associated proteins, Yaf9, Taf14, and Sas5. In previous research involving these chromatin-associated proteins, it was discovered that when an individual *YAF9* or *TAF14* gene is deleted in *S. cerevisiae,* the result is typically not lethal for the cells while still affecting various cellular processes. However, when both the *YAF9* and *TAF14* genes are deleted, cell viability becomes compromised, leading to lethality due to the disruption of essential processes such as chromatin remodeling, stress response, cellular growth, and transcriptional regulation. In the deletion of all three YEATS domain-containing proteins—Yaf9, Taf14, and Sas5—in *S. cerevisiae*, the cell becomes inviable due to disruption in critical cellular functions, resulting in inviability. Deficiency in essential chromatin remodeling and transcription initiation processes because of the absence of these proteins results in impaired gene expression regulation and stress response. This highlights the essential roles of these proteins in maintaining cellular stability.

The aim of this experiment is to evaluate the viability of yeast cells with different histone H3 or H4 lysine mutations in the *sas5Δ* background. From this experiment, when the histone tails of H3 and H4 are modified in yeast cells, changes in the growth of the cells can be observed, including differences for cell growth of AKY2672. While no visible difference in growth pattern for 5-FOA plates of AKY2672 with wildtype histones are seen in comparison to 5-FOA plates of AKY202 with similar histones, we can see visible reduction in growth patterns when comparing AKY202 with specific histone modifications with

AKY2672 with similar modifications. When comparing 5-FOA plates between AKY202 and AKY2672, we can observe reduction in growth pattern H3 acetylation and also methylation mutants for AKY2672 compared to AKY202 cells. This decrease is visible with H3K4,36,79R methylation modifications and H3K9,14,18,23,27R acetylation modifications. The 5 point H3 acetylation has an expected result as Sas5 has the highest affinity for H3k9ac, which is displayed in the decreased growth pattern when comparing AKY2672 with AKY202, highlighting Sas5 acting on H3 modification-dependently. The reduced growth pattern for AKY2672 cells with H3 methylation site mutations indicates a potential role in maintaining cell fitness with *sas5Δ* cells. These results might be further examined by replicating the observed effect through target mutations of all free lysine residues with SAS5Δ. Lack of visible growth patterns for H4 acetylation mutation sites for 5-FOA plates between AKY202 and AKY2672 is likely due to Sas5 high affinity for H4 histone peptides in both modified and unmodified states.

While there are observable changes in growth for *S. cerevisiae* cells with modified histones without Sas5, the changes are not signs of significant impact when compared to other YEATS domain-containing proteins in *S. cerevisiae*. This is likely due to the Taf14 and Yaf9 exhibit high specificity for certain histone modifications, particularly acetylation, such as H3K9ac and H3K27ac, and crotonylation like H3K9cr. Despite being essential for the structural integrity of the SAS complex, its absence does not impact cell growth when faced with histone modifications in multiple locations when compared to Taf14 and Yaf9. This is likely due to speciality in selective binding as well and lack of involvement in multiple transcription-related complexes, when compared to other YEATS domain-containing proteins.

The Spot dilution assay experiment was replicated 3 times (Additional figures of spot assay in Addendum no. 1). Some variation was observed across the replicate assay likely due to technical inconsistencies during the preparation. Further replication of the experiment maybe warranted to support the reliability of findings.

While previous studies of *sas5Δ* show changes in size of the catalytic unit of the complex where Sas5 belongs to, SAS complex, deletion of several key proteins involving the complex, such including Sas2 and Sas4 protein, would likely inhibit the chromosomal function in transcription regulation and silencing at gene loci like HML and telomeres. This could reveal how such disruptions affect histone acetylation and chromatin modification, as well as potentially impair ASF1-dependent chromatin assembly.

Future experiments that can be performed, taking the current results in mind, are observing phenotypic changes in dual combination deletion of Sas5 protein with one other

YEATS domain-containing protein along with histone modifications to observe both viability and impact for those particular sites. Additionally, a likely experiment is to use a similar technique of yeast spot assay to evaluate growth under various stress conditions could reveal how individual H3 modifications with *sas5Δ* affect chromatin dynamics and cellular resilience. Another experiment can be in further investigating and observing potential impact on H3 methylation and Sas5 or SAS complex by mass spectrometry to map Sas5 interactions with H3K4me3 and H3K36me3. Lastly, another experiment that can be performed is Fluorescence-Activated Cell Sorting (FACS) to analyze cells based on fluorescence to study the cell cycle by analyzing cells based on their DNA content.

SUMMARY

When researching chromatin-associated proteins influence with modified histones, *S. cerevisiae* is a simple eukaryotic model suitable for displaying the changes in cell growth and viability. The aim of this study was to investigate the viability of yeast cells carrying different histone modifications in combination with Sas5 deletion. This is from an ongoing research regarding the purpose and the extent of influence of YEATS domain-containing proteins in budding yeast. By using yeast spot assay to observe and compare the phenotype of histone mutations in combination with SAS5 deletion.

By replacing H3 histones in *S. cerevisiae* with H3 mutant with mutated methylation and acetylation sites in N-terminal tail, we observed a decrease in the cell growth for *sasΔ* cells. While not lethal, the result of the growth pattern indicates how Sas5 plays a role in yeast cell fitness when experiencing methylation and acetylation mutation in H3 histones. Replacing H4 histones in *S. cerevisiae* with mutated acetylation sites in N-terminal tail doesn't produce a similar decrease in cell growth for *sasΔ* cells as for H3 histone mutants, which displays Sas5 affinity for both modified and unmodified H4 peptides and its effect on yeast cell fitness.

Future research into Sas5 is likely to investigate deeper into Sas5 interactions with H3 methylation and acetylation by targeting mutations of all free lysine residues with SAS5Δ. This can also help strengthen the results of this experiment and investigate the decreased viability of yeast cells with H3 methylation and acetylation mutations with SAS5 deletion. Using spot dilution assay to more variable dilutions to compare the stress of the cells under these mutations. Then reviewing the cells with particular histone mutations along with SAS5 deletions, the use of FACS analysis and comparison of cell cycle single mutants and multiple mutants. This can help to review the influence of lack of SAS5 with histone mutation on the cell cycle in detail and analyze replication stress.

Addendum

Addendum no. 1 to "Combinatorial effect of histone H3 and H4 mutations with deletion of YEATS domain protein Sas5 in *Saccharomyces cerevisiae*"

Additional figures of Spot assay analysis of parental, AKY202, and *sas5*Δ, AKY2672, yeast cells

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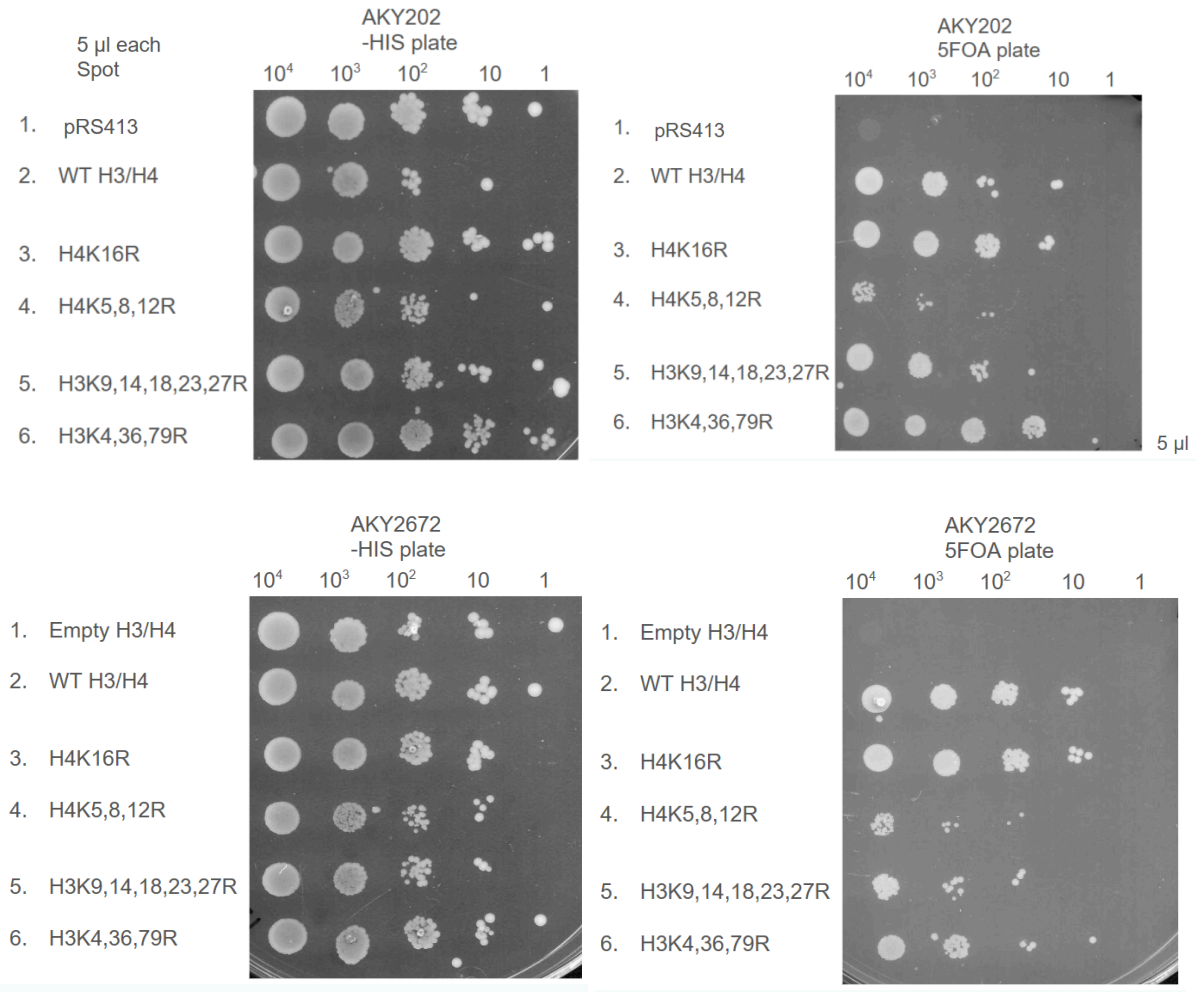

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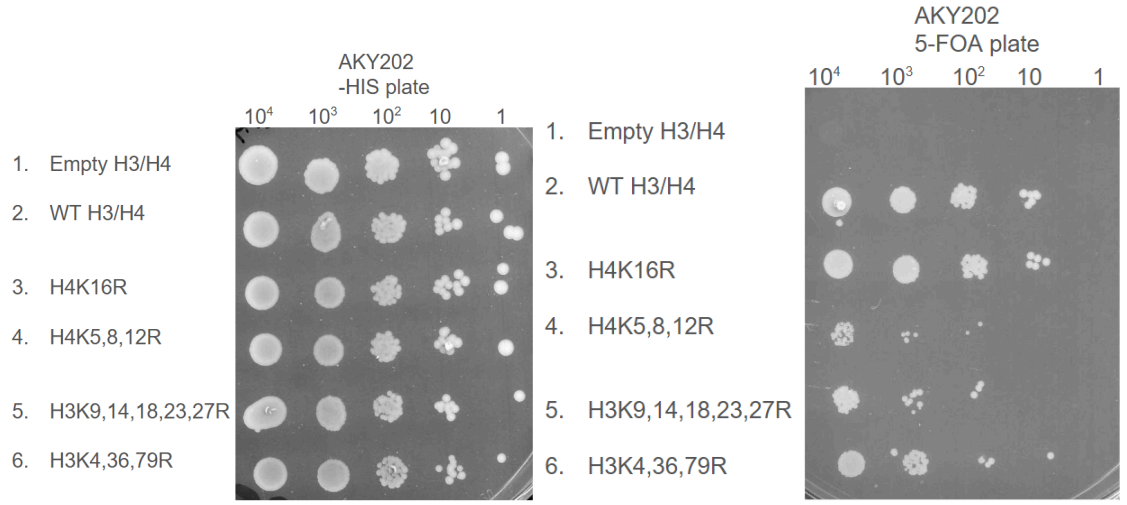

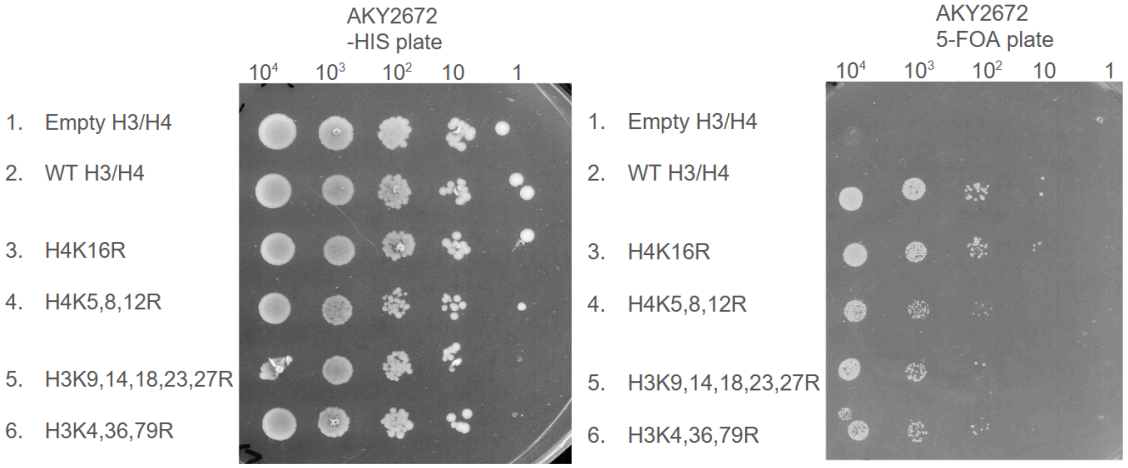

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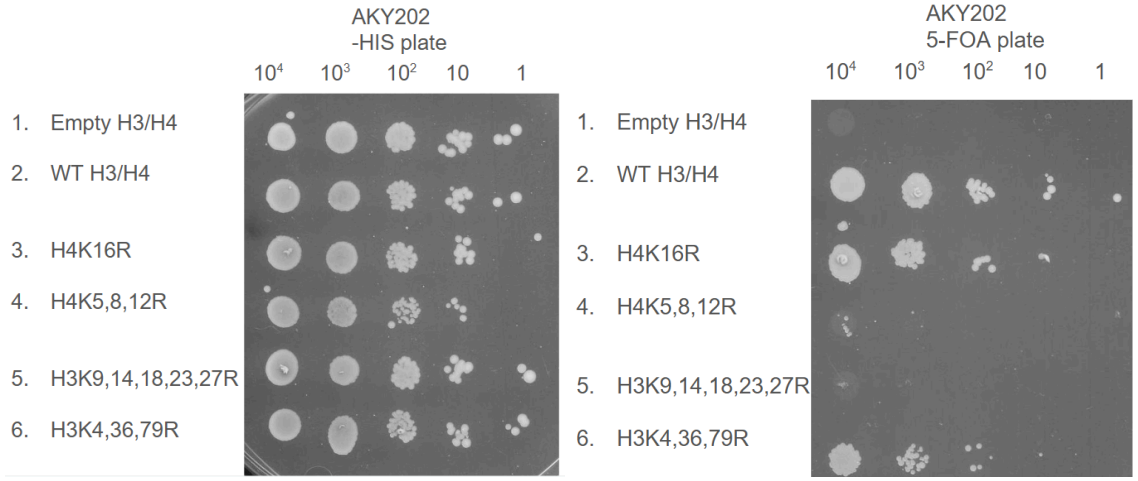

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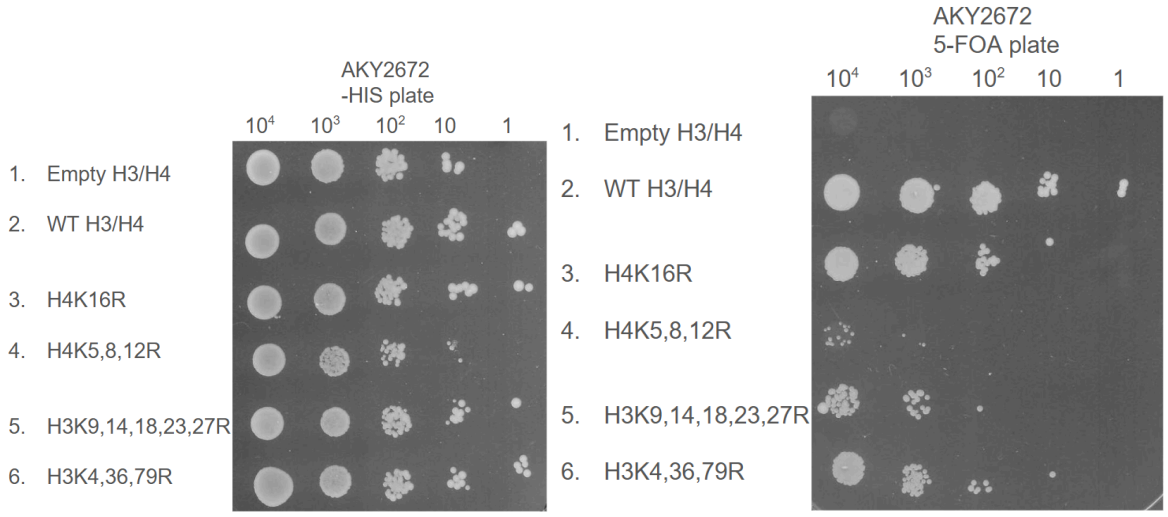

Addendum no. 2 to "Combinatorial effect of histone H3 and H4 mutations with deletion of YEATS domain protein Sas5 in *Saccharomyces cerevisiae*"

supplementary table of primers:

SAS5KO_5-1569	TTTTTGGTGCCATATAATAGACGCTCTTTTATGGATCAT
SAS5KO_3-1570	CTACTTTAGCTTGTTATGAGCCTTCACTGGCTCGTCCTT
SAS5_ctrl-1571	GCTTTATCCACGGGTGTTACCAG
FBA1_CTRL_2	CGAACTCCAAAATGAGCTATC

Addendum no. 3 to "Combinatorial effect of histone H3 and H4 mutations with deletion of YEATS domain protein Sas5 in *Saccharomyces cerevisiae*"

Yeast strains

Strain	Genotype	Source
AKY202 *MAT A*	*hht1-hhf1::LEU2 hht2-hhf2::kanMX YCp50:hht2-hhf2* (URA3)	Laboratory collection
AKY2671 *MAT A*	*hht1-hhf1D::LEU2 hht2-hhf::kanMX sas5::TRP1* YCp50:hht2-hhf2 (URA3)	This study
AKY2672 *MAT A*	*hht1-hhf1D::LEU2 hht2-hhf::kanMX sas5::NatMX6 YCp50:hht2-hhf2* (URA3)	This study
AKY2673 *MAT A*	*hht1-hhf1D::LEU2 hht2-hhf::kanMX sas5::HGB YCp50:hht2-hhf2* (URA3)	This study

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