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PyroTag: Pyrosequencing as a Powerful Tool to Study the Diversity of Microbial Community

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Introduction

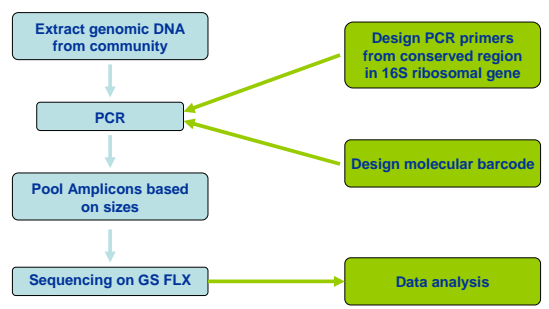
At JGI, major efforts have been spent on using the 16S RNA sequencing to study the composition of bacterial community. Currently, 16S rRNA genes from a microbial community are amplified with conserved PCR primers and the mixed amplicon is cloned and sequenced using traditional Sanger sequencing method. Using Roche GS FLX amplicon sequencing method, individual amplicon can be sequenced directly on the platform, producing hundreds of thousand ~250 bp tags of the full length 16S rRNA gene. We call this pipeline PyroTag. The benefit of the PyroTag technology includes higher resolution and less bias than traditional PCR and cloning based sequencing. The disadvantage is that the read length is short as we used FLX reagent kit for the study.

The aim for this study is to systematically test the effect of amplicon length and 16S rRNA regions targeted on diversity coverage of complex microbial community. The goal is to develop a standard PyroTag pipeline to provide to broad range JGI users.



Molecular barcodes have been used to distinguish different samples, amplicon lengths, and different targeted 16S regions. In this study, we used the bacterial community from termite gut as the targeted environmental sample. Strategy and detailed experiment design as well as preliminary results will be presented.

Experiment Design



Conclusion

PyroTag is a powerful approach to study bacterial community diversity. Comparing to traditional cloning and Sanger Sequencing, it tremendously reduces the workload and provides higher resolution. It is also less biased than the traditional approach because no cloning is involved. It has the throughput traditional approach can not match.

Molecular barcodes provide an effective way to study multiple communities in one experiment setting. Neighboring hypervariable regions can be targeted separately or together when they are within the sequencing read length.

We are testing the Titanium platform for this purpose. The long read length of the platform should provide more flexibility.

Method

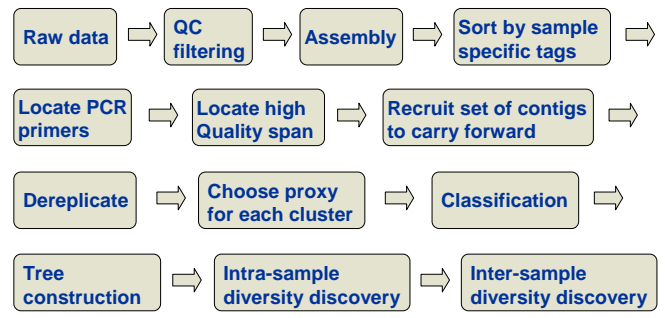
Genomic DNA extraction:

- Termite hind guts were collected on the site (Costa Rica)
- Community genomic DNA was extracted by regular phenol-chloroform extraction method
- Same pool of genomic DNA was used for all 16S region PCR amplifications
- Diluted genomic DNA (5ng/ul) was used for PCR

PCR and pooling:

- 25 cycles of optimized PCR reaction
- Standard PCR clean-up
- PCR product quantification
- Same number of molecules were pooled
- Similar length or different length

Data Analysis pipeline (under development):

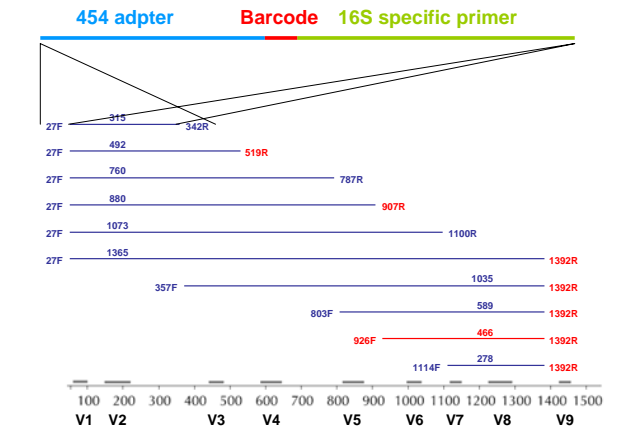


454 sequencing:

- Standard Amplicon sequencing on GS FLX

PCR primer and molecular barcode design:

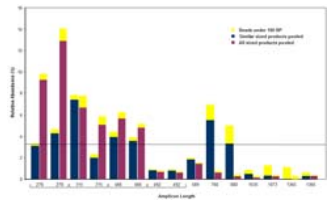
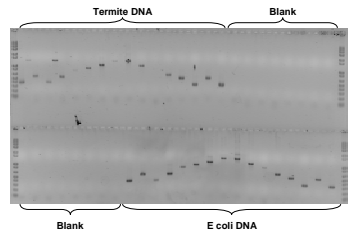
- Unmodified 454 A and B primer sequence
- Molecular barcode after TCAG key
- 16S specific forward and reverse primer after barcode



Results

PCR product validation:

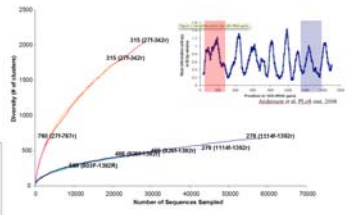
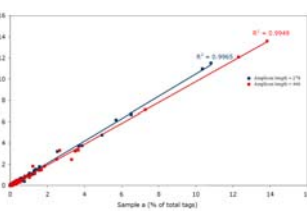
All amplicon sizes worked well under our PCR condition



emPCR biased against long fragments when different sizes were mixed
Amplicon sizes up to 900 bp can be sequenced
Similar sized fragments can be mixed in emPCR stage and sequenced as a pool

Pipeline highly reproducible:

Amplicon size does not have major effect on reproducibility.



Rarefaction curve:

Results showed that amplicon size appears to have minimal effect on diversity estimates.
In contrast, 16S HVR has major effect on diversity estimates.

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