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Newsletter # 5: To counter select, or not to counter select?

That is the question:

Whether 'tis nobler in the mind to suffer

the slings and arrows of outrageous counter selectio ,

Or to take arms against a sea of troubling sequences

And by opposing select only the ones that are specific.

All aptamer companies, we included, will tell you that one of the advantages of aptamers is our ability to introduce counter selection for specificity into the process. In general, the statement and the concept is true, but counter selection is something that must be approached with caution, and must be applied intelligently or you will end up with aptamers that have neither affinity nor specificity.

Successful selection is similar to bacteria growing on media. The observation of a colony is the result of exponential growth by one bacteria. The difference with aptamer selection is that the total number of sequences is not increasing, but individual sequences are growing in terms of their proportion within the population. When we do next generation sequencing, even though we capture millions of sequences, we are still only sampling a very small portion of a library of 10^{15} sequences. A million copies represents only 0.0000001% of the entire initial library. To see an increase in copy number for a single sequence means that this sequence has to increase exponentially. For selection to succeed, it is important that the selection pressure is strong in one uniform direction.

Let us consider counter selection in the development of aptamers for a single glycosylated amino acid on a protein. The protein with the glycosylated amino acid is used for positive selection, and the same protein without the glycosylation is used for counter selection .First, we need to do a few rounds of positive selection with just the modified protein, because if we start with counter selection, and we happen to lose the most specific aptamers, we will not have any sequences left in the population to select from. We want to increase the copy number of the specific aptamers before we introduce counter selection.

There are many possible epitopes for which aptamers are selected within the modified protein. The site containing the modified amino acid may only represent one epitope. It is improbable that the site containing the modified amino acid represents the epitope with the highest affinity. There are likely other epitopes in the protein for which aptamers in the random library bind with a higher affinity than any sequence that binds to the modified amino acid epitope. Thus, as a result of positive selection both specific and non-specific aptamers will increase in copy number.

Then we introduce counter selection by exposing the selected library to the normal protein and we only retain those sequences that do not bind. But there's the rub!. Not all the copies of those sequences that bind to the normal epitopes will find their epitope and bind to it. Some of these will still be retained from the counter selection, and they will be selected for again in the next round of positive selection with the modified protein. This maintains at least some competition against the specific aptamers and inhibits their ability to increase in proportion.

The whole situation becomes worse if we cannot be certain that all of the modified protein contains the modified amino acid, and all of our normal protein does not. For these reasons, counter selection for specificity for small molecules works much better. With small molecules, the contrast between positive and negative epitopes is clear and strong.

I do not mean to say that selecting aptamers for single modified amino acids within a protein is impossible, it is just difficult.

There are approaches that we use:

1.) We track the trajectories of the increase in copy number over selection rounds. We will also include a selection channel whereby we reverse the process and select for the normal protein, as positive, and the modified protein as counter.

2.) A better approach is to use interaction among proteins as the selection strategy. If there is a protein that binds to the target site, only when it is modified then we use this interaction to elute aptamers bound to this site alone. This represents a positive and clean selection for the specificity that we desire. This is a great approach for identifying aptamers that will interfere with ligand/receptor interactions.

The key message here is that the selection strategy has to be carefully and intelligently designed for each target, and monitored dynamically throughout the selection process. Time spent on this phase is the most important step.

Hamlet did spend a lot of time thinking, but perhaps things would have had a better outcome if he had developed a different strategy in the first place.