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Newsletter # 7: Aptamer free/Free selection

We have worked for several years to develop a method for the selection of aptamers that did not require immobilization of the target or the oligonucleotide library. After trying numerous approaches that did not work, and were increasingly complex, we have developed a relatively simple method.

First, why is free/free selection important?

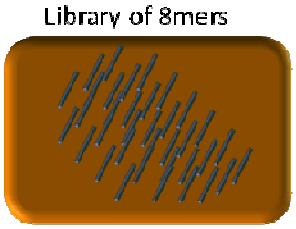
Aptamer selection requires partitioning of bound oligonucleotides from unbound ones. The use of nitrocellulose comes close to a free/free system but as anyone who has actually used this system can attest that there are many drawbacks. The principle behind nitrocellulose based selection is that proteins are retained on nitrocellulose while single stranded oligonucleotides pass through. This excludes the use of this approach for small molecules. We have found that a portion of most proteins will pass through nitrocellulose, and that nitrocellulose will bind some oligonucleotides based on their secondary structure. We abandoned using this method years ago because it was simply too unreliable. This meant that we had to immobilize target molecules. I think of molecules as represent a charge field in a three-dimensional space. Immobilizing a molecule by conjugating it to a solid support necessarily affects this charge field. This means that certain epitopes are lost and that novel epitopes that may be present only as a function of the conjugation are created. With small molecules conjugation can entirely remove any epitopes that would be present in the free form. For proteins, immobilization can affect folding.

Another significant problem with the need to immobilize targets was that this act implicitly removes complexes. Once the target molecule is immobilized, then there is a need to wash out all non-immobilized targets as their presence would prevent effective partitioning between bound and unbound sequences. This washing will also remove anything that is complexed to the target. Only if the nature of the complex is known, it can be rebuilt.

How does it work?

It is a two-step process. We want to focus selection on the random region of the library, so we add blockers to bind to the primer recognition sites.

A library of these blocked sequences is exposed to a lawn of immobilized random eight nucleotide sequence oligomers.



We use a library of 8mers for a large number of mathematical and biochemical reasons. One mathematical reason is that the total possible number of sequences is 65,536. We can achieve many duplications of each possible sequence on a 1 cm^2 gold chip. We block the remaining surface with thiolylated PEG molecules. We call this surface an immobilization field. Those sequences in the blocked library that do not bind to this surface are discarded. We continue only with those sequences that have the capacity to bind. We elute these sequences from the surface, and combine them with the target molecule. In the second phase of selection we expose this mixture to the immobilization field and this time we select for sequences that do not bind to the surface.

The change from a sequence that is able to bind to the surface to a sequence in the presence of a target molecule that now cannot bind to the surface indicates that the process of binding has made the sequence less available for binding to the lawn of 8mers.

We have successfully completed several projects with this approach as well as developed aptamers for our own targets. We analyze these sequences across selection rounds with the same next generation sequencing software that we have pioneered with immobilized targets.

We have filed for patent protection on this platform, but will apply this approach to the identification of aptamers for clients without changing our industry leading IP policy. We consider this as an exciting development.