



# Profiling the expression patterns of hundreds of microRNAs with the HS 4800™ Pro Hybridization Station

Exiqon is a Danish biotechnology company that provides products and services for the life sciences, research and drug discovery industries all over the world. It specializes in microRNA (miRNA) analysis and produces unique, locked nucleic acid (LNA™)-based high affinity arrays for miRNA profiling. Tecan HS 4800™ Pro Hybridization Stations were used during the development of these arrays and Exiqon now relies on the HS 4800 Pro systems to provide miRNA profiling and analysis services.

MicroRNAs have recently generated a great deal of interest and are now recognized as important post-transcriptional regulators of gene expression in a variety of biological functions (reviewed in 1-3). However, the actual functions of miRNAs and the mechanisms by which they are controlled remain unclear. Many organisms express hundreds of miRNAs that vary in abundance across cell and tissue types. The total number of miRNAs in vertebrate genomes is currently unknown, but hundreds have already been isolated and the final numbers may reach over 1,000. A great deal of research remains to be carried out into these molecules, including their possible roles in human diseases and potential for novel therapeutic tools.

Exiqon, a biotechnology company based in Copenhagen, Denmark and Woburn, USA, produces the miRCURY™ LNA microarrays, which were specifically developed for miRNA expression profiling. Using a high affinity nucleotide analogue, locked nucleic acid (LNA™), Exiqon creates oligonucleotide probes with greatly increased affinity for their complementary targets, due to the increased melting temperature ( $T_m$ ). This has proved to be particularly useful for detecting short nucleic acid targets such as miRNAs, as standard oligonucleotide technologies have insufficient specificity and affinity for these molecules. The miRCURY™ LNA arrays have almost 1,500 LNA™-based capture probes, making it possible for researchers to profile all miRNAs from vertebrates, invertebrates,

plants and viruses (as registered in the miRBase 8.1 database at The Wellcome Trust Sanger Institute, UK) and to cross-profile between different species. The microarrays also contain a number of capture probes (miRPlus™ probes) for detection of miRNAs that are not included in miRBase. The microarrays have low sample usage requirements, being able to work with as little as 1 µg total RNA, and do not require miRNA enrichment.

Exiqon's miRCURY™ LNA arrays were developed using a Tecan HS 4800 Pro Hybridization Station, which offers fully automated, highly sensitive microarray processing with high reproducibility and flexibility. "We started using the HS 4800 Pro during the development of our array products because we needed an automated system for hybridizing and washing the slides," explained Ina Dahlsveen, Product Manager for Services at Exiqon. "It is very important to have as little variability between slides as possible, and using the HS 4800 Pro improves our reproducibility and minimizes technical issues that can arise during manual hybridization. Although our slides are compatible with the manual systems available, these are less efficient for processing large numbers of slides."

In May 2006, Exiqon started providing miRCURY™ LNA array profiling services, as Peter Roberts, Brand Manager at Exiqon, explained: "The services were actually set up in response to specific demand. After we launched our microarrays, we were getting at least one or two enquiries every week asking if we could run an analysis service, despite the fact that we never advertised one. So when we set the services up, it was a natural progression for us to incorporate the Tecan hybridization stations with them."

"Obviously with services you also need high throughput, as well as good reproducibility," Ina continued. "We soon acquired a second HS 4800 Pro system, and we will get a third station in the near future. Most of the samples we receive are human, but we also get many samples from mouse and rat, and our newest array contains capture probes for miRNA from all types of organisms. We have received

samples from plants, zebrafish, *C. elegans*, domestic cow and so on – there are customers out there working on all kinds of wonderful organisms. Our turnaround time is variable depending on the size of the project; at the moment, an average project involves about 10 to 12 slides and takes about two weeks from when we receive the samples until we deliver the results. Our services include data analysis, depending on the customer's requirements. Every project is different so we try to be as flexible as possible and customize our services for each customer and their samples."

"The arrays work very well with the Tecan hybridization stations, there is very good compatibility between them," added Peter. "We have had a few instances where customers that are using our arrays for the first time come across some technical problems but, by taking a Tecan hybridization station on-site and running the arrays with the customer, the problems have been solved."

The compatibility is so convincing that Exiqon's US office in Woburn, Massachusetts, routinely sends Tecan hybridization stations to its new miRCURY™ LNA customers to aid them in setting up new array procedures. Mikkel Nørholm, Manager of Technical Sales Support at Exiqon in the US, explained: "When a laboratory starts using a new technology, it normally takes some time

to get everything working perfectly. During that process it's really helpful to have a gold standard, something that you know works. Combining a Tecan hybridization station with our predeveloped miRCURY™ LNA protocol means that even for inexperienced users, the procedures work straight away."

The program for Exiqon's automated microarray hybridization protocol was originally developed in collaboration with Erasmus MC, based in Holland. "The reproducibility you can get with the hybridization stations, even across different laboratories, is so high that we could simply install the program exchanged by e-mail and it worked instantly!" said Mikkel. "This is also a major benefit for our customers as they can start on their experiments much more quickly after receiving the program from Exiqon electronically."

#### References:

1. Bartel DP (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**(2): 281-297
2. Shivdasani RA (2006). MicroRNAs: regulators of gene expression and cell differentiation. *Blood* **108**: 3646-3653
3. Wang Y *et al.* (2007). MicroRNA: past and present. *Frontiers in Bioscience* **12**: 2316-2329

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