# Investigating telomeric protein-protein interactions using the Yeast Two Hybrid System

**Ami Gisby*, E. Raymond, EM. Holstein, D. Lydall**

Newcastle University

## Aims;

Our aim is to use the Yeast 2 hybrid system[1] to detect protein-protein interactions between 13 different telomere associating proteins in *saccharomyces cerevisiae.* The 13 proteins we looked at were as follows; Cdc13, Stn1, Ten1, Rad17, Mec3 & Ddc1, Exo1, Sgs1, Dna2, Rad24 and RPA1, 2 & 3.

## Introduction;

Telomeres are regions of DNA that contain repetitive nucleotide sequences at the ends of chromosomes. Telomeric DNA consists of single-stranded 3' overhangs that are elongated by the enzyme telomerase and serve to protect chromosome ends from degradation. As well as being elongated telomeres have associating proteins that serve to protect telomeres from being recognised and treated as double strand breaks. Dysfunctional telomeres can lead to the DNA damage response and genomic instability, which is potentially lethal.[2]

The yeast two hybrid system detects protein-protein interactions using the separated DNA-Binding (DB) and Activation (AD) domains of a transcriptional activator protein, such as GAL4 as seen in Figure 1. In vivo, the GAL4 DB and AD are in physical contact with each other and so activate transcription of the reporter gene, however these two domains can be separated and subsequently fused to two separate proteins, known as the Bait and Prey. Transcription of the reporter gene lacZ only occurs when both the GAL4 AD and DB domains are in physical contact meaning the proteins that they are fused to are interacting.

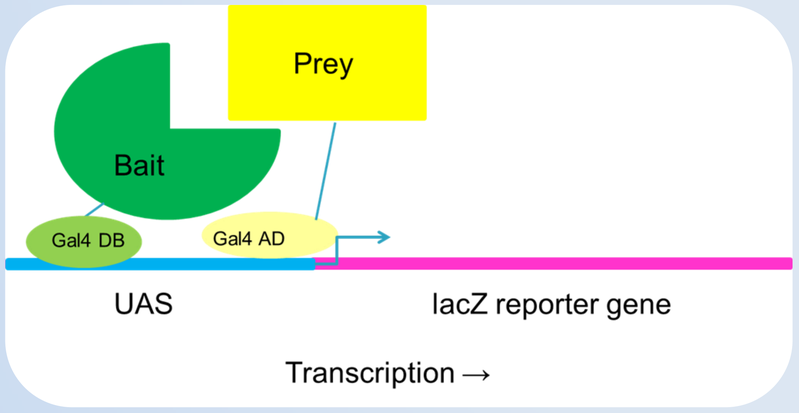


Figure 1; Showing the interaction between the Bait and Prey proteins when they are fused to their respective DB and AD domains, allowing transcription of lacZ to occur.

## References;

1. S. Fields & O. Song, 1989, A novel system to detect Protein-protein interactions, Nature; 340(6230):245-6.
2. JM. Dewar & D. Lydall, 2012, Similarities and differences between 'uncapped' telomeres and DNA double strand breaks, Chromosoma;121:117-130.

*BSc Biochemistry undergraduate, a.l.gisby@ncl.ac.uk

## Experimental Procedure

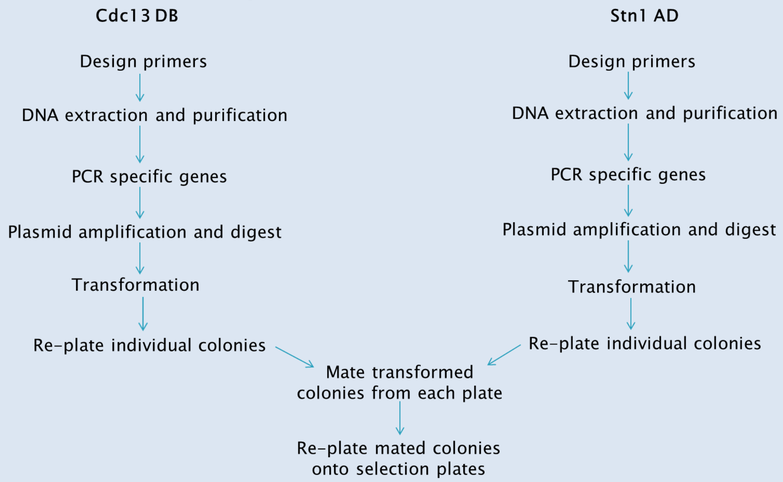


Figure 2; flow diagram showing each step of the procedure for two of the genes Cdc13 & Stn1.

## Method;

1. Design of forward and reverse primers for the 13 genes to amplify them using polymerase chain reaction (PCR). Three primers designed for each gene of interest are as follows; forward overlapping sequences with GAL4-DB, forward overlapping sequences with GAL4-AD and reverse primers complementary to the plasmid.
2. A DNA extraction from the DLY640 wild type strain was preformed.
3. The plasmids pDL1432 and pDL1435 were amplified using E. coli and digested at the Sma1 cleavage site. Both plasmids contained a promoter region, an Ampicillin marker and a gene encoding one domain of GAL4 transcriptional activator protein, followed by the Sma1 cleavage site. pDL1432 has a leu marker, allowing the transformed yeast to grow on selective –leu media, whereas the pDL1435 has a trp marker, allowing the transformed yeast to grow on selective –trp media. PDL1435 also contained a wild type Cyh2 gene.
4. Transformation of the yeast strains DLY7352 and DLY7353 allowed digested plasmids and amplified genes to be taken up by the cells .
5. Each of the 13 genes were transformed so each Mata strain could be mated with each Matα to detect any interactions when grown on selection plates. Summary of the method can be seen in figure 2.
6. All the markers mentioned in the plasmids are used to select for mated strains that interact, by plating the yeast onto 6 selective plates as follows;

- -leu-trp - control plate used to show that mated strains contain both plasmids pDL1432 and pDL1435.
- -leu-trp+Xgal – colonies turn blue if interactions occur
- -his-leu-trp – Grow in the lack of histidine if interactions occur
- -ade-leu-trp – Grow in the lack of adenine if interaction occur
- -his-leu+CHX & -ade-leu+CHX show presence of pDL1435 which has a wild type CYH2 genotype, causing death when grown on cycloheximide.

## Results;

Only 11 of the genes Exo1, Rad24, DDC1, Rad17, Stn1, Cdc13, Ten1, Mec3, RPA1, RPA2 & RPA3 successfully mated as seen on the control plate -leu-trp in figures 3 & 4.

-leu-trp+ Xgal plate shows growth for every hybrid with 11 protein-protein interactions being identified as shown in figure 3 by the blue colour of the colonies. These interactions were as follows;

- Cdc13DB →DDC1, Rad17 & Stn1 AD
- Exo1 DB → Rad17 & Stn1 AD
- Rad24 DB →Rad17 & Stn1 AD
- DDC1 DB → Rad17 & Stn1 AD
- Rad17 DB → Stn1 AD
- Stn1 DB → Rad17 AD

-his-leu plate showed that RPA1 interacted with everything but Mec3 & Ten1.

-his-leu+CHX & -ade-leu+CHX showed RPA1DB interacted with every protein but itself.

-ade-leu-trp showed limited growth at Cdc13 and RPA1 , suggesting a possible interaction.

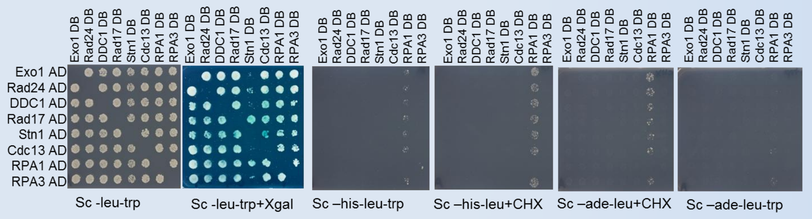


Figure 3; shows the 8 hybrids grown on the 6 selection plates as mentioned in the method.

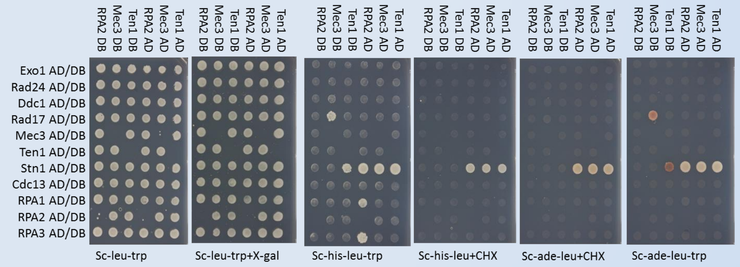


Figure 4; showing the interactions of Mec3, Ten1 & RPA2 with the other 11 proteins on the 6 selection plates as mentioned in the results.

## Conclusions;

Protein-protein interactions found at telomeric ends in the wild type DLY640 strain are;

- RPA1 interacted with every other protein but itself, Ten1 and Mec3
- Stn1 interacts with Ten1, RPA2 and Mec3
- Rad17 interacts with Mec3
- RPA2 interacts with RPA1 and RPA3.

Using the se findings future research will be carried out measuring protein-protein interactions in presence of uncapped telomeres . This will then allow DNA damage dependant protein-protein interactions to be identified.