MicroRNA Purification for Diagnostic Development

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Purpose

Purify MicroRNAs from plasma samples to develop a diagnostic blood test that tests for Aortic Aneurysm presence, growth, and location.

Background

Aortic Aneurysms

Aortic aneurysms are abnormal bulges in the walls of the aorta that can cause significant health complications depending on their size and location. As aneurysm size grows, tension along the vessel increases. This can cause the walls of the aorta to either dissect or rupture. Both can create false lumen. This can divert the blood flow away from important areas of the body, form blood clots, and increase internal bleeding. Testing for aneurysms is expensive, and they are often discovered accidentally.

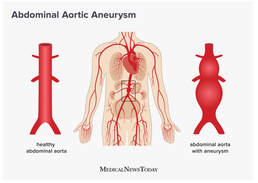

Figure 1 adapted from Medical News Today. Abdominal aortic aneurysm depiction

microRNA

MicroRNAs are small, single-stranded non-coding RNA sequences of approximately 22 nucleotides. They are used for gene regulation by either blocking translation or degrading the target messenger RNA sequence. Certain microRNAs are associated with aortic aneurysm growth (miR-133a, miR-133b, miR-331-3p, etc.

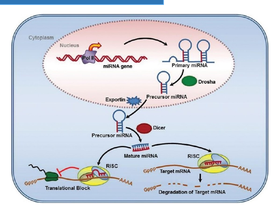

Figure 2 adapted from Pulmonary Vascular Research Institute. Depiction of microRNA function in gene regulation.

microRNA Purification

There are many types of nucleotide purification including precipitation purification, antibody purification, and charge-purification. The protocol used in experimentation is charge-purification. Post isolation of the nucleotide sequence, ethanol addition will shift the charge to be temporarily positive. Nucleic acids will then bind to the fiber glass filter. Centrifuging the column will remove the ethanol. The purified RNAse free water will reverse the charge back to the original, eluting the microRNAs.

Methods

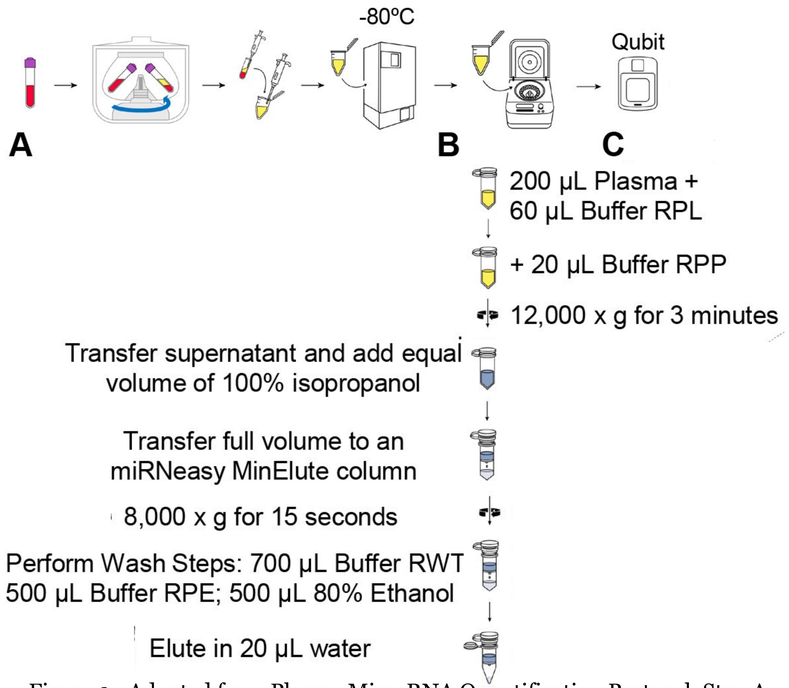

Figure 3 : Adapted from Plasma MicroRNA Quantification Protocol. Step A involves isolating the plasma samples from the blood and was done prior to experimentation. The samples are stored at -80°C until purification. Purification involves addition of buffers, ethanol, and DNAse/RNAse free water. Centrifuging and pipetting techniques were used. Qubit analysis was used for initial quantification in concentration (ng/ul).

Results

Sample Number	Concentration (ng/ul)	Sample Number	Concentration (ng/ul)
1	654	7	607
2	1350	8	509
3	1270	9	3800
4	802	10	1030
5	N/A	11	506
6	N/A	12	1290

Figure 4: Qubit Analysis readings of eluted microRNA from plasma samples. Samples are standardized against dyes, which then are tested for fluorescence. 2 ul of the eluted microRNA are added in with Qubit working solution to test for concentration in ng/ul.

Discussion

Data Interpretation

The charge purification methods used in experimentation yielded results in which the total microRNA present in each sample could be determined. Potential limitations of the project could be due to storage of RNAs because of degradation over time. Variation in data could be due to different samples used as human biology varies from different human subjects.

Future Work

Future work involves the next steps in the development of the diagnostic blood test. Normalizing the concentrations, selecting endogenous and exogenous controls, generating cDNA, and utilizing Digital Droplet PCR for quantification of each microRNA in samples. Finally, a Receiver Operator Curve will be performed to determine indicators of aortic aneurysms.

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