High throughput screening of yeast telomere mutants

Project funded by:
Newcastle University
Research Scholarship

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Introduction

Telomeres are complex structures that protect the ends of chromosomes and are highly conserved amongst organisms with linear chromosomes.

Cancer cells need special mechanisms to protect their telomeres.

We used budding yeast to mimic the effects of telomere damage.

Aim

To investigate the effects of loss of Cdc13 (a DNA binding protein) function on the fitness of yeast cells with individual genes knocked out.

Methods

Utilised a wild type *CDC13* strain and a temperature sensitive *cdc13-1* mutant strain.

Robotics equipment was used to carry out a series of steps (outlined in figure 1) resulting in mutant strains combining the *CDC13* yeast and the *cdc13-1* mutants with a library of gene knockout mutants.

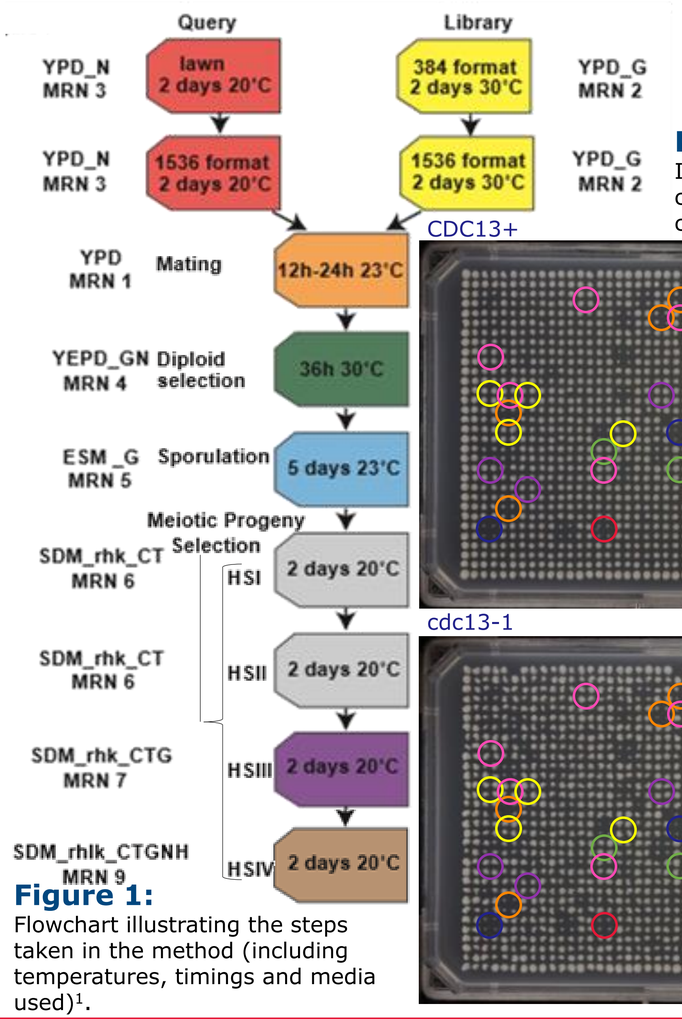

Figure 1:
Flowchart illustrating the steps taken in the method (including temperatures, timings and media used)[1].

Figures and Results

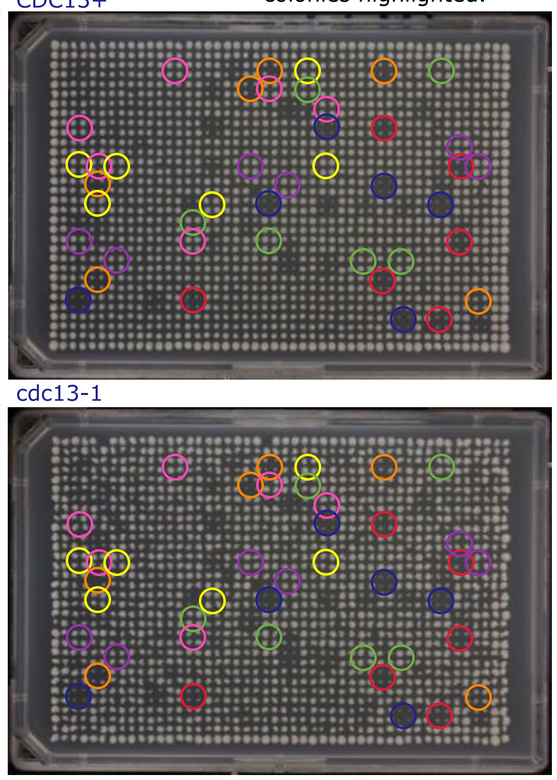

Figure 2:
Images of the final culture plates with key colonies highlighted.

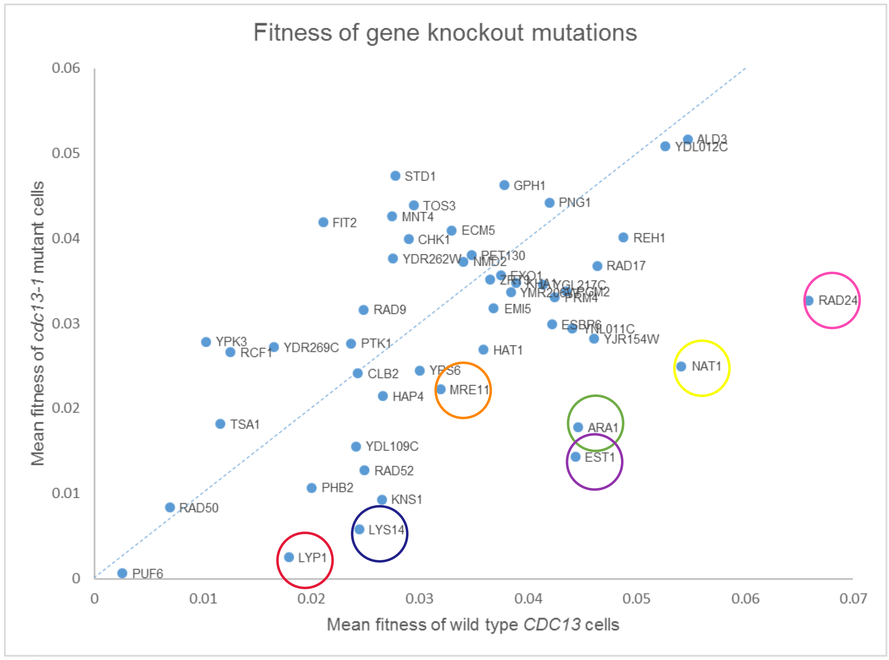

Figure 3:
Graph showing the mean fitness of gene knockout colonies on the final culture plates, with the wild type *CDC13* strain against the mutant *cdc13-1* strain.

- *Cdc13-1* mutant colonies grow more poorly than *CDC13* wild type colonies (figure 2).
- Similar gene knockout mutants (such as *LYS14*, *LYP1* and *MRE11*) died out on both plates (figure 2).
- *RAD24*, *NAT1*, *ARA1* and *EST1* knockouts had far greater fitness in the wild type than in the mutant strain (figure 3).

Conclusions and Future Work

- *LYS14* gene knockouts require lysine to survive, which the HSI-HSIV medias lack, and LYP1 knockouts are killed by thialysine which is present in the HSI-HSIV medias. *MRE11* knockouts are slow growing, meaning the quick transfer from one media to the next may have limited growth. The fact these gene knockouts died out in both strains illustrates that the experiment worked as expected.
- *RAD24* knockouts may have had poorer fitness in the *cdc13-1* strain due to damage caused by this mutation requiring *RAD24* to allow cells to respond to it[2]. *EST1* knockouts likely had poorer fitness in the *cdc13-1* strain as it is sicker than the wild type, thus needing more telomere maintenance which *EST1* is part of[3].
- No clear reason why *ARA1* or *NAT1* knockouts would have greater fitness in the wild type strain, so further research into these genes could be insightful.
- Future work could include exposing these mutant yeast strains to wider temperature ranges, to investigate in depth the *cdc13-1* strain's temperature sensitivity.

References

1) Modified from an image provided by Dr Eva-Marie Holstein, clinical trials administrator at Newcastle University. 2) Zubko MK, Guillard S, Lydall D (2004). "Exo1 and Rad24 Differentially Regulate Generation of ssDNA at Telomeres of Saccharomyces cerevisiae cdc13-1 Mutants." Genetics 168(1):103-15. 3) Evans SK, Lundblad V (1999). "Est1 and Cdc13 as comediators of telomerase access." Science 286(5437):117-20.