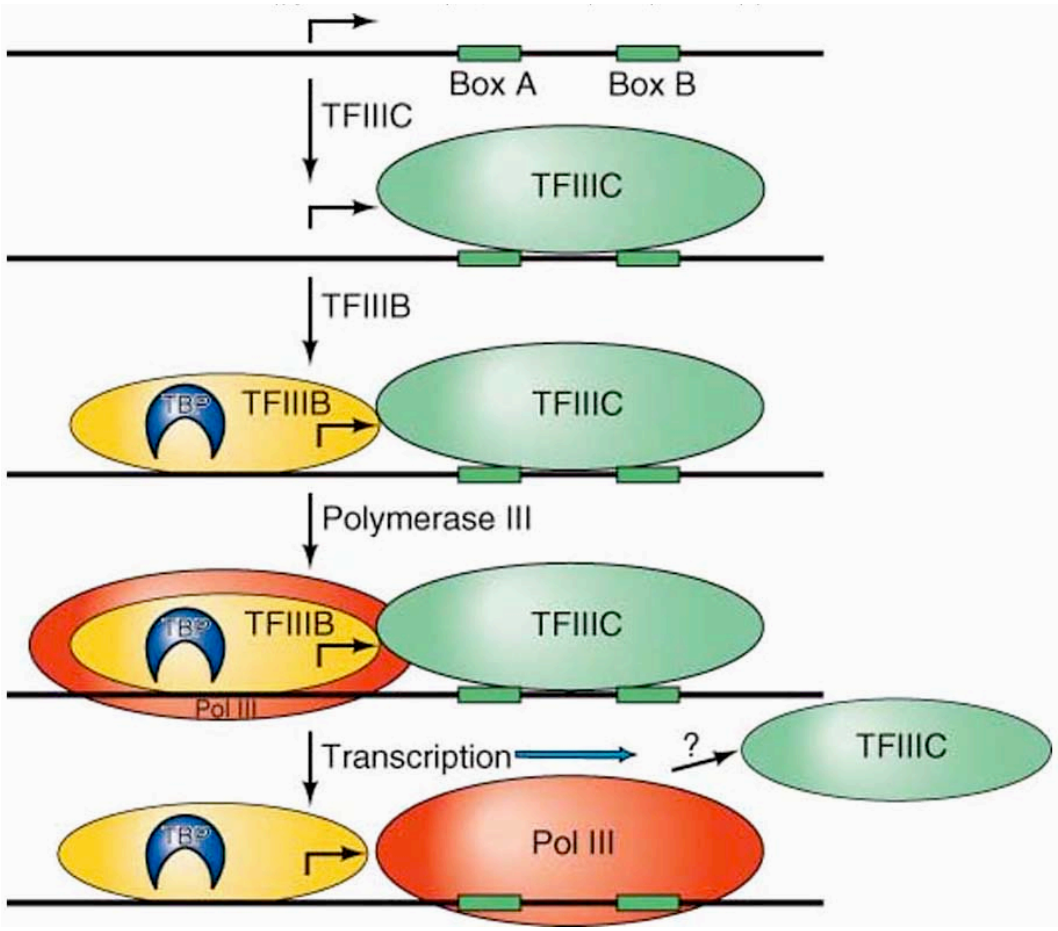


Figure 2. Assembly of RNA Polymerase III Transcription Machinery at tRNA genes. $TF_{III}C$ recognizes the B-Box first, then the A Box. $TF_{III}B$, including the TATA binding protein are then recruited followed by Polymerase III. It may not be necessary for $TF_{III}C$ to remain bound to the DNA to facilitate transcription.

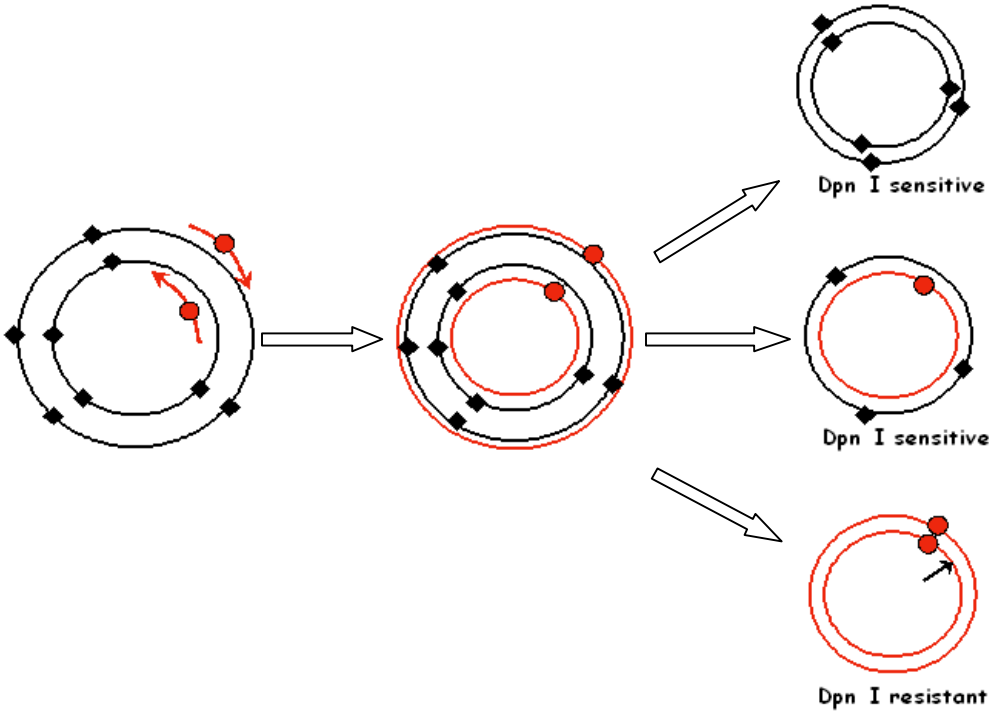


Figure 3. Site Directed Mutagenesis. Specific additions, changes, or deletions can be made to a given plasmid DNA sequence with PCR using primers containing the desired mutation (red circles). These sequences can then be integrated into homologous regions of the yeast genome. *In vivo* production of the mutant forms produces plasmids lacking methylations (black diamonds) found on the template plasmid that was propagated in *E. coli*, allowing for degradation of the template DNA.

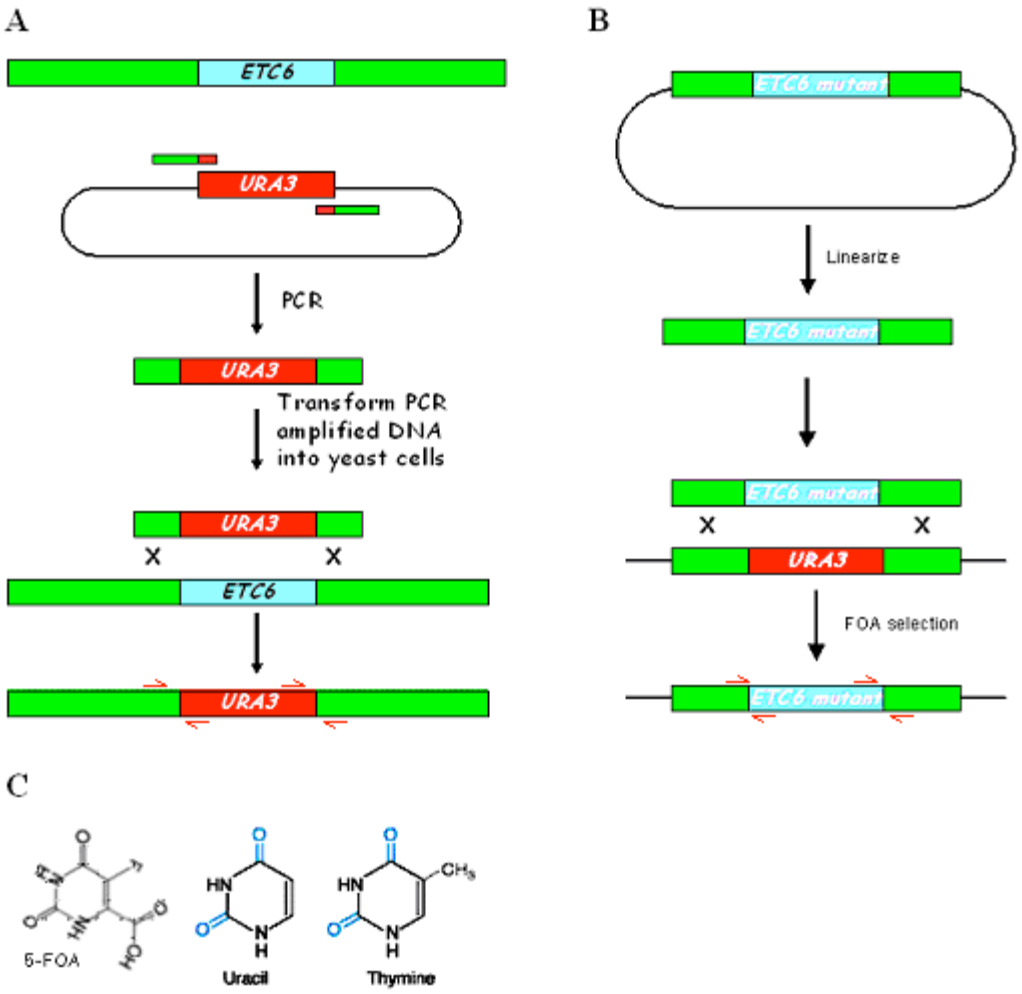


Figure 4. Integration of mutant *TFC6* by *URA3* counter selection.
A. The *URA3* gene is amplified by PCR using primers that add a tag that is homologous to the region flanking the genomic sequence to be replaced. Transformation with this PCR product results in *ETC6::URA3* strains that can be selected on minimal media lacking uracil. B. The mutant form of the gene knocked out with *URA3* is created via site directed mutagenesis. The resulting plasmid is linearized and transformed into the *ETC6::URA3* strain. Strains that have proper recombination events can be selected on media containing 5-fluoroorotic acid, a compound toxic to yeast with a functional *URA3* gene. Red arrows indicate location of PCR primers used to verify proper integration. C. Structure of *5-FOA* and its similarity to the structure of other nucleotides. *5-FOA* is incorporated into the uracil synthesis pathway by the enzyme encoded by URA3, resulting in improper production of uracil.

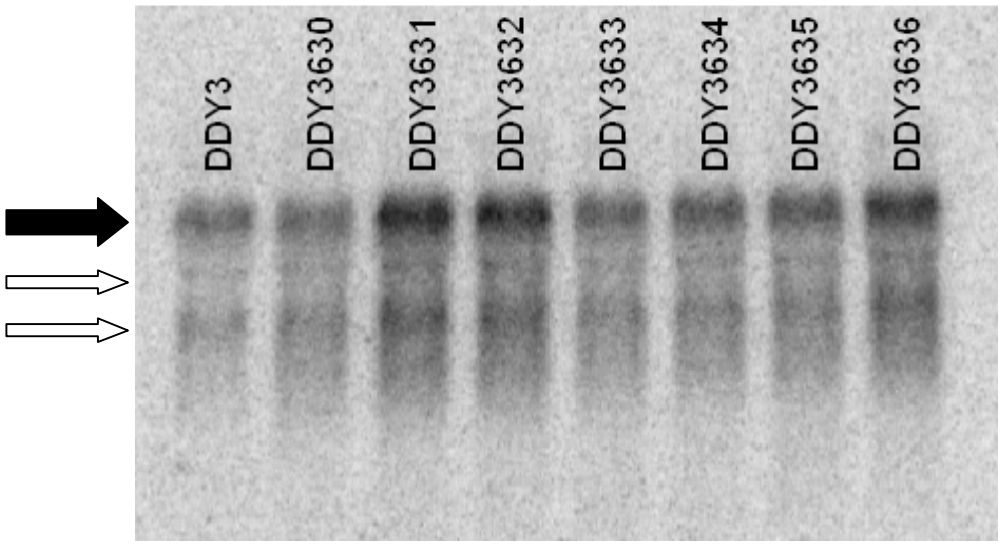
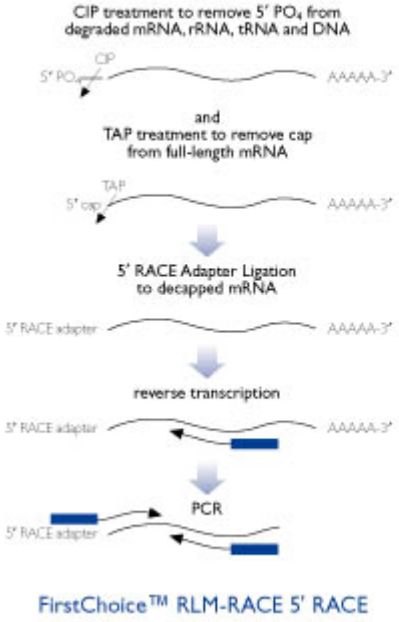
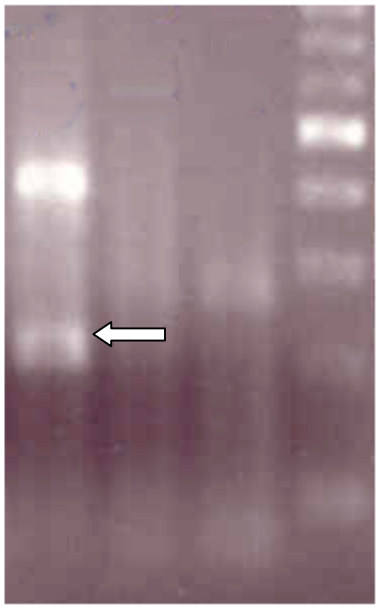


Figure 5. Northern Blot Analysis of *ETC6* Mutant Strains.
An equal amount of RNA from each strain was electrophoresed on a denaturing gel, and blotted to a nitrocellulose membrane**.** The membrane was then incubated overnight with a ^{32}P Uridine labeled RNA probe specific for *TFC6*. DDY3 is wild type yeast. DDY3630 (lane 2) was a control that had the wild type copy of *TFC6* integrated into the TFC::*URA3* strain. Strains 3631-3636 are all independent isolates containing the mutagenized copy of *ETC6*. The black arrow indicates the TFC6 signal. The white arrows indicate uncharacterized bands which may alternate TFC6 transcripts that correspond to the novel transcription start site.

A.



B.



C.

TGTCACTTTCCTCACNNNNNNNNANNNATTCAATACAAAATCATCACCGTCGGTGTCATCAACATTATTGACT
TGTGTAGTATTTATCACATCATCGCGACCAGCATGCACTAATTGTGCGAAATTTGCAACTGCTTTCTTAGATG
CGTTTCTCCTCGGCCTTTTACTGCCTGAGTTTTCAGATACTGGGCT**T**TTTCATCAAAGCCAGCAAACGCAGCG
TCTGGATCCGCGAAGGGCGAATTCCAGCACACTGGCGGCCGTTACTAGTGGATCCGAGCTCGGTACCAAGCTT
GGCNTAANCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC
GGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGC
CCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGANTCGGCCAACGCGCGGGGAGAGGCGGTTT
GCGTATTGGGCGCTCTTCCGCTTCCTCGCTAACTGACTCGCNGCGCTCGGTCGTTCGGNNGCGGCGAGAGGTA
TCAGCTCACTCAAAAGG

Figure 6. Mapping the Transcription Start Site of *TFC6*. A. Flowchart of the Ambion RLM-RACE® Protocol (from www.ambion.com) B. DNA gel showing results of nested PCR reactions. The larger, more intense band, was a PCR artifact, while the smaller band (indicated by the arrow) Resulted in genuine *TFC6* sequence. C. Raw sequencing data of the smaller band from B cloned into a TOPO vector. Red lettering indicates the 45 base pair 5' RACE Adaptor. The large blue T residue was the location of the cap site in two clones sequenced, while the underlined T residue was the cap site in one clone. This suggests a transcription start site 77 or 78 base pairs upstream of the *start codon* as currently annotated.

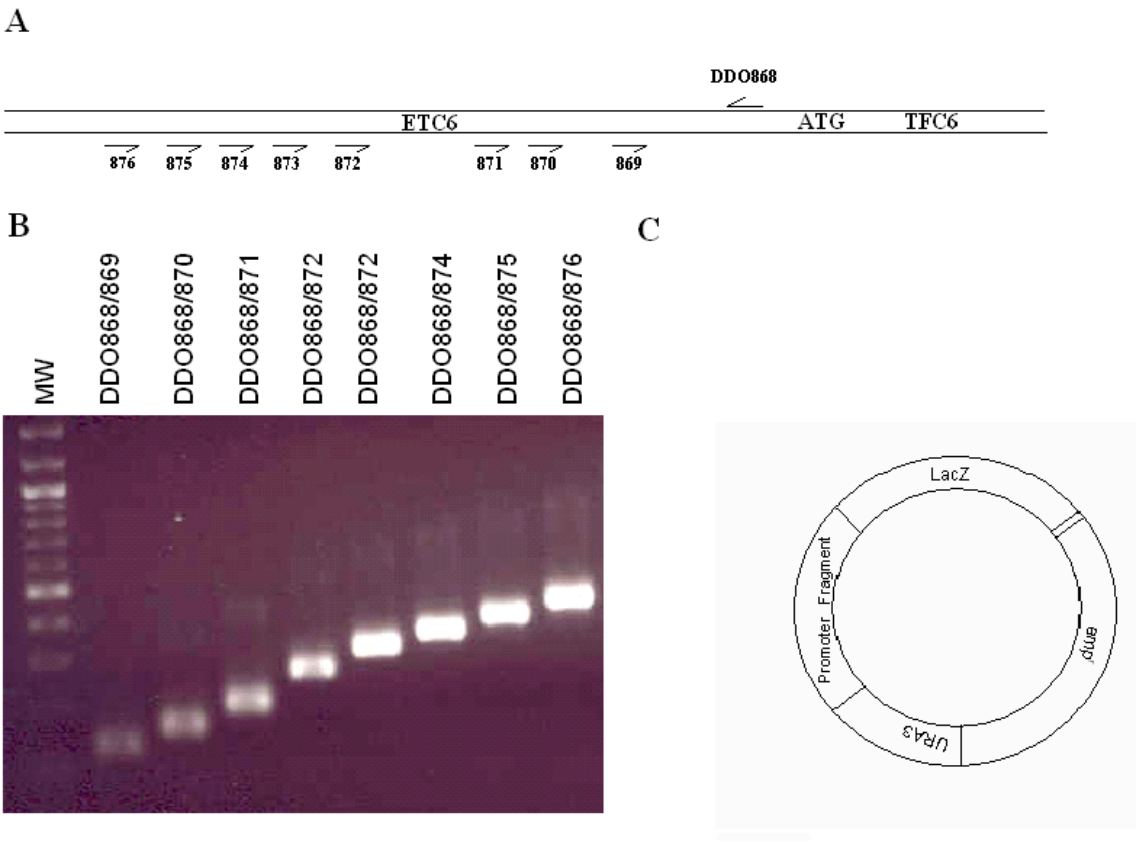
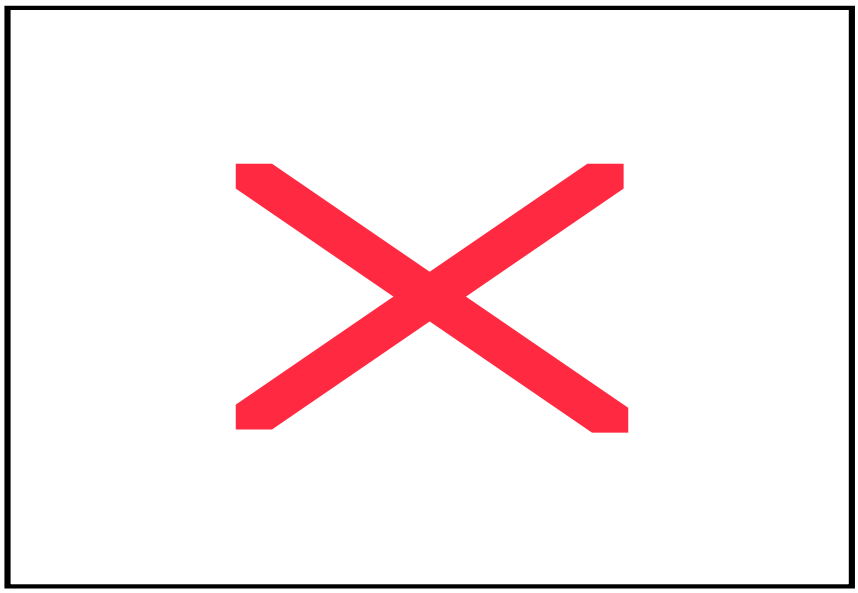


Figure 7. Construction of LacZ Reporter Assay Plasmids.
A. Location of primers to create serial deletions of the *TFC6* promoter region. B. PCR products of indicated primer pairs from a DDY 624 genomic DNA template.
C. Generalized scheme of LacZ reporter plasmid

A.



B

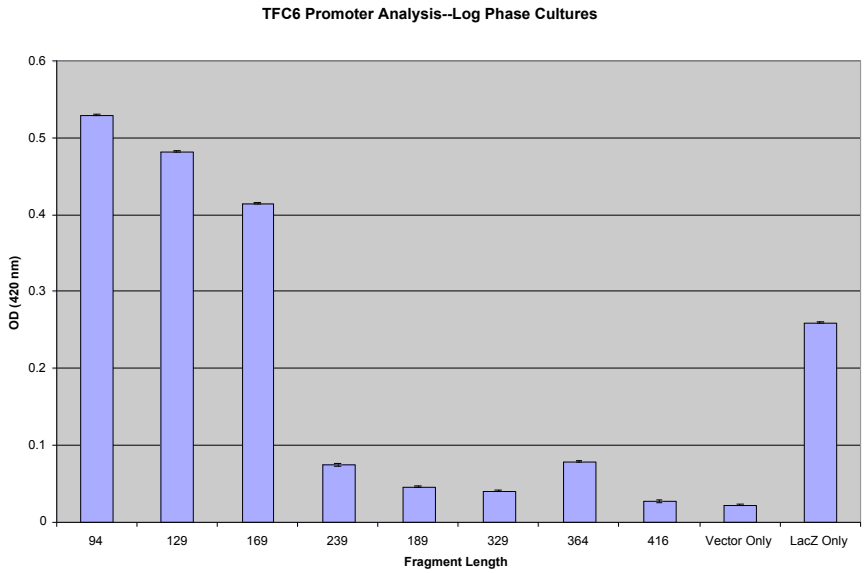


Figure 8. *TFC6* Promoter Analysis--Beta-galactosidase Activity Assay
A. Beta-galactosidase activity levels of overnight stationary phase wild type yeast cells transformed with a plasmid expressing the *LacZ* gene under the control of varying lengths of the *TFC6* promoter. This experiment was only conducted once. B. Beta-galactosidase activity levels of Log phase wild type yeast cells transformed with a plasmid expressing the *LacZ* gene under the control of varying lengths of the *TFC6* promoter. The values shown are averages of two replicates.

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