Agilent case study

Cellular Metabolism Reveals Insights into Biology and Potential for Therapeutic Discovery

Imagine you've been reading a really great book, but for some reason you're unable to finish it. Maybe you lost the book, or maybe you get to the best part only to find someone tore out the key chapter. Frustrating, to say the least, but you can probably get another copy.

An analogous situation can sometimes occur during the biological process of translation, when an mRNA is "read" by a ribosome to produce a protein. If the mRNA contains an ill-placed termination codon, translation gets halted prematurely and a functional protein – perhaps an essential one – does not get produced. The consequences of this situation can be much more than frustrating; the lack of that protein may lead to a serious disease.

Scientist Syed Ali and his team want to make sure cells tell the whole story.

"Our work begins with powerful informatics tools that help us identify target genes," Ali explained. "We then synthesize small RNA fragments called antisense oligonucleotides, or ASOs, that are designed to modulate expression of the target protein. These ASOs are carefully examined, using tools such as qPCR and western blots, to ensure they are having the desired effect."

But while target effect is a good sign, it is not the ultimate goal. It is imperative that the ASO works as a therapeutic agent; that is, it has to alleviate the disease state and improve patient health.

"At this point, the biology becomes our primary focus," Ali continued. "We look closely at the candidate ASO in terms of functional activity to understand how it impacts key cellular processes such as basic metabolism. This is where Seahorse XF plays a vital role – we closely examine mitochondrial bioenergetics and the glycolytic pathway as important measures of the impact of the ASO on overall cell health."

Syed Ali, Scientist

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Ali and his team moved to adopt the Seahorse XF because their review of relevant literature citations suggested it was the "gold standard" platform for these purposes. Of particular interest was the Cell Mito Stress Test, which provides them with much-needed insights into basal respiration, ATP production, maximal respiration, and spare respiratory capacity.

"Most published studies report these four parameters as a way of getting a broad, yet detailed, view of cellular function," he said. "When we first brought the Seahorse XF online, we quickly saw results that confirmed it was the right technology for us – we were able to reproduce results that had been reported by other groups, so that helped us align our work with what was being reported in the literature. When we saw that, we truly believed in this technology."

Ali also reports that few groups are using the Seahorse XF platform to investigate the efficacy of therapeutic ASOs the way they are, which has led them to explore new ways to use the data it provides to guide their work.

"As we push our research into new directions, we've come to rely on the Seahorse XF more and more," he said. "For example, we've had to develop our own methods for delivering ASOs into our *in vitro* systems, and Seahorse technology has become an integral part of those efforts. Getting reliable information about things like ATP production is important for our work, and the results we see should be consistent. If the data is well clustered, that helps us determine whether we're looking at something we can trust."

Beyond the critical metabolic insights it delivers, Ali expressed appreciation of the Seahorse XF from a practical standpoint. "I had to develop an image-based assay to count our cells in a 96-well plate format, which was a challenge," he explained. "We now have a high-content imager that allows us to easily place the Seahorse plate in to count the cells, which is very convenient. Also, it's useful that the Seahorse software has a user-friendly interface that allows us to directly export our results into Excel, so it's easy for us to extract our data in a readily usable form."

"In our industry, especially for small start-up companies like us, time is of the essence," he continued. "Whatever works is what we use. As long as the Seahorse XF continues to deliver gold-standard results, it will have a place in our lab."

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