Multi target Ligand-Guided Selection (LIGS) to generate aptamers against B-cell biomarkers

Nucleic Acid Aptamers (NAAs) are single-stranded ribo- and deoxyribo-oligonucleotide molecules, which fold into complex functional three-dimensional structures. These three-dimensional structures can bind to target molecules with high affinity and specificity. Nucleic Acid Aptamers are selected through an iterative process called Systematic Evolution of Ligands by Exponential enrichment (SELEX). Here, we implemented Ligand-Guided Selection (LIGS), a variant of SELEX, to elute aptamers against multiple biomarkers expressed in B-cells, i.e., CD19 and CD20. During LIGS step, specific aptamers against CD19 and CD20 were competitively eluted using two specific monoclonal antibodies against CD19 or CD20 at 25°C. The eluted multiple libraries were then sequenced using the Illumina high-throughput (HT) DNA sequencing platform. The resulting sequences were analyzed using a novel bioinformatics workflow designed using GALAXY, an online bioinformatics server. A total of 18 candidates were identified against CD19, and 13 candidates were identified against CD20 based on defined enrichment values. The potential hit sequences were subsequently synthesized and screened against CD19 and CD20 positive or negative cell lines to identify aptamers candidates against respective markers. Collectively, this study establishes LIGS as a state-of-the-art screening technology that can be used to generate highly specific aptamers against multiple receptor-proteins in their native state expressed on one cell population.