Microarrays or NGS for expression analysis?

What are the differences between NGS and microarrays?

The key difference between the two technologies center on how the mechanisms by which transcription is measured.

In a microarray, a design is made for probes to specific locations in a species transcriptome, and after hybridization abundance measurements are made by means of fluorescence.

With NGS approaches, the output in the form of counts of short sequence reads, come from actual expressed sequence of the transcriptome selected during the library preparation stage.

Are the results comparable?

For a well-defined organism (e.g. humans) either technology will provide good measures of transcript abundance at the gene level, however the underlying nature of where the measurements are made is different.

If we approximate the output from two exons of a gene been an NGS library and an Affymetrix GeneST array, we see that the NGS measurements will come from transcript sequences along the whole length of the gene, but with the microarray, there are a defined number of probes designed towards the 3’ end of the gene.

Are people still using microarrays?

Microarrays are a well-established technology with a proven track record spanning two decades of use in the laboratory. Their advantages include:

- A less complicated and less labour-intensive sample preparation.
- Simpler and less labour-intensive data analysis with many well-established tools that are usable by the non-expert practitioner.
- Arrays are typically cheaper when working with large numbers of samples and can provide higher throughput.

Multiple publications show good concordance between the techniques.

When highlighting similarities and differences in differentially expressed genes (DEGs) in experiments performed on NGS and array platforms, Rao et al. 2019 state that:

"Approximately 78% of DEGs identified with microarrays overlapped with RNA-Seq data .... RNA-Seq data showed additional DEGs."

Wales Gene Park Tech Note  Microarrays or NGS for expression analysis?  Last revised - October 2020
Benefits of NGS approaches

NGS approaches have the advantage of offering a lot more flexibility in what can be achieved than an array experiment.

Advantages include:

- As the technology does not rely on transcript-specific probes and sequences whatever RNA transcripts are present within a sample, NGS approaches can be used to detect novel transcripts. It is possible to undertake analyses to detect novel transcripts, gene fusions or even identify sequence variants within the transcriptome (SNVs, small insertions and deletions).

- NGS approaches achieve a higher specificity and sensitivity over microarrays. They can therefore detect a larger number of differentially expressed genes, with marked sensitivity improvements in genes with low expression levels.

- The detection of rare and low-abundance transcripts is possible by increasing the number of reads produced covering each section of the transcriptome. The technology can therefore be used to detect expression changes in weakly expressed genes, detect rare transcripts or detect single transcripts per cell.

- Microarrays have limitations in the detection sensitivity with low and high expressed transcripts. Low expressed transcripts are often hard to distinguish against the background noise signal and very high signals are often saturated because of measurement limitations. NGS approaches are described as having a wider dynamic range producing a signal which is much more digital in nature (based on counting sequence reads) and with more accurate outputs at the extreme high and low expression levels.

- NGS approaches will work with smaller amounts of starting material.

How can I choose?

The primary determinants of which method to use will likely be the previous experience of the researcher, the funds available and how they plan to undertake the analysis of their experiments (i.e. do you plan to use or have access to expert bioinformaticians).

Aside from these constraints, the aims of the project can drive a decision. You should ask yourself things like:

- Is genome information available for my species of interest?

- Do I want to identify novel genes and/or other genomic features?

- Do structural alterations interest me, for instance gene fusions and alternative transcripts?

For projects focusing on identification of novel SNVs or high-resolution comparison of gene expressions, NGS will be the sensible choice.

References

https://doi.org/10.3389/fgene.2018.00636