LC Sciences

TECHNICAL BULLETIN

Probe Content Selection - microRNA Microarrays

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μParaflo® technology, with its flexible (or programmable) *in situ* synthesis process removes technical, as well as, cost barriers in probe content selection. The following list provides several commonly used methods for selecting biological contents.

1. Use standard arrays as provided by LC Sciences. MiRNA contents of these arrays are selected from the latest version, or any previous version of miRBase database[1]. Species-specific arrays, as well as multiple species arrays, are available for all known species with published miRNA sequences in miRBase. Multiple species arrays are useful when miRNA sequence information of study subject is not complete or not even available. Standard arrays are mostly used by medical researchers who want to focus their studies to validated and/or annotated miRNA sequences.

2. Add custom sequences to standard arrays. Many researchers collect sequences from the most updated literature, computational predictions, and/or sequencing experiments and add them as custom sequences to standard arrays of corresponding species. The custom sequences are not limited to miRNAs. For example, piRNAs, snoRNAs, tRNAs and other small RNAs have been incorporated.[2] This approach is very useful in discovery studies.

3. Use deep sequencing result as array contents. Deep sequencing yields results that cover genome-wide miRNAs without the need for any prior sequence knowledge. However, it costs substantially higher and takes longer time to complete than array assay. More importantly, its sample preparation involves significantly more steps than array assay and therefore results in additional profiling distortions.[3,4] Integration of the two complementary technologies makes a comprehensive, effective, as well as, efficient tool for performing profiling studies on a large number of samples without worrying about missing any sequencing information. For most applications sequencing data is produced in a single sequencing run (e.g. using one lane on Illumina GAIIx Genome Analyzer) on a pooled sample combining all representative sample types involved in a corresponding project. Sequencing data are analyzed and array probe sequences are produced through Seq-Array℠ software pipeline (LC Sciences).

In additional to above biological contents, an array also contains control probes for chip quality analysis, spiking-in controls, internal positive controls, and sometimes negative controls as well. More than 12 spike-in controls are used in each assay. Corresponding perfect matched and single-base mismatched detection probes are implemented for hybridization specificity assessments. Standard internal positive controls include 5 to 6 probes targeting at different sections of 5S ribosomal RNA of corresponding species. Most probes are repeated three times or more. Synthesized probes consist of natural DNA and modified nucleotide residues to produce uniform binding coefficients across all probes including short and/or low CG sequences.

1. Griffiths-Jones S. (2004) **The microRNA Registry**. *NAR* 32(Database Issue), D109-11.

2. Vagin, V.V., Sigova, A, Li, C., Seitz, H., Gvozdev, V., and Zamore, P.D. (2006) **A distinct small RNA pathway silences selfish genetic elements in the germline**. *Science* 313, 320-24.

3. Monya, B. (2010) **MicroRNA profiling: separating signal from noise**. *Nat Meth* 7, 687-692.

4. Linsen, S.E. et al. (2009) **Limitations and possibilities of small RNA digital gene expression profiling**. *Nat Meth* 6, 474–476.

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