The Importance of Negative Aptamer Selection

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Aptamer Selection

Aptamers are DNA- or RNA-based ligands capable of selectively binding practically any molecular target. They are commonly identified by an in vitro method of selection referred to as Systematic Evolution of Ligands by Exponential enrichment or "SELEX". The process begins with a very large pool (~10^{13} to 10^{15} unique sequences) of randomized polynucleotides, which is generally narrowed to just a few aptamer binders per molecular target (3,10). Figure 1 shows a simplified schematic for repeated rounds of SELEX of DNA aptamers. In this design, the aptamer library is exposed to the immobilized target. Non-binders are eluted, binders are eluted and amplified, and the resulting pool is re-exposed to the immobilized target. Once multiple rounds of SELEX are completed, the best DNA sequences are identified (based on sequencing, bioanalysis, and other analytical methods) and tested for affinity and selectivity.

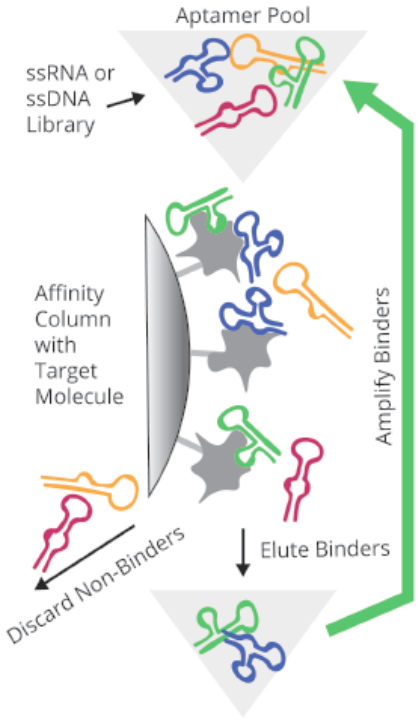


Figure 1. Traditional SELEX

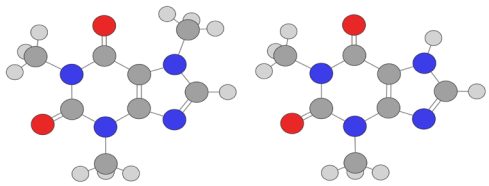
Over the years, many variations of the SELEX process have been developed to speed the enrichment process or improve selection for success in specific applications (8). Despite the many differences in aptamer selection strategies, most are incorporating negative selection (counter selection) steps to reduce the enrichment of aptamers to unwanted targets and improve aptamer selectivity and affinity.

The Origination of Negative Aptamer Selection

The first modification made to the SELEX process in the early 1990s was negative selection against selection components. In an early stage in the SELEX process, the oligonucleotide library was added to the SELEX system without the target. This eliminated sequences binding directly to the purification support. Affinity of the final aptamers was 10-fold higher when a negative selection step was conducted (1). More recently, researchers developing selective aptamers to ibuprofen performed negative selection with bare magnetic beads following the fourth round of SELEX. Eliminating ssDNA with affinity to the bare beads reduced the number of sequences by 56%. (7) Negative selection against generic SELEX components has become fairly routine. As selection has evolved, researchers have gone beyond negative selection for generic SELEX components and added negative selection (counter selection) steps that actively seek to remove sequences binding to unwanted targets from the oligonucleotide pool.

Achieving Selectivity for Highly Similar Molecules

Negative selection has been effectively used to generate aptamers without co-affinity for molecules that are highly similar in structure to the target. In the early 1990s, Jenison used negative selection with caffeine to generate aptamers that were selective for theophylline and did not bind caffeine, differing by a single methyl group (1,5).



Caffeine Theophylline

Researchers developing enantioselective aptamers to ibuprofen performed counter selection following the eighth round of SELEX using anti-inflammatory drugs structurally similar to ibuprofen. Beads coated with fenoprofen, flubiprofen, and naproxen eliminated 22%, 23%, and 13% of ssDNA sequences, respectively, demonstrating that negative selection for several targets can be conducted simultaneously. Binding studies were performed following selection to identify aptamers with enantioselectivity for (R)-ibuprofen and (S)-ibuprofen (7).Researchers in Hungary selected DNA aptamers to human respiratory syncytial virus (RSV) using immobilized inactive RSV. Following negative selection against antibody modified beads used for immobilization, a second negative (counter) selection step was conducted to eliminate oligonucleotides that bind human rhinovirus (HRV), a virus that commonly coinfects the respiratory tract. The resulting aptamer selectively detected RSV in complex matrices (9). Negative selection is a powerful tool in the development of selective aptamers.

Selective Binding in Complex Matrices

The use of purified targets, strategic negative selection, and appropriate selection buffers can be combined to discover aptamers that selectively bind targets in complex samples for detection or purification. Unfortunately, developing purified targets for selection can sometimes be a challenge. The researchers in Hungary who developed selective aptamers to the RSV virus utilized a virus-specific antibody to immobilize RSV virus that was only partially purified (9) (This technique is also useful for discovery of a selective aptamer to pair with a known antibody.) Researchers at York University in Toronto showed that negative selection is not required to develop selective aptamers from a complex sample if the target protein is over-expressed in the sample. In a titration experiment conducted with cell lysate, library enrichment for the target protein occurred without negative selection when the target protein was >0.8% of the total protein in the lysate. This suggests that for certain applications, such as development of aptamers for production purification, purified target and negative selection may not be required, simplifying and speeding the aptamer selection process (4).

Selective Binding to Unique Cell Types

Aptamers with selectivity for known cell surface markers have demonstrated unique utility in tissue staining, cell imaging, and in vivo cell targeting. For known cell surface markers, researchers are utilizing whole cells during the selection process to generate aptamers with selectivity to sites that are available when proteins are bound to the cell surface. More recently, researchers have turned to whole cell SELEX to identify aptamers with selectivity for unique cell types without prior knowledge of a cell surface protein for targeting. Several groups have used positive selection with malignant cell lines and negative selection with non-malignant cell lines to avoid enrichment of aptamers to unwanted targets expressed on both target and normal cells, generating highly selective aptamers (2).

Researchers in China selected for aptamers to nasopharyngeal carcinoma (NPC) cells. The NPC 5-8F cell line was used in the first round for positive selection. In each subsequent round, nonmalignant human nasopharyngeal epithelial cells, NP69, were used for negative selection, followed by positive selection with 5-8F cells. A total of 25 rounds were completed. The process yielded an aptamer with excellent selectivity for NPC cells, no recognition of normal cells, and minimal recognition of other malignant cell types. Using this selective aptamer for affinity purification of the unknown target, the aptamer target was determined to be CD109 via mass spectrometry. Negative selection is a critical element in the selection of aptamers to low-abundance biomarkers (6). See the review by Catuagno for several more examples of the use of cell-SELEX and negative selection to identify selective aptamers for biomarker discovery and detection.

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Aptamer Discovery Design

As researchers work to develop aptamers that can differentiate between highly similar compounds and utilize aptamer selection to discover low-abundance cell surface markers, negative selection has become increasingly important. Determining the need for negative selection, the targets for negative selection, and the timing and number of rounds of negative selection are all critical decisions in aptamer discovery design. For more information regarding negative selection and aptamer discovery design for a specific application or project, please contact a Base Pair aptamer specialist at info@basepairbio.com.

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