Cellular Pathways Regulated by Ubiquitin Signalling

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Introduction

- Ubiquitin is a protein found in many organisms from yeast to humans. See figure 1 below for the structure.
- Ubiquitin modifies and regulates functions of other proteins in fundamental processes such as the cell division cycle. It has been linked with common diseases of the cell cycle such as cancer.
- It targets proteins by forming chains on their surface through lysine residues. Ubiquitin contains 7 lysines (K6, K11, K27, K29, K33, K48 and K63). (*1*)
- Most ubiquitin chains target proteins for degradation, except K63 chains which regulate cell signalling. However little is known about the functions of K63 signalling. *(2)*

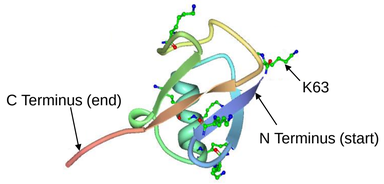

Figure 1. Ubiquitin protein structure, modified from *(3)*

Aims

The aims of this project were to use the model yeast organism *Saccharomyces cerevisiae* to construct/obtain strains and plasmid DNA to allow studies of K63 function using large-scale genetic analysis.

Specific objectives were:
- Remove the ubiquitin encoding genes 1 and 2
- Analyse ubiquitin encoding gene 3
- Isolate key plasmids from pre-existing strains.

Results

Ubiquitin Encoding Gene 1 Was Successfully Removed From The Genome

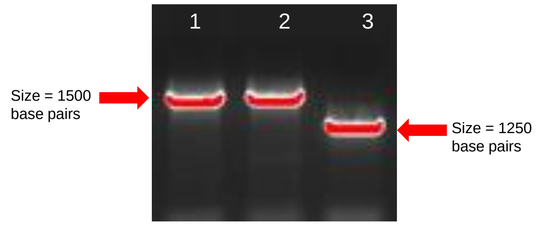

Figure 2. A disruption cassette was used to remove the ubiquitin encoding gene 1. Gene deletion was confirmed by PCR of two possible strains (lanes 1 and 2) and a wildtype (lane 3). PCR of the deletion produces a band of 1500 bp whereas the wildtype gave a band of 1250 bp.

Ubiquitin Encoding Gene 2 Was Successfully Removed From The Genome

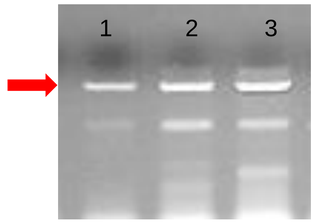

Figure 3. A disruption cassette was used to remove the ubiquitin encoding gene 2. Gene deletion was confirmed by PCR of three possible strains (lanes 1-3) a band at the expected size was obtained (1500 – 1750 bp) . The arrow indicates the band of interest.

Ubiquitin Encoding Gene 3 Was Successfully Analysed

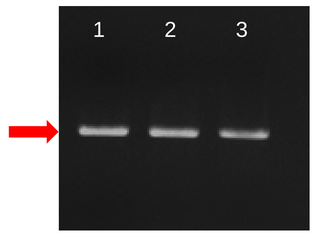

Figure 4. PCR was used to amplify the ubiquitin encoding gene 3 region from three strains (lanes 1-3) The correct band was obtained (size expected 1000-1250 bp) indicated by the arrow. The PCR fragment was then purified and the DNA sequence analysed.

Key Plasmids Were Successfully Isolated

Figure 5. A plasmid isolation technique was used to remove plasmids from relevant yeast strains. The plasmids were analysed on a gel which confirmed successful isolation of two separate plasmids (P1 and P2) as two different band patterns were observed.

Conclusions

Ubiquitin encoding genes 1 and 2 were successfully removed from the genome.

The sequence of ubiquitin encoding gene 3 was successfully analysed.

The essential plasmids for further work were successfully isolated.

Future Directions

The two remaining ubiquitin genes will be deleted and the final plasmids will be constructed using the plasmids isolated from this project.

The strains and plasmids produced will next be used for large scale genetic analysis using state-of-the-art robotics screening and gene mutation libraries of the essential and non essential yeast genes to understand K63 functions.

Keywords

- **Disruption Cassette –** a fragment of DNA which is used to replace a gene
- **Genome –** Entire collection of an organisms DNA
- ***K63*** – the 63rd amino acid in the protein is lysine
- ***Plasmid*** – circular DNA which replicates independently from the chromosome
- ***PCR*** – The polymerase chain reaction, a common technique used to make copies of specific DNA fragments
- ***Wild type*** – an unmutated strain of organism.

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