Characterization of Potential G-Quadruplex (G4)-Binding Proteins

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ABSTRACT

G-quadruplexes (G4s) are unique, four-stranded secondary DNA structures that form in guanine-rich genomic regions. They play important roles in gene regulation and genome stability. G4s appear to regulate maize genes, suggesting potential agricultural significance. We suspect our antibody, which we refer to as "G4X," should have G4-DNA-binding activity. The G4-binding proteins, like the G4X, are antibodies designed to recognize G4 structures selectively, facilitating their detection and isolation in biological systems. This study tested how well G4X can bind to G4 DNA using immunodepletion with G4-forming oligonucleotides and magnetic beads. We investigated how different concentrations of G4X affected DNA capture efficiency. G4X was also used for cellular staining, allowing visualization of G4 structures within a biological context.

METHODS

For each of the six folded oligo samples (5K, 5L, 10K, 10L, 18K, 18L), 150 µL of each oligo was added to separate tubes. A serial dilution of G4X antibody in Antibody Storage Buffer (ASB) was prepared by adding undiluted SG4 to tube D1 and transferring 4 µL from one tube to the next, creating a dilution series from D0 to D4. The dilution tubes were kept on ice for 1 hour. Afterward, 27 µL of each oligo sample was added to the corresponding tubes, followed by 4 µL from each G4X dilution. The reaction tubes were incubated for 90 minutes at 37°C, 2 hours at room temperature, or overnight at 4°C to allow the antibody to bind. Anti-Flag beads were washed with buffer, resuspended in 170 µL of wash buffer, and 5 µL of beads were added to each reaction tube. After a 2-hour incubation at room temperature, the beads were separated using a magnetic rack. The supernatant (30 µL) was collected into labeled tubes for analysis. DNA concentration in the supernatant was measured using a Nanodrop spectrophotometer, and the results were recorded. These steps were repeated for each of the six oligo samples.

BACKGROUND

- G-quadruplexes (G4s) are stable secondary DNA/RNA structures found in plant genomes (Griffin & Bass, 2018).
- Thousands of G4 motifs are in genes linked to energy stress responses like hypoxia and nutrient deprivation (Andorf et al., 2014).
- G4 structures require K^+ for folding, while Li^+ does not support G4 formation, making it a key control.
- Our G4X protein binds G4s and has a FLAG tag for purification.
- FLAG-tagged beads bind G4X, allowing the removal of G4-bound oligos.
- If G4X is functional, G4 oligos (in K^+, not Li^+) will be depleted by bead removal.
- DNA absorption spectroscopy (A260) will monitor oligo concentration—decreased A260 indicates G4 depletion.

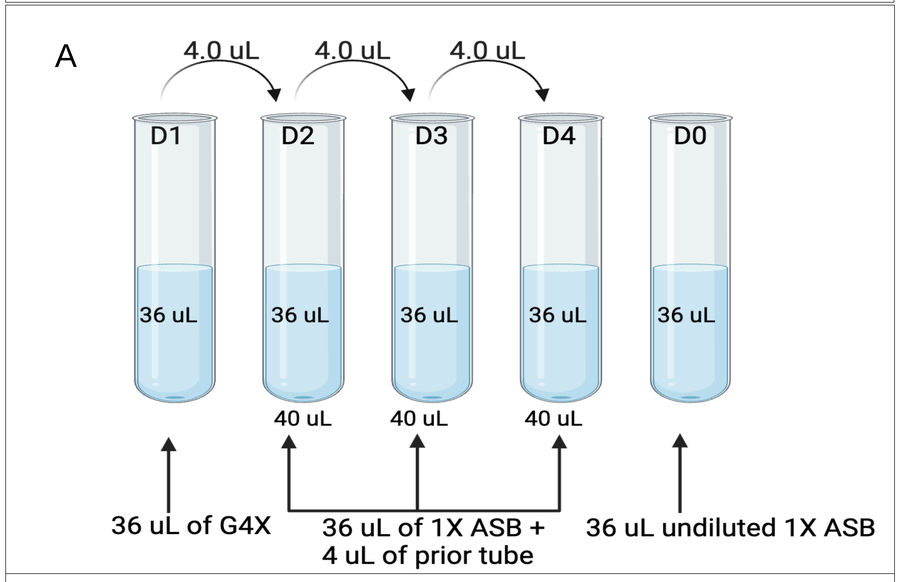

Figure 1: (A) Serial dilution of G4X antibody in Antibody Storage Buffer (ASB), where 4 µL from one tube is transferred to the next, creating a dilution series from D1 to D4. The dilution series is kept on ice for 1 hour to maintain antibody stability.
(B) Binding of the G4X antibody to the protein quadruplex (G4) with corresponding magnetic beads.

RESULTS

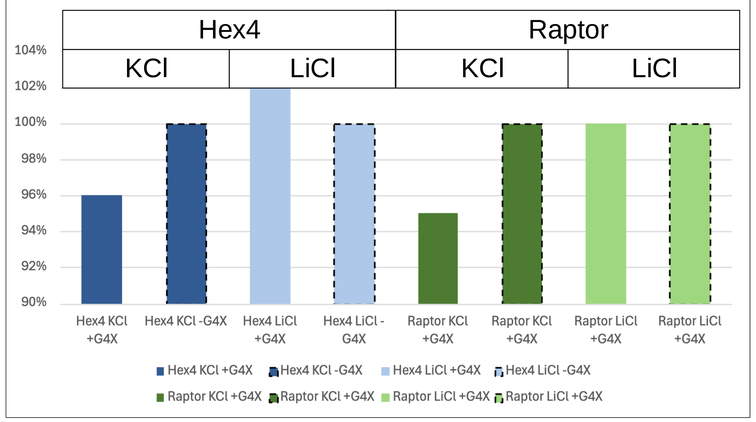

Figure 2: Graph showing the depletion of KCl values, with the "KCL-G4X"' and "KCl Raptor" tubes indicating no DNA loss. Meanwhile," KCl Hex4" and "KCl Raptor" showed DNA depletion.

CONCLUSION

- The research identified a clear trend in the data, with KCl, acting as the control, not showing immunodepletion, consistent with its expected role.
- This outcome confirms that the control condition (LiCl) functioned as intended.
- Moving forward, we plan to integrate additional research to enhance our understanding of stress responses like hypoxia and nutrient deprivation.
- The use of fluorescent dye attached to our G4X will enable us to visualize and better characterize cellular structures, particularly telomeres, and their role in response to environmental stress.
- This approach will not only improve our understanding of telomere dynamics but also offer new insights for agricultural applications, helping to enhance crop resilience and advance genetic research.

REFERENCES

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