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Top 5 Advantages of Metagenomic Next-Generation Sequencing for Microbial Detection

Metagenomic next-generation sequencing (mNGS), also known as shotgun metagenomic sequencing, is a relative newcomer to labs but is quickly becoming an indispensable tool for researchers by providing state-of-the-art, comprehensive analysis of both microbial and host genomes.

How is mNGS performed? At a basic level, separate DNA and RNA sequencing libraries are constructed from a single sample, which may contain mixed populations of microbes. High-throughput sequencing is conducted using these libraries, and data are analyzed bioinformatically, assigning taxa to their reference genomes to identify which microbes are present and in what proportion. A wide array of sample types can be analyzed by mNGS, including plasma, cerebrospinal fluid, joint aspirates, tissue samples, respiratory samples, and ocular fluid.[1,2]

mNGS has multiple advantages over alternative methods for microbial detection, such as culture, PCR, and 16S/18S sequencing. Here are our top five reasons to consider trying out mNGS in your next study.

Advantage 1:

Unmatched comprehensive sequencing analysis of ALL microbial and host DNA/RNA

Unlike other molecular assays (quantitative PCR and 16S/18S ribosomal RNA sequencing) or culturing, mNGS captures the sequences of all DNA or RNA present in a sample, providing a high-throughput technique to sequence not only an entire microbiome, but also the human host genome or transcriptome, all from a single sample.[1]

mNGS identifies the full spectrum of microbes, including bacteria, viruses, fungi, and parasites.

By way of comparison, PCR and 16S/18S ribosomal RNA sequencing (16S/18S sequencing) are both more targeted, requiring specific primers and probes, which narrows the output to only a subset of all genomic content.[1,3]

In addition, 16S/18S sequencing can only detect bacterial and eukaryotic organisms, excluding viruses from this analysis. mNGS identifies the full spectrum of microbes, including bacteria, viruses, fungi, and parasites.[1]

Advantage 2:

Highly quantitative results with the appropriate controls and software

Sequencing outputs from mNGS are not only comprehensive, but also quantitative. Quantitative results are useful for monitoring the trajectory of infections over time, assessing treatment efficacy, and distinguishing contaminants from pathogens. However, culture alone can lead to a distorted view of abundance, particularly for polymicrobial mixtures and unusual or fastidious organisms.[4]

In a head-to-head comparison of quantitative PCR (qPCR) and Arc Bio's GalileoTM Viral Panel, a metagenomic next-generation sequencing reagent and bioinformatics pipeline, mNGS fared very well. Between the two methodologies, the positive percent agreement was 84.9% (73/86), and the negative percent agreement was 90.7% (233/257) for detection of DNA viruses in plasma, with most discrepancies at very low titers.[5] In addition, there was only a 0.25 log10 difference in viral quantitation results between Galileo and qPCR. Galileo even revealed seven subsequently confirmed viruses that were not initially tested for by qPCR.

In another study, Arc Bio's Galileo™ Viral Panel also performed exceptionally well, with 100% agreement as compared with qPCR in detecting viruses in plasma, and even revealing an additional 75 pathogens.[6] Quantitatively, the two technologies also agreed well, with a 0.45 log10 viral load difference between Galileo and qPCR. However, culture alone can lead to a distorted view of abundance, particularly for polymicrobial mixtures and unusual or fastidious organisms, and PCR only quantifies targeted organisms.

Advantage 3:
Detects novel and unculturable species

mNGS has a much greater capacity to identify novel species as compared to 16S/18S sequencing, and novel species simply cannot be detected with qPCR.[7-9] In a direct comparison to 16S rRNA sequencing, mNGS identified additional relatively lower abundant, yet biologically meaningful genera.[10] mNGS also allows you to detect the microbes in your sample that are unculturable or that require lengthy culture periods.[11,12] Furthermore, mNGS has been used to identify novel pathogens, including emerging viruses.[8,9]

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Advantage 4:
mNGS surpasses 16S/18S rRNA sequencing with higher taxonomic resolution

The taxonomic resolution with mNGS is superior to that of 16S/18S sequencing. While 16S/18S sequencing can typically only achieve resolution at the genus/species level, mNGS detects many more individual species, and even strains.[3,13,14] 16S/18S sequencing is limited in that it relies on the conserved and variable regions of the bacterial 16S or eukaryotic 18S rRNA gene for taxonomic classifications.[14] mNGS provides coverage of genomic regions outside of the small rRNA gene, resulting in a more comprehensive output.[14]

Advantage 5:
Unbeatable microbial yield from a single, low-volume sample with rapid turnaround time

mNGS is not as time-consuming as you might think. A single low-volume primary specimen draw allows you to detect everything in your sample within 24 to 30 hours.

Especially when using an integrated mNGS approach, as with Arc Bio's Galileo ONE platform, the start-up time for running mNGS in your lab is greatly reduced. Galileo ONE overcomes limitations in technical and computational expertise required to conduct mNGS analysis, providing controls, reagents, software, and bioinformatics algorithms to analyze the results.[5]

Metagenomic next-generation sequencing is a research tool that has come into its own, making it an ideal addition to your genomic toolbox. It brings a wealth of data to your study that has been missed by traditional, targeted methods.

Curious about how using metagenomics could enhance your sample analysis? Contact us at **sales@arcbio.com** or **(617) 475-5240** to learn more about the Galileo ONE platform, an integrated, turn-key solution for comprehensive microbial detection and quantification.

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