

# The Effect of the Copy Number of the Telomerase RNA Gene on the Elongation of Telomeres in *Saccharomyces cerevisiae*

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**The Effect of the Copy Number of the Telomerase RNA Gene on the Lengthening of  
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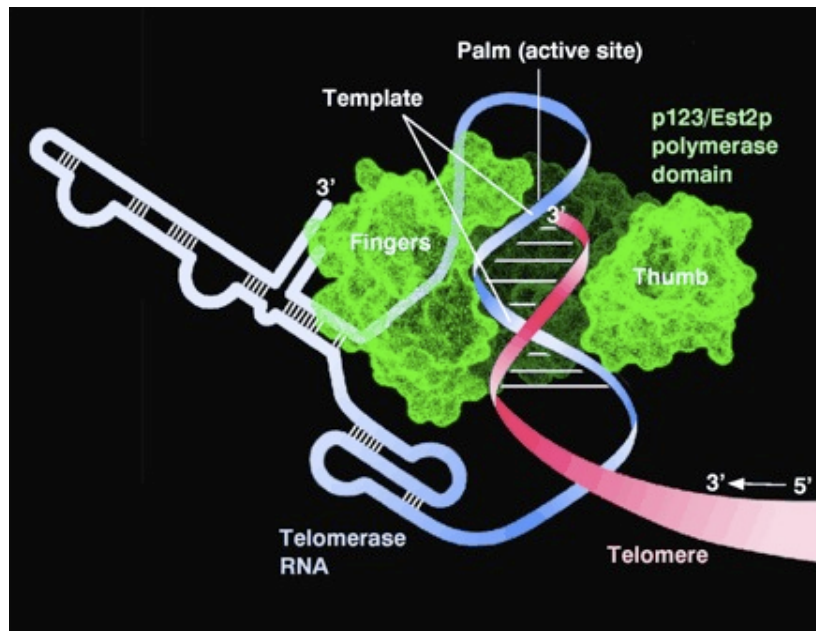


Figure from [http://www.mun.ca/biochem/courses/3107/images/telomerase\\_model.gif](http://www.mun.ca/biochem/courses/3107/images/telomerase_model.gif)

**Rebecca Sherwood**

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## **Table of Contents**

Abstract.....	6
Introduction.....	7
Materials and Methods.....	13
Results.....	21
Discussion.....	29
References.....	32

**List of Figures**

Figure 1: Telomerase Complex and Recruitment Pathways.....12

Figure 2: *De novo* Telomere Addition Assay.....20

Figure 3: *De novo* Telomere Addition Assay Results.....25

Figure 4: Native Telomere Lengths I.....26

Figure 5: Native Telomere Lengths II.....27

Figure 6: Real Time PCR.....28

## **List of Tables**

Table 1: Plasmids.....	17
Table 2: Yeast Strains.....	18
Table 3: RT PCR Primers.....	19

## Abstract

Telomeres are repeated sequences at the ends of chromosomes, which promote chromosome stability by preventing the loss of necessary nucleotides from the DNA with successive rounds of replication. Telomeres are elongated by the enzyme telomerase, which has both a protein component and an RNA component. In the yeast *Saccharomyces cerevisiae*, the *TLC1* gene encodes the RNA component of the enzyme. Telomerase RNA interacts with several proteins to perform its function, including the Ku protein, which binds to the end of the DNA and helps to recruit telomerase to the chromosome thereby facilitating the lengthening of chromosome ends. Ku interacts with telomerase RNA at the site of a 48-nucleotide stem-loop on the RNA's structure. Previous experiments have shown that yeast strains engineered to carry two copies of the *TLC1* gene exhibit higher levels of telomerase RNA than those that have only one copy of the gene. Also, a yeast strain carrying a copy of the mutant *tlc1Δ48* gene, which contains a deletion of the 48-nucleotide stem-loop, contains lower levels of telomerase RNA than a strain with the wild type *TLC1* gene. This series of experiments is investigating whether the copy number of the telomerase RNA gene affects the elongation of telomeres in *S. cerevisiae*. In order to determine this effect, the *de novo* telomere addition of four strains was examined, as were the native telomere lengths of these strains. The assay indicated that the efficiency of telomere elongation was unchanged by increasing the copy number of the wild type gene but was increased upon increasing the copy number of the mutant gene. Analysis of the native telomere lengths showed that increasing the copy number of either the wild type or the mutant gene allowed the cells to maintain their telomeres at a longer length.

## **Introduction**

### *Telomeres and the Telomerase Complex*

DNA polymerase is limited in that it can only replicate DNA in the 5' to 3' direction and requires an RNA primer for the synthesis of each strand. This results in a gap at the 5' end of the DNA strand with every round of replication, as the RNA primer must be removed, and DNA polymerase is incapable of filling this gap with the appropriate nucleotides. Some organisms, such as bacteria, circumvent this difficulty by having circular chromosomes. However, for organisms with linear chromosomes, this presents a problem since it could result in the loss of nucleotides, which may be parts of essential genes. Telomeres are repeated sequences added to the ends of chromosomes, which promote chromosome stability by preventing the loss of necessary nucleotides with successive rounds of replication. Telomeres can also be added to sites of double strand breaks as a way to minimize the instability caused by the damage (Pennaneach, Putnam, & Kolodner, 2006).

The nucleotide sequences, as well as the maintenance, of the telomeres vary among species. In humans, the telomeres consist of TTAGGG repeats (Blackburn, 2001). Telomerase, the enzyme responsible for elongating telomere ends, is a complex composed of both a protein subunit and an RNA component. The protein subunit, referred to as TERT and encoded by the *hTERT* gene, is a reverse transcriptase (Nakamura et al., 1997). The RNA component is called TERC and is encoded by the *hTERC* gene (Feng et al., 1995). TERC acts as the template for the addition of the nucleotides by TERT. In most human cells, telomerase is not active because the transcription of the *hTERT* gene is suppressed. Therefore, telomeres become shorter with



each round of replication. In contrast, *hTERT* is transcribed and, subsequently, telomerase is active in the ovaries, testes, and developing stem cells (Smogorzewska & de Lange, 2004).

In *Saccharomyces cerevisiae*, the telomere sequence is more flexible, consisting of TG<sub>1-3</sub> repeats (Blackburn, 2001). In this unicellular organism, telomerase actively extends the telomeres before each cell division in order to maintain telomere length. The enzyme is similar to human telomerase in that it is a complex of a protein and an RNA molecule. Est2p is the catalytic component of the enzyme, which functions as the reverse transcriptase (Lingner et al., 1997). The RNA component of telomerase, TLC1 RNA, provides a template of CA repeats so that Est2p can add the telomeric TG<sub>1-3</sub> repeats to chromosome ends (Singer & Gottschling, 1994).

#### *Telomeres and Disease*

Telomeres arose as such an interesting research topic because they have been linked to several human diseases. Dyskeratosis congenita is a genetic disorder, in which afflicted individuals have abnormal nail growth and skin color, and bone marrow failure. This disease can be inherited as either an X-linked mutation or an autosomal dominant mutation. When it is caused by an autosomal gene, it has been found that the mutation is in the TLC1 RNA gene (Chen & Greider, 2004). These findings are significant since it may be possible to search for ways to treat the disease by increasing transcription from the wild type gene to produce more functional TLC1 RNA (Chen & Greider, 2004).

A more publicized link between telomeres and disease has been their association with cancer. The telomeres in cancer cells are short but stable, indicating that these cells have found ways to maintain telomere length in order to become immortal. There are

two mechanisms by which cancer cells are able to do this. One involves the reactivation of telomerase, and the other is by crossing over between existing telomeres (Cech, 2004). The continued investigation of telomerase activation may result in the discovery of possible targets for therapies to combat the growth of cancer cells.

#### *Telomerase Regulation and Recruitment*

There are a variety of proteins involved in the regulation of telomere length in *S. cerevisiae*. Rap1p is a protein that binds to the telomeric repeats and interacts with Rif1p and Rif2p, which also associate with the chromosome ends. These proteins restrict the accessibility of the telomere end to telomerase and are thus regarded as negative regulators of telomere length (Levy & Blackburn, 2004). Tel1p and Mec1p, on the other hand, are checkpoint kinases, which are positive regulators of telomere length, perhaps because they make the telomere more accessible to telomerase (Ritchie, Mallory, & Petes, 1999).

In addition to the proteins involved in regulating access to the telomeres, there are also proteins, which are integral in the recruitment of telomerase to the chromosome end. TLC1 RNA is essential not only because of its function as the template for Est2p, but also because it contains the binding sites for several of the proteins, which are critical for proper telomere elongation. Among these proteins is Est1p, which binds to the RNA's structure. Est1p interacts with Cdc13p, which is a protein that binds to the single stranded DNA at the ends of chromosomes. In this way, the interaction between Est1p and Cdc13p acts as a pathway through which the telomerase enzyme can be recruited to chromosome ends (Evans & Lundblad, 1999).

Another key protein is Ku, a heterodimer, consisting of Yku70 and Yku80 subunits. It is a DNA end-binding protein, which has been implicated in both non-homologous end-joining and telomere addition (Stellwagen, Haimberger, Veatch, & Gottschling, 2003). Ku binds preferentially to the double stranded DNA at the ends of chromosomes and can also bind transiently to broken DNA ends to promote their repair. It is similar to Cdc13 in that it recruits telomerase to telomere ends; however, as is shown in Figure 1, instead of interacting with a telomerase bound protein to accomplish its function, Ku binds directly to a 48-nucleotide stem loop in the TLC1 RNA's structure (Peterson et al., 2001).

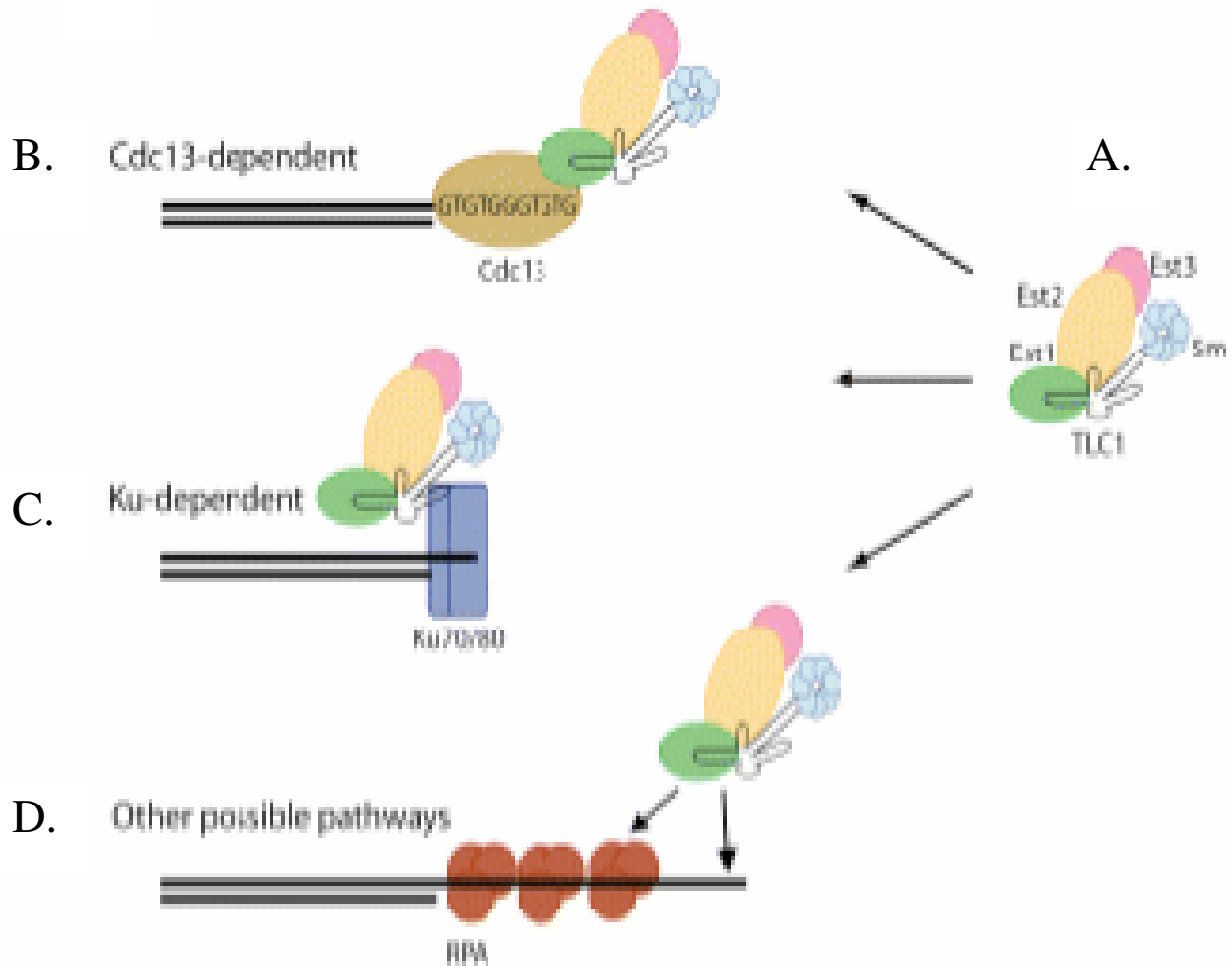
#### *TLC1 RNA*

TLC1 RNA is clearly an integral part of the telomerase complex; therefore, several studies have focused on the importance of this molecule and the implications mutations in it may have for telomeric addition. Previous experiments have quantified the amount of TLC1 RNA to be about 29 molecules per haploid yeast cell (Mozdy & Cech, 2006). Telomerase elongates telomeres during late S phase and G2/M phase in the cell cycle (Diede & Gottschling, 1999). Therefore, since *S. cerevisiae* contains sixteen linear chromosomes, there are thirty-two chromosome ends to be elongated after replication in S phase, more than the number of TLC1 RNA molecules available. Because of this, not every chromosome's telomere is elongated with each cell division; instead, telomerase is preferentially recruited to shorter telomeres, since these require elongation the most (Sabourin, Tuzon, & Zakian, 2007). Another result of this is that diploid cells with only one copy of the *TLC1* gene proved to have shorter telomeres as a result of having less TLC1 RNA in their cells (Mozdy & Cech, 2006). Heterozygotes

with one wild type copy of the gene and one mutant copy of the gene lacking the template RNA also had shorter telomeres most likely because this mutant *tlc1*  $\Delta$ template RNA competed with the wild type RNA for telomeric proteins and substrates (Mozdy & Cech, 2006).

In addition to the deletion of the template region of the RNA, the deletion of the 48-nucleotide stem loop has also been studied. Since these mutants lack the stem loop required for interaction with the Ku protein, they are not recruited to telomere ends as efficiently. It has been shown that this deletion results in the maintenance of shorter telomeres (Peterson et al., 2001). In this study, strains have been constructed with either an increased copy number of the wild type *TLC1* gene or the mutant *tlc1*  $\Delta$ 48 gene, in order to determine the effects that these genotypes would have on the length of telomeres and the efficiency of elongation.

# Figure 1



**Telomerase Complex and Recruitment Pathways.** (A) This figure shows the components of the telomerase complex (TLC1 RNA, Est 1, Est 2, Est3, and the seven Sm proteins) and their binding sites on the TLC1 RNA. (B) Telomerase can be recruited to chromosome ends by the interaction between Cdc13 and Est 1. (C) Another recruitment pathway involves the interaction between the TLC1 RNA stem loop and the Ku protein. (D) It is proposed that there may be other undiscovered pathways through which telomerase can be recruited to the chromosome end. Figure from Pennaneach, et al. *Molecular Microbiology* (2006) 59(5), 1357-1368.

## Materials and Methods

### *Plasmids and Plasmid Minipreparation*

The three plasmids used in this experiment are listed in Table 1. The first, pRS314, is a centromeric plasmid with a *TRP1* marker (Sikorski & Hieter, 1989). The second, pSD107, is the pRS314 plasmid with the *TLC1* gene inserted into Not1/Xho1 sites. The third plasmid, *ptlc1*Δ48, is pSD107 with base pairs 288-335 of *TLC1* deleted corresponding to the 48-nucleotide stem loop.

In order to use these plasmids in subsequent steps, they were isolated from bacteria using an alkaline lysis procedure (Sambrook & Russell, 2001).

### *Yeast Strains and Transformations*

The yeast strains used in these experiments are listed in Table 2. UCC5706 is a yeast strain with a *trp1* mutation. It has a wild type *TLC1* gene and a deletion of the *RAD52* gene to eliminate homologous recombination. Its seventh chromosome has also been altered to contain an *ADE2* gene followed by a short telomeric sequence, an HO endonuclease site, and a *LYS2* gene so that this strain can be used in the *de novo* telomere addition assay described later. YJA020 also has a mutation in the *trp1* gene. It contains the mutant *tlc1*Δ48 gene, instead of the *TLC1* gene, and a deletion of the *RAD52* gene.

These two strains were used in the transformations, which led to the four different strains used in these studies. UCC5706 and YJA020 were both transformed with pRS314 to create empty vector control strains using a high efficiency LiAc transformation procedure (<http://www.fhcrc.org/science/labs/gottschling/yeast/ytrans.html>). These strains were named YRS003 and YRS004, respectively. UCC5706 was also transformed

with pSD107, and YJA020 was transformed with *ptlc1Δ48*, giving the two experimental strains. These strains were named YRS001 and YRS002, respectively.

#### *De Novo Telomere Addition Assay*

In order to test the efficiency of telomere elongation in the strains listed above, a *de novo* telomere addition assay was performed (Figure 2). On the first day of this three-day assay, four 5ml YC-lys-trp cultures were inoculated with single colonies of YRS001, YRS002, YRS003, and YRS004 and grown on a wheel in a 30°C incubator overnight. The next morning, the cultures were diluted to a cell density of  $0.8 \times 10^6$  cells/ml and transferred into 500ml flasks containing 125ml YEP + 2.5% raffinose media. The flasks were incubated on a 30°C shaker overnight. On the third day, the cultures were diluted to a density of about  $8 \times 10^6$  cells/ml, and then 125μl of nocodazole were added to each flask to arrest the cells in the G2/M phase of the cell cycle. The flasks were incubated at 30°C for two and a half hours to allow the cells to arrest. 25ml of each of the four cultures was transferred to a Falcon tube and centrifuged to serve as the 0 hour time point. The remaining volume of each culture was centrifuged and then resuspended in 100ml YEP + 3% galactose + 100μl nocodazole. The galactose induces cleavage at the HO site, while the nocodazole maintains the cell cycle arrest. The four flasks were incubated at 30°C on a floor shaker, and time points were collected in Falcon tubes every hour for four hours. Each time point was centrifuged to pellet the cells and then stored at -20°C (Diede & Gottschling, 1999).

#### *Analysis of the Time Points from the de novo Telomere Addition Assay*

After all of the time points were collected, the DNA was extracted from the cells by using a rapid genomic DNA isolation procedure (Hoffman & Winston, 1987). The

isolated DNA was then digested with SpeI overnight, and the resulting samples were analyzed on a 1.3% agarose gel the next day. The DNA from the gel was transferred to a nylon membrane creating a southern blot (<http://www.fhcr.org/science/labs/gottschling/misc/southern.html>), which was then probed at 42°C overnight with an *ADE2* probe. It was then visualized by washing the blot in CSPD and incubating it in a cassette with Kodak BioMax x-ray film.

#### *Analysis of Native Telomere Lengths*

In order to analyze the native telomere lengths of YRS001, YRS002, YRS003, and YRS004, a rapid genomic DNA isolation procedure was performed on colonies grown from glycerol stock solutions of the four strains (Hoffman & Winston, 1987). The DNA was digested overnight with XhoI and analyzed on a 1% agarose gel the next day. A southern blot was created from the gel and probed overnight at 42°C with the Y' probe, which recognizes the Y' sequence at the end of approximately half of the yeast's chromosomes. It was visualized with CSPD and Kodak BioMax x-ray film. The blot was then stripped of this probe (<http://www.fhcr.org/science/labs/gottschling/misc/southern.html>). It was reprobbed overnight at 37°C with a degenerate CA oligonucleotide probe, which detects all of the chromosomes' telomeres by directly recognizing the telomeric TG<sub>1-3</sub> sequence. After washing the southern blot, it was visualized with CSPD and Kodak BioMax x-ray film.

#### *RNA Preparation and Quantitation by Reverse Transcriptase PCR*

In order to quantify the amount of TLC1 RNA present in YRS001, YRS002, YRS003, and YRS004, the RNA was isolated from each of the strains (<http://www.fhcr.org/science/labs/gottschling/yeast/rprep.html>). The concentration of RNA was



determined by their ultraviolet absorbances, and each of the samples was diluted with water to a concentration of 2 $\mu$ g/ $\mu$ l. An equal mixture of the TUB2cDNA and TLC1-1147 rev primers listed in Table 3 was made, and 2 $\mu$ l of this mixture was added to each 10 $\mu$ l RNA sample. These samples were put in the PCR machine for 3 minutes at 95 $^{\circ}$ C. During this run, a mixture was made containing 2 $\mu$ l 10x RT buffer, 1 $\mu$ l dNTP mix, 1 $\mu$ l RNase inhibitor, 1 $\mu$ l reverse transcriptase, and 3 $\mu$ l water for each sample. After 3 minutes, the samples were removed from the PCR machine and the 8 $\mu$ l mixture described above was added to each RNA sample. They were again put in the PCR machine to carry out the reverse transcriptase reaction at 42 $^{\circ}$ C for 1 hour and 92 $^{\circ}$ C for 10 minutes.

Real Time PCR was performed in triplicate for each sample, and separately for the TUB2 primers and the TLC1 primers. In order to carry out this reaction, 5 $\mu$ l of the cDNA obtained in the reverse transcriptase reaction was combined with 6 $\mu$ l of a mixture of either the TUB2Fwd and TUB2Rev primers or the TLC1-352 fwd and TLC1-649 rev primers, and 9 $\mu$ l SYBR Green. The samples were run in a real time PCR machine with an annealing temperature of 52 $^{\circ}$ C.

Table 1. Plasmids

<b>Plasmid</b>	<b>Features</b>	<b>Source</b>
pRS314	Empty vector with <i>TRP1</i> marker	Sikorski & Hieter, 1989
pSD107	Wild type <i>TLC1</i> gene, <i>TRP1</i> marker	Scott Diede of the Fred Hutchinson Cancer Research Center
ptlc1Δ48	Mutant <i>tlc1Δ48</i> gene, <i>TRP1</i> marker	Suzanne Peterson of the Fred Hutchinson Cancer Research Center

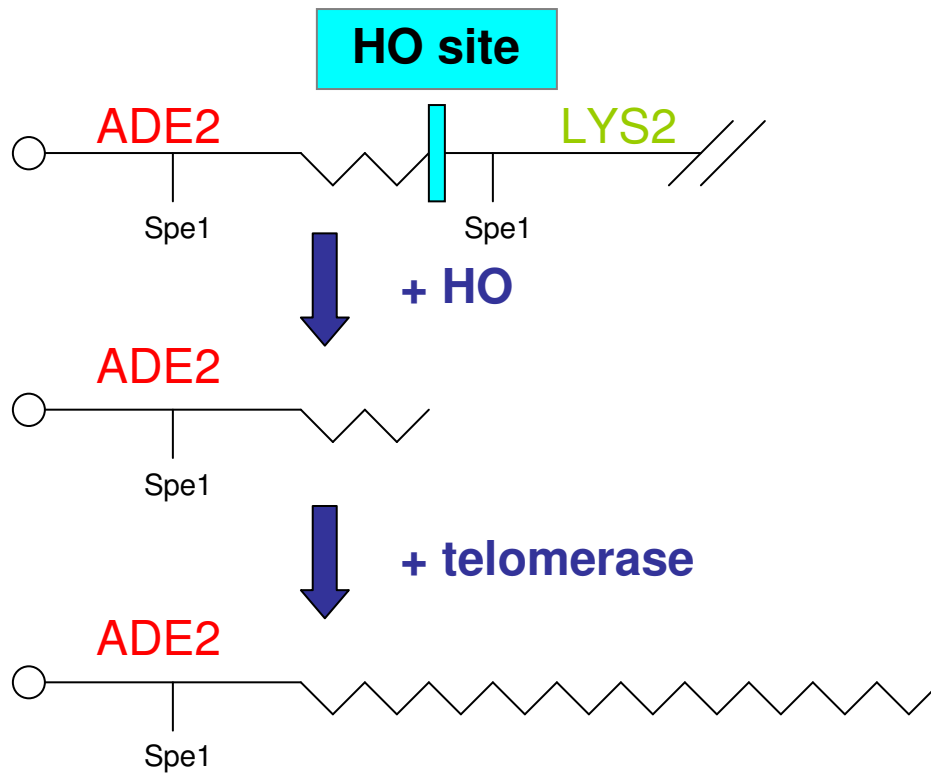
Table 2. Yeast Strains

<b>Yeast Strain</b>	<b>Genotype</b>	<b>Source</b>
UCC5706	Mat a – <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1: GAL1-HO-LEU2 VII-L::ADE2-TG(1-3)-HO site-LYS2 rad52::hisG</i>	Scott Diede of the Fred Hutchinson Cancer Research Center
YJA020	Mat a – <i>ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1: GAL1-HO-LEU2 VII-L::ADE2-TG(1-3)-HO site-LYS2/+ tlc1Δ48 rad52</i>	Julian Arthur of Boston College
YRS001	UCC5706 + pSD107	This study
YRS002	YJA020 + p <i>tlc1Δ48</i>	This study
YRS003	UCC5706 + pRS314	This study
YRS004	YJA020 + pRS314	This study

Table 3. RT PCR primers

<b>Name</b>	<b>Sequence (5' → 3')</b>
TUB2cDNA	TCCAAACCTTGAGGAGCGACAGAA
TUB2Fwd	TATCTGTGGTGAGCACGGTTTGA
TUB2Rev	ATGACGCTGTCTACAAGCTCAGCA
TLC1-1147 rev	AAATGCATCGAAGGCATTAGG
TLC1-352 fwd	CACTGCTATTGCATTTAGTTGC
TLC1-649 rev	ATAAAGTGACAGCGCTTAGCA

## Figure 2



***De Novo* Telomere Addition Assay.** The figure above depicts the *de novo* Telomere Addition Assay. The top diagram shows the construction of chromosome VII with the *ADE2* gene, the short telomeric sequence, the HO endonuclease site, and the *LYS2* gene. In the first step, the cells are transferred into galactose media. HO, which is under a galactose-induced promoter, then cleaves the chromosome, exposing the shortened telomere end, which can then be elongated by telomerase in the next step. Adapted from Diede & Gottschling, 1999.

## Results

### *De Novo Telomere Addition Assay*

A *de novo* telomere addition assay was performed to analyze the efficiency of elongation in the four experimental strains. The Southern blot depicting the results of the assay is shown in Figure 3. The 1kb ladder in the first lane is followed by the five time points for strain YRS003, which has a wild type *TLC1* gene and is transformed with an empty vector plasmid (Table 2). These lanes represent wild type telomere addition. The zero time point appears to have no telomere because, at this point, the chromosome has yet to be cut by the HO endonuclease; therefore, the DNA is in the upper most band of the blot. At each successive time point after this zero hour, the band around the 750 base pair mark becomes broader, indicating that the telomeres are being elongated. A similar pattern of addition is seen in the next five lanes, which represent the time points of YRS001, which contains the wild type *TLC1* gene both in the chromosome and on a plasmid (Table 2).

YRS004, which has the mutant *tlc1Δ48* gene, shows decreased efficiency. This is evident when these time points are compared with those for the wild type strain; it is clear that the band in the later time points does not become as broad in the mutant strain. The last five time points represent telomere addition in YRS002, which contains a copy of the mutant *tlc1Δ48* gene both in the chromosome and on a plasmid. The telomere addition of this strain is increased significantly when compared with the addition of YRS004. It appears as if the bands of the three and four hour time points could even be heavier than those for wild type; however, when the 1650 base pair loading controls of the strains are

compared, it is clear that the concentration of DNA is greater for those time points of YRS004 than it is for those of YRS003 or YRS001.

#### *Native Telomere Lengths*

The native telomere lengths of the strains were analyzed by southern blot. Figure 4A is visualized with the Y' probe and shows the native telomere lengths of the four experimental strains, as well as the untransformed parent strains. The parent strains were analyzed to confirm that the addition of the empty vector plasmid did not have an effect on telomere length. The first three lanes show the native telomere lengths of three individually prepared genomic preps of UCC5706, which is the untransformed parent strain of YRS003 and YRS001. The next three lanes represent the telomere lengths of YRS003, which are comparable to those of UCC5706. YRS001, whose native telomeres are depicted in the following three lanes, has slightly longer telomeres than UCC5706 and YRS003.

The next three lanes represent the genomic preps obtained from YJA020, and those are followed by YRS004. These strains both have the *tlc1Δ48* mutation and, therefore, have shorter native telomeres, as is supported by previous literature (Peterson et al., 2001). The last three lanes show the native telomere lengths of YRS002, which has the *tlc1Δ48* gene in the chromosome and on a plasmid. The telomere lengths of this strain are slightly longer than those of YJA020 and YRS004. Figure 4B, like Figure 4A, is also a Southern blot visualized with the Y' probe. This blot includes one genomic prep of each of the four experimental strains in order to compare the native telomere lengths of these strains more closely.

Figure 5A is the same Southern blot as is shown in Figure 4A, but it has been stripped of the Y' probe and visualized with the CA probe. In contrast to the Y' probe which shows approximately half of *S. cerevisiae*'s telomeres, the CA probe shows all of the telomeres and also has an internal loading control. This ensures that the shifts in the bands are due to differential telomere lengths and are not due to unequal running of the samples on the original gel. This figure, like Figure 4, shows that the telomeres of YRS001 are slightly longer than those of UCC5706 and YRS003. Similarly, the telomeres of YRS002 are longer than those of YJA020 and YRS004. Figure 5B is analogous to Figure 4B in that it offers a closer comparison of the four experimental strains, but it is visualized with the CA probe.

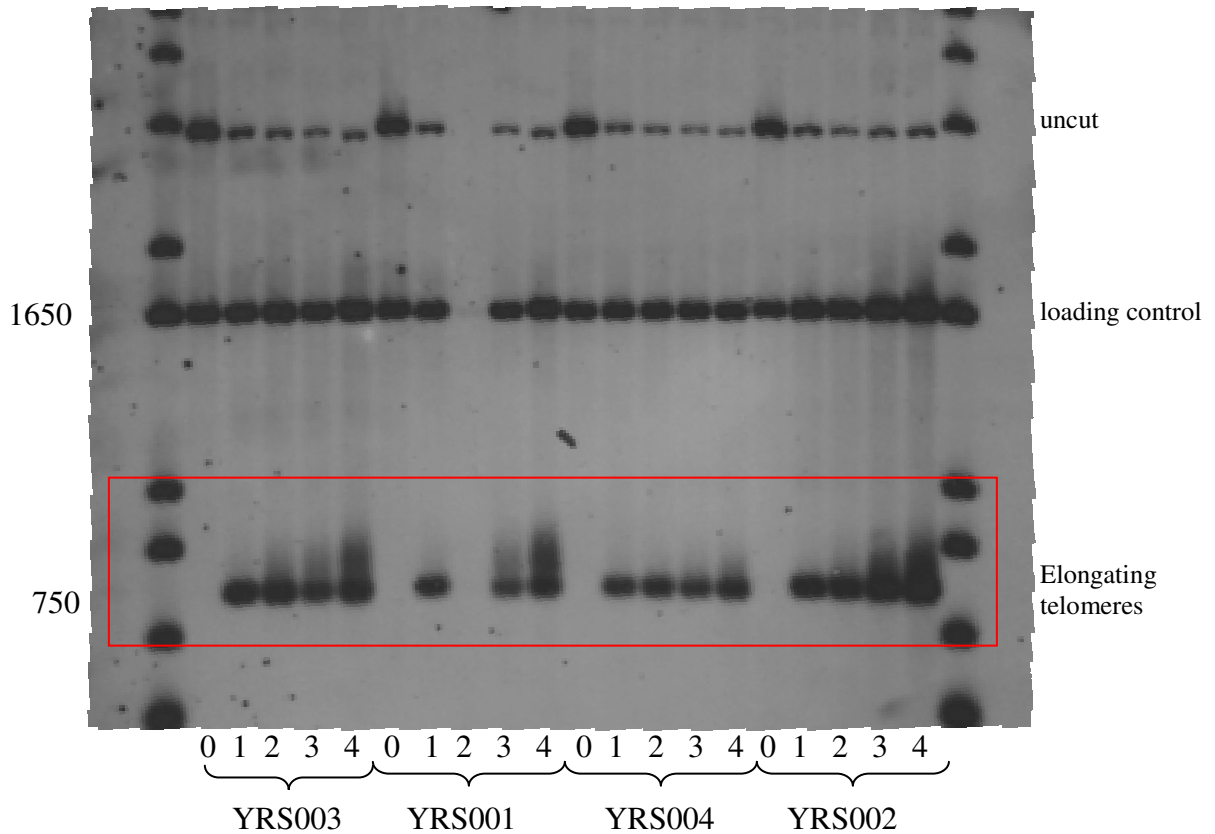
#### *Real Time PCR*

Real time PCR was performed in order to determine if the differences in the efficiency of telomere elongation observed in the *de novo* telomere addition assay were due to different levels of telomerase RNA in the strains. Figure 6 shows the results of the real time PCR performed in triplicate for two cDNA samples obtained from individual RNA preps for each of the four strains, for a total of 42 reactions. For each of these samples, the PCR was run using TUB2 primers, which would serve as an internal control, and TLC1 primers, which would give the relative level of telomerase RNA in each of the samples. As is evident in the figure, the procedure did not yield results. Several of the samples did not cross the threshold value indicated by the horizontal red line in the figure. Even for those samples that did cross the threshold value, the data is still not useable. Since the PCR was run in triplicate for each cDNA sample, it would be expected that the identical samples should cross the threshold at similar times. However,



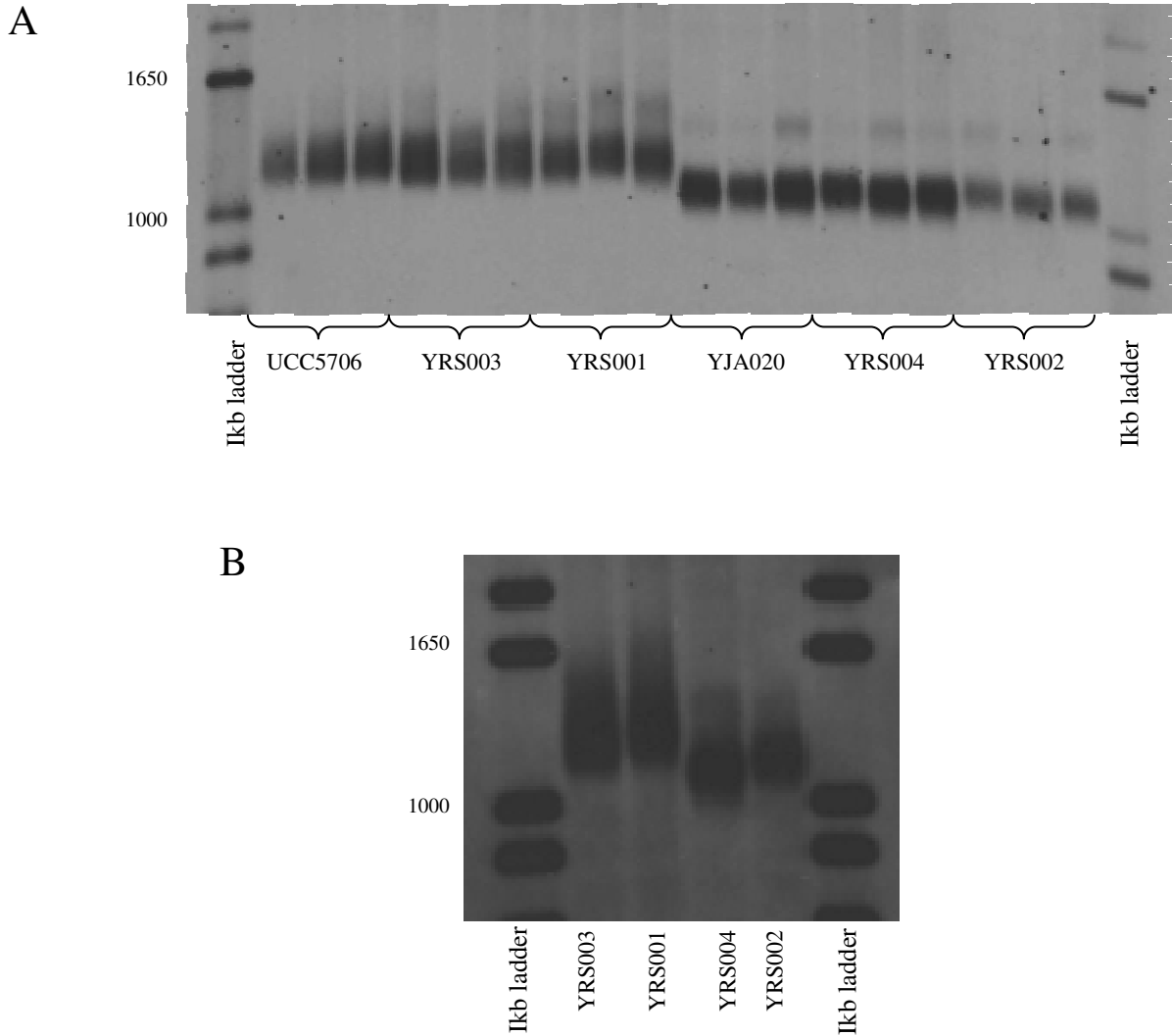
the time at which these samples crossed the threshold value was too disparate to make any conclusions.

# Figure 3



***De Novo* Telomere Addition Assay Results.** This Southern blot shows the results of the *de novo* telomere addition assay performed on the four experimental strains. After the time points were collected, genomic preps were performed and the DNA was digested with *SpeI*. The resulting samples were run on a 1.3% agarose gel, which was then transferred to a nylon membrane. The blot was incubated with the *ADE2* probe at 42°C overnight. The telomere bands are surrounded by the red box in the figure.

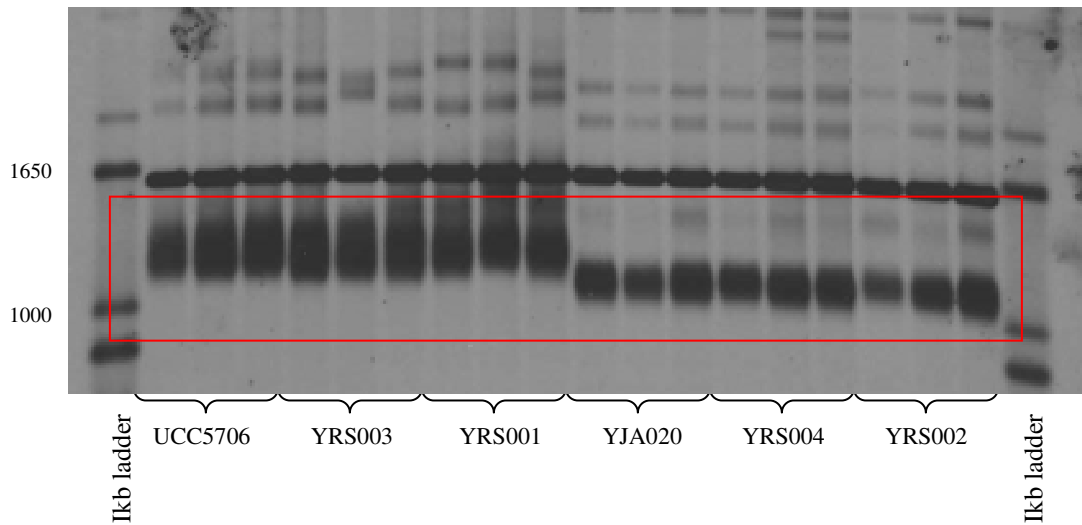
# Figure 4



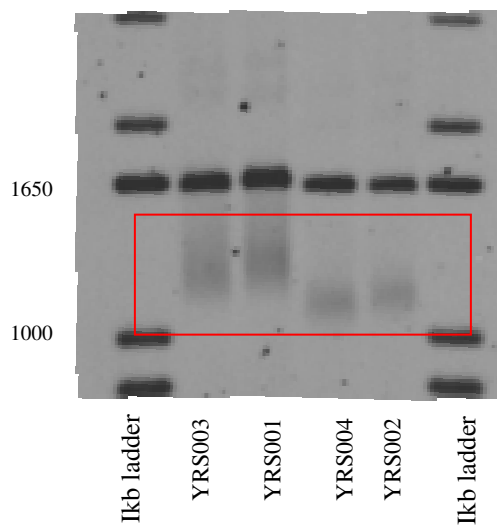
**Native Telomere Lengths I.** (A) This Southern blot depicts the native telomere lengths of the untransformed parent strains and the four experimental strains. Genomic preps were performed on cultures of each strain and the DNA was digested with XhoI. The samples were run on a 1% gel and then transferred to a nylon membrane. The blot was incubated with the Y' probe at 42°C overnight. (B) This Southern blot shows the native telomere lengths of the four experimental strains. It was created in the same way as (A).

# Figure 5

A

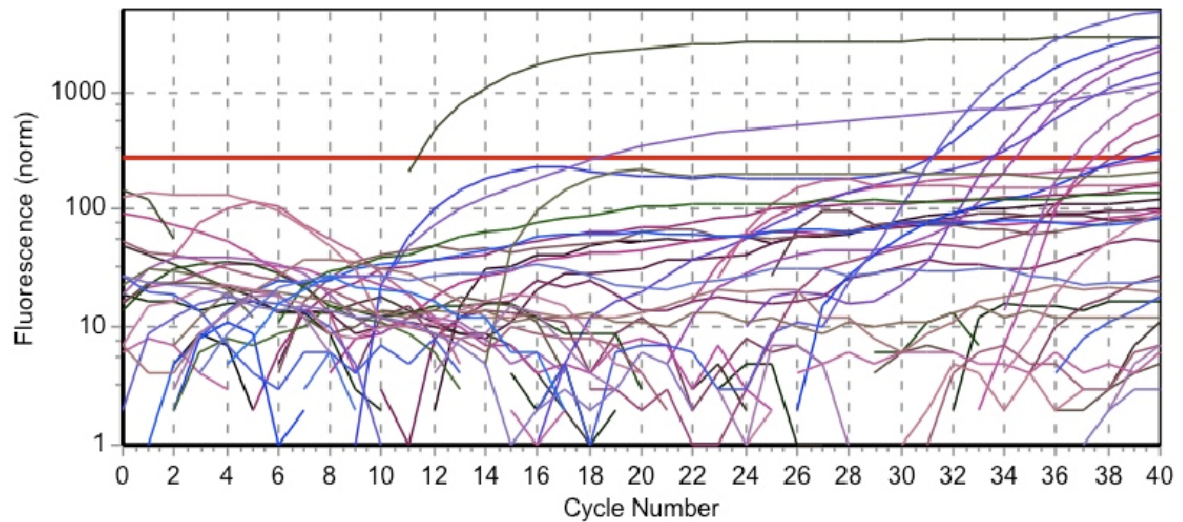


B



**Native Telomere Lengths II.** (A) This Southern blot depicts the native telomere lengths of the untransformed parent strains and the four experimental strains. The blot in Figure 4A was stripped and incubated with the CA probe at 37°C overnight. (B) This Southern blot shows the native telomere lengths of the four experimental strains. The blot in Figure 4B was stripped and incubated with the CA probe at 37°C overnight. The telomere bands are surrounded by the red box in the figure.

# Figure 6



**Real Time PCR.** This figure represents the 42 RT PCR reactions, which were performed on the cDNA samples obtained from the experimental strains. Three identical reactions for each of the two cDNA samples from each of the strains were run using either the TUB2 primers or the TLC1 primers. The red line indicates the time at which each sample crosses the threshold value.

## Discussion

### *Differential Efficiencies in Telomere Elongation and Native Telomere Length*

The results from the *de novo* telomere addition assay (Figure 3) indicate that an increased copy number of the wild type *TLC1* gene does not increase the efficiency with which the telomeres are lengthened when compared to a strain with a single chromosomal copy of the wild type gene. However, increasing the copy number of the mutant *tlc1 $\Delta$ 48* gene does increase the efficiency of telomere elongation when compared to a strain with a single chromosomal copy of the mutant gene.

A possible explanation for these observations involves the level of telomerase RNA in each of the strains. Since the telomerase enzyme is composed of both an RNA component and protein components, all of these elements must be present to form the complete enzyme complex. As explained in the introduction, there are approximately 29 *TLC1* RNA molecules present in a wild type strain of *S. cerevisiae* (Mozdy & Cech, 2006). This is fewer than the number of chromosomes present at the time in the cell cycle when telomerase is active. Therefore, telomerase RNA could be the limiting component of the enzyme complex. If this were the case, then increasing the copy number of the gene would theoretically increase the number of telomerase RNA molecules per cell thereby allowing more enzyme complexes to form. Since the wild type strain is capable of being recruited through the Ku-dependent pathway, it does not have a problem finding the ends of chromosomes in order to lengthen the telomeres. Therefore, increasing the number of complexes does not increase the efficiency of telomere elongation. However, the mutant strain cannot be recruited by the Ku protein because it lacks the 48-nucleotide stem loop structure with which Ku interacts. As a

result, the mutant strain may have difficulty finding chromosome ends and thus has significantly reduced efficiency of telomere elongation. Increasing the copy number of this mutant gene may increase the number of the mutant *tlc1Δ48* RNA molecules available to form telomerase complexes. Although this mutant form of the enzyme is not recruited as efficiently, the increased number of complexes makes it more likely that they will be able to find the ends of the chromosomes.

The possible explanation given above for the differences observed in the *de novo* telomere addition assay may also explain the differences in the native telomere lengths in the strains (Figures 4 and 5). The telomerase complex preferentially elongates the shortest telomeres in the cell (Sabourin et al., 2007). Therefore, if there is a limited number of telomerase complexes per cell, as is indicated by the finding cited above that there are only 29 TLC1 RNA molecules per cell, then these enzymes will be recruited to the shorter telomeres, and will be unable to elongate every telomere in each round of replication. If increasing the copy number of the *TLC1* gene subsequently increases the number of complexes in each cell, then it is possible that there are now enough complexes to elongate each chromosome's telomere with each round of replication. As a result, even though the increased number of complexes would not increase the efficiency of telomere addition, this would enable the cell to maintain its native telomeres at a longer length.

This trend is also observed in the mutant *tlc1Δ48* strain. The native telomere lengths of this strain are shorter than wild type; however, increasing the copy number of the mutant gene, increases the length at which the cell can maintain its telomeres. It is

possible that this is due to the increased number of telomerase complexes present in these cells.

### *Future Directions*

Although it can be speculated that increasing the copy number of the telomerase RNA gene increased the number of telomerase RNA molecules present in each strain, this possibility was not confirmed in this study. This hypothesis may have been confirmed if the real time PCR performed on the strains had produced useable data. Therefore, a future direction for this project could be to obtain conclusive results from the real time PCR quantification of the telomerase RNA in each strain. There are a few points at which the experiment may have gone wrong, resulting in the failure of the procedure. First, since RNA is relatively unstable, it is preferable to perform the reverse transcriptase reaction soon after obtaining the RNA preps. The reverse transcriptase reaction in this experiment was run a few weeks after the RNA was isolated from the cells. Even though the absorbance readings indicated that there was still RNA present in the samples, this time delay may have negatively impacted the results. The samples should have been analyzed by gel electrophoresis to confirm the integrity of the RNA. Another possible source of error was the annealing temperature used in the real time PCR reaction. The TUB2 and TLC1 primers had slightly different annealing temperatures. The reaction was run with an intermediate annealing temperature, but since it was not ideal for either primer set, this may have affected the efficiency of the PCR. Therefore, if this procedure were to be repeated, the real time PCR should be performed for each primer set individually so that the reaction could be run at each primer set's ideal annealing temperature.



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