

**FACTORS THAT INCREASE THE EFFICIENCY OF DNA REPAIR  
WHEN USING TRANSCRIPT RNA AS A TEMPLATE**

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**FACTORS THAT INCREASE THE EFFICIENCY OF DNA REPAIR  
WHEN USING TRANSCRIPT RNA AS A TEMPLATE**

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## ABSTRACT

Interaction with various chemicals and environmental agents can cause damages to DNA. In this study, we particularly focus on double stranded breaks (DSBs) and a repair mechanism that uses transcript RNA to repair the break. A previous study has shown that the frequency of DSB repair by transcript RNA in the *cis* system is increased in cells with a genotype of *spt3 rnh1 rnh201*. This research focuses on determining factors that increase the efficiency of DSB repair when using transcript RNA as a template. Cells with the genotype of *spt3 rnh1 rnh201* in a *cis*-system were mutated and transformed with gene over expression library in two separate experiments. We have found 13 candidates from 20,000 mutated cells that can improve the efficiency of repair using transcript RNA, as well as 58 potential candidates out of 3,784 cells transformed with the yeast gene over expression library.

## INTRODUCTION

All living organisms contain genetic material encoded in DNA, which make up the composition and function of the body. The genome is comprised of genetic material and contains both coding and non-coding sequences of DNA. A genome's stability is dependent on its ability to maintain integrity and prevent future discrepancies from proceeding to new generations [12]. Interaction with various chemicals and environmental agents, however, can cause damages in the DNA, which can result in a double stranded break (DSB). Unrepaired damages can lead to mutations, which can potentially cause diseases. Several factors such as proofreading enzymes, DNA repair enzymes, and checkpoints exist to prevent or correct DSBs from occurring; however some damages go undetected by these mechanisms. DSBs are usually repaired precisely by a process known as homologous recombination, which uses identical or nearly identical molecules of DNA to repair the broken end of DNA. Although this mechanism of DNA-DNA recombination is commonly used, RNA viruses have shown to perform homologous recombination using RNA molecules [3]. With this knowledge, researchers questioned whether RNA molecules could be used to repair DNA DSBs of an organism.

The purpose of the study that I have been working on with graduate student Chance Meers, was to determine other factors that can improve the efficiency of DSB repair by transcript RNA. Two experiments were conducted, the first consisting of random point mutations in the yeast genome, while the second experiment will consist of insertions of a plasmid into a yeast cell with over expressed random genes found in the

yeast genome. As seen in the Keskin *et al's* study, altering the genes *RNH1 RNH201 SPT3* and *RAD51* allow DNA to use transcript RNA as a template. With the yeast genome containing 5,300-5,400 genes the likelihood of finding mutations that can improve the efficiency of repair is good [5]. The over expression of certain genes in the yeast genes can also promote increased frequency of repair [9]. Keskin *et al's* study explains that the abundance of transcript RNA in our cells can now be put into use [3]. Therefore the results from this study can potentially be used to treat diseases, caused by DNA double stranded breaks, by the method of using transcript RNA.

## LITERATURE REVIEW

Previous studies have shown that a DNA DSB, in *Saccharomyces cerevisiae* and human cells, can be repaired using synthetic RNA oligonucleotides as a template [10,11]. As a result, Keskin et al's study has developed a mechanism for repairing a DNA DSB using homologous recombination in which endogenous transcript RNA is used as a template [3]. In this study, a spliced, intron less, antisense *his3* transcript from chromosome III was used as a template to repair a broken *his3* allele on chromosome XV. This method of repair is known as a *trans* system because the antisense transcript used to repair the break comes from a different chromosome, chromosome III instead of chromosome XV. Another method of repair known as the *cis* system was performed to compare the frequency of repair to the *trans* system. In the *cis* system, a spliced, intron less, antisense *his3* transcript from chromosome III was used as a template to repair the same but broken *his3* allele on chromosome III [3]. During the development of these systems to study RNA driven DSB repair, several elements needed to be considered.

First it was essential to ensure that the DSB repair was mediated by transcript RNA rather than the complementary DNA (cDNA). Unfortunately, due to the abundance of Ty retrotransposons in yeast cells, there is a high chance that the spliced antisense *his3* RNA will be converted into complementary-DNA (cDNA) by Ty1 reverse transcriptase. This conversion causes the cDNA to repair the homologous broken *his3* sequence rather than the initial endogenous transcript RNA [6,13]. Since the function of the *SPT3* gene is known to activate Ty transcription to produce the reverse transcriptase enzyme, it was deleted to terminate this process [3]. The results of Keskin et al's study

proved that deleting the *SPT3* gene decreases the likelihood of reverse transcription, which produces the cDNA, and therefore will prevent cDNA from acting as a template for repair[3].

To continue the repair process of a DSB using transcript RNA as a template, the formation of an RNA-DNA heteroduplex is required [3]. According to Cerritelli's et al's study, the genes coding for Ribonuclease (RNase) H1 (*RNH1*) and the catalytic subunit of RNase H2 (*RNH201*) are responsible for cleaving the RNA strand of RNA-DNA hybrids. Therefore deleting these genes can further promote the repair of a DSB using transcript RNA as a template by allowing the formation of a RNA-DNA hybrid.

In order to test the impact of the deletion of *RNH1* *RNH201* and *SPT3* in Keskin at al's study, two experimental yeast cell systems, *cis* and *trans* were used (figure 1a,b)[3, 6,13]. As mentioned above, the *cis* system used a spliced antisense *his3* transcript from chromosome III to repair a DSB located inside the intron of the same *his3* locus [3]. While the *trans* system used a spliced antisense *his3* transcript from chromosome III to repair a DSB in a different *his3* allele on chromosome XV [3]. In Keskin's study, two assays of *trans* and *cis* were performed using two yeast strains containing the wild-type *SPT3* gene. The experiment containing the wild type *SPT3* strain concluded that the DSB in *his3* is repaired "exclusively via cDNA pathway"[3]. As expected, there was also a higher frequency of His<sup>+</sup> colonies in the *trans* system due to the continuous generation of the *his3* transcript in the presence of galactose [3]. The second experiment with deleted *SPT3* *RNH1* and *RNH201* genes showed that the *cis* system produced much higher frequency of His<sup>+</sup> colonies than the *trans* system, which concludes that DSB repair was

not mediated cDNA but rather by an RNA transcript [3]. These results are consistent with the findings, which suggest that donor sequences closer to the DSB repair the break more efficiently than farther donor sequences [7,8]. Overall this data concludes that transcript RNA can be used to repair a DSB in *cis*-system *rnh1 rnh201* and *spt3 rnh1 rnh201* [3].

As for determining the genes that could disrupt the process of using transcript RNA as a template for repair, it is also essential to find genes that could enhance this process. The results from Wabha et al's study conclude that the presence of the Rad51 recombinase protein can directly promoted the DNA-RNA hybrid [3, 14]. Moreover, Keskin et al's study shows that DSB repair was increased by a factor of 3 in *cis*-system *rnh1 rnh201 spt3 rad51* compared to *rnh1 rnh201 spt3* cells [3].

## METHODS

The strains used in this study are found in table 1. The medium used in this study consisted of YPD, YPGalactose, His<sup>-</sup>, and Ura<sup>-</sup>.

### **Pronging technique experiment 1**

Each mutated clone was transferred, using a sterile toothpick, from the YPD media onto a MICROTTEST™ tissue culture plate with 96 wells filled with deionized water (figure 2). The controls were placed in the first four wells of the first column, while the remaining wells each received one mutated colony. Once all 96 wells were filled, a 48-pinned pronger was used to transfer the diluted colonies from the MICROTTEST™ tissue culture and plated onto YPD media, YPGalactose (YPGal), and His<sup>-</sup> (figure 3). Following 48 hours of growth, the yeast colonies were displaced from YPGal media onto His<sup>-</sup> media using the replica plating method.

### **Pronging technique experiment 1**

Each transformed clone was transferred, using a sterile toothpick, from the YPD media onto a MICROTTEST™ tissue culture plate with 96 wells filled with deionized water (figure 6). The controls were placed in the first five wells of the first column, while the remaining wells each received one mutated colony. Once all 96 wells were filled, a 48-pinned pronger was used to transfer the diluted colonies from the MICROTTEST™ tissue culture plate onto Ura<sup>-</sup> and His<sup>-</sup> (figure 7). Following 48 hours of growth, the yeast colonies were displaced from Ura<sup>-</sup> onto YPGal media by replica plating. Following an

additional 48 hours of growth, the repla plating method was used to displace the colonies from YPGal media onto *His<sup>-</sup>* media (figure 7).

## RESULTS

Two experiments were performed to research for factors that can improve the efficiency of DSB repair by transcript RNA. The first experiment used ethyl methylsulfonate (EMS), a mutagenic organic compound, to produce point mutations in the genotype of *spt3 rnh1 rnh201* cells of the *cis*-system. The second experiment consisted of the introduction of plasmids into the yeast cell for the over expression of genes found in the yeast genome.

In the first experiment, the three controls that were used consisted of a YS486 strain containing His<sup>+</sup>, two HK454 strains with *rnh201Δ spt3Δ saeΔ*, and a YS486 strain with 0% EMS *rnh1Δ rnh201Δ spt3Δ* (table 1). The cells were initially plated on YPD media, a medium lacking histidine (His<sup>-</sup>), and YPGal medium. With the YPD media known to be a complete medium for yeast growth, growth was seen in every colony following pronging. The YS486 strain containing His<sup>+</sup> was a positive control, was the only strain that was able to grow on the medium lacking histidine (figure 3). As described in Keskin et al's study, the YPGal medium induces a DSB by activating the expression of HO endonuclease and then activating the *his3* antisense from chromosome III to induce repair (figure 1b)[3]. As a result, slow growth of cells was seen on the YPGal medium (figure 3). Following the replica plating of cells from the YPGal medium onto His<sup>-</sup> medium, we searched for higher frequency of His<sup>+</sup> papilla. Colonies that experienced more growth than the HK454 strains with *rnh201Δ spt3Δ saeΔ* and YS486 strain with 0% EMS *rnh1Δ rnh201Δ spt3Δ* controls were identified as candidate colonies (figure 4).

Finally, patch screenings of the candidate clones were performed to confirm that the increased growth was due to the mutation rather than from experimental errors (figure 5). Patches of the candidate were formed by first plating them on YPD media to allow for growth. Once there was a significant amount of growth, the new candidate colonies were dispersed to grow in patches. The patches were then transferred onto YPGal medium to induce DSB repair, and then replica plated onto His<sup>-</sup> with some control patches to observe and compare repair (figure 5). Lastly, if there was significant growth compared to the control, the candidate clone was confirmed to have a mutation that increased the frequency of DSB repair in yeast using transcript RNA as a template for repair. (figure 5). In this first experiment, 13 candidates out of 20,000 have been found. No candidates were found in the 2,640 mutated screen that I performed.

In the second experiment, the three controls that were used consisted of a His<sup>+</sup> AS3, two AS1 strains, one AS2  $\Delta$ *spt3* strain, and a AS4 strain (table 1). The cells with transformed plasmids and the controls were initially plated onto Ura<sup>-</sup> medium, which was used as a control to determine the efficiency of the addition of the plasmid containing the *URA3* gene, and His<sup>-</sup> medium which was used as control to determine whether repair was occurring prior to activation of the YPGal promoter (figure 7). We saw complete growth on the Ura<sup>-</sup> medium, and only the control His<sup>+</sup> YCP50PK plasmid was growing on the His<sup>-</sup> medium (figure 7). The cells were replica plated from Ura<sup>-</sup> onto YPGal medium, to induce a DSB by activating the expression of HO endonuclease followed by activating the *his3* antisense from chromosome III to induce repair and to express the gene on the plasmid of the over expression library (figure 7). Following the replica plating of cells from the YPGal medium onto His<sup>-</sup> medium, we some His<sup>+</sup> papilla. Clones that

experienced more papilla than the AS1, AS2, and AS3 controls were identified as candidate colonies (figure 8). Finally the same patch screenings performed in the first experiment will be done to the potential candidates of this experiment to confirm that the increased growth was due to the mutation rather than from experimental errors (figure 5). Once the candidates are confirmed, their DNA will be extracted and sequenced to determine which yeast gene increases the frequency of repair of a DSB when over expressed. So far 44 library screens have been made, with 58 potential candidates. Patch screenings will still need to be performed for this experiment.

## DISCUSSION

The purpose of this study was to identify new factors that could increase the efficiency of DNA repair when using transcript RNA as a template. This study has shown the discovery of potential factors that can increase the efficiency of transcript RNA as a template for repair. Through EMS mutagenesis, the study has found 13 candidates that improve the efficiency of repair when using transcript RNA out 20,000. 58 potential candidates out of 3,783 have been found from yeast over expression library experiment. This study suggests that there are numerous potential candidates that may exist to improve this mechanism of DSB repair. As mentioned before, the yeast genome contains ~6,000 genes and therefore the experiments of this study will allow for the discovery of more factors regulating this repair mechanism. The results of this study could potentially increase the efficiency of repair so much that it would be more favorable to use RNA transcript as a method for repair rather than cDNA. As Keskin *et al*'s study explains, the abundance of transcript RNA in our cells can now be put into use "...RNA may have a marked impact on genomic stability and plasticity"[3].

## FIGURES AND TABLES

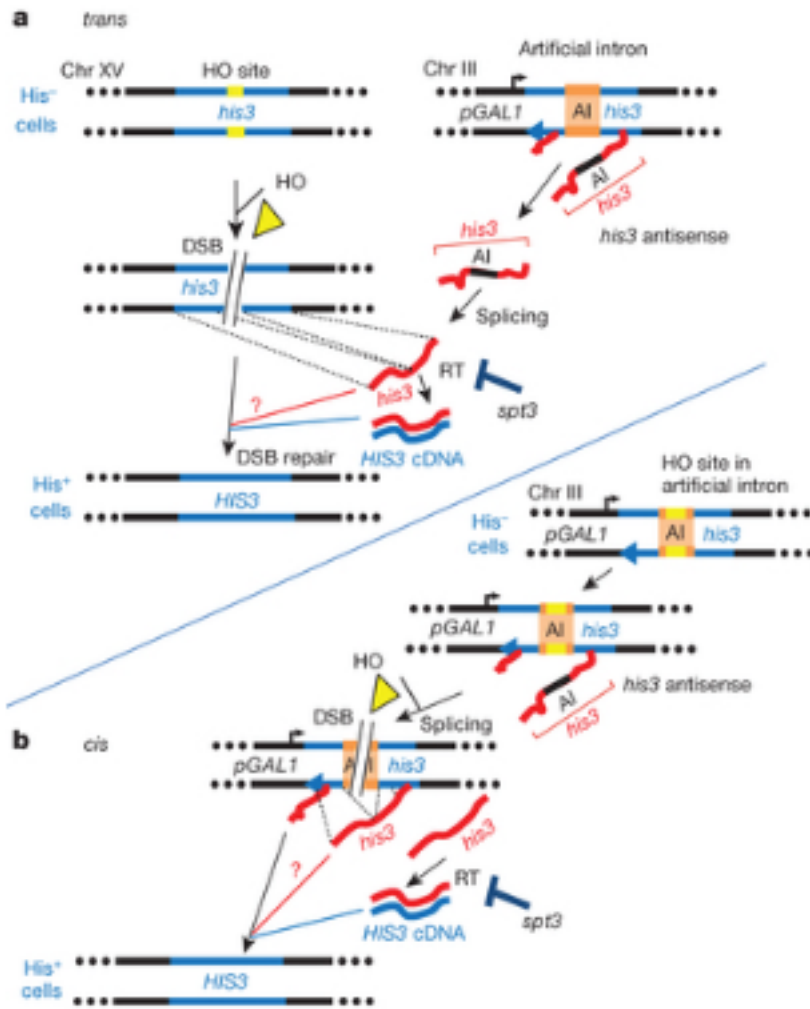
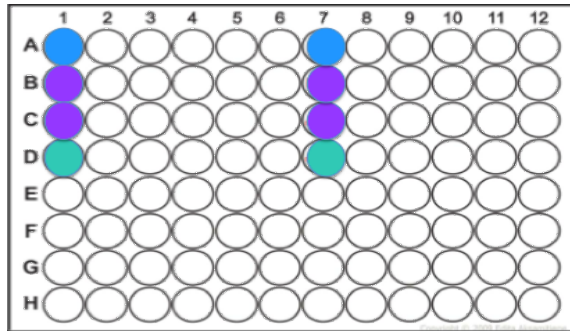
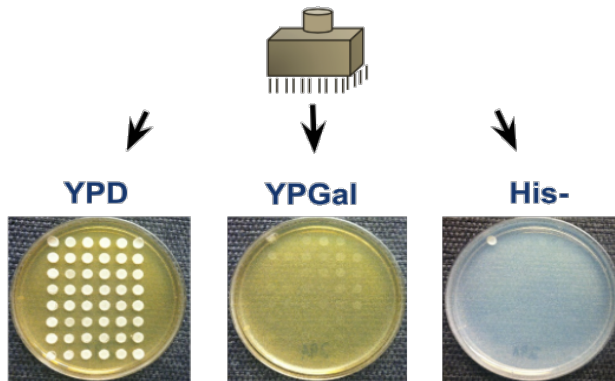


Figure 1. a,b Scheme of *trans* (a) and *cis* (b).

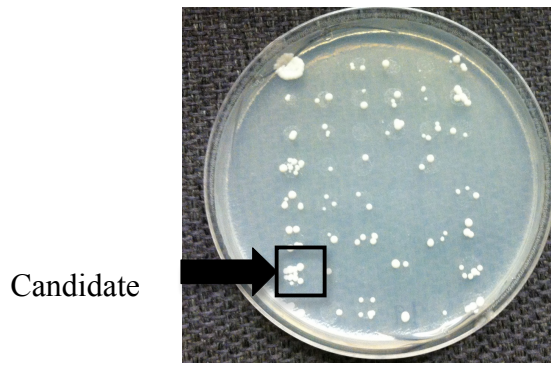
- YS486 HIS+ (after repair)
- HK454 (*rnh1Δ rnh201Δ spt3Δ sae2Δ*)
- YS486 0% EMS (*rnh1Δ rnh201Δ spt3Δ*)



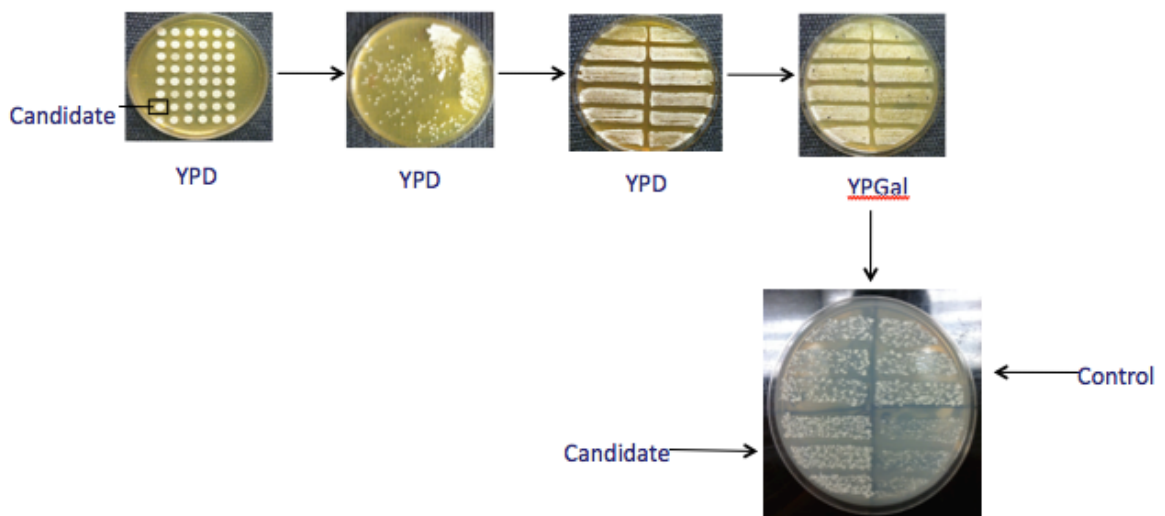
**Figure 2.** The plate that the mutated colonies were transferred to. Of the 96 wells, each well received one mutated colony. Wells 1A, 1B, 1C, 1D and 7A,7B, 7C, 7D received a YS486 strain (after repair), two YS486 strains with 0% EMS (*rnh1Δ rnh201Δ spt3Δ*), and a HK454 strain (*rnh1Δ rnh201Δ spt3Δ sae2Δ*) respectively.



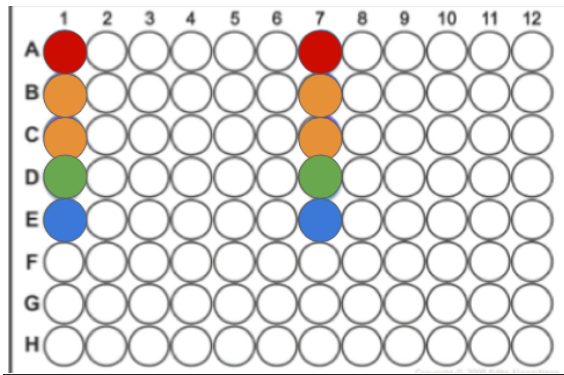
**Figure 3.** This figure displays the three different mediums, YPD, YPGal, His<sup>-</sup> that the mutated colonies were transferred to.



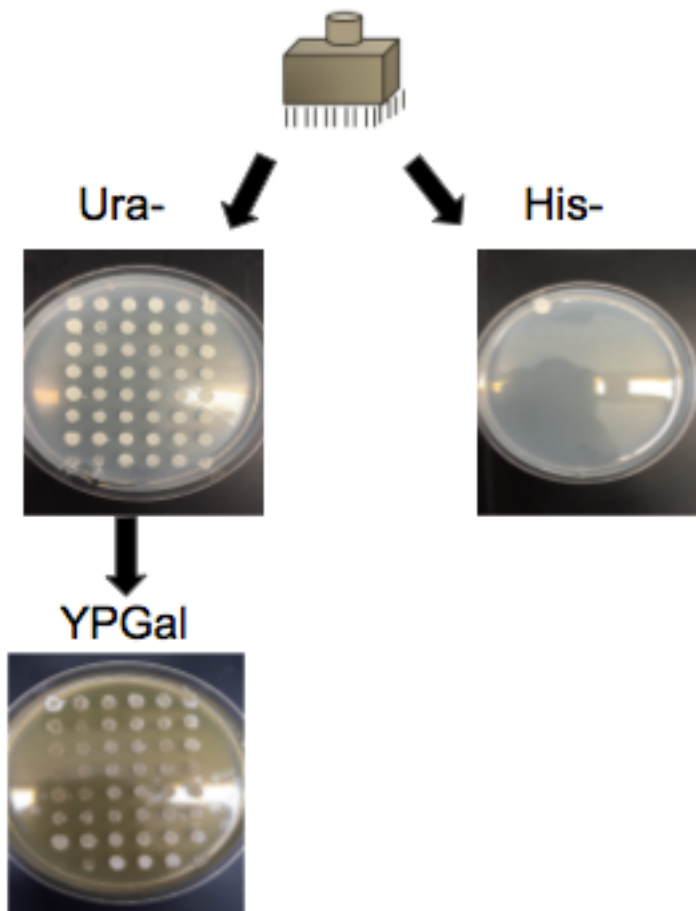
**Figure 4.** A candidate colony that shows significant growth compared to the controls.



**Figure 5.** This figure displays patch screening of the candidate. First, the candidate is plated on YPD medium to allow for growth. Once there is a significant amount of growth, the new candidate colonies are spread to grow in patches. The patches are then transferred onto YPGal to induce repair, and then displaced onto His<sup>-</sup> with some control patches to observe repair. Finally, if there is significant growth compared to the control, the candidate is confirmed to have a mutation that increased repair and growth.



**Figure 6.** The plate that the colonies were transferred to. Of the 96 wells, each well received one mutated colony. Wells 1A, 1B, 1C, 1D, 1E and 7A,7B, 7C, 7D, 7E received a YS486 His<sup>+</sup> plasmid, two YS486 YCP50PK plasmids, a YS440 YCP50PK plasmid, and a YS486 Mud2 plasmid respectively.



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**Figure 7.** The two mediums that the colonies were first transferred to, Ura- and His-. Colonies from the Ura- medium were then replica plated onto YPGal.

Strain	Relevant genotype	Source
FRO-1073	<i>hoΔ hmlΔ::ADE1 mataΔ::hisG hmrΔ::ADE1 ade1 leu2::pGAL1mhis3AI-ADE3 lys5 trp1::hisG ura3-52 ade3::GAL::HO</i>	[3]
FRO-1075,1080 ( <i>trans</i> )	FRO-1073 <i>his3::HOcs</i>	[4]
YS-164, 165	FRO-1075, 1080 ( <i>HIS3::HOcs</i> ):: <i>TRP1</i>	[3]
YS-166, 167	YS-164, 165 <i>pGAL1-mhis3AI::CORE</i>	[3]
YS-172, 174 ( <i>cis</i> )	YS-166, 167 <i>pGAL1-mhis3AI::HO</i>	[3]
YS-278, 281	YS-172, 174 <i>YCLWTy2-1::CORE</i>	[3]
YS-291, 292 ( <i>cis</i> ) WT	YS-278, 281 <i>YCLWTy2-1Δ</i>	[3]
YS-440	YS-291,1a <i>SPT3::G418 Cis</i>	[3]
YS-441	YS-292 1a <i>SPT3::G418 Cis</i>	[3]
YS-486, 487 ( <i>cis</i> )	YS-291, 292 <i>rnh1Δ::NAT rnh201Δ::hygMX4 spt3Δ::kanMX4</i>	[3]
CM106	YS-486, 2 micron	[4]
CM103	YS440, 2 micron	[4]
AS1	CM106+YC50PK (empty vector)	This Study
AS2	CM103+ YC50PK (empty vector)	This Study
AS3	CM103 <i>pGal1-mhis3AI::his3+YCp50pk</i>	This Study
AS4	CM106 + <i>pAC3013</i>	[3]

**Table 1.** These are the yeast strains and plasmids that were used in this study.

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