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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED DEVELOPMENT OF A SYSTEM TO INVESTIGATE THE MUTATIONAL SPECTRUM OF SPONTANEOUS FORWARD MUTATION AT CAN1 OF SACCHAROMYCES CEREVISIAE

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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DEVELOPMENT OF A SYSTEM TO INVESTIGATE THE MUTATIONAL SPECTRUM OF SPONTANEOUS FORWARD MUTATION AT *CAN1* OF *SACCHAROMYCES CEREVISIAE*

By

Richard Michael Carr

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TABLE OF CONTENTS

LIST OF FIGURES

1 INTRODUCTION

Mutation is a process by which the genetic information contained in the nucleic acid sequence is altered. Many molecular events have been characterized that lead to mutation. Molecular models of mutational events have been generated based on prokaryotic systems. Up until recently most studies of mutagenesis were directed toward prokaryotes. The DNA chromosomal architecture in eukaryotes and prokaryotes are inherently different and thus models based on one system may not be applicable to the other. Recent advances in recombinant DNA technology and methods of DNA sequencing have made it possible to study *in vivo* mutagenesis in eukaryotes.

When studying the frequency and types of mutations found in eukaryotic systems it is important that the methods one uses do not interfere with the processes of mutation thus producing artifacts. Several widely used approaches to the study of *in vivo* mutagenesis in eukaryotes were based on untested assumptions. Shuttle vectors carrying specific target genes have been introduced into eukaryotic hosts. Cells containing mutations in a target gene were selected for and sequenced for evaluation. The basis of this method was that the autonomous shuttle vector mutated in the same manner as the host chromosomal DNA. This presumption may be false because the plasmid is essentially prokaryotic in nature (the target gene often came from a prokaryote) and it replicated independently of the host genome and thus may not be subjected to the same mutational processes. Other investigations have used integrating vectors with target genes that were isolated, retrieved

and analyzed for their molecular basis of mutation. In these studies it has not been determined whether the integration of the foreign (usually prokaryotic) DNA into the host genome alters the process of mutagenesis. The method used in this study called, double-strand gap repair, is an error-free process (Orr-Weaver et al., 1981; Orr-Weaver and Szostak 1983; Plewa et al., 1987; Plewa et al, 1989b). This procedure employs a specific gapped homologous target gene on a shuttle vector which is repaired by copying the missing sequence directly from the chromosomal gene. In this way the information is transferred from chromosomal DNA that had already mutated and thus no artifacts were created.

The target gene used in this research was *CAN1* of *S. cerevisiae* which codes for arginine permease (Hoffmann, 1985). L-canavanine is a toxic arginine analog that is imported into the cell via the *CAN1* encoded permease. Because *S. cerevisiae* can synthesize arginine a mutation in the *CAN1* gene will block the uptake of canavanine and thus enable the organism to survive on canavanine containing media (Ahmad and Bussey, 1986; Broach et al., 1979). This gene is particularly suited for the study of forward mutation because it provides a large 1.8 kb target and allows for the rapid positive isolation of mutants. To gain a representative analysis of mutational events within the *CAN1* locus, I isolated one thousand independently arising spontaneous mutants.

Before the mutations could be recovered by gapped plasmids an initial progenitor plasmid was created. My role in this process was generating restriction maps of the *CAN1*

containing plasmid TLC1, the isolation of the smallest fragment containing an intact *CAN1* gene, and the eventual transfer of this gene into the pMH158 vector.

Another project I was a participant in was the testing of a new glass bead transformation procedure to see if it altered the mutational frequency or if it affected the fidelity of the gene conversion process (Plewa et al., 1989a). These controls were very essential because if a procedure changed the frequency of mutation in the system being studied then this method could not be used to study mutational spectra. Likewise if the new technique altered the fidelity of the gene conversion event it could not be used in this project because double strand gap repair is dependent on this process.

2 MATERIALS AND METHODS

2.1 Isolation of *CAN1*⁻ Mutants

Two ml of sterile YEPD media was placed in a sterile test tube and inoculated with a single colony isolate of *S. cerevisiae* strain XY729 and incubated at 30°C in a shaking water bath for 48 hr. The culture was vortexed and 1 ml was placed in a sterile 1.5 ml capacity microfuge tube which was then centrifuged at high speed for 2 min. Following centrifugation the supernatant fluid was decanted, the cells were resuspended in 1 ml of 0.9% sterile saline solution, centrifuged at high speed. The supernatant fluid was removed and the procedure was repeated. The cells were resuspended in 1 ml of sterile saline and three aliquots of 100 µl were plated on three separate SC -arg +can (SAC) plates and incubated at 30°C for 3 days. Five separate colonies were picked from each plate and each colony was streaked onto labelled SC -leu, SAC, and YEPD plates respectively. Plates were then incubated at 30°C for 3 days. If colonies grew on both the SAC and the YEPD plates but not the SC -leu plate then these cells were labeled as *leu2-3, can1*⁻. Colonies that grew on all three plates were not used because I needed *LEU2* as a selective marker for transformation. *Can1*⁻ cells were transferred to YEPD slants, incubated at 30°C for 3 days, numbered from 1 to 1000, sealed with parafilm and stored at 4°C.

2.2 Elution of DNA from an Agarose Gel Using an Extraphor

An agarose gel was run, it was stained for five minutes with 20 µl of ethidium bromide, the desired pieces were cut out. The Hamilton syringe was rinsed several times with

ethanol. The elbows of the extraphor to be used were rinsed several times with ethanol then with sterile water. The extraphor was filled with 1× TBE as described in the extraphor operating manual, the stopcock was closed before proceeding. The elbows were rinsed with 1× TBE using the syringe and making sure that no bubbles were left in the elbow. The gel pieces were placed in the wells. The Hamilton syringe was rinsed several times with ethanol and then with sterile water. 50 μl of 3 M sodium acetate + bromphenol blue were drawn up in Hamilton syringe. The tip of the syringe was slowly placed at the bottom of the elbow, the solution was carefully layered in bottom of elbow, the syringe was removed so that the solution was not disrupted. The lid was placed on the extraphor and run at 5 v/cm for 30-45 min (time depended on thickness of gel and DNA content). The 1× TBE buffer was withdrawn with a pasteur pipette attached to a large plastic syringe. The lid was now removed. The Hamilton syringe was rinsed several times with ethanol and then sterile water. The syringe was slowly lowered into the elbow all the way to the bottom. 50 μl of solution was carefully drawn up (often needed to remove a larger volume), the syringe was slowly removed from the elbow. The syringe was slowly emptied into a sterile eppendorf tube to prevent DNA shearing. The syringe was rinsed with ethanol and then sterile water several times before using for another sample.

2.3 Concentrating DNA Samples

The samples were brought up in twice the volume of sample in ice cold 100% ethanol. The samples were placed in a -22°C freezer for 15 min. The samples were spun for 30 min at high speed in a Sorvall RC-5B centrifuge. The ethanol was poured off, the tube

was tapped and placed upside down on tissue. 100 µl of cold 95% ethanol was added to each sample, poured off and kept upside down. The samples were desiccated 3 min. 30 µl of TE (pH 7.4) was added to the first sample, it was vortexed, centrifuged briefly and transferred to the next tube containing similar DNA and repeated. Extracted DNA was stored in the refrigerator.

2.4 Cleaning Glass Beads

The beads were soaked overnight in concentrated HCl. The beads were placed in a filter flask and the fluid was sucked off. The beads were rinsed with 0.5 M HCl. The beads were rinsed with distilled water until a pH ~4 was reached. The beads were rinsed with 0.5 M NaOH and rinsed with distilled water until a pH of approximately eight was reached. The beads were dried in an oven until they rolled easily. The beads were dispensed into containers, autoclaved and placed in a drying oven until easy to work with.

2.5 Glass Bead Mediated Transformation

Cells were grown in YEPD media for 3 days. Cells were centrifuged, washed twice with selective media containing 1 M sorbitol and resuspended in the selective medium + 1 M sorbitol at one tenth the original volume. Aliquots of 200 µl were placed into 15 ml Falcon tubes and 0 - 10 µg DNA was added to each tube. Carrier DNA when used was 50 µg of sonicated calf thymus DNA suspended in selective media + 1 M sorbitol. Sterile, clean 0.3 g portions of glass beads (0.45 - 0.52 mm diameter) were added to each tube. The tubes were vortexed at the highest speed for 30 sec. As much liquid was removed as

possible and plated on selective medium + 1 M sorbitol. The plates were incubated for 7 days at 30°C.

2.6 Optimum DNA Concentration for Glass Bead Mediated Transformation

The general glass bead transformation procedure was followed. *S. cerevisiae* strain XY729 was used and SC -leu was the selective medium. Six samples were analyzed and the concentrations of pMH158 DNA included 0, 5, and 10 μg. Carrier DNA (50 μg sonicated calf thymus DNA) was added to one tube for each DNA concentration.

2.7 Mutation Frequency for Glass Bead Mediated Transformation

The general glass bead transformation procedure was used with this experiment. *S. cerevisiae* strain XY729 was used and SC -arg was the selective medium. The concentration of *Xho*I-restricted pMP4 DNA included 0, 1, 2.5. 5 and 10 μg. Each DNA concentration was analyzed three times in competent yeast cells and plated on SAC plates. Two controls were conducted; untreated resuspended cells were diluted by 10^5 in 0.9% sterile saline solution and spread on YEPD plates, also 200 μl of resuspended cells were vortexed with the glass beads, diluted by 5×10^5 in 0.9% sterile saline + 1 M sorbitol and plated on YEPD + 1 M sorbitol.

2.8 Mitotic Gene Conversion Frequency for Glass Bead Mediated Transformation

Yeast strain D_7 which is heteroallelic at *TRP5* was used to test if the glass bead transformation procedure altered the frequency of gene conversion. SC -trp was used as

the selective medium. The cells were grown to a titer of 5×10^7 cells/ml. The concentration of *Xho*I-restricted pMP4 DNA included 0, 1, 2.5. 5 and 10 μg. Each DNA concentration was analyzed five times in competent yeast cells and plated on SC -trp + 1 M sorbitol. The control was untreated resuspended cells diluted 10^5 in sterile 0.9% saline solution, three aliquots of 100 μl were plated on YEPD and three aliquots of 300 μl were plated on YEPD.

2.9 Restriction Mapping of DNA

DNA for restriction maps was used at concentrations from 250 - 450 ng. The type of restriction enzyme(s) were matched with the appropriate salt buffer. When digesting DNA a total volume of 20 μl was used. The volume of DNA was determined and the buffer for each enzyme was added at one tenth the total volume. Two μl of each restriction enzyme buffer was added and 1 - 2 μl of each restriction enzyme was added. The volume of the DNA, enzymes, buffers and TE buffer (pH 7.4) equaled 20 μl. The buffers were added first followed by the DNA and then the restriction enzymes. The digests were incubated at 37°C for a minimum of two hours per enzyme. In the case where two enzymes were used and one of them was *Pst*I this enzyme was always allowed to restrict first because it did not react well when another enzyme was used first. After incubation the solution was heated to 65°C for 15 min to inactivate the enzymes. When a preparative digest was used 5 μg of DNA was used and the total volume was 100 μl, of which 8 μl were enzymes, 10 μl was restriction buffer and the rest was DNA and TE buffer pH 7.4.

2.10 Agarose Gel Electrophoresis

Gels of either 0.7% or 1.2% agarose were used. Lambda *Hind*III digests and 123 bp ladder were used as standards. Wells (in an eight lane gel) were filled with 22 μl of solution to be analyzed. Gels were run at either 3.75 v/cm for 2 h or at 5 v/cm for 1-1.5 h. Gels were stained with 20 - 50 μl of ethidium bromide for 15 - 30 min. Stained gels were visualized at 308 nm using a uv-opaque plexiglass shield (Maniatis et al., 1982).

3 RESULTS

3.1 Isolation of *can1*⁻ Mutants

I isolated 1000 independently arising *can1*⁻ spontaneous mutants. The procedure selected for independently-arising mutants and daughter mutants would not be selected. The cell cultures were grown to a titer of approximately 2×10^8 and concentrated 10-fold. From each tube three aliquots of 100 μl were plated onto SAC medium. From each plate five colonies were isolated. Since the mutation frequency of *CAN1* is $\sim 1 \times 10^{-6}$, approximately 1,000 mutants per tube would be expected. Only 15 *can1*⁻ mutants per tube were collected thus there was a very low chance of selecting daughter mutant cells. Another important criterion was that the cells did not have a *leu* suppressor mutation or were not *leu*⁺ revertants. This characteristic was important because strain XY729 has a *Leu2-3* mutation and thus cannot live on plates without leucine present but when it is transformed by the gapped plasmid shuttle vector it can grow on plates without leu because the plasmid carries *LEU2* and this allows for the selection of transformed cells. The actual numbers of *leu* suppressor mutations or revertants was very low. Occasionally a single colony would grow on a SC-leu plate this probably did indicate the presence of a single true revertant.

3.2 Glass Bead-Mediated Transformation

The initial glass bead transformation experiment was conducted to test for the optimum conditions for the highest rate of transformation. The experiment was designed with three

paired samples, 0, 5 and 10 μg of pMP158, respectively. To one sample at each concentration 50 μg of carrier DNA was added. PMH158 is a plasmid with a *LEU2* and transformants were selected on SC -leu. The negative controls showed only a few colonies able to grow on SC-leu plates while the samples transformed with 5 and 10 μg of pMH158 DNA gave scores of *LEU* transformants. In addition the same frequency of transformant colonies per plate were observed with or without the carrier DNA.

3.3 The Effect of Transformation on Forward Mutation at *CAN1*

The next experiment for the glass bead transformation method was designed to determine if an alteration in the mutational frequency could occur as a result of transformation by linear double-stranded DNA. The spontaneous frequency of forward *can1*^{r} mutants in untreated cells were compared to the frequency of mutations in treated cells in which varying amounts of linear DNA were added. The results indicated that no significant difference in the mutation frequency at *CAN1* among the DNA treated cells occurred (Figure 1). Two additional controls were also conducted with this experiment; one measured the original titer of the cell suspension and the other determined the percent killing by the procedure itself. Vortexing with glass beads alone was found to kill approximately 75% of the cells when plated on nonselective media (YEPD) plus 1 M sorbitol.

3.4 The Effect of Transformation on Mitotic Gene Conversion

An experiment to determine if the gene conversion process was affected by the introduction of linear DNA via the glass bead method was also run. It was necessary to determine this because double strand gap repair depends on gene conversion for the accurate transfer of the sequence information from the chromosome to the gapped plasmid. The test used a diploid strain of yeast – D_7 – which has two heteroalleles in *TRP5* (*trp5-12; trp5-27*). Mitotic gene conversion at a heteroallele may result in a *trp*$^+$ cell. By comparing the numbers of *trp*$^+$ convertants in competent cell suspensions exposed to varying amounts of DNA the spontaneous *trp*$^+$ conversion frequency could be compared to the competent cells exposed to varying amounts of linear double-stranded DNA. The results indicated that no significant alteration in the frequency of gene conversion took place (Figure 2).

3.5 Restriction Map of *CAN1* for Incorporation into pMP5

The initial restriction analysis of TLC1 (Broach et al., 1979) was carried out with the enzymes *Bam*HI, *Cla*I and *Hind*III. The results of the agarose gel showed that there was one unique *Bam*HI site in TLC1. From a computer search of the *CAN1* gene it was determined that this unique site occurred 5' to the *CAN1* promoter. The *Cla*I enzyme made three cuts which resolved four bands and *Hind*III made two cuts resolving three DNA restriction fragments bands. When both *Hind*III and *Bam*HI were used to digest the same DNA sample a pattern as in the *Hind*III enzyme digest was resolved. The *Bam*HI and *Cla*I restriction pattern demonstrated that the largest fragment of the *Cla*I digest was reduced

and that a new small band appeared for a total of five bands. When the DNA was cut with both *Bam*HI and *Cla*I four bands appeared (Figure 3).

To recover a restriction fragment between 3 and 8 kb in size that contained *CAN1* from TLC1, I had to determine restriction sites that flanked the gene. The 17 kb TLC1 was not a sequenced plasmid. The *CAN1* gene sequence was analyzed using DNA Star computer software and restriction enzymes that did not cut within the gene were used in the mapping of restriction sites that flanked *CAN1*. TLC1 was restricted with *Bam*HI and then cut the DNA with each of the following enzymes; *Bgl*II, *Pvu*II, *Xho*I, *Pst*I, *Nru*I, and *Sal*I. From the *CAN1* sequence information I knew that *Sal*I restricted once within *CAN1* and was used as a diagnostic. A *Bgl*II digest gave a pattern of three fragments, *Pvu*II, *Xho*I, *Sal*I and *Nru*I each gave two band patterns, and *Pst*I left four pieces (Figure 4).

To determine which fragment of each digest contained *CAN1*, *Bam*HI-restricted TLC1 was separately digested with each of the above enzymes and then subjected to another cleavage with *Sal*I. In each case the largest molecular weight fragment contained the *CAN1* gene. This was determined by comparing the agarose gel illustrated in Figure 4 with that illustrated in Figure 5 and looking for the DNA fragment that was reduced when treated with *Sal*I. The smallest fragment that contained the *CAN1* gene was the 6.5 kb fragment of the *Pst*I restriction digest. The 6.5 kb *Pst*I, *Bam*HI fragment was isolated by making *Bam*HI and *Pst*I digests of large amounts of TLC1 and removing the DNA from a preparative agarose gel (Figure 6).

Further restriction digests of the isolated fragment were attempted using the enzymes *Cla*I, *Eco*RI, *Eco*RV, *Hind*III, *Pvu*II and *Sma*I. *Cla*I, *Eco*RI and *Eco*RV cut the fragment while the other enzymes had no effect (Figure 7). Another agarose gel with an *Eco*RV digest of the 6.5 kb piece and an *Eco*RV, *Sal*I digest was run of the fragment to determine in which fragment the *CAN1* was actually located. The results from the gel show that the *CAN1* gene was located in the largest piece from the digest (Figure 8).

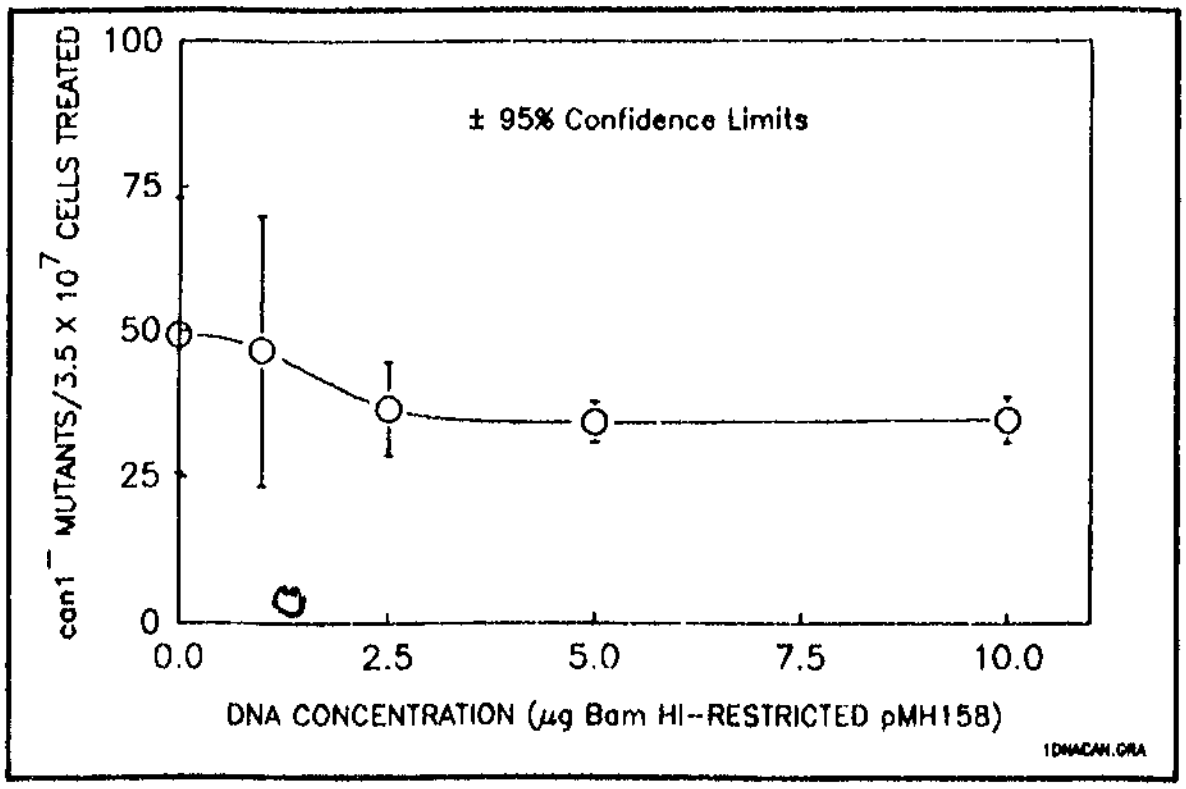

Figure 1 Effect of double-stranded linear DNA on the frequency of forward mutation at *CAN1* on chromorome 5.

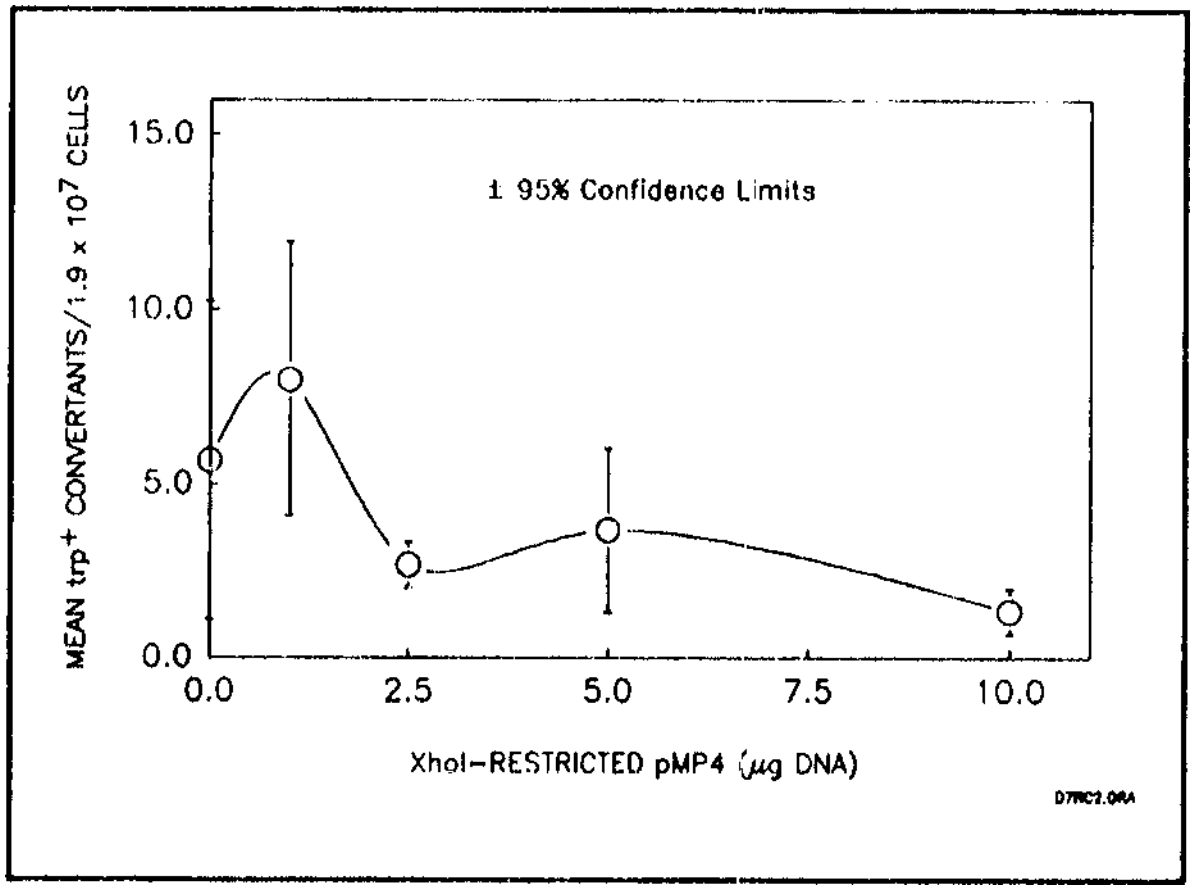

Figure 2 Effect of double-stranded linear DNA on the frequency of gene conversion at *trp5-12/trp5-27*.

Figure 3 Restriction analysis of plasmid TLC1. Lane 1 lambda *Hind*III DNA size standard, lane 2 *Bam*HI digest, lane 3 *Cla*I digest, lane 4 *Hind*III digest, lane 5 *Bam*HI and *Hind*III digest, lane 6 *Bam*HI and *Cla*I, lane 7 *Cla*I and *Hind*III digest, and lane 8 DNA standard 123 bp.

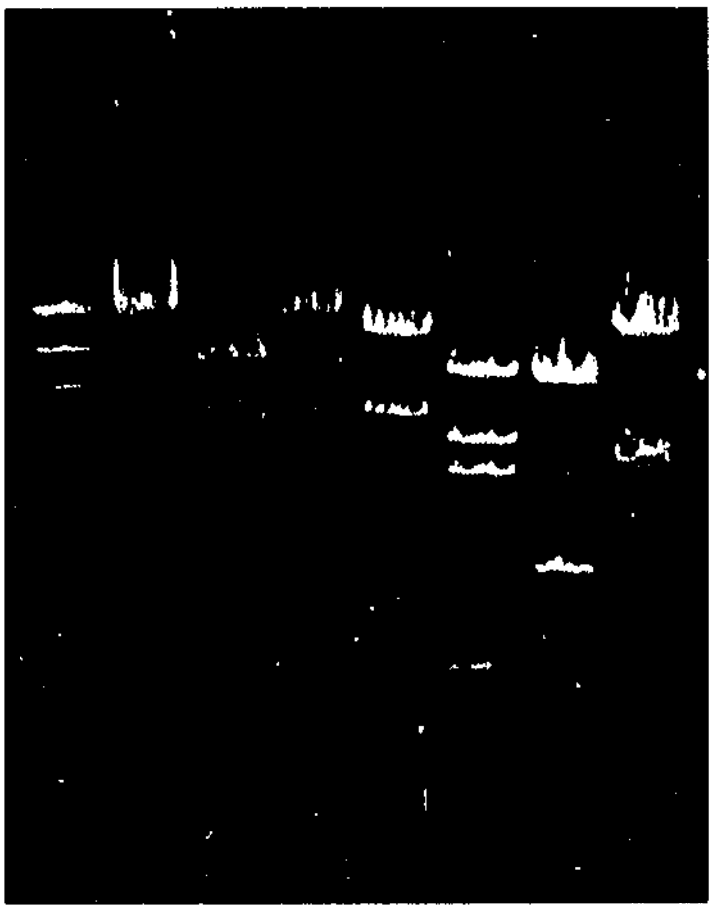

Figure 4 Restriction digest of plasmid TLC1 with unique restriction enzymes found in the pMH158 cloning window. Lane 1 lambda *Hind*III DNA size standard, lane 2 *Bam*HI digest, lane 3 *Bam*HI and *Bgl*II, lane 4 *Bam*HI and *Pvu*II, lane 5 *Bam*HI and *Xho*I, lane 6 *Bam*HI and *Pst*I, lane 7 *Bam*HI and *Sal*I, and lane 8 *Bam*HI and *Nru*I.

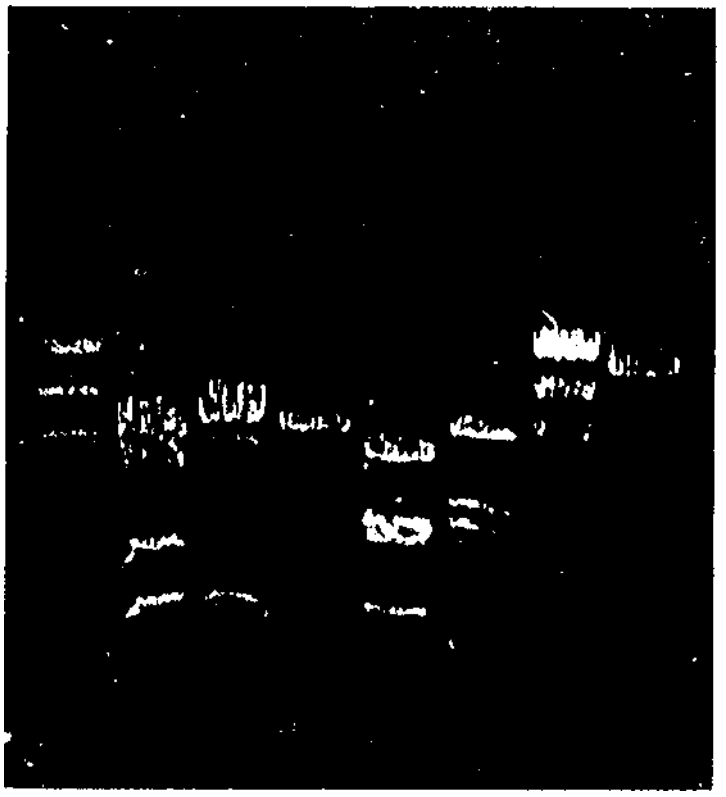

Figure 5 Use of *Sal*I diagnostic to determine the *CAN1*-containing DNA restriction fragment. Lane 1 lambda *Hind*III DNA size standard, lane 2 *Bam*HI, *Bgl*II and *Sal*I digest, lane 3 *Bam*HI, *Pvu*II and *Sal*I, lane 4 *Bam*HI, *Xho*I and *Sal*I, lane 5 *Bam*HI, *Pst*I and *Sal*I, lane 6 *Bam*HI, *Nru*I and *Sal*I, lane 7 lambda *Hind*III DNA size standard, and lane 8 *Bam*HI digest.

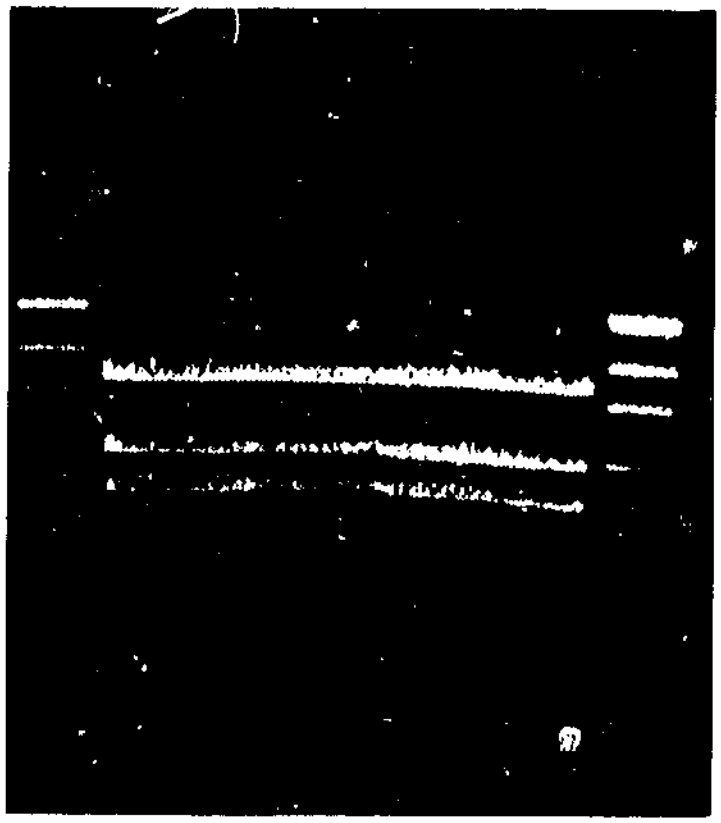

Figure 6 Preparative agarose gel for the isolation of *Bam*HI - *Pst*I restricted plasmid TLC1 DNA fragment. The top preparative DNA band represents a 6.5 kb fragment that contains *CAN1*.

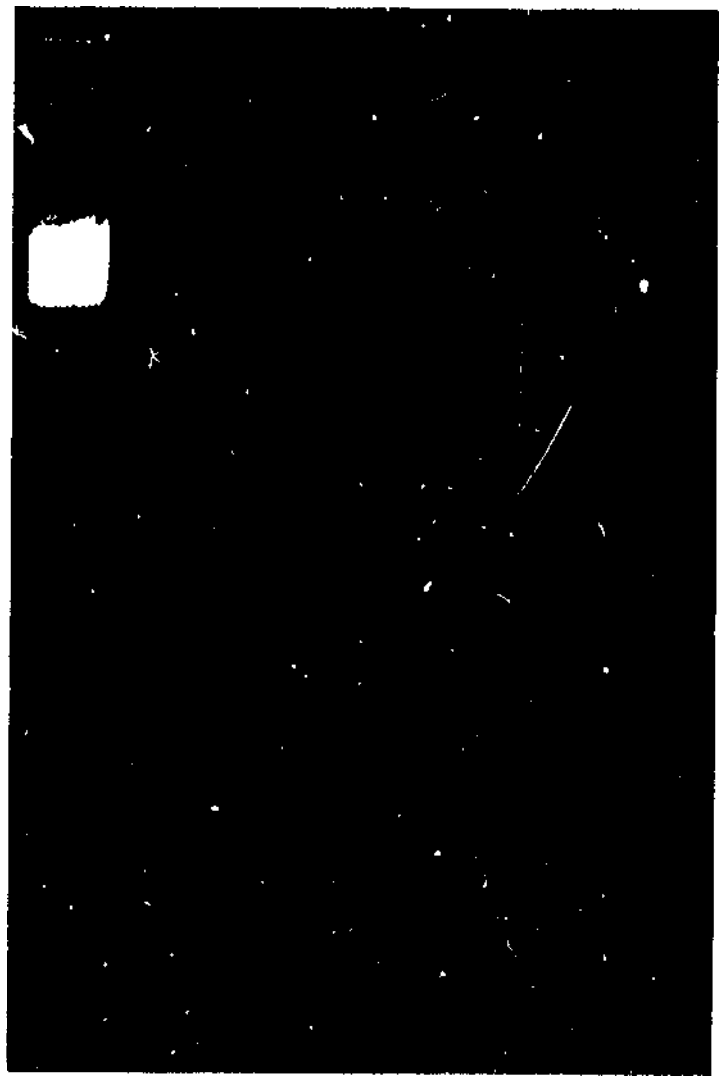

Figure 7 Restriction digest of 6.5 kb DNA fragment. Lane 1 DNA 123 bp standard, lane 2 *Cla*I digest, lane 3 *Eco*RI digest, lane 4 *Eco*RV digest, lane 5 *Hind*III digest, lane 6 *Pst*I digest, lane 7 *Sma*I and lane 8 undigested 6.5 kb DNA fragment.

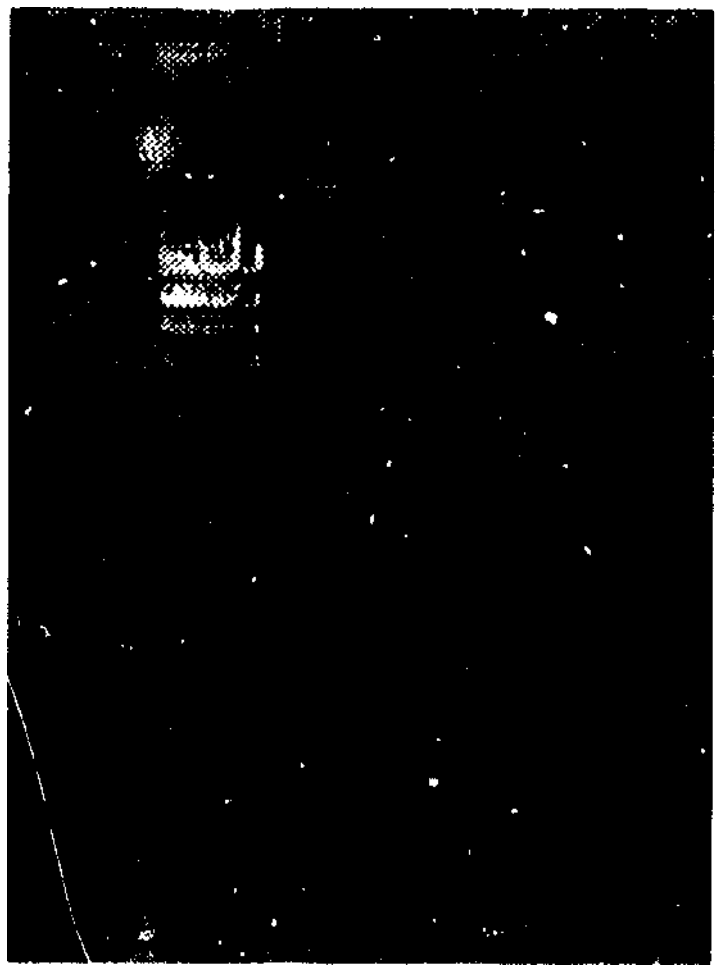

Figure 8 Determination of *CAN1* containing DNA fragment of *Eco*RV restriction digest. Lane 3 lambda *Hind*III DNA size standard, lane 4 *Eco*RV digest, lane 5 *Eco*RV and *Sal*I digest, and lane 6 DNA 123 bp standard.

4 DISCUSSION

In the study of a mutation spectrum it is essential that the mutants collected were random and independently arising. This is necessary so that one could get a representative picture of the process of mutation in the gene being studied. A daughter mutant is the offspring from a previous mutant and does not represent an independent mutation. Daughter mutants must be selected against because they distort the results of the study by artificially over-representing a single mutational event.

In this experiment leucine revertants and cells carrying leucine suppressor mutations were also selected against. It was important that these types of cells were not used because the original strain is a leucine auxotroph. This characteristic was used to select cells that were transformed with the plasmid that contains *CAN*1 and *LEU2*. The plasmid used in this study has an intact functional leucine gene and thus when a cell is transformed with the plasmid it becomes able to survive on media that does not contain leucine. I isolated 1,000 *can*1 mutants that could not grow on the SC -leu plates using the procedure described. This process insured that my selective marker was intact.

To make gapped plasmids to study the mutational spectrum at the *CAN*1 gene a progenitor plasmid (pMP5) first was constructed. The first steps in producing pMP5 involved making restriction maps of the TLC1 plasmid and the eventual identification and isolation of the *CAN*1 containing fragment to be used as the insert with pMH158 as the

vector. It was recognized from the *CAN*1 sequence that there was a unique *Bam*HI site at the beginning of the 5' flanking sequence of the gene (Figure 9). The initial restriction maps were generated with *Cla*I and *Hind*III. The use of other non-unique enzymes such as *Eco*RI and *Eco*RV were aborted because these enzymes cut in many places in the pMH158 plasmid and thus would make the insertion of the *CAN1* gene into the plasmid much more difficult. Enzymes that did not cut within the *CAN1* gene or enzymes that cut at unique sites in the cloning window of pMH158 were found by looking at the *CAN1* and the pMH158 sequences with a computer scan.

The enzymes tested produced no fragments smaller than the 6.5 kb *Pst*I, *Bam*HI-cut TLC1 fragment. Ideally this piece was still to large and added an additional 4.5 kb of unneeded and unsequenced DNA to the plasmid. This 6.5 kb fragment was inserted into pMH158 that was restricted with *Pst*I and *Bam*HI. The search continued for other enzymes that would trim the fragment down to a reasonable size but none were found thus the 6.5 kb piece was used to create the progenitor plasmid, pMP5 (Figure 10). After the plasmid was made it was tested for the ability to transform yeast strain XY729 and *E. coli* strain BNN45. This plasmid will be used to make gapped plasmids by which the mutated sequences in the *CAN*1 gene will be mapped.

In the study of molecular spectra via gap plasmid repair efficient and rapid methods of transformation are needed. The new glass bead transformation procedure is such a method. Although this method of transformation is less efficient then the traditional method using

LiCl and polyethylene glycol it is much easier and quicker to use (Constanzo and Fox, 1988). The old procedure required a specific cell titer and took nearly eight hours to complete with many steps where contamination could take place. The glass bead method can be run any time within 36 hours of logarithmic or early stationary phase, and takes from 45 - 60 min to run. One minor problem with this procedure is that the cells take up to 7 days to grow on the media with 1 M sorbitol added. This is still advantageous because many transformations could be run while waiting for the colonies to come up.

When introducing a new technique it was necessary to test the process for the creation of artifacts. The first test implemented was used to find the ideal conditions for the procedure itself. The next two tests acted as controls to show that the transformation procedure did not increase the frequency of mutation and that the fidelity of the gene conversion event was not affected. These controls were important because if either the frequency of mutation or the fidelity of the gene conversion event was altered then this process could not be used to study mutational events at the molecular level.

The eventual goal of this research project will be a comparison of the molecular events that are associated with mutation in Eukaryotic systems with models based on Prokaryotes. My participation in this project involved the collection of the mutants to be sequenced, the creation of a progenitor plasmid from which gapped vectors will be made to isolate and study the altered genetic information of the mutants, and the testing of a new method that will greatly increase the efficiency and speed with which this process can be studied.

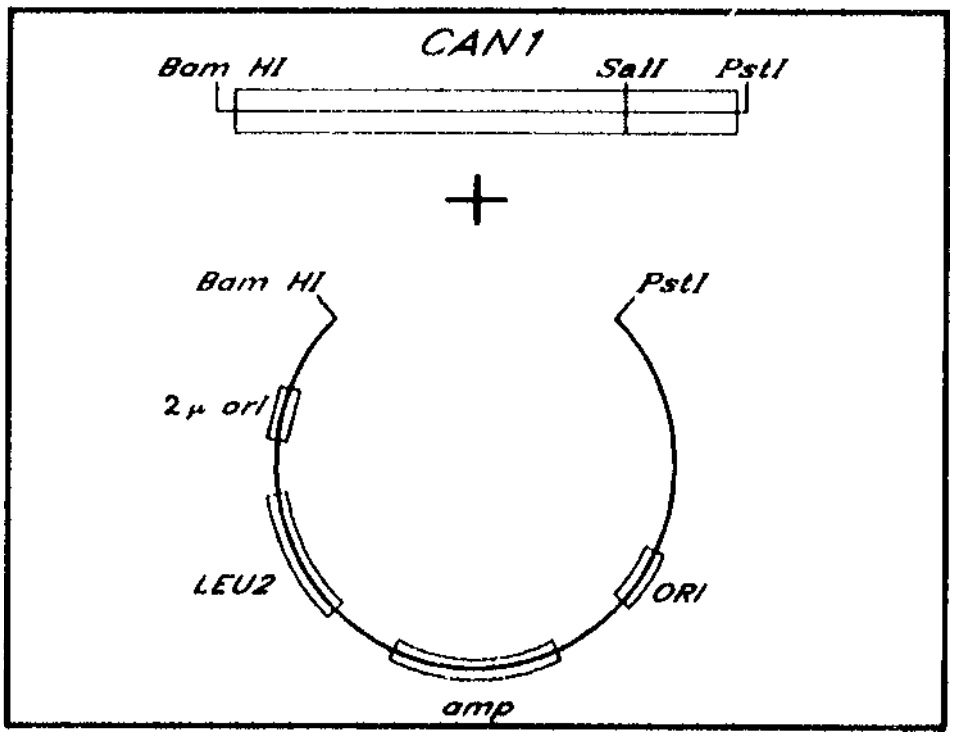

Figure 9 *CAN1* insert and pMH158 vector for the generation of the progenitor shuttle vector.

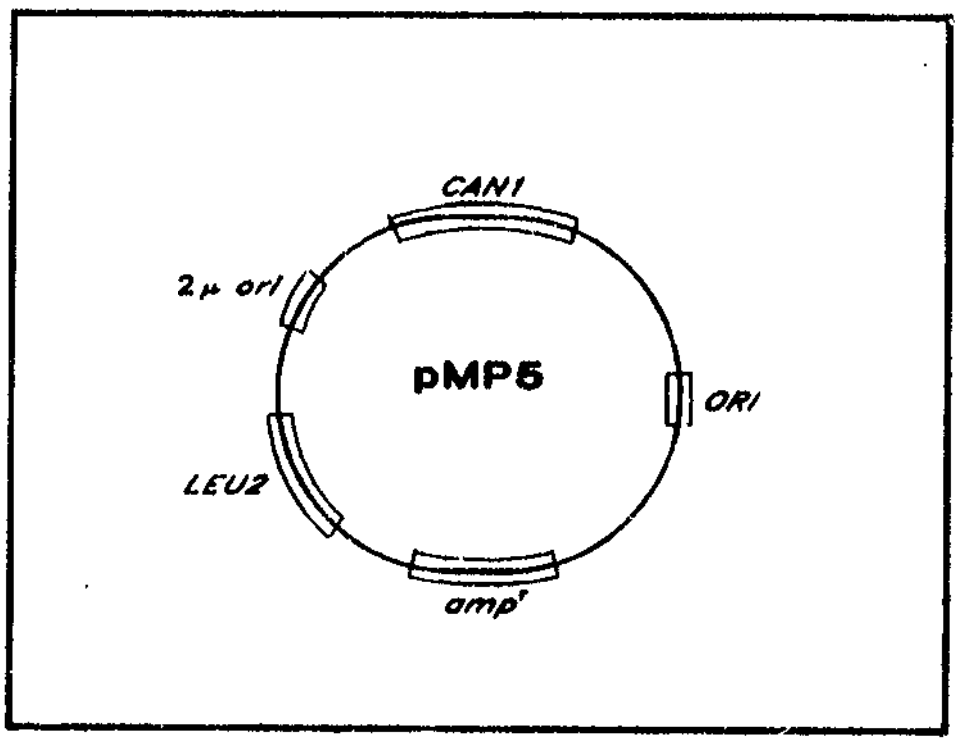

Figure 10 The shuttle vector pMP5.

5 LITERATURE CITED

Ahmad, M. and H. Bussey. 1988. Yeast arginine permease: nucleotide sequence of the *CAN1* gene. Current Genetics 10:587-592.

Broach, J., J. Strathern and J. Hicks. 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of *CAN1* gene. Gene 8:121-131.

Costanzo, M.C. and T.D. Fox. 1988. Transformation of yeast by agitation with glass beads. Genetics 120:667-670.

Hoffmann, W. 1985. Molecular characterization of the *CAN1* locus in *Saccharomyces cerevisiae*. J. Biol. Chem. 260:11831-11837.

Maniatis, T., E. Fritch and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Orr-Weaver, T., J. Szostak and R. Rothstein. 1981. Yeast transformation: A model system for recombination. Proc. Natl. Acad. Sci. 78:6354-6358.

Orr-Weaver, T. and J. Szostak. 1983. Yeast recombination: The association between double-strand gap repair and crossing over. Proc. Natl. Acad. Sci. 80:4417-4421.

Plewa, M.J., D. Kalinowski and F. Larimer. 1987. Development of pMP4, a shuttle vector for use in defining frameshift mutations at the *his4-38* region in yeast. Environ. Mutagenesis Supp. 9:103.

Plewa, M.J., S. Taylor, A. Kumar and R. Carr. 1989a. Double-strand gap plasmids do not alter the rate of mutation or gene conversion in *Saccharomyces cerevisiae*. Environ. Molecular Mutagenesis IN PRESS.

Plewa, M.J., D.P. Kalinowski and F.W. Larimer. 1989b. Development of a double-strand gap repair system to analyze the mutational spectrum in yeast chromosomes. Submitted

APPENDIX

Media for *CAN1*

YEPD Complete Media - Plates and Broth

	1 liter
Bacto-peptone	20 g
Bacto-yeast extract	10 g
Dextrose	20 g
Agar (for plates only)	20 g
dH_2O	1000 ml

Autoclave 20 minutes.

SC -arg +can Selective Media

	1 liter
Dextrose	20 g
Yeast nitrogen base	6 g
Agar	20 g
dH_2O	960 ml
Add under sterile conditions:	
Threonine stock solution	7.5 ml
Tryptophan stock solution	10 ml
SC -arg stock solution	40 ml

Autoclave 20 minutes. Cool and add 10 ml of Canavanine stock solution.

SC -arg Stock Solution (25x)

	360 ml
Adenine sulfate	240 mg
Histidine	240 mg
Isoleucine	540 mg
Leucine	360 mg
Lysine	360 mg
Methionine Stock	12 ml
Uracil	240 mg
dH_2O	348 ml

Filter sterilize and refrigerate.

Methionine Stock Solution (25x)

	200 ml
Methionine	4 g
dH_2O	200 ml

Filter sterilize into a sterile 100 ml bottle.

Threonine Stock Solution (25x)

	200 ml
L-threonine	4 g
dH_2O	200 ml

Filter sterilize into a sterile 100 ml bottle.

Tryptophan Stock Solution (25x)

	200 ml
L-tryptophan	400 mg
dH2O	200 ml

Filter sterilize into a sterile 100 ml bottle.

Canavanine Stock Solution (0.4%)

	25 ml
L-canavanine sulfate	1 g
dH_2O	250 ml

Filter sterilize and refrigerate.