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DNA Aptamers Against Airway Mucin Proteins for Therapeutics and Diagnostics

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Background

- Mucus is a protective function of the body made up of mucin proteins, inorganic salts, and water.
- MUC5AC contributes to making a thicker mucus layer, providing shelter for viruses or bacteria.[1]
  - Elevated MUC5AC levels are associated with impaired mucus clearance and development of chronic obstructive lung diseases, including Chronic Obstructive Pulmonary Disorder (COPD)[1,2,3]
- Current treatments that break down mucin proteins involve the use of enzymes or chemical treatments. Treatments are non-specific, result in adverse side effects, and can cleave protective mucins, leaving cells vulnerable to infection.[4]
- Aptamers are synthetic, single-stranded DNA or RNA oligonucleotides that are selected to bind strongly and specifically to target molecules.

Figure 1. MUC5AC, secreted from goblet cells, makes up airway mucus that form hydrogel transported by cilia along airway surface.[1] Potential applications: The MUC5AC aptamer attached to a protease binds to MUC5AC and directs the protease for efficient cleavage or the fluorescently labeled MUC5AC aptamer tags MUC5AC.

References

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2. Song, D. *et al.* Modeling Airway Dysfunction in Asthma Using Synthetic Mucus Biomaterials. *ACS Biomater Sci Eng* **7**, 2723–2733 (2021).
3. Chatterjee, M., van Putten, J. P. M. & Strijbis, K. Defensive Properties of Mucin Glycoproteins during Respiratory Infections—Relevance for SARS-CoV-2. *mBio* **11**, 10.1128/mbio.02374-20 (2020).
4. Gupta, R. & Wadhwa, R. Mucolytic Medications. in *StatPearls* (StatPearls Publishing, 2023).
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Intent & Motivation

Intent: To select for a DNA aptamer that binds to the mucin protein MUC5AC within mucus samples for therapeutic and diagnostic applications.

Motivation: Selected aptamers can be used in therapeutics to delivered engineered proteases to cleave mucin proteins and in diagnostics to tag mucin proteins in mucus.

Methods

One Pot Selection

- Mucin protein is immobilized on PCR tube using a SpeedVac Concentrator
- Initial Bowser DNA library: 65 nt DNA library with a 25 nt random region.[5]
- Incubate DNA with immobilized protein on the side of the tube → remove non-bound DNA → carry out PCR in the same tube

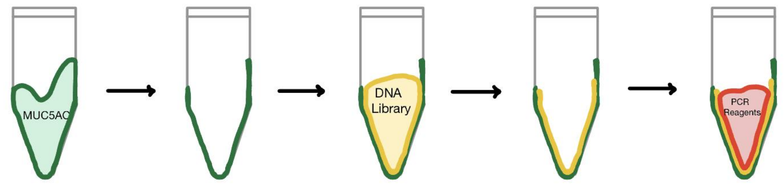

Figure 2. In tube selection process from Scoville et. al. against semi pure MUC5AC to generate aptamer candidates.[6]

In Tube ELISA Assay

- Confirms protein is immobilized on PCR tube and accessible to DNA library following immobilization on the SpeedVac Concentrator for 4 hours

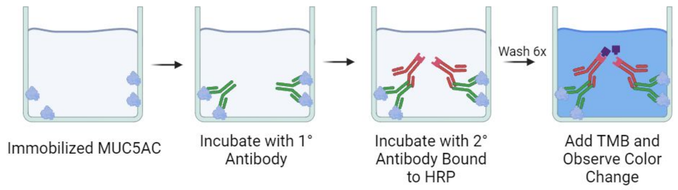

Figure 3. Indirect ELISA with primary and secondary antibodies to visualize protein presence. HRP oxidized TMB substrate to produce blue color change.

Amplification of single stranded DNA

Asymmetric PCR and Gel Extraction

- QIAGEN (silica bead) Gel Extraction Kit
- Cycle Course PCR used to determine number of cycles needed for asymmetric PCR

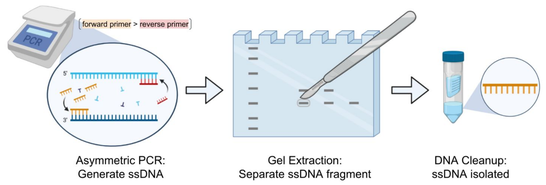

Figure 4. ssDNA is generated using asymmetric PCR. Asymmetric PCR products are run on a gel, and ssDNA fragments are extracted and purified.

Results

In-Tube ELISA of MUC5AC

- Left tubes: Positive Samples containing MUC5AC protein immobilized via SpeedVac
- Right tubes: Negative Controls containing nuclease free water
- Blue color change indicates presence of MUC5AC protein

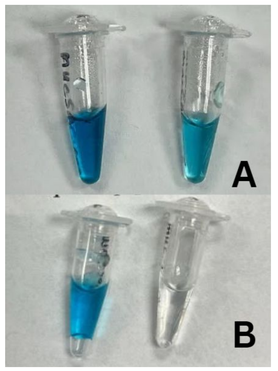

Figure 5. First ELISA test with non-specific binding, showing a color change in the negative control (**A**). Optimized protocol with more washes and a blocking agent showed expected results (**B**).

One Pot Selection

- Performed selection in tube with immobilized protein and single stranding regeneration method.
  - Approach: Symmetric PCR → cycle course PCR → Asymmetric PCR → Gel Extraction
- Cycle course PCR issues due to incorrect mastermix amount used
- DTT and BSA additives did not improve PCR.

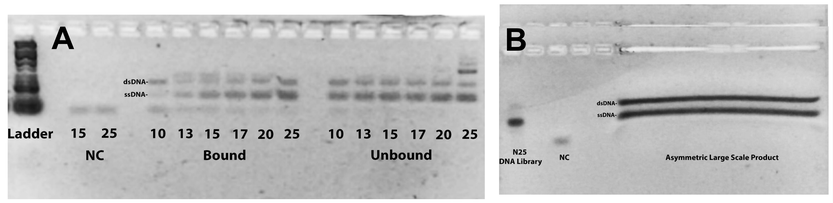

Figure 6: 4% agarose gel stained with GelRed of asymmetric cycle course PCR products of bound and unbound DNA post One Pot selection along with ladder and negative control (water). Optimal PCR cycle number was found to be 20 cycles (**A**). Gel of asymmetric large scale PCR (20 cycles) of bound DNA post One Pot Selection with a negative (water) and positive (DNA library) control. Single stranded DNA was extracted and purified with gel extraction kit (**B**).

Future Work

- Continue One Pot Selection against MUC5AC including negative and counter selections.
- Investigate alternative nitrocellulose filter selection method.
- Gel shift assay to assess binding affinity of potential aptamer.

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