What the Taq? The Influence of Different Hi-Fidelity Taq Polymerase on 16S rRNA Sequencing

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Abstract

Background.

Methods.

Results.

Conclusions.

Introduction

Materials & Methods

Human and Mock Samples: A single fecal sample was obtained from 4 individuals who were part of the Enterics Research Investigational Network (ERIN) and the processing and storage of these samples have been published previously (Seekatz et al., 2016). Clinical data and other types of meta data were not utilized or accessed for this study. All samples were extracted using the MOBIO™ PowerMag Microbiome RNA/DNA extraction kit (now Qiagen, MD, USA). The ZymoBIOMICS™ Microbial Community DNA Standard (Zymo, CA, USA) was used in this study and is made up of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Lactobacillus fermentum*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus subtilis* at equal genomic DNA abundance (http://www.zymoresearch.com/microbiomics/microbial-standards/zymobiomics-microbial-community-standards).

PCR Protocol: The five different high fidelity (HiFi) Taq DNA polymerase that were tested were AccuPrime™ (ThermoFisher, MA, USA), KAPA HIFI (Roche, IN, USA), Phusion (ThermoFisher, MA, USA), Platinum (ThermoFisher, MA, USA), and Q5 (New England Biolabs, MA, USA). The PCR cycle conditions were the same for every primer (Kozich et al., 2013) (https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP_v4.md). If the HiFi Taq had a specific activation time that was different then 2 minutes that was used instead. The 30 cycle default was used but the cycle conditions started at 15 and increased by 5 up to 35 cycles and was used for both fecal and mock samples. The fecal PCR consisted of all 4 samples at 15, 20, 25, 30, and 35 cycles for each Taq (total samples = 100). Although, the mock communities also had 4 replicates used for 15, 20, 25, and 35 cycles, 10 replicates were used for 30 cycles for all Taq (total samples = 130). For all the mock community samples there was not enough PCR product at 15 cycles for adequate sequencing.

Sequence Processing: The mothur software program was utilized for all sequence processing steps (Schloss et al., 2009). The protocol followed was similar to what has been previously publsihed (Kozich et al., 2013) (https://www.mothur.org/wiki/MiSeq_SOP). Two major differences from the stated protocol were the use VSEARCH instead of UCHIME for chimera detection and the use of the OptiClust algorithmn instead of average neighbor for OTU generation (Edgar et al., 2011; Rognes et al., 2016; Westcott & Schloss, 2017). Sequence error was determined using the seq.error command on mock samples after chimera removal and classification to the RDP to remove non-bacterial sequences (Schloss et al., 2009; Cole et al., 2013; Rognes et al., 2016).

Statistical Analysis: All analysis was done with the R (v 3.4.2) software package (R Core Team, 2017). Data tranformation and graphing was completed using the tidyverse package (v 1.1.1) and colors selected using the viridis package (v 0.4.0) (Garnier, 2017; Wickham, 2017). The total number of OTUs were analyzed using an ANOVA with a tukey post-hoc test. For the fecal samples the data was normalized to each individual by cycle number to account for the biological variation between different people. For both error and chimera analysis, samples were tested using Kruskal-Wallis with a Dunns post-hoc test. Where applicable correction for multiple comparison utilized the Benjamini-Hochberg method (Benjamini & Hochberg, 1995).

Reproducible Methods: The code and analysis can be found here https://github.com/SchlossLab/Sze_PCRSeqEffects_XXXX_2017.

Results

Insert Sub title here

54 # Discussion

Conclusion

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Table 1:

Table 2:

Figure 1: .

Figure 2: .

Figure 3: .

Figure 4: .

Figure 5: .

Figure 6: .

Figure 7: .

Figure S1: .

Figure S2: .

Figure S3: .

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