Identification and Characterization of the YPL150W Gene Function in *Saccharomyces cerevisiae*

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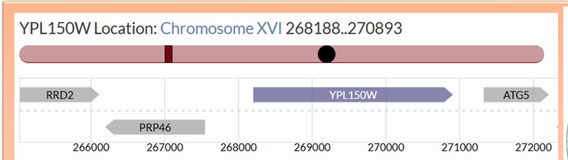
ABSTRACT

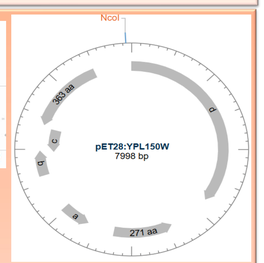
This project aims to characterize the function of the unannotated gene YPL150W in Saccharomyces cerevisiae. Although the yeast genome is fully sequenced, approximately 30% of its genes lack functional annotation. **YPL150W**, located on chromosome XVI, is conserved in organisms such as *Homo sapiens*, *Danio rerio*, and *Mus musculus*, suggesting a **conserved biological role**.

Bioinformatic analysis using the NCBI Conserved Domain Database reveals significant sequence similarity between YPL150W and known serine/threonine kinases, indicating potential enzymatic activity involving **phosphorylation of hydroxyl-containing residues**. Preliminary evidence suggests YPL150W encodes a nonspecific serine/threonine kinase, though experimental validation is required.

Characterizing YPL150W will advance our understanding of **conserved eukaryotic signaling pathways** and may have implications for both basic biology and industrial applications.

Developed Protocol



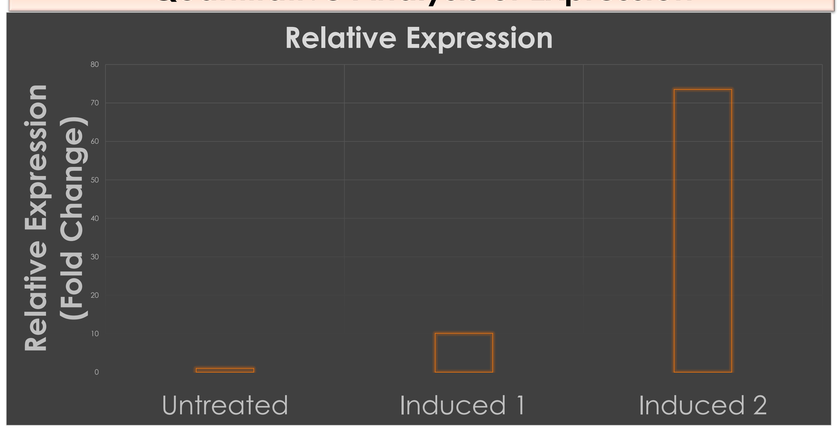


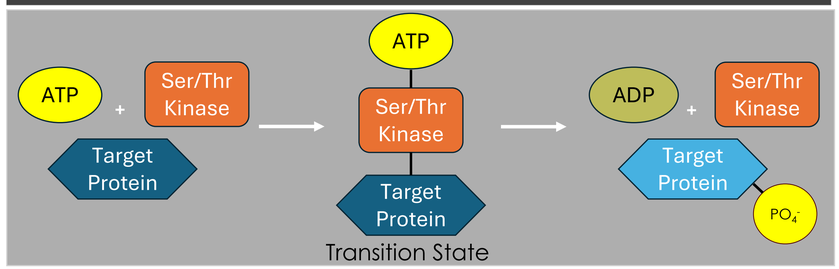
The gene coding for YPL150 is located on **chromosome XVI of *S. cerevisiae***. The DNA sequence was analyzed electronically to produce the mature mRNA sequence. The codons were optimized for efficient expression in a host organism of Escherichia *coli*. The resulting sequence was then digitally reverse transcribed to DNA.

The Biotech company Twist Bioscience synthesized the whole plasmid containing the DNA sequence that codes for YPL150W. This plasmid is also engineered to include an origin of replication in *E. coli,* an antibiotic resistance gene for selection, and a Histidine tag to facilitate protein purification.

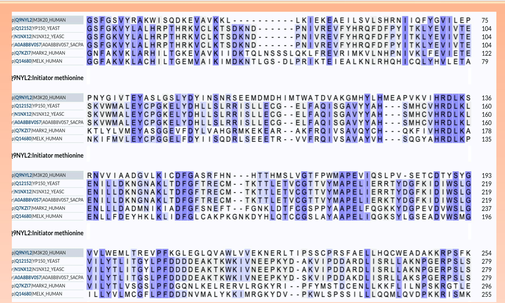
Genome DNA Sequence
↓
Translated Protein Sequence
↓
Optimized mRNA Sequence
↓
Optimized DNA Sequence
↓
Synthesize Expression Plasmid
↓
Overexpress Protein
↓
Validate Function

Quantitative Analysis of Expression





Sequence Alignment of YPL150W



Protein sequence alignment of the YPL150W protein from *Saccharomyces cerevisiae* reveals high similarity to homologous proteins in *Homo sapiens*, *Saccharomyces douglasii*, and other yeast species.
Analysis using UniProt indicates that key functional domains are conserved across these organisms, suggesting an **evolutionarily preserved role**, possibly in cellular signaling or structural regulation.

Overexpression of YPL150W Gene Product

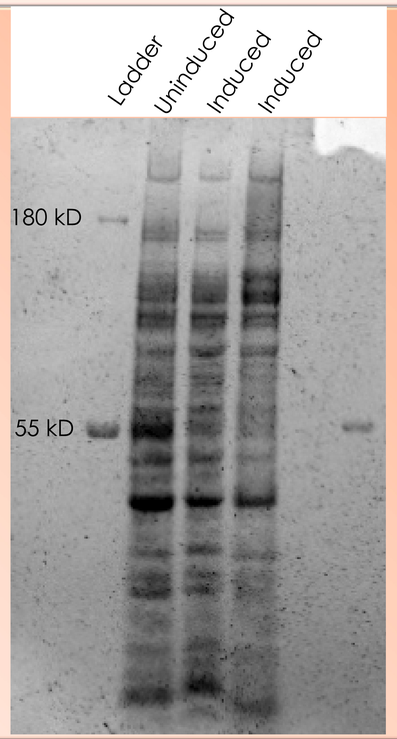
The plasmid pET28:YPL150W was transformed into an *E. coli* overexpression strain. The transformants were plated on an LB-agar plate containing kanamycin antibiotics.

Colonies were selected for further analysis.

Overnight cultures were used to inoculate 4.0 mL LB with kanamycin. After 4 hours, the culture was split into two fractions. One fraction was induced with 1 mM Isopropyl β-D-thiogalactopyranoside (IPTG), while the other was reserved as a control.

Coomassie total protein stain showed inconclusive overexpression of the YPL150W protein with the molecular weight of 200 kDa so His tag antibody western blot was performed.

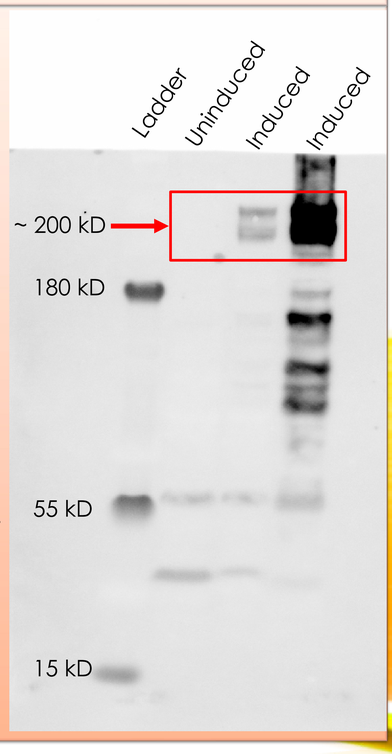
Lane 1: Standard Weight Ladder
Lane 2: Uninduced
Lane 3: IPTG Induced
Lane 4: IPTG Induced



Histidine Tag Antibody Western Blot

Lane 1: Standard Molecular Weight Ladder
Lane 2: Uninduced
Lane 3: IPTG Induced
Lane 4: IPTG Induced

Western blot analysis was performed using antibodies targeting the His-tag on YPL150W to **confirm protein expression**. Proteins from both induced and control samples were transferred from the SDS-PAGE gel to a membrane and probed with anti-His antibodies. Detection of a band at ~200 kDa **supported expression** of the His-tagged YPL150W protein.



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