

Summary

Using the latest technology, our laboratory extracts genetic material from submitted fecal samples, runs analysis to determine the presence and quality of 16S ribosomal RNA sequences, and uses next-generation sequencing (NGS) technology to produce 16S sequences for downstream analysis.



360 DIAGNOSTICS™

Microbiome: Next-Generation Sequencing

Laboratory mice are the most commonly used animal model in translational medical research.¹ In recent years, the connection between the gut microbiota on host physiology and the onset of diseases, including metabolic and neuronal disorders, cancers, gastrointestinal infections, and chronic inflammation, has become an important area of research interest.

One of the more recently recognized challenges with using mouse models in microbiome research is the abundant evidence that mouse phenotypes in disease models vary greatly between laboratories and have been found to be associated with differences in the microbiota.^{2,3}

More recently, the use of mass parallel sequencing, now more commonly referred to as next-generation sequencing (NGS), was developed to simultaneously sequence millions of individual DNA fragments from an individual sample or multiple samples.⁴

NGS technology was quickly extended to the sequencing of polymerase chain reaction (PCR) products generated by broadly reactive primers targeting variable regions of 16S rRNA genes.⁵ When compared to a database of bacteria

16S sequences and analyzed with bioinformatics tools, the result is the generation of a list of bacteria represented within a given sample and the abundance relative to the total number of sequences generated for that sample. The evolution of NGS reaction chemistries continues to improve 16S sequencing. Charles River uses the latest sequencing chemistry available that produces a 2×300 kb nucleotide read. Compared to older chemistries (2×250 kb), this roughly doubles the amount of generated 16S sequence, thereby increasing the accuracy of identification at the genus and species level.

NGS provides a unique tool for monitoring colonies with limited or complex consortia. It also circumvents the challenges of obtaining a relative abundance estimate in cultures that may have important growth requirements or nutrients while using culture techniques that are impacted by the competitive growth advantages of some bacteria over others.

Predictive “big picture” tools that utilize operating taxonomical unit (OTU) determination are used to generate alpha diversity, rarefaction plots and beta diversity principal component analysis (PCoA) available through QIIME™.

EVERY STEP OF THE WAY

References

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