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SCHOLARSHIP WEEK

Isolation of an Aptamer Selective to Glucose

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Abstract: Diabetes is a disease that hundreds of million people live with daily throughout the world. Although this disease is typically not fatal, it can be if not treated properly. The day to day life of a person with diabetes consists of blood sugar monitoring by finger pricks, insulin injections and strict diet. The research for a glucose aptamer would be the first step to eliminate the need for all of this. This project uses Systematic Evolution of Ligands by Exponential Enrichment, or SELEX, to select RNA that binds specifically glucose. The process is a cycle beginning with a PCR from a pool of millions and billions different DNA sequences, then transcription to RNA, negative selection, positive selection, and reverse transcription back to DNA. The conclusion of the reverse transcription is the beginning of the next generation where each generation becomes more selective to glucose. Eventually the RNA would be sequenced and converted to a riboswitch. A riboswitch is a sequence of untranslated mRNA that can bind a specific ligand, in this case glucose, and transmit a signal to the expression platform to start the reaction to make a protein. For this project the riboswitch would begin the production of insulin only in the presence of glucose. By making insulin outside of the pancreas, diabetes patients would no longer need insulin injections or constantly monitor their blood sugar levels. The project is currently on its 25th generation and is continuing to move forward. Once we obtain a high ratio of positive over negtive cleavage percentages we will begin the process to clone DNA and individually test sequences to find an aptamer that cleaves only in the presence of glucose.

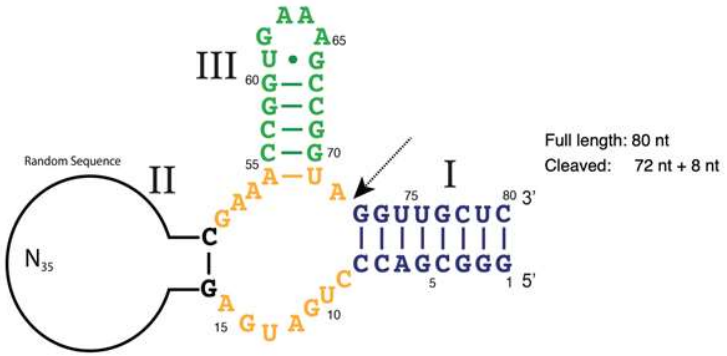

Figure 1: Hammerhead Ribozyme

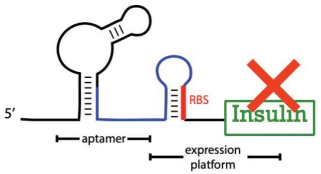

Figure 3: Hammerhead Ribozyme without glucose

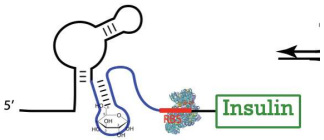

Figure 4: Hammerhead Ribozyme in the presence of glucose

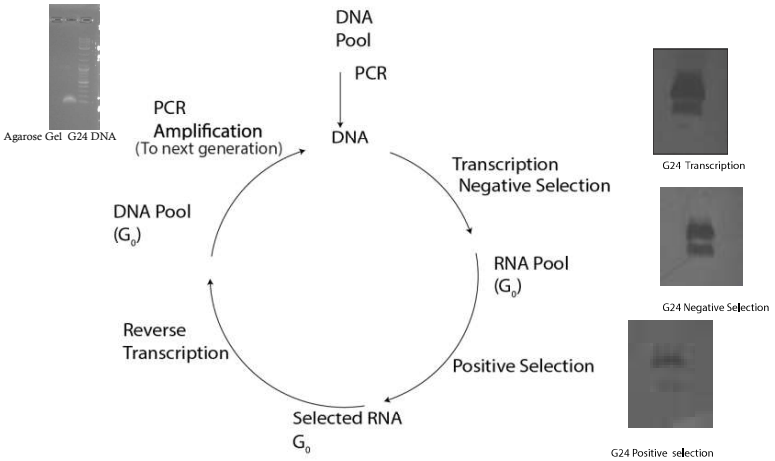

Figure 2: SELEX for glucose aptamer selection

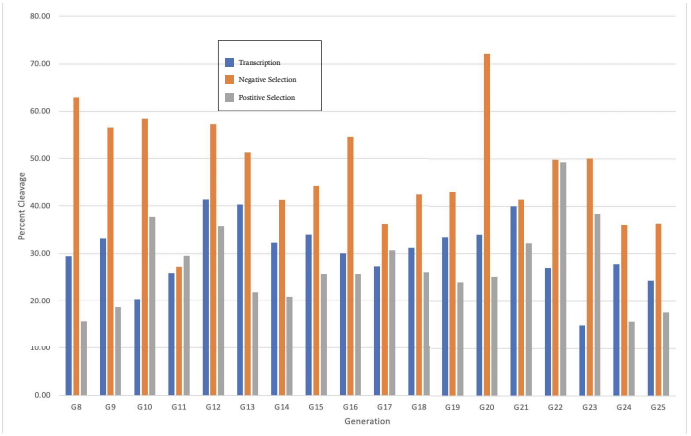

Figure 5: Aptamer Effiency over generations of SELEX.

Condition	Transcription	Negative	Positive
Magnesium	6mM	20mM	2mM
Tris	40mM	5mM	5mM
Ligand	-	20mM xylose, galactose and mannose	2mM Glucose

Table 1: Conditions of Magnesium Tris and Ligand for Data shown in Figure 5

Perspectives: The project is currently on the 26th generation undergoing SELEX. Once we obtain a high ratio of positive over negative cleavage percentages, we can begin the cloning and sequencing procedures. The aptamer once found, will be converted into a riboswitch. A riboswitch consists of the aptamer and an expression platform. The glucose will bind to the aptamer and then send a signal to the expression platform which will begin the production of insulin. This would allow insulin to be produced only in the presence of glucose and propose a significantly easier way to treat type 1 diabetes.

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