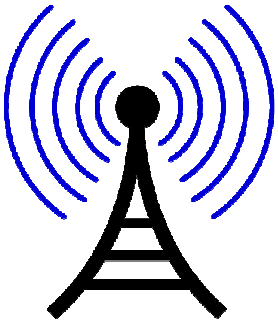
NeoVentures Biotechnology Inc.

5 York Street, Unit # 3,London, ON, N6A 1A1. Canada .519.601.7511

Newsletter #3: Signal detection with aptamers



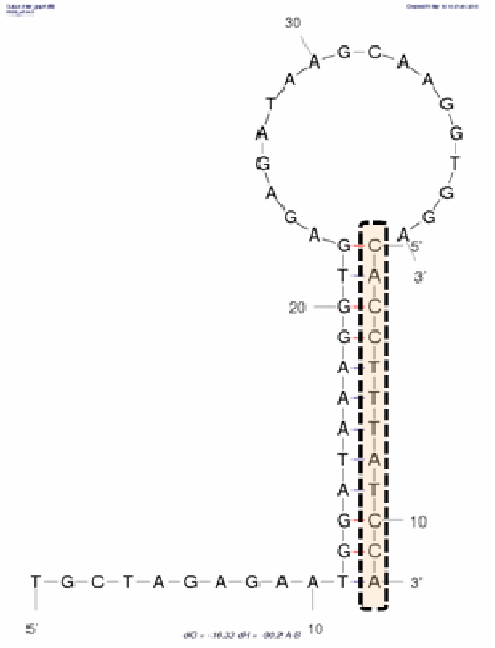
In this newsletter my intent is to review the various options that exist for signal detection in aptamer based diagnostics. I am going to avoid sophisticated applications such as single molecule cantilevers or channeled light waves. For now, let's stick to the basics.

It is straightforward to have a fluorophore or a biotin attached to either end of an aptamer. As a detection aptamer in sandwich system where the capture ligand is either an antibody or an aptamer both of these moieties enable direct measurement of the amount of detection aptamer bound to the target. With biotin, we apply a streptavidin/horseradish peroxidase conjugate for colour detection. The streptavidin portion of the conjugated protein binds to the biotin on the aptamer, while the HRP portion serves as the basis for the generation of colour.

Both systems work well. The use of colour is often favoured because instruments that are capable of reading colour absorption are less expensive than fluorescence readers. Sensitivity may vary depending on the matrix analyzed. It is important to remember that the colour system is an amplification of signal, as each HRP molecule bound can effect multiple colour changes.

One interesting application of fluorescently labeled aptamers is the use of fluorescence polarization. We have developed a commercial diagnostic kit for the prediction of breadmaking potential based on this approach (check out www.risingnumber.com for more information). We developed an aptamer that binds to a common domain across all groups of wheat storage proteins. We labeled this aptamer with a fluorophore. We combine it with the various extracted wheat protein fractions and measure the tumbling speed of the aptamer in each protein containing solution through the use of a hand held fluorescence polarization instrument. The proteins are much larger than the aptamer and thus the complex between the protein and the aptamer represents a slower tumbling speed than the aptamer alone. The beauty of this approach is that it does not require immobilization of the aptamer, reactions are performed in an entirely free system.

A key feature of aptamers is that they are single stranded oligonucleotides. This means that antisense constructs can be designed that will anneal at varying strengths depending on the degree of homology and the length of the antisense oligonucleotide. We can engineer antisense constructs with almost any binding strength for the aptamer that we want. We have found that we can obtain optimum sensitivity within detection systems when we use antisense constructs that have approximately the same binding affinity for the aptamer as the aptamer has for its target.



Designing antisense sequences that bind with a specific desired affinity is not trivial. We are in the process of developing algorithms that are useful for predicting k_D from melting temperature. This is a complicated process however, given that the secondary and tertiary structure of both the independent aptamer and the antisense construct must be taken into account. We generally try to make the antisense construct as long as possible to increase specificity and reduce affinity through the introduction of mis-sense nucleotides. The introduction of mis-sense nucleotides provides us with greater fine control on binding affinity than truncating or extending the antisense construct.

Antisense constructs make a lot of sense when working with small molecules. It is much less expensive to synthesize a labeled antisense that competes with the native target in a sample than it is to produce labeled target molecules. We have the same capacity for detection in terms of fluorescence or HRP based colour detection with antisense sequences as we do with aptamers.

We have developed a patented process whereby we can capture positive correlations between the displacement of the antisense from the aptamer in the presence of the target through the use of an immobilized third oligonucleotide which we call an anchor. This molecule has homology to an extension of the antisense that does not have homology with the aptamer. Thus, the more antisense displaced from the aptamer free in solution by the target, the more antisense available to bind to the anchor molecule. This approach has the advantage that we can design the binding affinity between the antisense and the anchor molecule with high affinity, thus allowing us to wash the system as thoroughly as we want. We have called this system "Shadow Sense". If anyone is interested in learning more about it, send us a note.

This newsletter provided a brief introduction to aptamer based detection platforms. Every system still requires considerable effort to optimize signal/noise ratios prior to commercialization. I think that we need to think differently about how we use aptamers in diagnosis and avoid being trapped by antibody based paradigms.