Deep Sequencing Offers New Insight Into Diverse Populations Of Microorganisms That Infect Patients Living With Cystic Fibrosis

Characterization of bacterial population changes with disease progression provides new understanding of total patient treatment.

Deep sequencing offers new insight into diverse populations of microorganisms that infect patients living with Cystic Fibrosis. Characterization of bacterial population changes with disease progression provides new understanding of total patient treatment. Deep sequencing is a powerful new tool to characterize the disease metagenome of the airways from patient living with Cystic Fibrosis. Previous sample preparation methods were hindered by the inability to remove unwanted nucleic acids (usually ribosomal RNA) that interfere with sequencing through reduction of quality and depth of data. Comparison of multiple rRNA removal methods were compared and validated by the analysis of high through sequence data from eight viromes, eight microbiomes, and ten microbial metatranscriptomes. Comparison of commercially available kits showed that the Ribo-Zero™ rRNA removal kit (Epidemiology) was the most effective in removing both microbial and host rRNA from the metatranscriptomes. Contaminating host material was reduced from 98% to less than 5% in the viral metagenomes, and from more than 99% to as little as 13% in the microbial metagenomes. When the samples were clustered into groups based on their metatranscriptomes. The CF4-A taxonomy is not shown here because an rRNA removal kit was used during sample preparation. The amount of Ribosomal RNA (rRNA) in each library before and after experimental subtractive hybridization. The efficiency of the rRNA-removal method varies between samples and with RNA integrity.

Introduction

Cystic Fibrosis (CF) affects approximately 30,000 individuals in the United States, and complications associated with the illness can lead to respiratory failure and death. Samples collected from CF patient airways often contain large amounts of host-derived nucleic acids that interfere with recovery and purification of microbial nucleic acids. Here, we present the methodology to study the microbial community in CF sputum using a metagenomic approach, and the gene expression dynamics of microbial assemblages associated with CF using a metatranscriptomics approach.

Importance of Metatranscriptomics

Deep sequencing offers new insight into diverse populations of microorganisms that infect patients living with Cystic Fibrosis. Characterization of bacterial population changes with disease progression provides new understanding of total patient treatment. Deep sequencing is a powerful new tool to characterize the disease metagenome of the airways from patient living with Cystic Fibrosis. Previous sample preparation methods were hindered by the inability to remove unwanted nucleic acids (usually ribosomal RNA) that interfere with sequencing through reduction of quality and depth of data. Comparison of multiple rRNA removal methods were compared and validated by the analysis of high through sequence data from eight viromes, eight microbiomes, and ten microbial metatranscriptomes. Comparison of commercially available kits showed that the Ribo-Zero™ rRNA removal kit (Epidemiology) was the most effective in removing both microbial and host rRNA from the metatranscriptomes. Contaminating host material was reduced from 98% to less than 5% in the viral metagenomes, and from more than 99% to as little as 13% in the microbial metagenomes. When the samples were clustered into groups based on their metatranscriptomes. The CF4-A taxonomy is not shown here because an rRNA removal kit was used during sample preparation. The amount of Ribosomal RNA (rRNA) in each library before and after experimental subtractive hybridization. The efficiency of the rRNA-removal method varies between samples and with RNA integrity.

Experimental Approach

Sputum samples were collected from Cystic Fibrosis in accordance with appropriate institutional Review Boards. Samples were labeled as “on treatment” “Post treatment” “stable” of “exacerbation”, the total RNA extracted using Trizol (Life Technologies) and Zymo Clean and Concentrator columns. Ribosomal depletion was compared using several rRNA removal kits (MicrobExpress and MicrobEnrich [Ambion], and Ribo-Zero Epidemiology rRNA removal kit [Epicentre, an Illumina Company]). Libraries were prepared for sequencing on a 454/GS/FLX sequencer.

Bioinformatics

(A) Taxonomy is assigned based on at least 50% query coverage and 80% alignment identity. (B) Bioinformatics pipeline for metagenomes. Taxonomy and functions are assigned based on the BLAST search against NT, NR and SEED database, with at least 60% query coverage and 40% alignment identity.

Conclusion

The microbial metatranscriptomics approach monitors the active community metabolism, as opposed to the metabolic potential encoded in the genomes. Of the three measures, the metatranscriptomes showed the greatest variation between patients and over time, thus is best able to capture the dynamic nature of these complex communities.