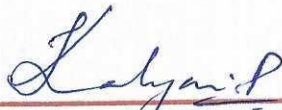


NGS Vs. Microarrays

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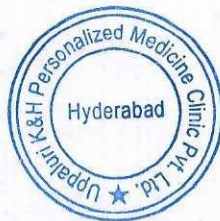


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Title: NGS Vs. Microarrays

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Objective: Advantages of NGS over Microarray.

- 1. Sequencing potential:** Next Generation Sequencing (NGS) has the potential to sequence the entire gene of interest or whole exomes or whole genomes (independent of selective gene targets). This helps in thorough analysis of the variant (with respect to its impact on the protein product) associated with any specific health condition. Microarray is limited by its ability to sequence only known set of gene targets/SNPs.
- 2. Sequencing Technology and variant detection:** Illumina sequencing technology, sequencing by synthesis (SBS), is a widely adopted next-generation sequencing (NGS) technology worldwide which uses four fluorescently- labeled nucleotides to sequence the tens of millions of clusters on the flow cell surface in parallel. The end result is true base-by-base sequencing that enables accurate data for a broad range of applications. The method virtually eliminates errors and missed calls associated with strings of repeated nucleotides. DNA Microarrays has a limited dynamic range of detection owing to both high background levels and saturation signals. Arrays can be used to calculate relative concentrations only in an indirect manner.
- 3. Methodological advancements:** The performance of Microarray was under shadow due to the several issues including the probe design, the reaction conditions during spotting, the hybridization and washing conditions. Furthermore, the suppression of nonspecific binding, the distance between the oligonucleotides and the surface also add to these factors. However, NGS technology offers an alternative method to prepare DNA fragments, utilizing an on-bead tagmentation library prep, which integrates the library preparation steps of DNA normalization, fragmentation, and size selection. Following tagmentation, a limited PCR is performed to integrate the adapters for sequencing and barcodes for sample indexing. This workflow is fast and simple, enabling sequencing-ready libraries to be generated in less than 90 min.
- 4. Coverage depth:** Depth of coverage is higher in NGS compared to DNA Microarrays. sequencing depth directly affects the reproducibility of variant detection and varies from 30x to 1000x and 5000x with decrease in error rates, based on various NGS applications.
- 5. Template volume for sequencing:** NGS requires only nano grams of sample DNA whereas Microarray requires micrograms of sample DNA (for whole genome tiling array)

6. **Precision:** The high confidence data of NGS is achieved through the counting of sequence tags whereas confidence of data from microarray is limited by competitive hybridization. This leads to high noise levels and higher number of false positives due to the vast parallel hybridization.
7. **Target Quantification:** DNA Microarray approach does not resolve the problem of multiplexing: the observed signal is still a composite of several components, so that quantification of specific targets remains uncertain. During NGS library preparation, with multiplex sequencing, individual "barcode" sequences are added to each DNA fragment so that each read can be identified and sorted before the final data analysis.
8. **Data analysis:** Microarray data are intensities from a laser reflectance that are treated as continuous data. Assumptions are made as part of the analysis that the logarithm of intensity is approximately normally distributed as this simplifies statistical analysis. Prediction algorithms are used.
With sequencing data, the data are the number of reads mapped to each feature. The data can be visualized through various tools and false positives can be eliminated. Hence more reliable.
9. **Yield:** Greater yields of sequencing data can be obtained from high throughput NGS technology compared to Microarrays.
10. **Detection of Fusions:** Rearrangements or Fusions can't be detected by DNA microarrays which is possible with Targeted NGS methodologies.

However, the two techniques, NGS and DNA Microarrays have benefits of their own and are can address different research needs.

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