Investigating the Mechanism of C9orf72 Mutations in Amyotrophic Lateral Sclerosis

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***Abstract* — The GGGGCC hexanucleotide repeat expansion of the *C9orf72* gene is the most common genetic cause for amyotrophic lateral sclerosis (ALS). This study examined the cloning of various 149 repeat expansions of *C9orf72* and studied their stability in yeast. Conclusively, results demonstrated significant, undesirable shrinkage in cloned repeat expansions and low stability of the repeat expansions in yeast, thus furthering our understanding of yeast models in ALS research.**

I. INTRODUCTION

The GGGGCC hexanucleotide repeat expansion located in the first intron of the *C9orf72* gene is the most common genetic cause for ALS / frontotemporal dementia. The repeat expansion is hypothesized to cause repeat-associated non-AUG translation (RAN-translation) that results in toxicity by aggregation of dipeptide repeat proteins (DRPs), contributing to ALS [1]. There is currently a lack of research into the exact mechanism by which the *C9orf72* gene partakes in RAN-translation. Thus, this study aimed to better characterize the mechanisms of the repeat expansion in the cloning process using 149-repeat expansions of *C9orf72* (C9149R).

Yeasts are favorable models for studying genetic disease due to their relative simplicity and high conservation of gene and protein translation with humans [2]. As genetic yeast screens are often executed over an 18-day period, this experiment observes the stability of repeat expansions in yeast over various time periods to determine if they are optimal for screening. It was hypothesized that stability of the repeat expansions in yeast would be low, as yeast is good at homologous recombination; this mechanism, which repairs harmful breaks in DNA by exchanging nucleotide sequences between two similar DNA stands, was expected to shorten the expansions' lengths and lessen their stability.

II. METHODS

Cloning of yeast C9149R plasmid: 149 repeats of the *C9orf72* repeat expansion (C9149R) were ligated into empty yeast p416 vectors under galactose-inducible (GAL1) or Glyceraldehyde-3-phosphate Dehydrogenase (GPD) promoters. The plasmids were transformed into Stbl3 *E.coli*, grown overnight, and then DNA was purified using a Qiagen mini-prep kit [3]. To find C9149R clones that retained the correct size (1230 bp), clones were run on 1% agarose gel (1% AGE) with the original C9149R as a positive control for non-retracted repeat size. 187 clones were screened in total.

C940R Stability in Yeast: 2-repeat (C92R) and 40-repeat (C940R) expansion plasmids were transformed into yeast and later grown in liquid media of SRaf-Uracil (-URA), SD-URA, and SGal-URA. Yeast DNA was harvested at five different time points: day 1, 2, 5, 7, 8. Three different protocols were used in order to maximize efficacy of DNA mini-preparation: Qiagen kit with glass beads [3], Zymo kit with unfiltered spin columns [4], and Zymo kit with filtered spin columns.

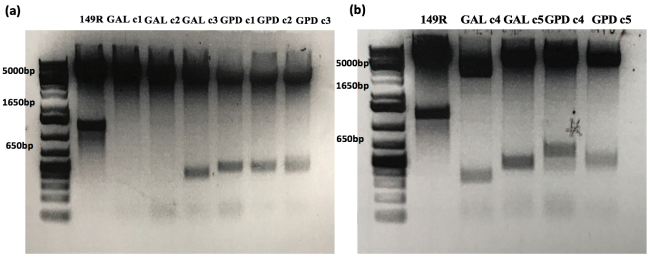

Figure 1: *Representative results of 187 clones.* ***(a)*** *1% AGE of 149R control; Three clones of GAL1 C9149R and of GPD C9149R.* ***(b)*** *149R control; Two clones of GAL1 and of GPD C9149R.*

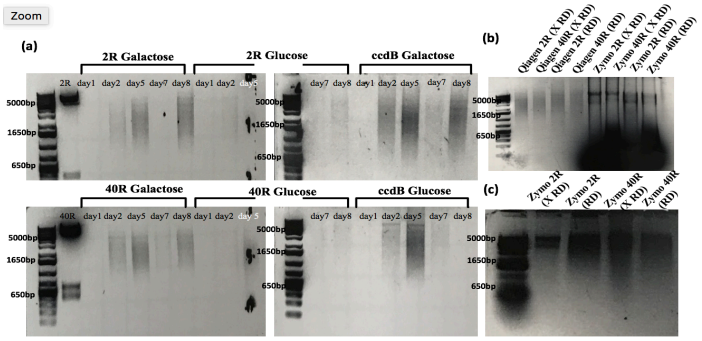

Figure 2: ***(a)*** *1% AGE of C92Rs and C940Rs mini-prepped with Qiagen kit;* ***(b)*** *left four mini-prepped with Qiagen kit and right four with Zymo kit + unfiltered spin columns. RD refers to being restriction digested at repeat expansion cut sites XhoI & HindIII-HF.* ***(c)*** *all four mini-prepped with Zymo kit + filtered spin columns.*

III. RESULTS & DISCUSSION

Cloning of yeast C9149R plasmid: As hypothesized, results in Figure 1 consistently showed that in comparison to the 149R control, the length of 149R clones had shrunk in both GAL1 and GPD yeast vectors. The shrinkage is suggested to lie in the process of DNA replication, as the DNA polymerase can slip from DNA strands due to the abnormally large repetitive nature of 149R. The high GC content of GGGGCC expansion is also thought to cause slippage, as GC bonds are stronger than AT bonds, making it harder for DNA polymerase to get through.

C940R Stability in Yeast: Figure 2a demonstrates smearing and low visibility. This occurrence is potentially attributed to undesired elements such as genomic DNA, proteins, and ribosomes not having been filtered properly. Because genomic DNA tends to be fragile, vortexing with glass beads may have fractured it into multiple fragments, leading to smearing in Figure 2a. This speculation is affirmed by the presence of intense DNA concentrations at the bottom of the gel in Figure 2b for clones mini-prepped with unfiltered spin columns, whereas these concentrations are not visible in Figure 2c after filtered spin columns.

IV. CONCLUSION

This study demonstrates that significant shrinkage occurs when C9149 repeat expansions are cloned in yeast, suggesting that repeat expansions are rather unstable in yeast. The results also affirm low stability of repeat expansions in yeast regardless of time period of replication. Unlike bacterial DNA, yeast DNA purification seems to require a gentler rupture of cell walls and more extensive filtration. Future steps must be taken to determine ways to lessen DNA polymerase slippage, making yeast an even more desirable medium for genetic studies.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

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