FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

DNA Aptamer Against Alzheimer's β-Amyloid 42 Protein

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Intent & Motivation

Intent: To select and characterize single stranded DNA aptamers that binds to the β-Amyloid 42 protein for future diagnostic applications.

Motivation: An aptamer-based diagnostic test could allow for early detection and treatment of Alzheimer's disease. A diagnostic aptamer test is a more accessible alternative for current antibody-based blood tests and other invasive tests which would allow for quicker disease detection and earlier diagnosis

Background

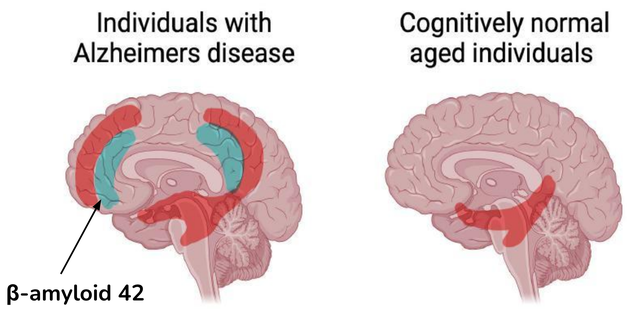

***Figure 1*:** Increased plaque buildup in Alzheimer's patients (left) vs healthy individuals (right)

- **Rising Prevalence:** Alzheimer's disease currently affects 6.9 million Americans over the age of 65 and is projected to double by 2060, indicating a need for effective diagnostics[1]
- **Limitations of Current Diagnostics:** Despite their accuracy, the invasiveness of spinal taps makes them an unsuitable option for elderly patients[2], warranting the need for less invasive diagnostic methods
- **Role of β-Amyloid 42:** Aggregated clusters of the β-amyloid 42 protein are strongly linked to Alzheimer's disease[3]
- **Recent Development of Blood Tests:** A multi-analyte blood biomarker test was created to assess the likelihood of amyloid plaques in patients with mild cognitive impairment or dementia symptoms. It algorithmically combines plasma Aβ42/Aβ40 and p-tau217/np-tau217 ratios[4]
- **Other Aptamers:** DNA aptamer, Aβ7-92-1H1, was identified after nine rounds of in vitro SELEX against β-Amyloid 42 monomers and was selected to identify β-Amyloid 42 aggregation[5]

Methods

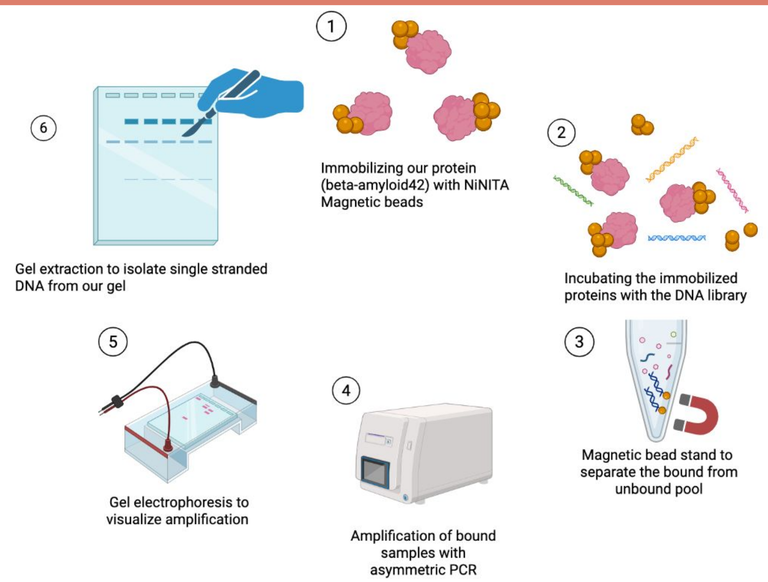

Figure 2: Steps of bead-based in vitro aptamer selection against β-amyloid 42

- DNA Library used (Yang and Bowser 2013)[6]:
  - 5'-AGCAGCACAGAGGTCAGATG (25N) CCTATGCGTGCTACCGTGAA-3'
- Incubated the DNA & protein in selection buffer at 25°C for 30 minutes
  - Selection buffer used: 1X PBS buffer with 5mM KCl & 3mM $MgCl^{2+}$ (PBS composition: 10mM phosphate, 150mM sodium chloride, pH 7.3 to 7.5
- For asymmetric PCR, we used 10uM forward primer and 1uM reverse primer
- Performed positive aptamer selection against recombinant his-tagged β-amyloid 42 protein that were immobilized on Ni-NTA magnetic beads
- Conducted negative aptamer selection against a protein-free tube to ensure specificity for only the target protein
- Performed symmetric PCR for 10 cycles prior to asymmetric PCR
- Performed cycle-course asymmetric PCR and gel electrophoresis to determine the optimal number of PCR cycles for sufficient DNA amplification without overamplification
- Tested DreamTaq MasterMix and Q5 high fidelity mastermix in control experiments to check for contamination
- Tested skipping gel extraction and instead did DNA cleanup with spin column to carry over double-stranded DNA for the next selection round

References and Acknowledgements

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All figures created in BioRender.

Results

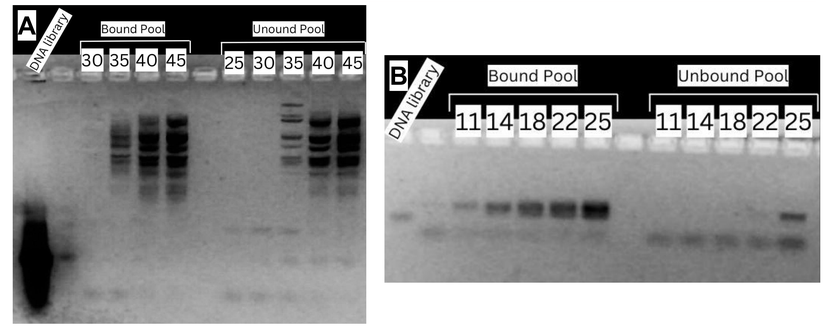

Figure 3: 3% agarose gel stained with GelRed, R3 Cycle course PCR gel
A: (using Q5 high fidelity mastermix) showing nonspecific amplification of our bound pool. Correct ssDNA banding appeared in the 35, 40, 45 cycle unbound pool. This was not seen in the bound pool.
B:(using DreamTaq mastermix) showing specific amplification of our bound pool. Correct ssDNA banding appeared in cycles 18, 22, and 25 of the bound pool cycles.

Low Yields from Gel Extraction:

- Low DNA yields from gel extraction prompted its substitution with DNA cleanup with spin columns prior to our next round of selection

Nonspecific Amplification:

- Ran tests to check for contamination in our samples and found issues with our DNA library (Yang and Bowser 2013) being contaminated
- Nonspecific amplification was attributed to skipping gel extraction protocols during prior rounds of selection

DreamTaq vs Q5:

- Switched from DreamTaq mastermix to Q5 high fidelity mastermix in our protocols
- Ran control tests and had more issues with nonspecific contamination with Q5 compared to DreamTaq

Future Work

- Perform a gel shift assay to visualize the interactions between aptamer candidates & β-Amyloid 42 on an agarose gel
- Identify aptamer candidates using DNA sequencing
- Start using a new library[7]:
  - library sequence: 5'- TCCGTGTGTAGTGTGTCTGNNNNNNNNNNNNNNNNNNNNNNNNCTCTTAGGGATTTGGGCGG - 3'
- Modified the new library by adding a 25-nt random region, replacing the 21-nt region with a GC-rich sequence to promote formation of secondary structures, which are more likely to act as aptamers.[8]
  - The random region ratio: 15% A, 30% C, 40% G, & 15% T
- This DNA library has been previously successful in selecting aptamers that bind to Interferon-gamma[9] and pluripotent stem cells[10]
- Planning to test Q5 Hot Start MasterMix to address nonspecific contamination issues