

Customer Application Stories

miRCURY LNA™ Products
for microRNA Research

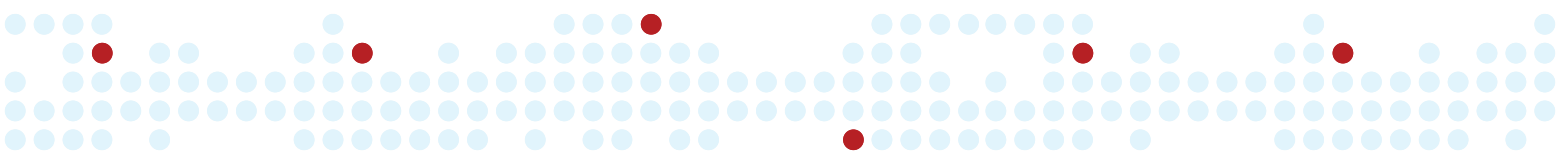


Dear microRNA Researcher

2 Exiqon | Customer Application Stories

Exiqon has a strong ambition that we pursue with persistence: we strive to deliver **best-in-class scientific support for the microRNA research community**.

On these pages, you can learn how researchers apply Exiqon's products and services in remarkable microRNA research projects.





Isolation

Expression
Analysis

Localization

Functional
Analysis

Determining the molecular mechanisms of nasopharyngeal carcinoma

miRCURY LNA™ microRNA Power Labeling Kits

Dr. Xin Li has just completed a three-year fellowship at the NIH and is planning to begin work at the Cancer Research Institute of Southern Medical University in Guangzhou, China.

What is your research area?

We are studying the molecular mechanisms of nasopharyngeal carcinoma, a cancer that occurs with high frequency in people from South Asia, especially South China. Our focus is on the role of specific candidate genes and important susceptibility genes in pathogenesis. Our studies have included functional analysis of candidate genes, animal models, and cancer stem cell research. Together with the Immunogenetics Section at the NIH, we have looked at microRNA expression and cDNA gene expression in cancer immunological therapy.

What has been your experience in microRNA profiling?

At the NIH, we labeled microRNAs in tRNA samples and then hybridized them to custom printed microRNA arrays.

What is your experience using the miRCURY LNA™ Power Labeling Kit from Exiqon?

I have used Exiqon's Power Labeling Kit and compared it with the products from other companies such as Ambion. Exiqon's product is impressive. The procedures are easy to understand and very fast. We were especially satisfied with the low amount of starting total RNA needed. We got beautiful results every time.

In your opinion what are the primary advantages of using miRCURY LNA™ Power Labeling Kit compared to other types of microRNA labeling kits?

The nasopharyngeal carcinoma samples we use are usually limited. The small starting amount required for Exiqon's kit was a critical factor to us. Furthermore, the Power Labeling Kit is very fast, thus our samples are labeled and ready for hybridization in just half a day.

How did you validate the results from the microRNA arrays?

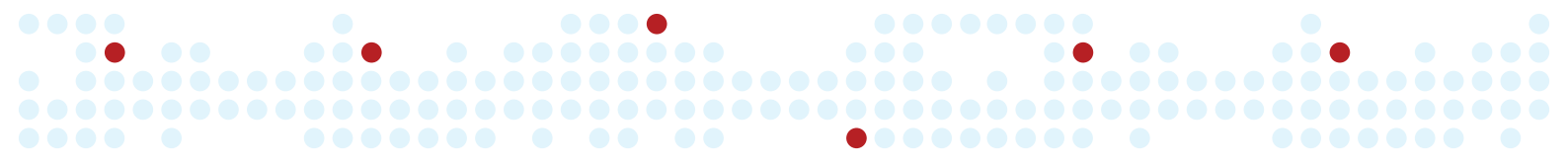
So far, we have used our own real-time qPCR in tissue and *in situ* hybridization in cell-culture, which takes a bit of time to optimize. We hope to use the miRCURY LNA™ *in situ* probes and real-time PCR kit in the future.

What are your future plans for microRNA research?

Soon I will return to China and finish the microRNA expression profile analysis in nasopharyngeal carcinoma, which has not yet to be reported so far. Then we hope to study the metastasis of this disease and cancer stem cells.



Dr. Xin Li,
from the Cancer Research
Institute of Southern Medical
University in Guangzhou,
China.





Isolation



Expression Analysis



Localization



Functional Analysis

Investigating the role of microRNA in iron homeostasis and related disorders



Prof Martina Muckenthaler,
from the University
of Heidelberg and
EMBL, Germany.

miRCURY LNA™ microRNA Microarrays

Prof Martina Muckenthaler is Head of Molecular Medicine at the University of Heidelberg. Her current research areas include the role of iron in health and disease, haematological malignancies and molecular diagnostics. Here, she describes part of her research on microRNA and how she has been able to use the technology incorporated in miRCURY LNA™ Arrays in her work.

Can you describe the current research going on in your laboratory?

My research focus is on regulatory mechanisms and gene networks involved in iron homeostasis and related disorders. The research group is integrated within the Molecular Medicine Partnership Unit (MMPU), an interdisciplinary cooperation between the European Molecular Biology Laboratory (EMBL) and the Medical Faculty of the University of Heidelberg. Within this unit we perform translational research that addresses regulatory circuits, such as those involved in non-sense mediated decay, cystic fibrosis or cancer.

How did your research lead you to the study of microRNAs?

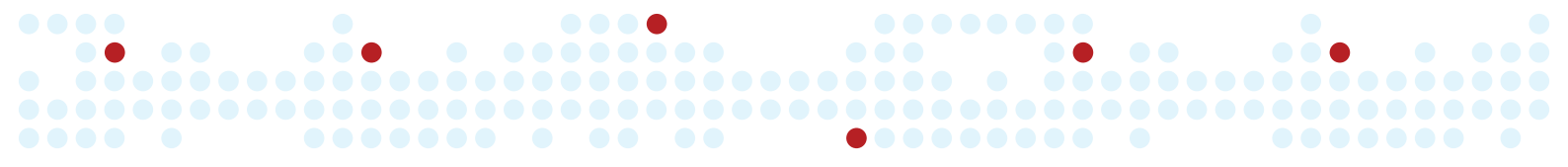
In recent years it became evident that miRNAs can be highly valuable as clinical markers for understanding molecular disease mechanisms and possibly for therapeutic intervention. miRNA response patterns are currently being evaluated for their role as biomarkers in acute childhood leukemia and breast cancer as well as for the understanding of regulatory processes involved in maintaining iron homeostasis.

We have a long-standing interest in post-transcriptional control mechanisms and regulatory networks involved in disease. Plus, we have access to clinical samples within the Department of Medicine and within the National Genome Network (NGFN-2), which funds our research. These two factors, added to our expertise in establishing specialized microarray platforms, made us decide to develop a sensitive array for genome-wide miRNA expression profiling (miChip).

For what reasons do you think there is so much current interest in microRNAs?

The discovery of miRNAs has revealed a new regulatory concept of gene expression. Several observations that could not be explained before by protein coding mRNAs can now be explained by mechanisms involving miRNAs. For example, miRNAs are frequently located within chromosomal regions that have been closely linked to cancer. These include minimal regions of amplifications or loss of heterozygosity, as well as chromosomal breakpoints. For example in the case of B-cell chronic lymphocytic leukemia (CLL) the 13q14 chromosomal region has long been scanned for the presence of oncogenes or tumor suppressor genes – without success. Recently, mir-15 and mir-16 were discovered within this chromosomal location and it turns out that these miRNAs play a role in enhancing apoptosis by decreasing the expression of the anti-apoptotic factor BCL-2.

In addition, experimental data indicates that cell-type specific miRNA expression remains largely unchanged in resulting tumors. It seems that tumors are better classified by miRNA expression than by mRNA expression patterns that are rather more complex.



This opens the possibility that miRNA expression patterns will be useful as clinical markers to be correlated with parameters like disease prognosis, therapy related toxicity, and the likelihood to develop secondary malignancies.

Cloning efforts and bioinformatic predictions suggest that miRNAs may regulate up to 20%–25% of mammalian genes. The accurate profiling of miRNA expression thus represents an important tool to investigate physiological and pathophysiological states. Both the qualitative and the quantitative expressions of miRNAs, therefore, are expected to exert a profound regulatory influence on the transcriptome of a given cell or tissue

What led you to try out LNA™-based arrays for microRNA profiling?

Well, first of all we didn't have much success with capture probes of conventional chemistry. We realized that, in contrast to our experience with mRNA expression profiling DNA-microarrays, we were not able to improve our results by playing with hybridization conditions, probe design or slide coating. We then hypothesized that LNA™-modified capture probes: (1) may result in a more sensitive detection of miRNAs in comparison to unmodified DNA-based capture probes and (2) can be designed such that an uniform melting temperature (T_m) can be applied to a genome-wide set of miRNAs by adjusting the LNA™ content and the length of the capture probes. T_m normalization of capture probes permits the establishment of normalized hybridization conditions suitable for all miRNAs, which otherwise cover a range of T_m 's between 45°C and 74°C.

In your opinion, what are the key differences between LNA™-based and DNA-based arrays when it comes to profiling microRNAs?

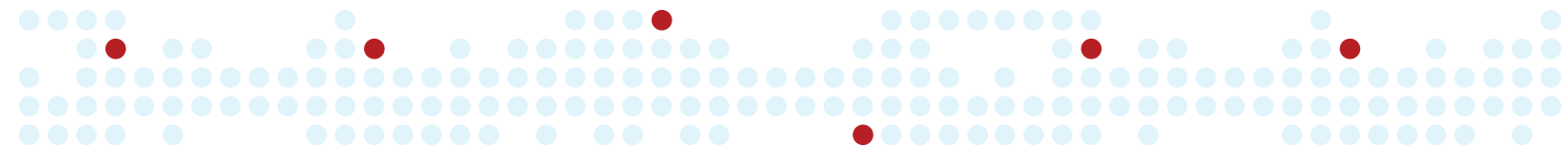
The main difference is selectivity and sensitivity. The biophysical properties of LNA™ allow the design of probe sets for uniform, high-affinity hybridizations. This yields highly accurate signals able to discriminate between single nucleotide differences and, hence, between closely related miRNA family members. The superior detection sensitivity eliminates the need for RNA size selection and/or amplification. The way we established our experimental protocols will greatly simplify miRNA expression profiling of biological and clinical samples. At the end of the day selectivity and sensitivity means faster processing of biological samples and more answers than questions when it comes to data analysis and interpretation.

Currently, what is the biggest challenge to your miRNA research?

Technically, we are able to produce miRNA expression profiles and deduce meaningful fingerprints. Although such fingerprints will be extremely useful for clinical diagnostics, they tell us little about the mechanism of pathogenesis. The next challenge is to identify bona fide target genes and understand the regulatory networks associated with the expression of miRNAs.

Finally, can you give us an indication of your near-future research plans?

Currently, we are intensively working on studying miRNA dependent responses in two of our major research projects: iron metabolism and childhood leukemias.

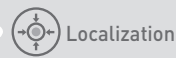




Isolation



Expression Analysis



Localization



Functional Analysis

Identification of molecular controls of stress resistance in agricultural studies



Dr. Shane Murray,
from the Centre for Proteomic and Genomic Research (CPGR) in Cape Town, South Africa.

miRCURY LNA™ microRNA Microarrays

Dr Shane Murray is a plant genomics expert at the Centre for Proteomic and Genomic Research (CPGR) in Cape Town, South Africa. The CPGR is a modern world class, high throughput biology research facility that provides state-of-the-art analytical services and technical expertise in the genomics and proteomics sectors.

What is the function of the Centre for Proteomic & Genomic Research? (Which areas of microRNA research are you involved in?)

The Centre for Proteomic and Genomic Research (CPGR) is a not-for-profit core research technology platform, funded by the South African government through its vehicles the Cape Biotech Trust and PlantBio. The mission of the CPGR is to stimulate excellent “omics” research, to support existing companies to facilitate the generation of new projects and to increase the number of suitably trained scientists in South Africa. While the CPGR has successfully used Exiqon’s miRCURY™ arrays to profile miRNA expression in both human and plant samples, my interest is in cereal crops, in particular maize. Maize is an important crop in Africa and identification of the molecular controls of resistance to biotic and abiotic stresses is a major research goal.

In your opinion, why should researchers perform a microRNA profile in plants?

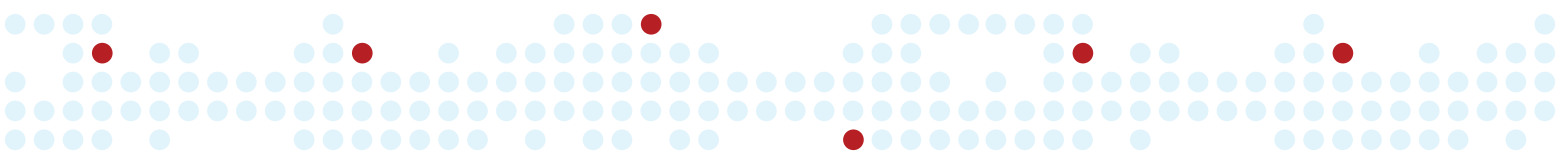
Profiling miRNAs in plants using an array-based approach is a good first approach. miRNA families are highly conserved, making it possible to identify known miRNAs expressed in different plant species under different conditions. miRCURY™ miRNA arrays contain probes for the vast majority of validated plant miRNAs (as listed in miRBase), which makes them a good tool for this purpose. In addition, profiling expression of miRNAs can complement traditional transcript profiling to further unravel control of gene expression and can help validate miRNAs identified through bioinformatics approaches.

What has been your experience in microRNA profiling in plants?

In collaboration with the Council for Scientific and Industrial Research (CSIR), we have conducted a proof-of-concept study, comparing miRNA profiles in maize flowers and leaves, using the miRCURY LNA™ array and labeling kit from Exiqon. We obtained data of very high quality. Our preliminary analysis indicates that miRNAs involved in development in both Arabidopsis and maize are differentially expressed in maize flowers.

What are your experiences of using the miRCURY LNA™ Array and the Labeling Kit from Exiqon with plant material?

See above.



In your opinion, what are the main advantages to using the miRCURY LNA™ Array and the Labeling kit compared to other types of microRNA array and labeling kits?

In my opinion, there are many advantages. Firstly, the miRCURY™ kit is really easy to use and we obtained good quality data. I particularly liked the spike-in controls, as a good method to determine the quality of the array. Secondly, the miRCURY™ arrays cover a wide range of species, allowing the identification of validated miRNAs in different species under different conditions. Thirdly, we use the Tecan HS4800Pro automated hybridization station for which Exiqon provides compatible protocols, further enhancing the ease of performing the assay and the quality of the data produced.

How do you validate the results from the microRNA arrays?

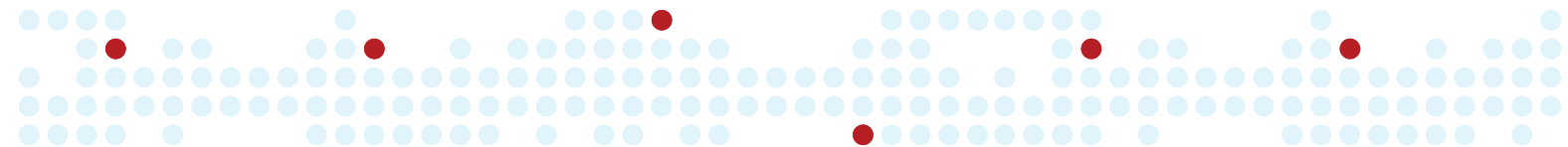
We are currently evaluating several qPCR approaches.

What is the biggest challenge when profiling microRNAs in plants?

This is difficult to say, as our proof of concept study went really well. Probably the same challenges apply as for full genome profiling: The necessity to grow and harvest plants under standard growth conditions, to extract good quality RNA (ensuring that small RNA species are not lost, in the case of miRNA profiling), to process the arrays routinely (we used a TECAN hybridization station, which reduces array-to-array variation) and to have access to good normalization and analysis packages for down-stream processing of the data.

How do you think the interest of microRNAs in plants will evolve in the next few years?

We believe interest in miRNAs in plants will definitely increase in the next five years, as more and more papers are published linking miRNA expression to a particular stress response. A number of other research groups in South Africa have already expressed interest in using the Exiqon arrays.

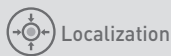




Isolation



Expression Analysis



Localization



Functional Analysis

Analyzing the role of microRNA in mammalian kidney development



Dr. Roman-Ulrich Müller,
from the Department of
Medicine IV, University
of Cologne.

miRCURY LNA™ microRNA Microarrays (Profiling Services)

Roman-Ulrich Müller works in the lab of Prof. Thomas Benzing, which was previously located at the Renal Division, University of Freiburg and is now located at the Department of Medicine IV, University of Cologne, and the Kidney Research Center Cologne. The lab focuses on the functional analysis of disease-relevant genes and their role in signal transduction in genetic kidney diseases. As part of this work they are studying microRNA expression in kidney development.

What is your research area?

We are working on kidney development in mammals and the role of kidney specific genes in the pathogenesis of congenital kidney diseases, e.g., nephronophthisis and FSGS.

What is your previous experience in microRNA profiling?

Until now we have been using small RNA cloning methods and small RNA Northern Blotting to detect small RNAs in our samples and establish miRNA-profiles in kidney development.

Why did you choose to use the miRCURY LNA™ Array microRNA profiling services from Exiqon?

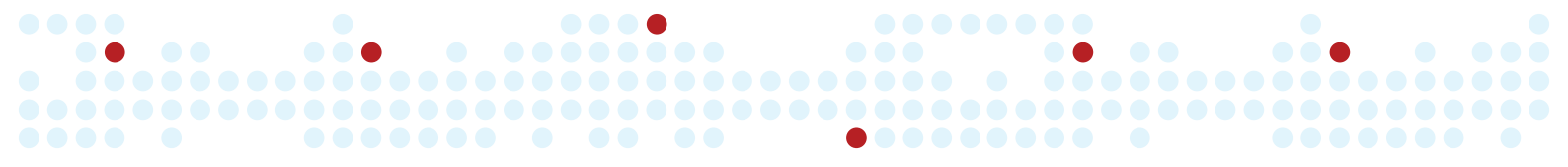
All the methods we have been using so far are pretty time consuming and are not exactly suitable for high-throughput profiling. As a lot of mammalian miRNA-sequences have now been published in miRBase, we judged that using micro-array technology would be the most favorable approach for our miRNA-projects.

In your opinion what are the main advantages to using the miRCURY LNA™ Arrays compared to other types of microRNA array?

Bearing in mind that some miRNAs only differ from each other in a single position, one major concern regarding miRNA-array technologies is specificity. In addition, some tissue specific miRNAs that are highly interesting can only be discovered using very sensitive assays as they are not abundant. miRCURY LNA™-arrays show important advantages compared to DNA-arrays both in terms of specificity and sensitivity as the modified nucleotides show a considerably higher mismatch penalty.

What do you feel are the main advantages of using a service provider, instead of performing experiments in your own lab?

In our lab we currently do not perform array hybridization and scanning ourselves and take advantage of service providers as to mRNA-arrays as well. Thus we did not want to establish the techniques and obtain the necessary reagents and machines ourselves, but decided to get started right away having Exiqon perform the necessary steps. Consequently we did not have to solve any of problems that always occur using new techniques as these had been well established at the Exiqon service facility.



How do you feel about the results you received from the microRNA profiling services?

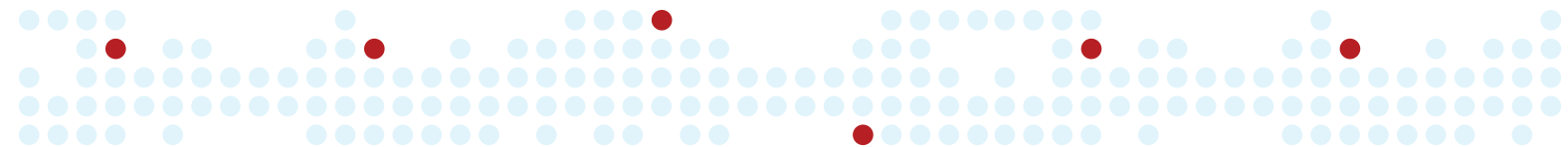
The results show some differentially regulated miRNAs and a nice overlap of expression regarding miRNAs encoded by a common primary transcript. Even miRNAs with a high level of similarity could be distinguished well using these arrays. Even though correlation coefficients of several replicates did not reach the levels that can be reached in whole genome chips (which is mainly due to the number of spots), we feel very confident about the results we were able to retrieve.

How did you go on to validate the results from the miRNA arrays?

As our main approach for validation, we chose miRNA *in situ* hybridization in cell-culture, whole-mount and whole organ experiments using miRCURY LNA™ *in situ* probes. The resulting data strongly confirms the results from the miRCURY miRNA-arrays and shows that LNA™ *in situ* hybridization is a nice way of validating data from screening and profiling approaches, that in addition provides further information about the localization of miRNA-expression at a suborgan level.

Can you disclose your near future research plans? Do you plan further miRNA arrays experiments, further validation, or functional analysis?

We are working on qPCR-approaches to further validate the miRCURY LNA™ array-data on a larger scale. In addition, we are going to compare the LNA™-array data to expression data obtained from DNA-arrays to further examine the advantages of LNA™-technologies. Regarding functional analyses, we will take a closer look at the localization of miRNAs in the kidney and on a subcellular level. Furthermore we are going to screen for mRNA-targets of the most abundant and differentially regulated miRNAs that we were able to identify and check for their function using sequence specific miRNA-inhibitors and lentivirally mediated miRNA-overexpression.

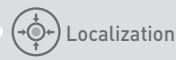




Isolation



Expression Analysis



Localization



Functional Analysis

Elucidation of the mechanisms of cervical neoplastic progression

miRCURY LNA™ microRNA Microarrays (Profiling Services)

Bali Muralidhar, previously working in Dr. Nick Coleman's group at the Medical Research Council Cancer Cell Unit in Cambridge, UK. The group is investigating novel approaches to cancer diagnosis. This involves two main areas:

The development of novel markers for improved screening for cervical cancer and colorectal cancer.

The mechanisms of cervical neoplastic progression.

What is the current research going on in your lab?

Basically we are a Papilloma Virus research lab that is looking into cervical cancer. We're looking for genes that could be responsible for progression of cervical carcinogenesis. We're also looking for biomarkers of progressive disease that could be used in clinical practice.

What made you want to study microRNAs?

We found that one of the major microRNA processing enzyme, Drosha, is a marker that we picked up in our W12 cervical carcinogenesis model as being important in the progression of cervical carcinogenesis through invasion and migration.

What is your previous experience in microRNA profiling?

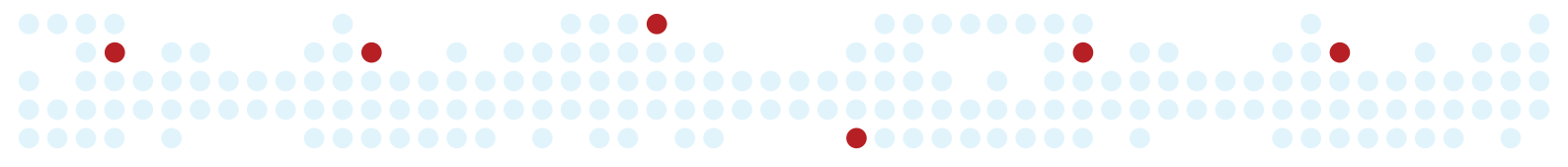
None! We have done a lot of array CGH in the lab, and a fair amount of gene expression arrays. But we had never done any microRNA profiling work at all, which is why we decided to use a service provider for our microRNA profiling studies.

Why did you choose to use the miRCURY LNA™ Array microRNA profiling services from Exiqon?

The main reason we chose Exiqon was because of the LNA™ technology. It seemed to be technically a much better system than was available on the market elsewhere, and the results that we could see on the Exiqon website seemed to be really good. We also spoke to people that had experience with other microRNA array platforms, and they suggested that we should use the Exiqon LNA™ technology. We looked into the different options and decided to go with Exiqon in the end.

In your opinion what are the main advantages to using the miRCURY LNA™ Arrays compared to other types of microRNA array?

Since submitting samples to Exiqon, we have also tried using a home-built DNA-based array for microRNA profiling. We found the Exiqon LNA™ array results to be much more reproducible, and we were able to validate the results by qRT-PCR1. When we ran some of the same samples using the home-built DNA-based array, there was much higher background on the arrays, and we got different results! We were not able to validate the differentially expressed microRNAs by qRT-PCR, so the changes that we saw on the home-built DNA-based array were probably not real.



What do you feel are the main advantages of using a service provider, instead of performing experiments in your own lab?

Service providers have a lot more experience in running these types of experiments, all the experimental conditions are consistently maintained and the results are much more reproducible. After an initial few glitches, our experiments are working well.

How do you feel about the results you received from the microRNA profiling services?

Really good. I like the layout of the final report of the results and I like the basic data analysis that is performed. I think the fact that Exiqon provides some bioinformatics is really valuable for researchers that may not have access to a bioinformatics facility themselves.

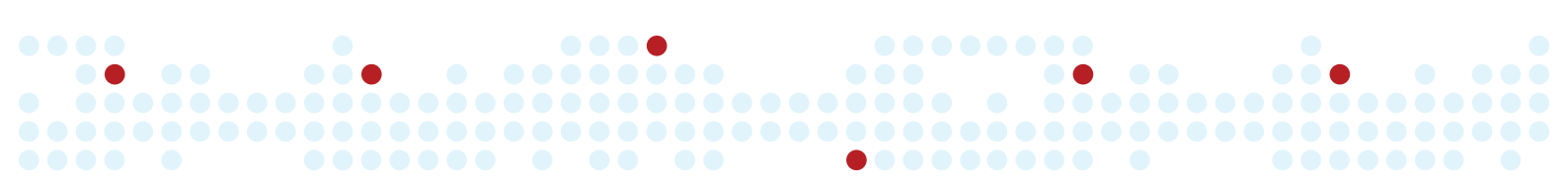
How did you go on to validate the results from the microRNA arrays?

We used TaqMan microRNA assays and were able to validate the differentially expressed microRNAs identified using the Exiqon LNA™ arrays¹.

Currently, what is the biggest challenge in your microRNA research?

Functional Analysis, definitely, and actually discovering potential targets of these microRNAs. It's all well and good to find out that microRNA x or y changes with treatment z, but you really need to identify the real biologically relevant targets of those particular microRNAs.

¹ Muralidhar B. *et al.* Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels. *J Pathol.* 2007 Apr 30;212(4):368-377





Isolation



Expression Analysis



Localization



Functional Analysis

Quantitation of microRNA expression levels in tumor angiogenesis

12

EXIQON | Customer Application Stories



Dr. Dorina Veliceasa,
from the Department
of Urology at the
Northwestern University
Feinberg School of Medicine.

miRCURY LNA™ PCR System

What is the current research going on in your lab?

We are interested in detecting changes in microRNA expression in endothelial cells that would target genes involved in the regulation of angiogenesis in the context of a tumor environment.

How did your research lead you to the study of microRNAs?

We are studying the transcriptional regulation of angiogenesis and we are interested in the identification of targets of different transcription factors. The microRNA field, it's such a hot topic. There are so many discoveries happening around the involvement of microRNAs in gene regulation. With over 800 annotated human microRNAs, and with the fact that each microRNA can have multiple targets within the cell, there are just so many areas to choose from.

What were the key factors for you in choosing a microRNA supplier and partner?

We started by looking at microRNA primer availability for the microRNAs we are interested in. Exiqon had the best primer coverage for the microRNAs we wanted to study.

What made you choose Exiqon?

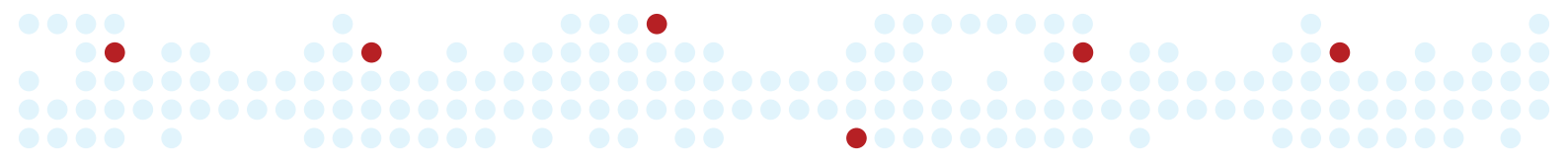
We spent some time learning about LNA™ technology, and we had a presentation at Northwestern by some Exiqon scientists. We had read a lot of information on the website, but the human contact was really helpful for us in choosing to work with Exiqon.

What were some specific challenges in your project?

At first, we used a qPCR approach, so it was really important to find good endogenous controls. Unfortunately, none of the typical controls worked very well in endothelial cells. We found a housekeeping microRNA which could be used as a control species in our model. But generally, the qPCR system, and the technology itself, is very good. After our qPCR work, we used some LNA™ probes for Northern blot, which gave us very good results. We've also used the new RNA isolation kit, and that worked well also.

How did you overcome them?

Mostly just a lot of testing and analyzing. We had to test many, many microRNA sequences to find the right control. Even a seemingly small change – as little as one cycle or even half a cycle – can have a big impact on normalization. As it turns out, let7a, whose regulation frequently changes in cancer models, was surprisingly very consistent between our experimental and control models.



What advice would you give to researchers who want to get started in microRNA research?

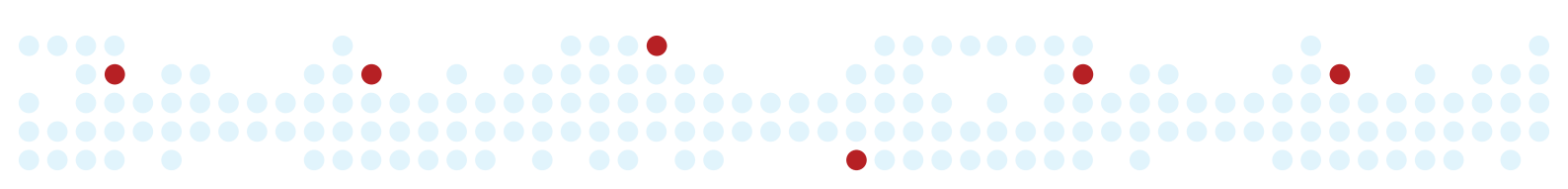
It's very important to have high quality RNA isolation. The Exiqon kit seems to be very good in our mammalian system (cells grown *in vitro*). For RT-PCR, it's crucial to include several controls for the PCR reaction. You need to have several experiments, duplicates and triplicates, RNA isolated from different experiments, etc., in order to get consistent data. It's especially important to have replicates to validate your data when you have very fine changes in the microRNA expression levels.

What would you tell a colleague about why they should work with Exiqon?

The primer coverage from Exiqon is very good, and it is updated frequently. The technology is very good, and not limited to one method – using LNA™ allowed me to avoid radioactive readout on my Northern blots. Also, the technical support is very good at Exiqon. Since microRNA was a completely new field for me when we started on this work, we had many questions along the way, and the scientists at Exiqon were very, very supportive. The response time, by phone or email, was short, and this was very helpful. In addition, our sales representative is great. He made it easy for us to get started in our projects with Exiqon's products.

Where will your research be showcased next? (Articles, conferences, posters, etc.)

We are currently preparing manuscripts to submit for publication. We will have a poster coming soon, and then an article to follow.



Investigating molecular mechanisms of hormone action in relation to disease states



Scientist Louisa Cheung,
from the Karolinska Institute,
Sweden.

miRCURY LNA™ PCR System

Louisa Cheung is finishing her Ph.D. in the Molecular Endocrinology lab headed by Professor Gunnar Nordstedt at the Center for Molecular Medicine, Karolinska Institutet, in Sweden. The lab is focusing on the analysis of molecular mechanisms of hormone action in relation to diseases. To better understand the underlying signaling pathways of the hormonal action they now study microRNA expression. Here she describes how the miRCURY LNA™ microRNA PCR System has benefited this work.

What is your area of research and microRNA interest?

My research interest is sex differences in liver on a molecular level. I'm studying how hormones contribute to the differences and how nutritional status would affect the sex differences.

What is your experience in performing microRNA research?

We have been studying sex differences in transcript profiles using microarray and then we are curious about the potential sex differences in the small RNA fraction. Therefore we started a screening using miRCURY LNA™ microRNA Array, and then we validated our results using miRCURY LNA™ microRNA PCR System.

For what type of research were you using detection of microRNA by real-time PCR? And for what part have you been using the miRCURY LNA™ microRNA PCR System?

We chose miRCURY LNA™ real-time PCR for the validation of microarray results and also to explore the potential regulation of the selected miRNAs. Northern blot was not considered since it is less accurate for quantification.

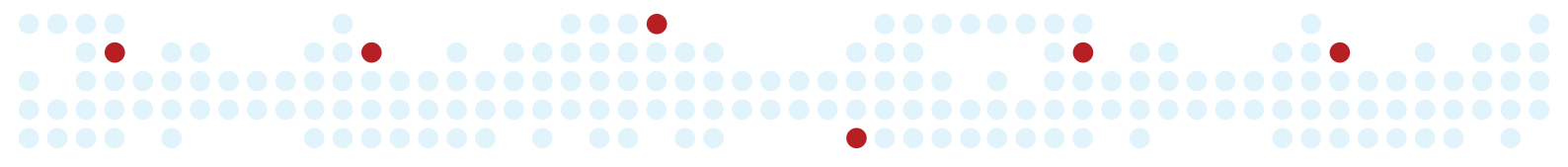
Since we performed the microarray experiment using miRCURY LNA™ microRNA Array, it seems natural to validate the results using miRCURY LNA™ microRNA PCR System.

What is your experience in performing quantitative PCR experiments before?

We have been studying the expression levels of many genes using SYBR® Green coupled with primers designed by ourselves. I have also performed miRNA quantification using TaqMan microRNA assays.

What do find are the main benefits of the miRCURY LNA™ microRNA PCR System?

The miRCURY LNA™ microRNA PCR System is easy to use and provides high sensitivity and reproducibility. It makes my validation faster, easier and more quantitative. With a small amount of miRNA, I could confirm my microarray results and explore the regulation by different treatments. I personally like the reaction mix protocol since the volumes to pipet provide good margin for potential pipetting error. It makes it easier to work on a large number of samples. The running protocol is fast, less than two hours including dissociation curve. The high sensitivity gives me the control to dilute my samples to adjust the optimal amount and I find the risk of obtaining false negative results being much smaller than with other systems.

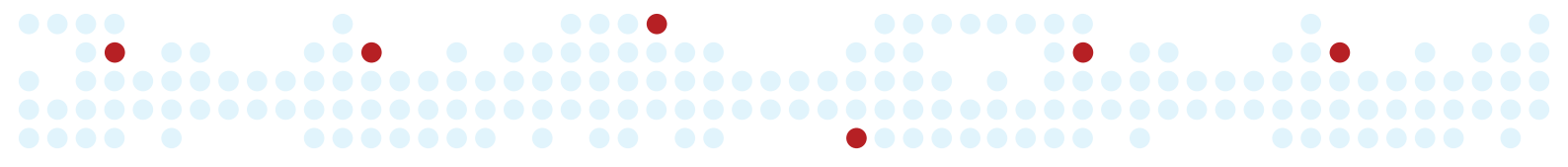


Did you validate your studies by other means?

We are using real-time PCR kits to validate our microarray data. There are some plans to further validate it using *in situ* hybridization.

When will we hear more about your studies?

I hope you can hear more from our study shortly. We are in the process of submitting a paper with all our findings in a peer-reviewed journal.





Isolation



Expression Analysis



Localization



Functional Analysis

Changes in Gene Expression in Response to Starvation



Dr. Tom Hamborg Nielsen,
from the Copenhagen University, Denmark.

MicroRNA Array Services

Tom Hamborg Nielsen, Associate Professor and group leader, and Maria Lundmark, Post Doctoral researcher, both work at Plant Molecular Biology Laboratory, Faculty of Life Sciences, Copenhagen University, Denmark.

What is the current research going on in your lab?

We are interested in understanding how plants respond to phosphate starvation by changing their gene expression. Phosphate is a major nutrient, required for growth of plants and all other living organisms. On a global scale phosphate availability is an important factor in plant productivity and ecosystems balance. Recent research in this field has revealed that the regulation of gene expression in response to P-starvation in plants involves several mechanisms forming an intricate network, where microRNAs play a key role. It is an exiting challenge to unravel these mechanisms, as it may allow new strategies for developing improved crops. We focus on the role of a group of transcription factors which are central to this regulatory system.

How did your research lead you to the study of microRNAs?

A key element in P-starvation dependent gene expression in plants is a specific MYB-related transcription factors called PHR1. The regulation mediated by PHR1 and its homologs is one of our main research interests. It was recently discovered that PHR1 regulates a family of microRNA, miR399. This miRNA family targets a ubiquitin ligase, which influences protein turnover, and, by a yet unknown mechanism, regulates the expression of genes encoding P-transport proteins, essential to the cellular uptake of P. Other non-coding RNAs act as mimicry, antagonizing the effect of miR399s. Clearly, miRNAs are essential to the response and we need to understand how they are involved in nutrient dependent gene expression. Data obtained by the array based miRNA analysis designed by Exiqon has now revealed that more families of miRNA are involved in this response.

What were the key factors for you in choosing a microRNA supplier and partner?

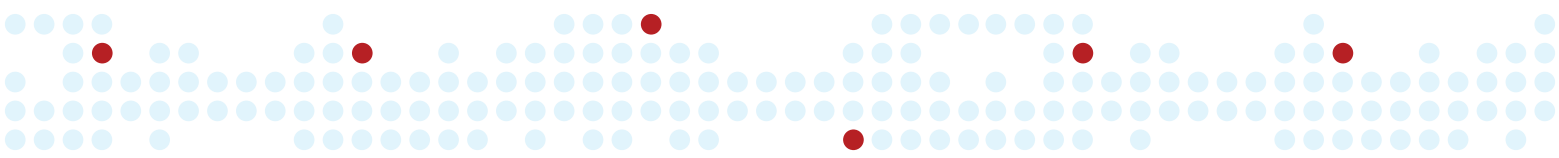
The novel miRCURY LNAT[™] Arrays were specifically designed for miRNA profiling in the model plant Arabidopsis, which serves as our experimental system. This opened new and unique possibility to discover all the miRNAs involved in the biological processes that we are studying. The miRNA Profiling Services at Exiqon has performed the technical aspects of the analysis, a task which requires time and specialised knowledge. This has allowed us to focus on the quality of biological aspects of the experiment. We find that this has provided a highly efficient approach.

What made you choose Exiqon?

The Arabidopsis microarrays designed by Exiqon are presently one-of-a-kind, and offered us a unique opportunity to analyze a biological problem by sensitive and specific detection of mature miRNA. Also the good personal contact to the company furthered the choice.



Dr. Maria Lundmark,
from the Copenhagen University, Denmark.



What were some specific challenges in your project?

The major challenge is to obtain high quality plant material to analyze and high quality RNA preparations. During P-starvation plants increase their activity of RNAses and the overall levels of RNA in the cells may be low in the P-starved plant material.

How did you overcome them?

Further biological characterization verified the quality of the analyzed experiments. We have good experience in purifying good quality RNA and the quality RNA was verified by appropriate analysis.

What advice would you give to researchers who want to get started in microRNA research?

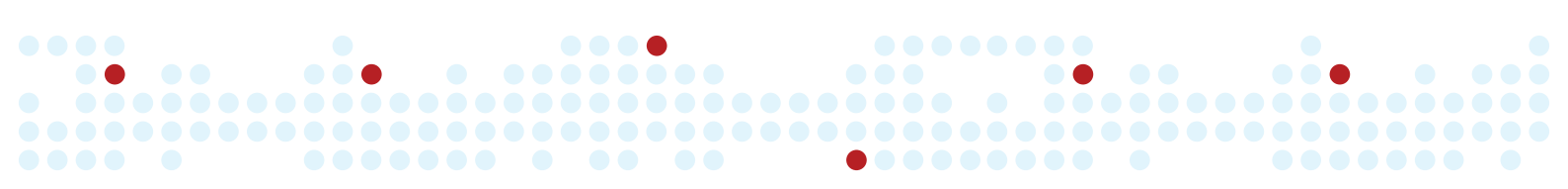
This is a field developing fast and it is recommendable to keep track of the technological developments within the field. For us, the new facilities developed by Exiqon have greatly facilitated an analysis in a broader perspective. It is really important to verify the microarray data by alternative methods. Using LNA™-primers offers an attractive way to achieve this.

What would you tell a colleague about why they should work with Exiqon?

We have experienced the collaboration with Exiqon as very good and highly qualified.

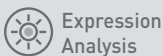
Where will your research be showcased next? (Articles, conferences, posters, etc.)

We have presented the data as a poster on an international plant biology conference (ASPB annual meeting, 2009) and we are next planning a method oriented paper in a scientific journal.





Isolation



Expression Analysis



Localization



Functional Analysis

The role of microRNA in skeletal muscle differentiation



Dr. Dylan Sweetman, formerly in the group of Dr. Andrea Münsterberg at the University of East Anglia School of Biological Sciences. Today, Dr. Sweetman is at the School of Biosciences, University of Nottingham.

miRCURY LNA™ microRNA Detection Probes for *in situ* hybridization

Dr. Dylan Sweetman, formerly in the group of Dr. Andrea Münsterberg at the University of East Anglia School of Biological Sciences. The group investigates cellular and molecular mechanisms that underlie embryonic development.

What is the current research going on in your lab?

We use the chick embryo as a vertebrate model organism. We are particularly interested in the signals that control cell fate decisions. For example, we are looking at developing somites, which give rise to skeletal muscle, cartilage, bone and tendon, and we have characterized the origin of cells that generate the vertebrate heart. Our work has provided insight into the mechanisms that are used to specify these different cell types and tissues. In particular, I am investigating the function of microRNAs in skeletal muscle differentiation.

What made you want to study miRNAs?

We decided to investigate microRNAs primarily because of the excellent work of Ronald Plasterk's group looking at microRNA expression patterns in zebrafish by *in situ* hybridization [Science. 2005 Jul 8;309(5732):310-1. Please see page 22]. It was clear from this study that some microRNAs show interesting muscle-specific expression patterns.

What is your previous experience in *in situ* hybridization?

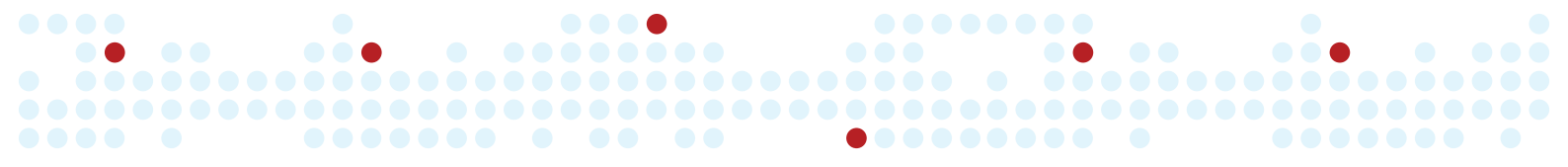
I have several years experience in mRNA *in situ* hybridization using traditional RNA antisense probes on whole mount samples from a variety of organisms, e.g. chicken, mouse and *Drosophila*.

Why did you choose to use the miRCURY LNA™ microRNA detection probes from Exiqon?

Basically no other technology has been demonstrated to work for microRNA detection *in situ*. Following the success of LNA™ probes as demonstrated in Plasterk's lab, we decided to take the same approach.

What label and detection method did you choose?

I have tried end labeling the LNA™ probe myself using DIG-UTP, but I found that the staining took several weeks to develop. That is why I started using the LNA™ probes pre-labeled with 5'- and 3'-DIG. The double DIG labeled probes give a much stronger signal, so staining requires a much shorter time. As well as the time saving, the double DIG probes usually result in a higher signal to noise ratio, although extensive washes with high detergent TBST buffer are also very important for removing background in my experience [see protocol].



What were the main challenges to develop a protocol for microRNA detection in your samples, and how did you overcome them?

Getting the technique working did require a certain amount of optimization from the original protocol. This involved mainly choosing appropriate temperatures for the hybridization and washing steps. I found that the temperatures need to be optimized for each probe, using 21°C below the estimated melting temperature as a starting point. You also have to be quite patient when waiting for the staining to develop!

What positive and negative controls do you feel are important for *in situ* hybridization experiments?

In general, I think that the specific labeling patterns that we observe are the best kind of a positive control. As you can see from the images we see very little background. We know that the labeling we observe is specific, because we see a different expression pattern for miR-1 and miR-206, which are highly related microRNAs (only 2nt difference between the mature microRNA sequences). In spite of the high degree of sequence similarity between the two microRNAs, it is evident from the *in situ* images that miR-1 is expressed in heart, whereas miR-206 is not. If I was setting up the technique from the beginning, I would recommend using a strongly expressed microRNA such as miR-206 or miR-124 that has a tissue specific expression pattern (rather than a widespread expression pattern) as a positive control. As a negative control we have chosen to use a probe designed to detect a plant microRNA.

How do you feel about the *in situ* hybridization results you obtained?

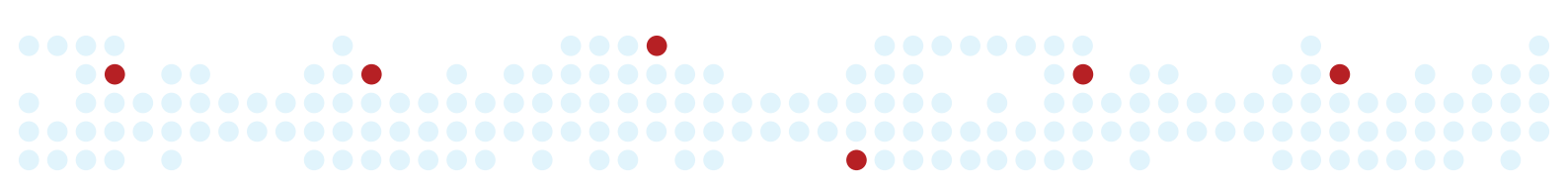
The results we have obtained using the LNA™ detection probes are excellent, and we have been able to publish the work (Dev. Biol. 2008 Jun 21) so we are very happy!

Will you go on to validate your *in situ* hybridization results using other techniques (qRT-PCR, Northern blot etc.)?

We plan to perform some Northern blotting of microRNAs in cell lines. We would also like to move on to study the function of these microRNAs, by introducing microRNA mimics and inhibitors by electroporation into somites.

Currently, what is the biggest challenge in your microRNA research?

The major challenge right now is to perform functional studies *in vivo* in the embryo using gain and loss of function approaches. We have been trying to construct a microRNA overexpression vector, but unfortunately this has proved quite time consuming to set up, and the correct processing of the microRNA should always be confirmed to make sure the mature product is generated.



Investigating the functional role of microRNAs in acute myeloid leukemia



Amanda Dixon-McIver,
from the Institute of Cancer,
Barts Hospital, in London,
UK.

miRCURY LNA™ microRNA Detection Probes for *in situ* hybridization

Amanda Dixon-McIver is finishing her Ph.D. under the supervision of Dr. Silvana Debernardi, in the Medical Oncology Laboratory headed by Prof. Bryan Young in the Institute of Cancer, Barts Hospital, in London, UK. The group has performed microRNA profiling, and Amanda is currently using LNA™ technology for *in situ* hybridization and microRNA knockdown.

Amanda Dixon-McIver is finishing her Ph.D. under the supervision of Dr. Silvana Debernardi, in the Medical Oncology Laboratory headed by Prof. Bryan Young in the Institute of Cancer, Barts Hospital, in London, UK. The group has performed microRNA profiling, and Amanda is currently using LNA™ technology for hybridization and microRNA knockdown.

What is the current research going on in your lab?

Our lab is interested in investigating the role of microRNAs in haematological malignancies, in particular acute myeloid leukaemia (AML). Previously, we have shown that miR-181a expression is strongly correlated with the AML morphological sub-type and with the expression of genes identified through sequence analysis as potential interaction targets¹. We are currently investigating the functional role of microRNAs in AML, by examining the effect of knocking down particular microRNAs.

What is your previous experience in microRNA knockdown?

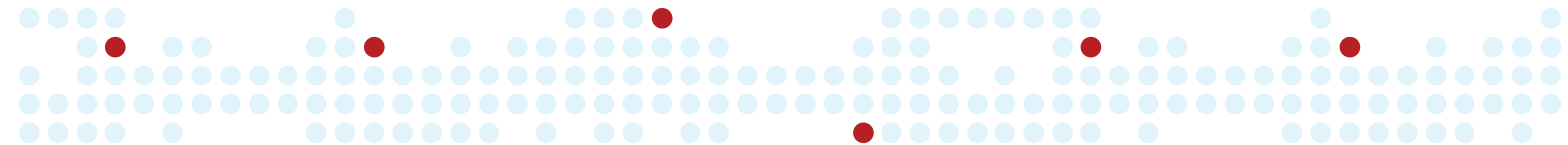
We have not had any prior experience with microRNA knockdown.

Why did you choose to use the miRCURY LNA™ microRNA Knockdown Probes from Exiqon?

We chose to use miRCURY LNA™ knockdown probes as we had previous experience with using LNA™ probes for *in situ* hybridization with great success². We felt that the LNA™ technology itself was the best available for our application. The LNA™ knockdown probes are also fairly easy to deliver into cells, and the published data available³ suggest that LNA™ knockdown is very effective. As a negative control for knockdown experiments, we use the scrambled knockdown probe available from Exiqon. It was useful to be able to obtain LNA™ knockdown probes with a fluorescent FITC label, to allow visual confirmation that electroporation had been successful. We were also able to counterstain the cells with DAPI to visualize the nucleus.

In your opinion what are the main advantages to using the miRCURY LNA™ microRNA Knockdown Probes compared to other types of microRNA inhibitors?

The main advantage is that it is fairly easy to get the probe into the cell and the published data available suggested that it is very effective.



Why did you choose to use electroporation to deliver the LNA™ knockdown probes into this cell type, and how difficult was it to set up and optimize the conditions?

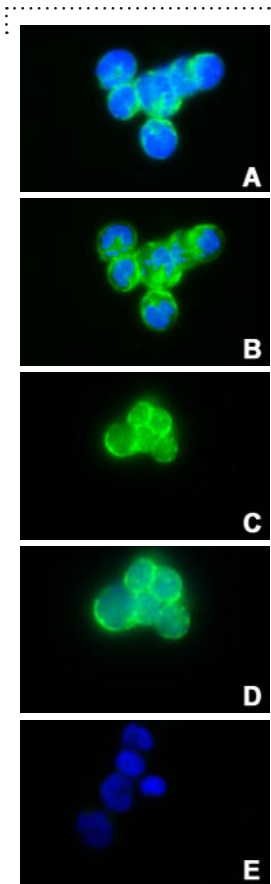
The cell line that we used for this study is Molt4, an acute lymphoblastic leukaemia suspension cell line. We chose to use electroporation to deliver the LNA™ knockdown probe, as we have found electroporation to be the most effective method for delivery into haematopoietic suspension cell lines in our lab. Electroporation of small RNA molecules can be performed using a square wave pulse electroporator, which results in minimal cell death when the electroporation conditions have been optimized.

Currently, what is the biggest challenge in your miRNA research?

The biggest challenge in our microRNA research is confirming that the knockdown has been successful. We are currently measuring microRNA knockdown by qRT-PCR, however this may not be the most appropriate method so we are investigating other techniques. We are also investigating an interesting cellular phenotype that we have observed when knocking down our microRNA of interest.

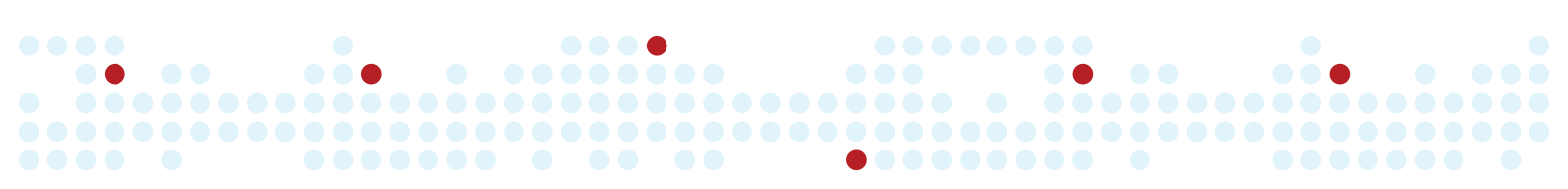
1 Debernardi *et al.* , MicroRNA miR-181a correlates with morphological sub-class of acute myeloid leukaemia and the expression of its target genes in global genome-wide analysis. *Leukaemia* (2007) 21, 912 – 916.

2 Dixon-Mclver A. *et al.* . PLoS ONE . 2008 May 14; 3 (5): e2141 .Distinctive patterns of microRNA expression associated with karyotype in acute myeloid leukaemia



Efficient uptake of fluorescently labeled miRCURY LNA™ knockdown probe into Molt4 acute lymphoblastic leukaemia suspension cell line. Cells were electroporated (300V, 100 nM miRCURY LNA™ knockdown probe, 5' FITC labeled, 107 cells) and images were acquired 2 hours post-electroporation using a confocal microscope.

A: 5' FITC labeled scrambled knockdown probe, FITC only.
B: 5' FITC labeled scrambled knockdown probe, FITC and DAPI.
C: 5' FITC labeled miR-specific knockdown probe, FITC only.
D: 5' FITC labeled miR-specific knockdown probe, FITC and DAPI.
E: Reagent only (no knockdown probe).



The role of microRNA in early developmental processes of vertebrate embryos



Dr. Parker Antin,
from the University of
Arizona, the United States.

miRCURY LNA™ microRNA Detection Probes for *in situ* hybridization

What is the current research going on in your lab?

Research in our laboratory is focused on understanding the molecular regulation of early developmental processes in vertebrate embryos. We primarily use the chicken embryo as a model organism, and approach research questions from the dual perspective of how individual molecules function and how their functions can be integrated into network models.

How did your research lead you to the study of microRNAs?

My lab runs a large scale *in situ* hybridization database project, funded by the NIH, to determine the expression patterns for all differentially expressed genes in the chicken embryo. In 2005, we got wind of LNA™ technology as a way to detect microRNAs. We contacted Exiqon, as well as other laboratories, and after some testing and initial negotiations, conducted a massive screen (140 unique probes) to detect and localize all chicken embryo microRNAs that were known at the time.

What were the key factors for you in choosing a microRNA supplier and partner?

Whether it is a microRNA supplier, or a partner, or both, you want an organization that's willing to work with you. At the time [we started investigating microRNAs], protocols were still evolving with regards to optimal conditions for *in situ* hybridization detection of microRNAs. Having a flexible, responsive partner was and is very important to us.

What made you choose Exiqon?

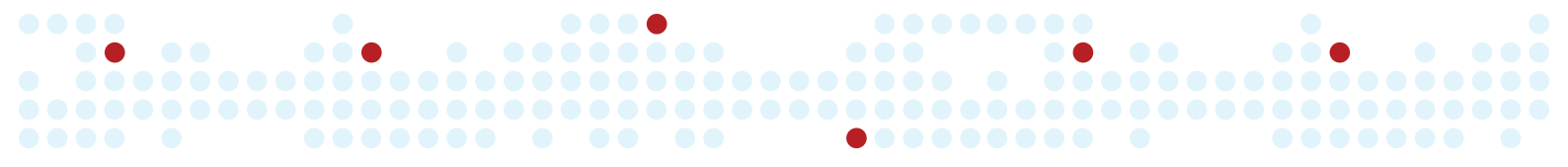
Exiqon demonstrated a superior level of both expertise and flexibility, including a desire to help us in our efforts to screen this biological system.

What were some specific challenges in your project?

It was a challenge to develop protocols to detect microRNAs in chicken embryos. We were really pushing the field, in terms of detection. It was completely unclear what ISH conditions would work, or if it could even be done in this system. We found surprises at every turn, down to the most detailed level.

How did you overcome them?

Mostly elbow grease, and collaboration with Exiqon. The company was very helpful, providing probe sets with different characteristics, and maintaining a high level of responsiveness to our needs. This allowed us to troubleshoot quickly, and reach a solid, successful result.

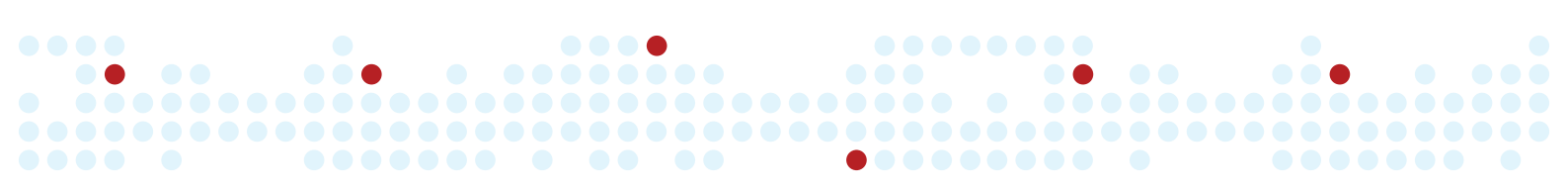


What advice would you give to researchers who want to get started in microRNA research?

The field is growing so quickly, that it can be a little daunting to get into at the moment. I will say that in our experience, Exiqon reagents stand out in front of other platforms from a technological standpoint. It depends on what projects you're doing, but everyone has shown now that LNA™ technology is superior to any other for the detection of small RNA species.

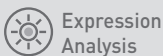
What would you tell a colleague about why they should work with Exiqon?

Number one, Exiqon is the source for LNA™ technology. They have the most expertise. They have worked really hard on product development, and it shows. Exiqon has done a good job establishing and maintaining direct access for researchers to sources of information, through their online technical repository, through one-on-one conversations with product managers and in-house scientists, and through the development of a network of experts for referral questions.





Isolation



Expression Analysis



Localization



Functional Analysis

Elucidating the mechanisms of microRNA regulation of animal development

24

EXIQON | Customer Application Stories



Professor Ronald H. A. Plasterk, formerly at the Hubrecht Laboratory, now Minister of Education, Culture & Science in the Netherlands.

miRCURY LNA™ microRNA Detection Probes for *in situ* hybridization

Prof Ronald H. A. Plasterk has recently become Dutch Minister of Education, Culture and Science. Before that he was Director of the Hubrecht Laboratory / Netherlands Institute for Developmental Biology in Utrecht, and Professor of Developmental Genetics at the University of Utrecht. Here he describes part of his research on small RNAs and how the miRCURY LNA™ Detection probes has benefited this work.

What is your research area?

We have for several years been studying the mechanism and regulation of DNA transposition by RNAi in the nematode *C. elegans* and zebrafish. Recently we have also been investigating the role of small RNAs – especially microRNAs - in animal development. In fact, out of the 20 people in our group 15 are now directly involved in this work.

Why did you choose to test the miRCURY LNA™ detection probes?

I attended a conference last Autumn and saw some very nice *in situ* images of microRNAs in plants. The group had used the miRCURY LNA™ detection probes from Exiqon. Immediately thereafter I got 3 probes targeting microRNAs that according to the literature were specific in brain and liver in zebrafish embryos. We made our experiments the same day we received the probes and we got some beautiful data and spectacular images.

What have been the main benefits of the miRCURY LNA™ detection probes?

The probes simply enabled this research – we did try DNA and RNA probes but they were clearly not as useful. The miRCURY LNA™ detection probes were very sensitive and specific and *in situ* hybridization data were generated very fast. A few months after our initial experiments we generated a complete catalogue of images showing the temporal and spatial expression patterns of 115 conserved microRNAs in zebrafish embryos. This work was published in *Science* this Summer¹ and a second paper will be published in *Nature Methods* January 2006.

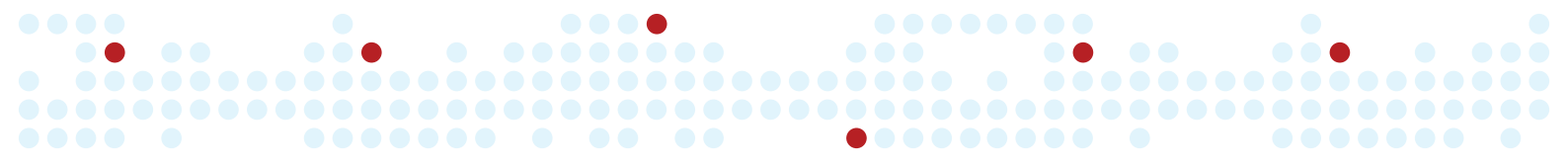
Do you think microRNAs are just the tip of the iceberg when it comes to small non-coding RNAs?

We have been cloning small RNAs and only a small subset is microRNA. There are clearly many others and the copy numbers are high. Currently we don't know much about these molecules.

Can you disclose your near future research plans?

We are expanding the catalogue of expression patterns in mouse and especially human brain and if possible link these to human diseases. We use morpholinos to knockdown microRNAs studying the phenotypic effect and miRCURY LNA™ detection probes to verify the knockdown. So small RNAs remain an important research area of our group.

¹ Wienholds *et al.*, *Science*, 2005, 309, 310-311.



High-throughput functional screening of microRNA regulation of muscle cell differentiation



Dr. Annick Harel-Bellan,
from the Institut Andre Lwoff,
Paris, France.



Dr. Anna Polesskaya,
from the Institut Andre Lwoff,
Paris, France.

miRCURY LNA™ microRNA Knockdown Libraries

Dr. Annick Harel-Bellan (AHB) is Directeur de Recherche at the Institut Andre Lwoff in Paris. She heads a group working on epigenetics and cancer (Laboratoire Epigenetique et Cancer). Dr. Anna Polesskaya (AP) is a senior scientist and longstanding member of this group.

What is the main focus of the research conducted in your lab?

(AHB-AP) We are interested in understanding the mechanisms of epigenetic control of cell fate and the underlying regulatory mechanisms that govern cell differentiation.

What made you interested in microRNA?

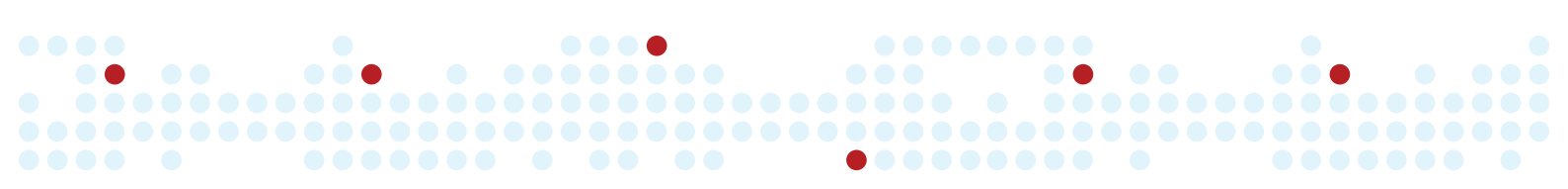
(AHB) We have been interested for many years in exploring the mechanisms of cell fate control during the terminal step of muscle cell differentiation. In particular we are studying the role of microRNAs and other small ncRNAs in the regulation of gene expression during myogenesis. We have discovered that miR-181 is differentially expressed during differentiation of our murine cell culture model. *In vivo*, mir-181 is poorly expressed in adult muscle tissue, but strongly induced in regenerating muscle. In order to explore miR-181 function we designed a loss-of-function assay based on antisense inhibitors containing LNA™ (we were the first to do that). The experiments showed that mir-181 plays a crucial role in switching on the early programme of gene expression during myogenesis by allowing expression of MyoD, the key transcription factor during muscle cell differentiation. mir-181 exerts this effect by attenuating translation of hoxA11 mRNA, that encodes a transcription factor that inhibits myoD transcription. Mir-181 is therefore essential to the establishment of terminally differentiated muscle cells, but is probably not required for the maintenance of this cell type (Naguibneva *et al.*, Nature Cell Biol., 2006).

What is the advantage of using LNA™ in microRNA inhibitors?

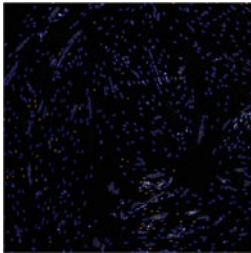
(AHB) As we showed in Naguibneva *et al.* (A LNA™-based loss-of-function assay for microRNAs. Biomed Pharmacother. 2006 Aug 28;), LNA™ microRNA antisense inhibitors are efficient and specific with long lasting effects.

You are currently engaged in a large project aimed at identifying novel microRNA involved in muscle cell differentiation. Could you describe the experimental approach chosen for this project?

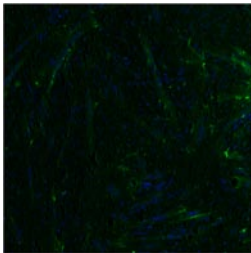
(AP) We have chosen a novel human skeletal myoblast cell line as our model system. These cells were transfected in 96 well plates with the Exiqon miRCURY LNA™ KD library of microRNA inhibitors. Differentiation was induced 24 h later, and the efficiency of differentiation was evaluated by immunofluorescent staining at 7-day time point. In a parallel approach, the expression of miRNAs during terminal differentiation of these cells was profiled by Exiqon array services.



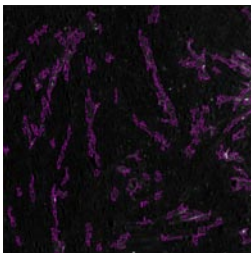
Automated image analysis based on MHC and multinucleated cells



Hoechst 33342 (note the multinuclear myotubes)



Myosin Heavy Chain



Recognition of MHC-positive multinuclear myotubes by ArrayScan

What did the expression profiling tell you?

[AHB] The data shows surprisingly dramatic changes over time. A large number of microRNAs are differentially regulated and they fall in different categories of expression kinetics: Generally we observe up- and downregulated microRNAs, but a few microRNAs appear to be transiently differentially regulated during the process. Interestingly, many of the differentially regulated microRNAs are related – either belonging to the same family or closely associated on the chromosome, and possibly deriving from the same primary transcript.

How do you monitor the effect of the KD probes on differentiation?

[AP] During myogenesis, individual myoblast cells fuse to form long polynucleate cells. To enable high throughput automated detection of these myotubes, we stain them with Hoechst 33242 (nuclear stain) and antibodies against a late marker of terminal muscle differentiation, MHC (Myosin Heavy Chain). See images. The images are acquired by an automated microscope and sophisticated image analysis software is used to detect and quantify phenotypic changes such as decreased/increased efficiency of differentiation, increased proliferation or cell death, and other phenomena caused by the KD of specific miRNAs in differentiating myoblasts.

Setting up large scale parallel transfections and automated image analysis seems like a major challenge. What type of expertise and equipment was required?

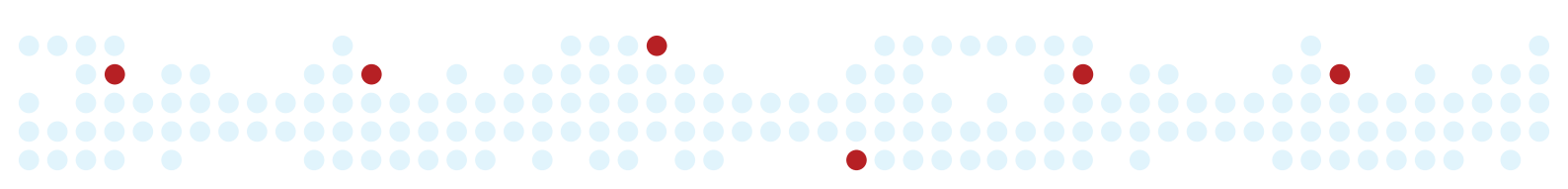
[AP] The RNAi group in our laboratory helped us use their pipetting-diluting robot MLStar, (Hamilton) for plating and transfection of the human myoblasts. To acquire and analyze the images, we have set up collaboration with the I-Stem Institute in Evry, France. The specialists of I-Stem have helped us to develop the techniques for the automated image analysis using the ArrayScan (Cellomics).

How did you set up the transfection conditions?

[AP] To determine the reagents and conditions to efficiently transfect human myoblasts, we have used the AllStars hs Cell Death control siRNA from Qiagen. Afterwards, we have used the LNA™ inhibitors of miRNAs which are known to be important for terminal muscle differentiation (miR-181, miR-206, miR-133) to set up the working conditions for the KD screen.

How did you setup the image analysis?

[AP] Here again, we have used the LNA™ inhibitors against miR-181, miR-206, and miR-133 in increasing concentrations, to obtain different levels of inhibition of terminal differentiation. The multinuclear, MHC-positive myotubes were recognized by the image analysis software, and the level of inhibition of terminal differentiation was quantified, and compared to visual observation, and Western blot analysis of MHC expression.



With a project of this scale surely you must have encountered a series of problems?

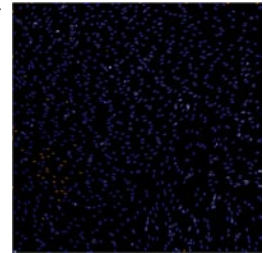
(AP) We have had problems with edge effects, likely due to the evaporation of the media during the long incubation of the plates (7 days). Increasing the volume of the differentiation media from 100 to 200 ml resolved this problem. Using robots for transfection meant that we could not plate the cells first and transfect them afterwards, because of mechanical damage to the cells in the middle of wells caused by ejection of transfection mixture from the pipette tips. Therefore, we had to set up a reverse transfection protocol adding the transfection reagents first and the cell suspension afterwards. But it worked out surprisingly well. Unfortunately, the long incubation times combined with the use of robots also increased the risk of contamination, which was important in our original screen (3%). In the ongoing secondary screen, done to confirm the original "hits", we have paid special attention to the problem of contamination.

The primary screen was recently performed and the preliminary results look exciting.**Can you describe the general nature of the results and how you plan to go from here?**

(AP) We were very (pleasantly) surprised by the large number of "hits". As many as 120 LNA™ inhibitors have been included in the secondary screen, based on the variety of strong phenotypical changes observed in the cells. 55 of the "hits" have been detected in our miRNA profiling of myoblasts, as being differentially regulated between myoblasts and myotubes. The vast majority of these "hits" are novel candidates for a role in skeletal myogenesis, though we also identified some previously-described "myoMIRs", such as miR-133 and others. In order to weed out false positive hits we will analyze the results of a secondary, confirmation screen, and we will then re-synthesize the LNA™ inhibitors for the most promising miRNA "hits" to test the kinetics and the dose-dependence of the candidate miRNAs in terminal differentiation. In a parallel approach we will try to ascertain whether the KD phenotype can be rescued with synthetic (mimic) miRNAs. We have previously used this approach with success (Naguibneva *et al.*, Nature Cell Biol., 2006). The confirmed and validated novel miRNAs will be analyzed in silico to predict their specific targets with a role in terminal muscle differentiation.

What is the value of performing a genome wide screen with microRNA inhibitors in parallel with microRNA expression profiling?

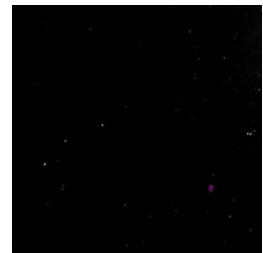
(AHB) The power of the functional screen is that it leads you directly to hits that are functionally important. However, essential microRNA regulation might go unnoticed in a screen due to functional redundancies of individual microRNA, ie., microRNA belonging to families or coregulated microRNA that together play an important regulatory function. Expression profiling generates a different but complimentary set of data that is a useful cross-reference when evaluating screening data. In addition, it is useful to pinpoint microRNA families and transcriptional clusters that merit closer investigation even if they did not show up in the KD screen. We plan to do this using experimental 3rd generation inhibitors specially designed by Exiqon.

Inhibition of differentiation with a-miR-206

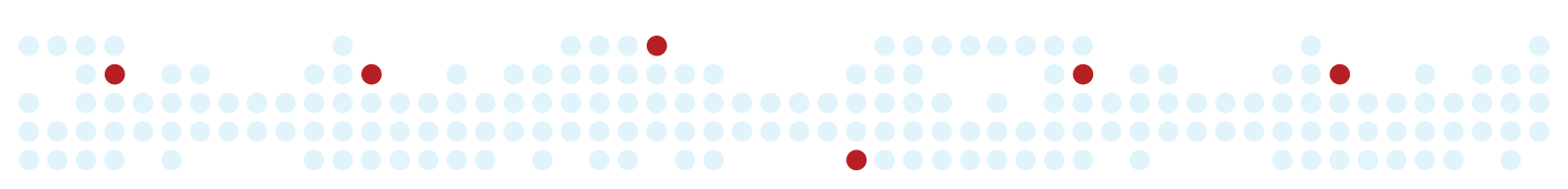
Hoechst 33342 (no multinuclear cells)



Myosin Heavy Chain (not detected)



No MHC-positive multinuclear myotubes detected by ArrayScan



Why is screening with a complete library important – couldn't you have performed a restricted screen against differentially expressed microRNA detected in your expression profiling data?

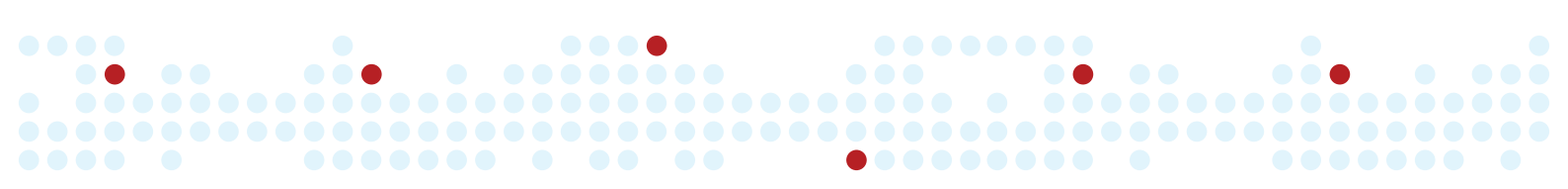
[AP] Many of the LNA™ inhibitors that significantly changed the phenotype of human myoblasts in our KD screen target microRNAs that appear not to be differentially expressed. This observation suggests that constitutively expressed miRNAs can nevertheless be essential for terminal myogenic differentiation. The truth is that a large proportion of the hits were not obvious candidates based on the expression profiling data.

Based on your experience do you have any helpful advice for colleagues contemplating using KD libraries?

[AP] KD libraries represent a very powerful tool for the discovery of novel miRNA-dependent pathways. It is important to apply the KD screening to an efficient and robust cell culture model with a quantifiable read-out that can be adapted to high-throughput conditions.

What is the current limitation with functional analysis tools and what type of novel tools would you like to see developed?

[AP] The profiling of miRNA expression during terminal differentiation shows that the members of a number of miRNA families are regulated in the same manner. The LNA™ inhibitors of individual miRNAs belonging to these families did not induce any phenotypic changes in our KD screen, which can be a result of functional redundancy between the miRNAs of the same family. It will be very useful to include the inhibitors of the whole families of miRNAs (such as let-7, miR-29, miR-30 and others) in KD libraries in the future.



Contact information

Outside North America

Business hours

8:30 a.m. - 4:30 p.m.
Central European Time (GMT +100)

Mailing address

Exiqon A/S
Skelstedet 16
2950 Vedbaek
Denmark

General inquiries and technical assistance

Phone: +45 45 65 09 29
Fax: +45 45 65 04 59
Email (form): exiqon.com/contact

How to order

Phone: +45 45 65 09 29
Fax: +45 45 66 18 88
Buy online: exiqon.com/ls
Email (form): exiqon.com/contact

North America

Business hours

8:30 a.m. - 4:30 p.m.
Eastern Standard Time

Mailing address

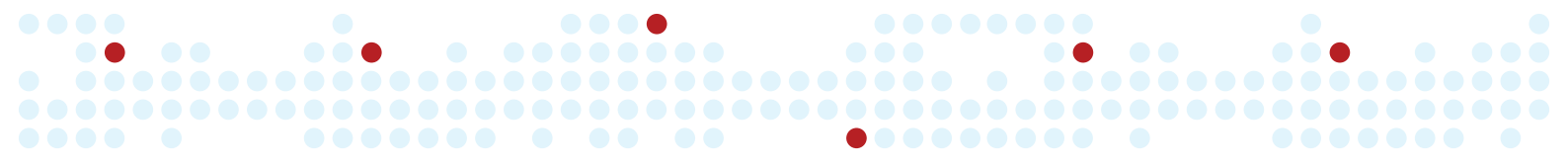
Exiqon, Inc.
14 F Gill Street
Woburn, MA 01801
United States

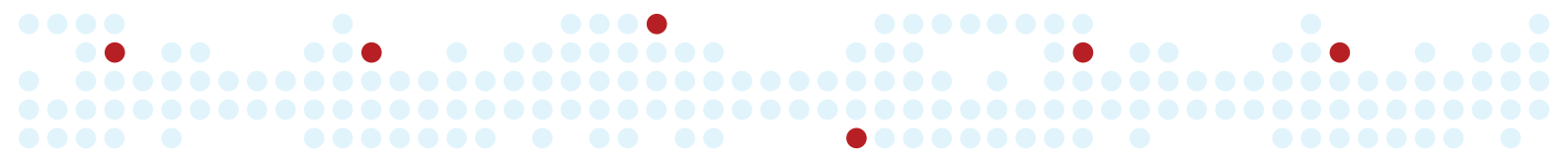
General inquiries and technical assistance

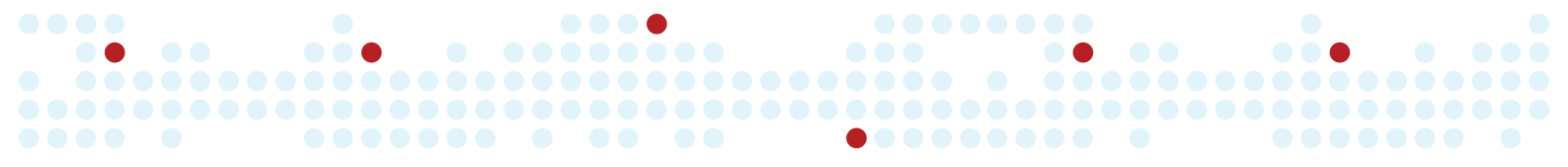
Phone: +1 781 376 4150
Fax: +1 781 376 4152
Toll free (US & Canada):
+1 888 miRCURY
Email (form): exiqon.com/contact

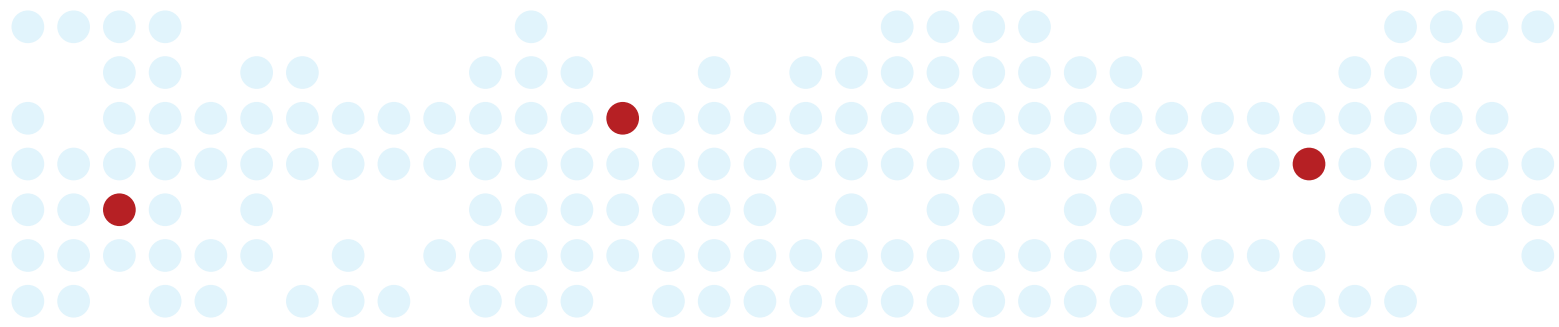
How to order

Phone: +1 781 376 4150
or +1 888 miRCURY
Fax: +1 781 376 4152
Buy online: exiqon.com/ls
Email (form): exiqon.com/contact









exiqon.com/ls

EXIQON
Seek Find Verify

