We also evaluate multiplexing capabilities using PacBio® high-throughput sequencing of the complete 16S rRNA gene Circular Consensus Sequence (CCS) Reads. Further 16S analysis is carried out using PacBio’s rDNA tools. CCS reads are exported, quality filtered, and where required output sequences may be classified using a number of communities. Ambigous rDNA populations representing soil, fecal, and mock communities. CCS Sequencing and Analysis Workflow

Circular Consensus Sequence (CCS) Reads
- PacBio’s long reads and circularized templates provide high-quality consensus from multiple passes over the same molecule.

Analysis Workflow
- CCS reads are exported, quality filtered, and where required binned by barcode using the “Reads of Insert” program in the Mothur package (http://www.mothur.org). The complete rDNA tools analysis pipeline with custom scripts is available for download on GitHub: https://github.com/PacificBiosciences/rDnaTools.
- Analysis of 16S CCS sequences may be carried out with standard tools from the Mothur package (http://www.mothur.org) combined with Python custom scripts. The complete rDNA tools analysis pipeline with custom scripts is available for download on GitHub: https://github.com/PacificBiosciences/rDnaTools.

rDNA Tools Pipeline

Accurate Single Molecule 16S Sequences
- The PacBio SMRT Portal Reads of Insert protocol generates highly accurate CCS reads. Filtering parameters are adjustable according to project needs.
- The accuracy of SMRT sequencing depends on filtering criteria according to project needs. Filtering parameters are adjustable to generate highly accurate CCS reads.
- The output of high-quality CCS reads enables highly accurate filtering.

Output of 99.9% predicted accuracy filtering

16S Analysis of BEI Mock Communities
- Full-length 16S amplification was performed on two mock microbial community samples from BEI Resources.
- Microbial Mock Community B, Even, containing DNA from 20 bacterial strains at ~equimolar concentrations.
- Microbial Mock Community B, Staggered, containing DNA from 20 bacterial strains at up to 4 orders of magnitude differential in concentrations.
- SMRTBench™ Libraries were prepared following standard protocols.
- A single SMRT Cell was run for each BEI Mock Community using standard P6-C4 chemistry and protocols, with a three-hour collection time.
- Results of demultiplexing using SMRT Portal Reads of Insert protocol. All samples were represented by >2000 reads/cell with stringent filtering criteria.

Multiplexing 16S Samples with Barcodes
- 16S amplification was carried out on five metagenomic samples, three soil, one fecal, and one mock community, using PCR primers tagged with PacBio barcodes.
- Equimolar amounts of five barcoded amplicons were pooled for SMRTbell library preparation using standard protocols.
- Five SMRT Cells were run for each library using standard P6-C4 chemistry and protocols, with 3-hour collection times.

Conclusions
- SMRT Sequencing provides accurate, full-length 16S sequences for identification of community constituents to the species level.
- PacBio provides tools for 16S data analysis: Reads of Insert analysis for generation of accurate single-molecule sequences and barcode demultiplexing, and rDNA tools pipeline for 16S-specific filtering and clustering.
- With the PacBio RS II system, genomes at 0.01% relative abundance can be accurately identified with a single SMRT Cell.
- Multiplexing with PacBio barcodes offers cost-effective solutions for 16S sequencing.

References

Acknowledgements
The authors would like to thank Dr. John Crocker, Dr. Daniel Hickey, and the members of his team for sharing the results of their sequencing experiences. They would also like to thank the DOE Joint Genome Institute, Argonne National Laboratory, for allowing us to use their equipment. They would also like to thank the DOE Joint Genome Institute, Argonne National Laboratory, for allowing us to use their equipment.