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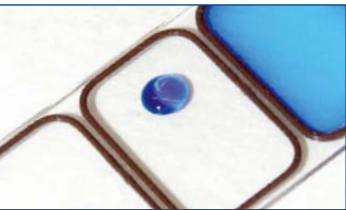
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Reprint

microRNAs – new stars on the horizon

Using established workflows for fresh insights into the field of gene regulation

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microRNAs – new stars on the horizon

Using established workflows for fresh insights into the field of gene regulation

In the future, the importance of miRNA studies will increase considerably. The standardized use of miRNA microarrays in research will make it possible to identify individual miRNAs as well as complete miRNA signatures with a high degree of accuracy. This approach is already being used with very promising results in the following fields of applied medicine – in cancer diagnostics, in the development of miRNA-based therapeutics and for the prognosis of therapy progress.



Dr. Carola Wagner & Dr. Ralph Oehlmann, IMGM Laboratories

miRNAs – a brief introduction

microRNAs (miRNAs) were first discovered in nematodes in 1993 and represent a major class of ribonucleic acids (RNAs). They possess regulatory properties and are involved in important biological processes such as cell division, cell differentiation, oncogenesis and apoptosis [1,2]. Their proven participation in the development of tumors make miRNAs key candidates for medical diagnostics, therapy-supporting prognosis and various other therapeutic approaches [3, 4, 5].

In recent years, more than 6,000 miRNAs have been identified in 60 spe-

cies and 12 types of virus (as at April 2008, Sanger miRBase 11.0, URL: <http://microrna.sanger.ac.uk>). To date, 678 miRNA sequences have been described in humans, some of which have been divided into families, such as the hsa-let7 family with its 11 miRNA members.

miRNAs belong to the class of non-coding RNAs and stand out due to their size (17-25 nucleotides (nt)). Detection of miRNAs poses a great technical challenge due to their shortness, their high degree of sequence conservation, their partly high copy number and the lack of a poly (A) tail [6].

Microarrays and quantitative real-time PCR, two proven technologies from the field of gene expression, were specially adapted for miRNA analysis. It has thus become possible to perform miRNAs detection on a global scale and to identify group-specific miRNA expression patterns [5].

The miRNA workflow – from detection to functional analysis

Depending on the question posed, either the identification of individual modulated miRNAs or the detection of entire miRNA signatures is of primary interest. An

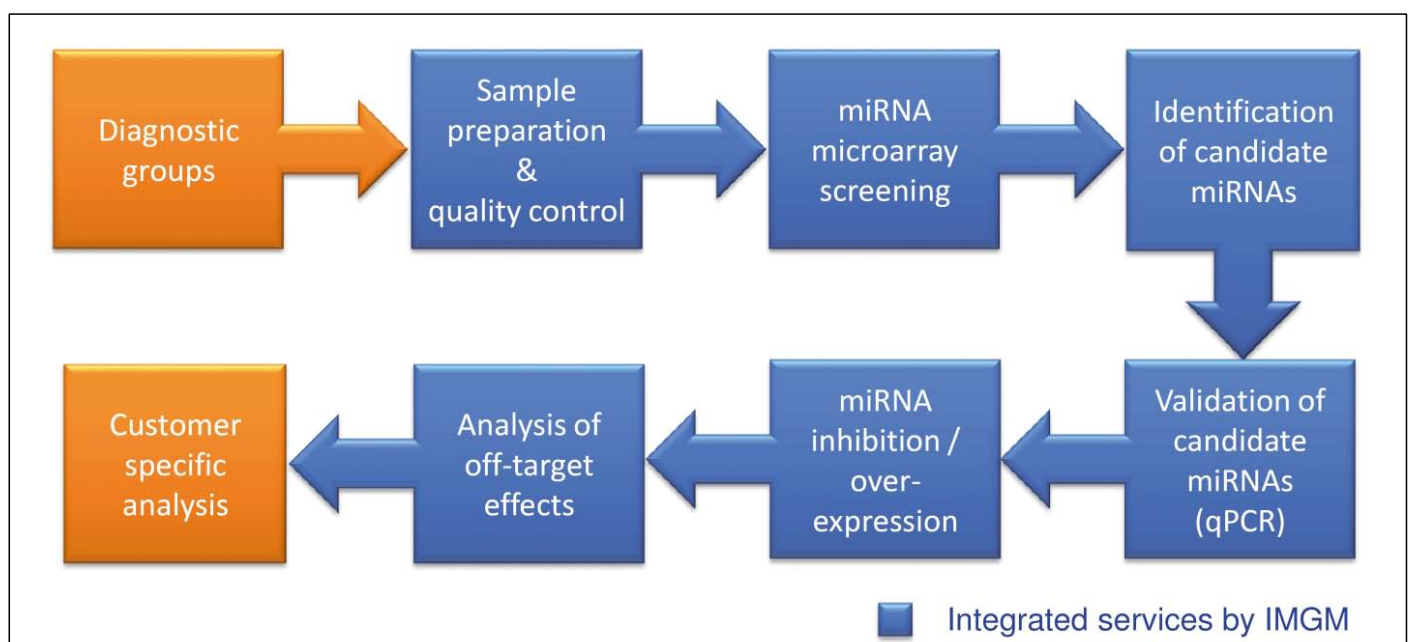


Fig. 1: miRNA workflow for the identification of differentially expressed microRNAs, their validation and functional analysis.

example of a miRNA-analysis workflow is shown in Fig. 1 and depicts the various stages of a typical miRNA experiment, comprising microarray-based screening, identification of relevant miRNAs, validation of miRNA candidates and their subsequent functional analysis.

miRNA microarrays currently constitute the most up-to-date and comprehensive screening method available for miRNA expression profiling. For the analysis of experimental groups (e.g. healthy versus diseased) they allow for the quick and reliable identification of differentially expressed miRNAs. The miRNA workflow is thus comparable to the well established approaches of gene expression analysis.

For successful detection of miRNAs, isolation of total RNA including all small RNA species is required. Suitable RNA of sufficient quality and quantity can be extracted from various starting materials (e.g. cells, tissue, blood and blood fractions), independent of their condition (e.g. fresh, frozen or formalin-fixed and paraffin-embedded).

Depending on the supplier of miRNA microarrays (e.g. Agilent, Ambion, Invitrogen), different strategies regarding probe design and sample labeling are followed in order to guarantee a highly specific, sensitive and robust detection. The identification of differentially expressed miRNAs is performed through the application of proven statistical algorithms for normalization, filtering and significance testing (Fig. 2). In general, it is recommended to validate miRNAs identified by microarray-based screening. Quantitative real-time PCR is the preferred method (gold standard) for miRNA hit validation. Only after successful validation of microarray results, functional analysis of miRNAs will be promising.

It is known that individual miRNAs influence the regulation of large numbers of target genes characterized by a significant degree of sequence homology. When a perfect sequence homology occurs, the functionality of miRNA is identical to small interfering (si)RNA. They cause an enzymatic degradation of the target gene transcript, which subsequently can no longer be translated. However, an imperfect sequence homology leads to a block in translation while keeping the transcript intact [1, 2]. Software tools like miRanda, TargetScan, miRTar or miRacle may be used to predict target genes regulated by specific miRNAs.

Functional analysis is performed by “over-expression” or “knock-down” of selected miRNAs in a cellular system. An integrated miRNA workflow for the identifi-

cation and functional analysis of miRNA is offered, for example, by the companies IMGm Laboratories and Sirion.

Outlook for miRNA microarray technology

There are still several challenges to be overcome before the full potential of miRNA microarrays as principal technol-

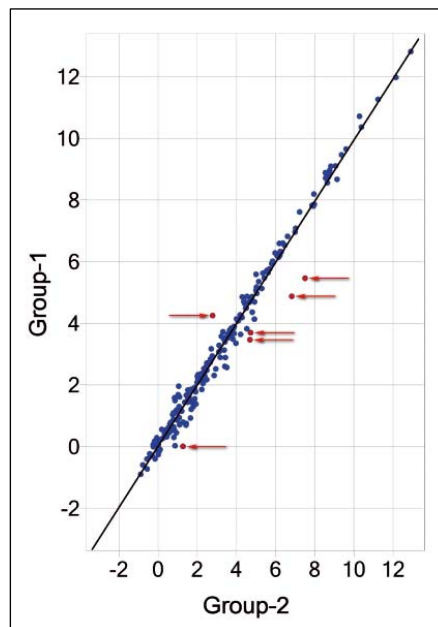


Fig. 2: Visualization of microarray results of two groups (mean value from 3 biological replicates) in a scatter plot. Points, which deviate distinctly from the 45°-line represent differentially expressed miRNAs. Statistical algorithms are used to assess the significance of differential regulation and to reduce false positives.

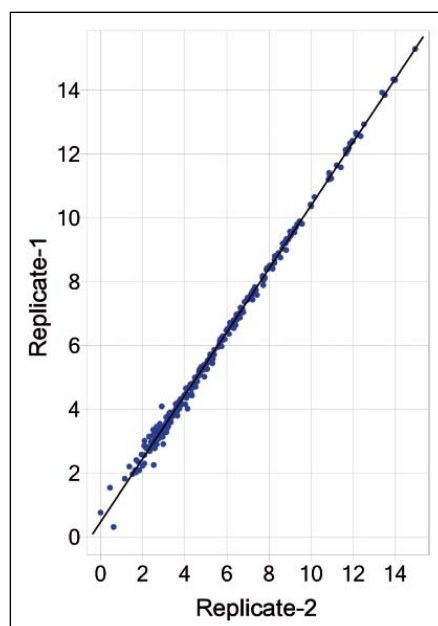


Fig. 3: Visualization of microarray results of two technical replicates in a scatter plot. The normalized log₂ signals are plotted against each other. The closer the points plot to the 45°-line, the more constant is the expression of the miRNAs represented in each case.

ogy for research and diagnostics can be realized [7].

In this respect, standardization and comparability of miRNA microarray experiments are of particular importance [8].

The standardized classification and nomenclature of miRNAs as stored in the Sanger miRBase database are a key contribution in this endeavor. Commercially available miRNA microarrays offer the possibility of generating technically reproducible data (Fig. 3) and, through their standardized content, provide excellent comparability.

Standardized workflows combined with technical expertise, as offered by the accredited service provider IMGm Laboratories GmbH, make it possible to carry out miRNA research in a fast and straightforward fashion.

The use of modern miRNA analytics in applied medicine is one of the hottest research topics in the pharmaceutical industry. Development in the coming years will show whether miRNA-based products will find broad application in diagnostics, therapeutics and prognostics. It will be interesting to see whether and to which extent miRNA research will play a future role in improving the human condition.

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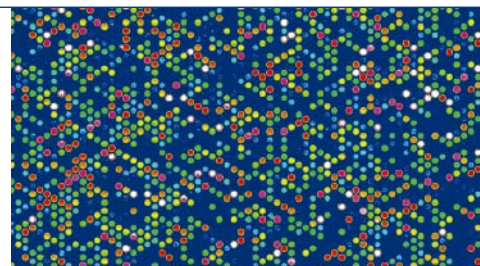
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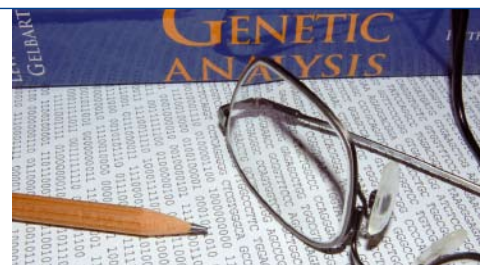
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