FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

Aptamer Selection for DNA Aptamers against Glioblastoma Biomarker

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Background

- Glioblastoma is an extremely invasive, deadly form of brain cancer
  - Median survival after diagnosis is just one year, with only 5% of patients living 5 years after their diagnosis
- Current diagnoses include expensive and invasive biopsies and CT scans, while treatments include surgical resection and adjuvant radio and chemotherapy [1]
- Aptamer use in glioblastoma diagnosis and treatment mostly unexplored due to lack of research [2]
- Whole-cell SELEX used to bind aptamers to human glioma cells, proving that aptamers can be used for glioblastoma targeted therapy, ultimately improving drug accumulation and tumor penetration [3]
- Project explored the use of the SELEX process to select an aptamer with the highest binding affinity to YKL-40, the chosen target protein
- YKL-40 is a biomarker for glioblastomas because it is overexpressed in said tumors and secreted in bodily fluids, like urine [1]

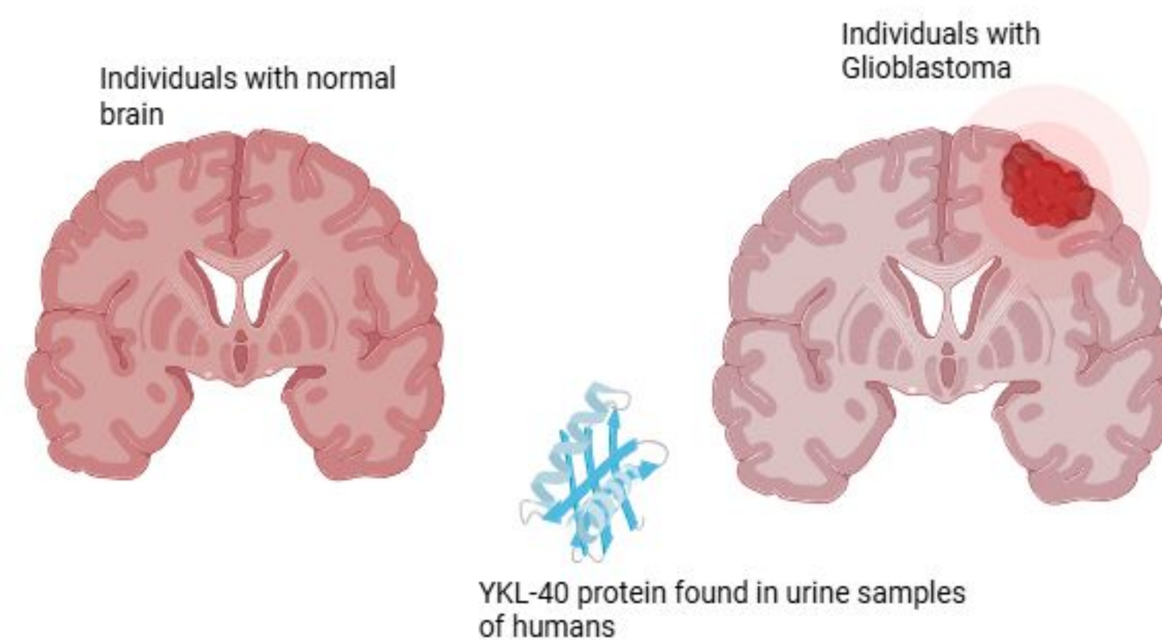


Figure 1: Normal brain (left) vs. presence of tumor in glioblastoma patient (right)

Intent & Motivation

Intent: To select and characterize a DNA aptamer against YKL-40, a biomarker of glioblastoma.

Motivation: Current diagnostics of glioblastoma are extremely invasive and expensive. The selected aptamer could be used as a screening tool for glioblastoma. Such a non invasive and accessible diagnostic tool would be beneficial to help patients get proper treatment and improve their prognosis.

Methods

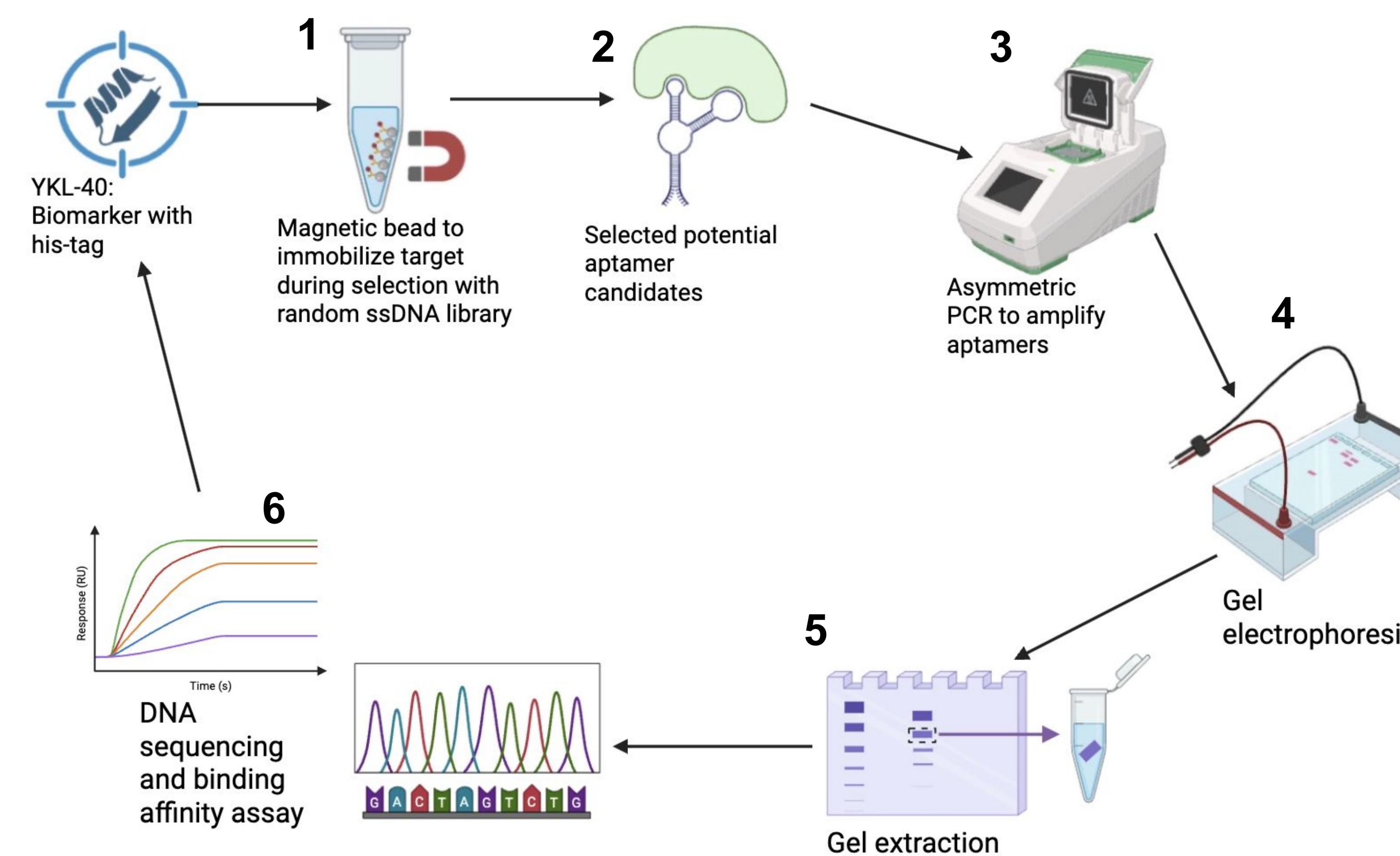


Figure 2: Overview of the SELEX workflow used to identify DNA aptamers against YKL-40. YKL-40 is immobilized on magnetic beads (1) and incubated with ssDNA library to enrich for binding sequences. Selected aptamer candidates (2) are amplified by asymmetric PCR (3), verified by agarose gel electrophoresis (4), and purified through gel extraction (5). The resulting pools undergo DNA sequencing and binding affinity assays (6) for downstream applications.

- Performed asymmetric PCR using the G-rich DNA aptamer library (63 nt long with a 25 nt long random region) to preferentially amplify single-stranded DNA for downstream selection
- Verified and purified ssDNA via agarose gel electrophoresis and gel extraction, isolating aptamer sequences for subsequent quantification and selection rounds
- Positive selection with PBS buffer was conducted to isolate binding nucleic acid sequences from library, incubated at 25ºC for 30 minutes
- Used magnetic beads to hold protein target during selection and wash away unbound aptamers using PBT buffer
- Process repeated with larger gel and negative selection conducted to remove sequences bound to non-targets
- The stringency of selection in future rounds will increase as the number of washes is increased and negative selection will occur every other round

Results & Discussion

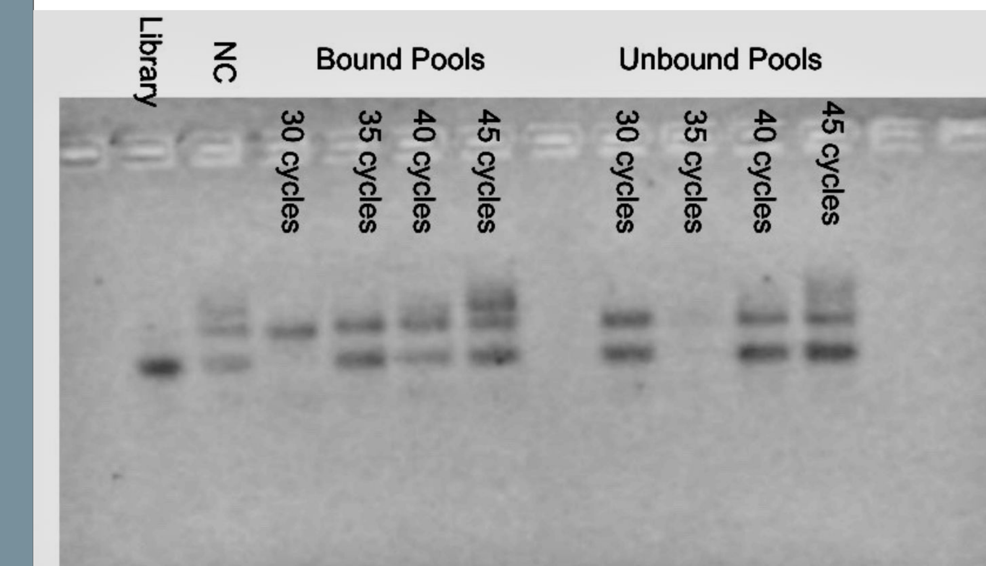


Figure 3: Cycle-course agarose gel, after one round of SELEX against YKL-40, showing asymmetric PCR amplification at 30-45 cycles for library, negative control (NC), and bound/unbound pools. Band intensity increases with cycle number, allowing identification of optimal amplification conditions.

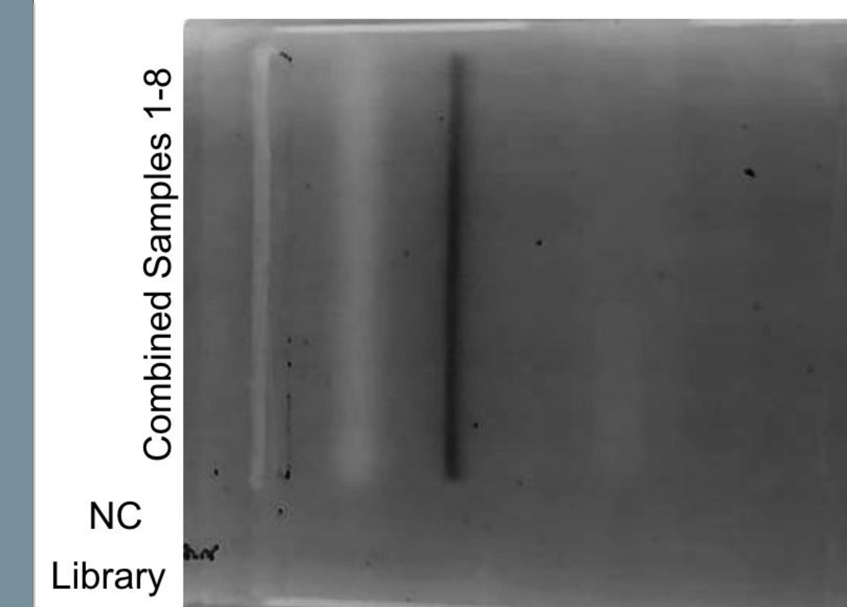


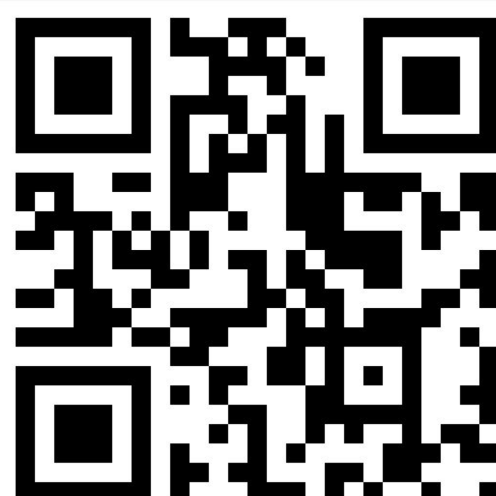
Figure 4: Large scale asymmetric PCR on our combined samples 1-8, ran at 90 V, 250 mAmps, for 45 minutes.

- Completed one round of selection against YKL-40 using the SELEX process, generating bound and unbound aptamer pools
- Performed one cycle-course PCR on these bound and unbound pools
- Samples were amplified at cycles 30, 35, 40, and 45 cycles and run on a large 4% agarose gel alongside a 250 nM library and NC
- The bound pool showed optimal amplification at 35 cycles, producing a strong band
- 30 cycles produced little to no amplification, while 40-45 cycles showed over-amplification, indicated by the smeared and more intense bands
- The unbound pool displayed a weaker overall amplification, consistent with early-round SELEX enrichment
- Figure 4 shows the completed large scale asymmetric PCR using our combined samples
- Sample bands are shown, however due to an issue with gel dye, library and negative control information are not visible

Future Work

- Complete rounds of selection to enrich for aptamers that bind strongly and specifically to YKL-40
- Carry out binding affinity assays and send selection pools for DNA sequencing to determine aptamer candidates
- Apply findings to develop an accessible, affordable, and non-invasive diagnostic for glioblastomas

References and Acknowledgements



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