Rapid 16S Next Generation Sequencing for Bacterial Identification in Polymicrobial Samples

Chaitali Parikh, Elena Bolchacova, Stephen Jackson

Thermo Fisher Scientific, 180 Oyster Point Blvd, South San Francisco, CA 94080

ion torrent
by **Thermo Fisher Scientific**

ABSTRACT

In order to identify prokaryotic species in a sample, it is often necessary to culture the sample for hours or days to increase the abundance of bacteria to assayable levels. This often precludes the rapid identification of infectious species. Furthermore, some species are not easily culturable. We have developed a facile research method for identifying bacterial species by 16S ribosomal RNA sequencing on the Ion Torrent platform. The Ion 16S™ Metagenomics Kit is designed to PCR amplify the hypervariable regions of the 16S gene of bacteria. We used this kit to construct libraries from 15 retrospective samples of synovial fluid with various bacterial species either spiked in or present at collection. Libraries were sequenced on the Ion PGM™ system and the data analysis performed using the Ion Reporter™ workflow which provides an automated analysis solution. Bacteria present in the samples were correctly identified in samples containing a single spiked-in species, mixed-species samples, and in infected samples. Thus, the Ion Torrent™ platform provides a mechanism for rapidly identifying bacteria that are present in research samples without culturing.

INTRODUCTION

Rapid and accurate identification of bacterial species in samples is a critical need in clinical microbiology. Traditional culture based and phenotypic methods are time-consuming and fail to identify unculturable or unusual species. Recent advancements in the field of Next Generation Sequencing may provide the tools to overcome these hurdles in the future.

The use of 16S ribosomal RNA in bacterial classification is widely recognized in research. It is especially important in samples of bacteria with unusual phenotypic profiles, rare bacteria, slow-growing bacteria, uncultivable bacteria and culture-negative infections. Bacterial 16S rRNA genes consist of 9 "hypervariable regions" (V1-V9) that can provide species-specific signatures. These are flanked by stretches of highly conserved regions which serve as primer-binding sites.

In this study the Ion 16S Metagenomics kit, consisting of primer pools to amplify multiple variable regions (V2, 3, 4, 6-7, 8 and 9) was used to construct libraries from 15 retrospective samples of synovial fluid with either single or mixtures of bacterial species. The libraries were sequenced on the Ion PGM and data analyzed via the automated Ion Reporter pipeline. With this workflow we were able to correctly identify the bacteria at either the genus or the species level in each sample.
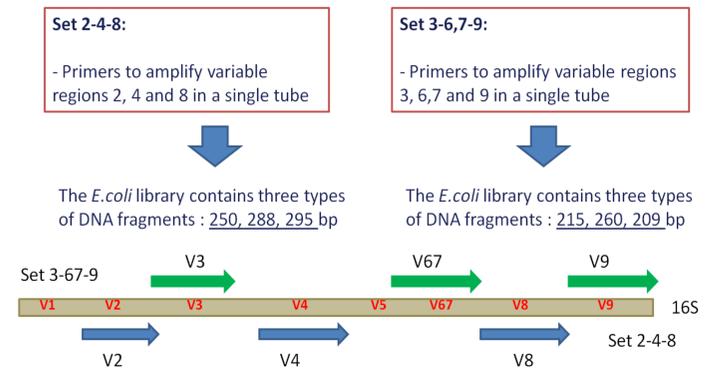

Figure 1. 16S Metagenomics Primer Pools
Two sets of primers pools can simultaneously be used to amplify 7 of the 9 hypervariable regions of the 16S rDNA gene in bacteria:
• Pool 1: Primer set to amplify V2-4-8
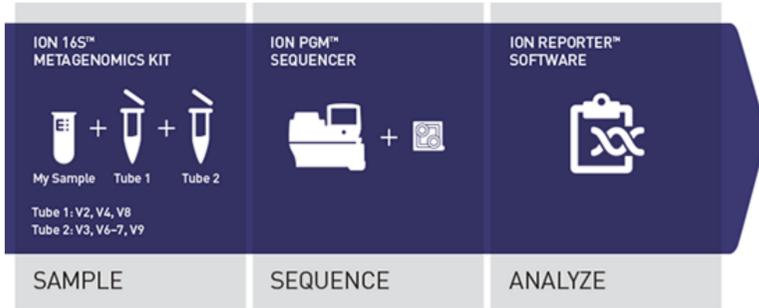• Pool 2: Primer set to amplify V3-6,7-9

Figure 2: Ion 16S Metagenomics Workflow
The two primer pools included in the kit are used to amplify 16S variable regions from samples. After generating amplicons, the Ion Plus™ Fragment Library Kit was used to ligate barcoded adapters and synthesize libraries. Barcoded libraries from all 15 samples were pooled and templated on the OneTouch2™ system followed by 400bp sequencing on the Ion PGM. Automated analysis, annotation and taxonomic assignment occurs via the Ion Reporter Software pipeline. Classification of reads is through alignment to either the curated MicroSEQ ID or curated Greengenes databases.
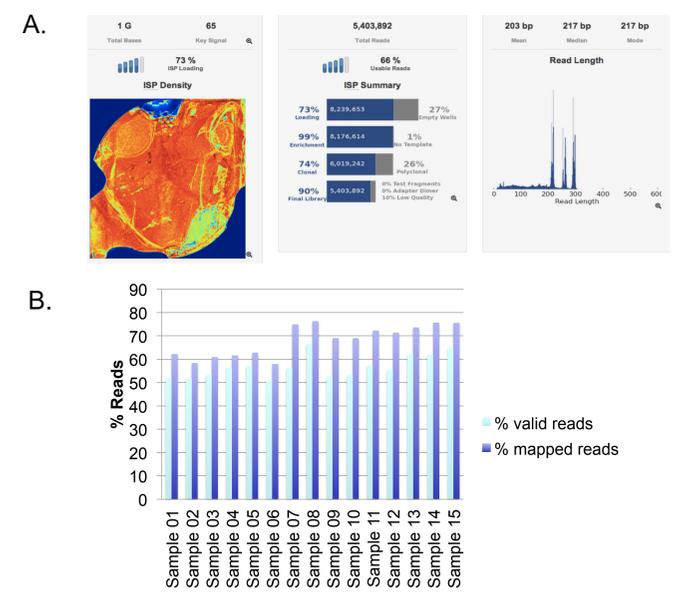

Figure 3: Sample multiplexing in a single sequencing run
A. Sequencing run metrics shows good chip loading resulting in over 5 million total reads after passing various filters. Read length histogram shows peaks at expected sizes.
B. Multiple samples barcoded and sequenced simultaneously on a single Ion PGM 318 chip resulted in sufficient number of reads. The number of total reads per sample was between 2.5x10^6 and 4.5x10^6. Approximately 50-65% of these reads passed stringency filters and of these, 60-75% mapped to the databases.
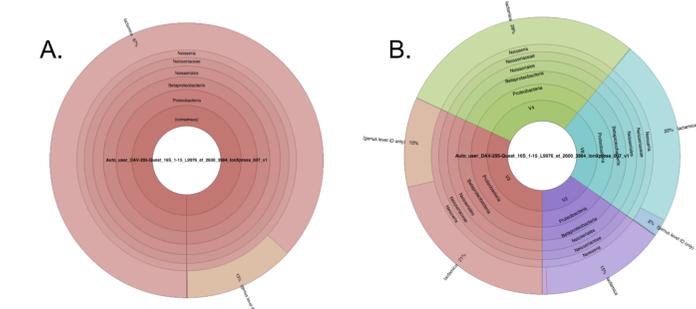

Figure 4: Example of data visualization with Krona charts
A. Consensus view of sample 07. Using stringent analysis parameters gives better resolution.
B. Visualizing results by primers. Surveying multiple V regions simultaneously gives greater confidence in making bacterial identification in this study.

Figure 5. Multiple primer sets allows for accurate identification in single and polymicrobial samples
With the 16S NGS workflow we were able to assign correct species or genus level identification in "mock" single or polymicrobial samples as well as in biological samples.

CONCLUSIONS

1) Using a Next Generation Sequencing approach, we demonstrate a rapid and sensitive method for the identification of bacterial species in polymicrobial samples.
2) Samples can be highly multiplexed to reduce time and cost.

For Research Use Only. Not for use in diagnostic procedures.

REFERENCES

For more information, please visit www.lifetechnologies.com.