MicroRNA profiling on a rapidly evolving microfluidic array

We present a microarray technology for researchers carrying out microRNA (miRNA) profiling, who are looking for a more flexible alternative to preprinted arrays. Geniom® One is a benchtop instrument for production and processing of arrays, allowing researchers to produce an miRNA profiling array any way they choose, whenever they want, with complete control over content. Newly discovered miRNAs and other small RNAs can be immediately incorporated into an experiment.

Geniom One: a flexible platform for miRNA analysis

Geniom One technology combines microfluidics and *in situ* oligonucleotide synthesis to provide the ultimate flexibility in the synthesis, hybridization and detection of customized microarrays. This feature of Geniom Technology makes it particularly attractive for meeting the challenges of gene expression profiling in rapidly developing fields such as miRNA research. The public miRNA registry miRBase[1] (http://microrna.sanger.ac.uk/) has been updated frequently since its introduction in December 2002: on average, new updates occur every 3 months. However, most high-content and high-throughput analysis systems for miRNA are unable to keep pace with this rapid development because they rely on batch production of oligonucleotide probes and attachment of these probes to solid substrates such as microarray slides or beads.

The Geniom product family overcomes these problems by providing a flexible solution for miRNA profiling that can respond on a day-to-day basis to developments in this dynamic field. Geniom One is a benchtop instrument for the production and processing of biochips. Each biochip consists of eight independent microarrays that are located in microfluidic channels, and each microarray can incorporate up to 15,000 features. These features can be individually designed in a fast and easy manner to accommodate practically any array-based experimental setup. At the most basic level, researchers can produce an array based on the most recent release from the Sanger miRBase, starting from the first day of that release. Additionally, because any sequence information can be converted into an miRNA microarray, researchers have the ability to incorporate proprietary sequences from miRNA discovery projects or computationally predicted novel miRNAs. Application of the platform is not just limited to miRNAs. Any other small RNAs—for example, piwi-interacting RNAs—can also be immediately incorporated into an array and profiled.

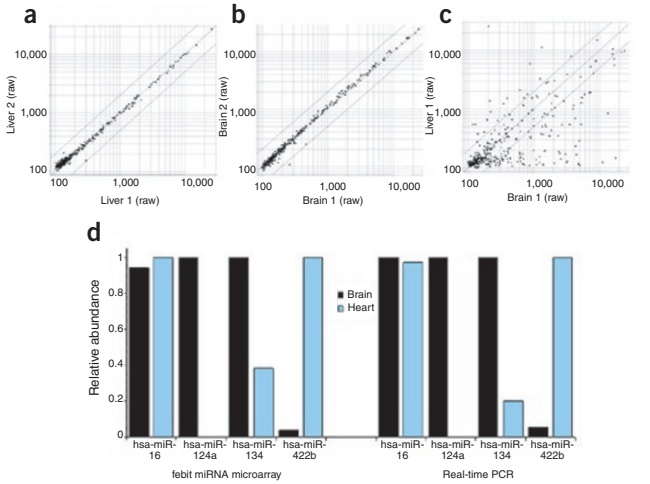

Figure 1 | Scatter plots and consistency charts. (**a**,**b**) Scatter plots showing technical replicates between mouse brain (**a**) or mouse liver (**b**) RNA. (**c**) Scatter plot illustrating differences between mouse brain and liver samples. (**d**) Consistency between data obtained using a preprinted array and that obtained using a febit microarray. Intensities shown are relative to the highest value for each miRNA, which was set to 1.

The Geniom One is essentially a fully independent microarray production and processing instrument. Apart from the degree of flexibility this offers the user, it also offers a secure system with respect to the content of each array. Critical information, that is, information relating to proprietary sequences, never has to leave the laboratory. Array design can be tested and optimized on an experiment-to-experiment basis.

Fast and optimized procedures give high-quality data

To combine Geniom's flexibility with speed, we optimized and streamlined sample-labeling and hybridization conditions. Using labeling

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technology from Genisphere Inc., we adapted to our platform a sensitive sample-labeling procedure requiring less than 1 h. The procedure is described in the febit protocol delivered with the FlashTag Biotin labeling kit. With our protocols, no small-RNA enrichment nor even the purification of mature miRNAs is necessary. This not only saves time but also limits sample loss, reduces the chance of introducing experimental artifacts and increases sensitivity. Consistent and reproducible data can be obtained with as little as 250 ng of total RNA. febit provides 15 artificial spike-in controls that can be used for data normalization. We designed these controls using sequences from *Arabidopsis thaliana* genes implicated in photosynthesis and tested them for cross-hybridization with human miRNA probes.

Geniom Technology shows a high discriminatory power

Analysis of miRNAs can be tricky because sequence families with differences as few as one nucleotide exist. Their specific analysis demands highly optimized hybridization conditions that can be used to distinguish between these homologous miRNAs.

The eight hsa-let-7 family members share 71–95% sequence identity, which makes their specific detection challenging (**Fig. 1**). Thus, they provide a good test case to investigate the discriminatory power of a detection system. For this analysis, we labeled synthetic RNA oligonucleotides for each family member, individually hybridized them to an array and calculated the relative cross-hybridization to probes for the other seven let-7 family members. The result indicated that the Geniom system has a good discriminatory power for all members (**Fig. 1**).

The human miRNAs hsa-miR-10a and hsa-miR-10b differ in only one nucleotide, which makes them the ideal model for testing the specificity of a system. For this analysis, we labeled synthetic RNA oligonucleotides for the two miRNAs, individually hybridized them to an array and calculated the relative cross-hybridization. The result showed that the febit system is able to discriminate as little as one-nucleotide difference (**Fig. 2**).

Flexible chip design allows for unique experiments

Geniom offers a user-friendly software interface for design of array content for profiling small RNAs. miRNA sequences are downloaded by the user and entered by 'cutting and pasting', and the array template is designed using febit's intuitive software. Arrays can then be synthesized and be ready to start the experiment in about 8 h.

Geniom's flexible chip design raises the possibility that the arrays could be used for several nonstandard experimental applications. For example, we have demonstrated in-house that the miRNA profile from the combination of a eukaryotic host and its viral pathogens can be analyzed in one array. Capture probes for pri- and pre-miRs can be designed and rapidly optimized using Geniom technology. Computationally predicted miRNAs can be experimentally verified, and this new information can immediately be translated into further experiments. New miRNAs or other small RNAs can be discovered by producing tiling arrays covering the region of interest. For example, we have demonstrated the presence of small RNA expression from the intergenic regions of bacteria.

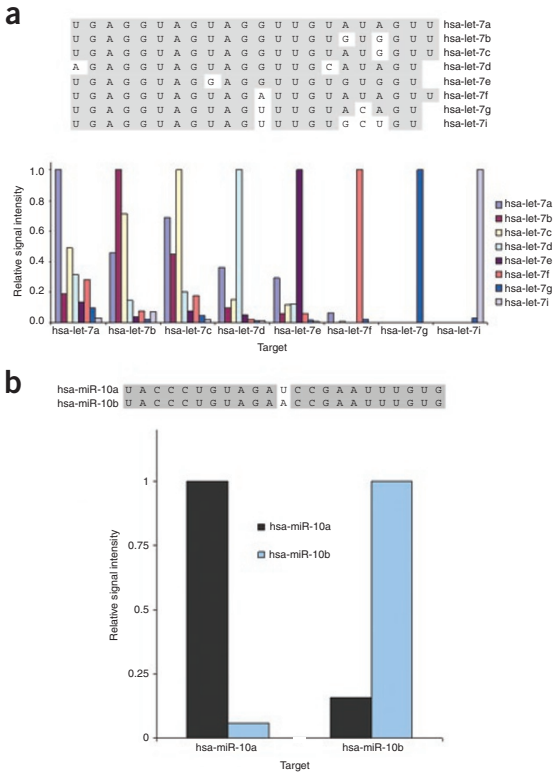

Figure 2 | Specificity test. (**a**,**b**) Synthetic RNA oligonucleotides corresponding to all eight members of the hsa-let-7 family (**a**) or to hsa-miR-10a and hsa-miR-10b (**b**) were hybridized to different febit arrays and the relative cross-hybridization was determined.

The Geniom technology is not limited to miRNA analysis: any nucleic acid can be analyzed with the same degree of flexibility. This opens a completely new field of miRNA analysis, for example, the co-analysis of miRNAs and their target mRNA from the same sample, using the same platform.

Summary

The Geniom technology provides a solution for a research field requiring increasingly flexible and customized solutions for high-throughput miRNA analyses. Preprinted arrays have unchanging content for significant periods of research time, whereas miRNA research is best served by platforms that can keep up with the pace of new miRNA discovery. Geniom One can keep up with that pace.

The day-to-day flexibility and iterative capabilities of the Geniom Platform offer an attractive option for researchers who are designing and optimizing content for diagnostic arrays.

For the occasional user, the Geniom technology is also available from febit as a miRNA profiling service, providing the same degree of flexibility and speed without the need to acquire additional equipment.

1. Griffiths-Jones, S. The microRNA registry. *Nucl. Acids Res.* **32**, D109–D111 (2004).