Methodology Challenges of 16S rRNA Bacteria Identification in Viperfish

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Summary

- Total DNA was isolated from viperfish stomach and intestine for Nanopore 16S rRNA sequencing
- Compared to similar studies using Illumina 16S V3 sequencing, our number of reads and sequence quality are lower

Abstract

Studying the gut microbiome of the deep-sea fish, such as the viperfish, may reveal how these organisms survive under the harsh conditions such as low oxygen and temperature. We isolated total DNA from the stomach and intestine of four viperfish, and prepared the samples for Nanopore 16S rRNA sequencing. Compared to a similar study utilizing Illumina 16S V3 sequencing, we had lower data output and quality. The presence of viperfish DNA likely inhibited the amplification during library preparation, and future efforts should focus on host DNA depletion.

Introduction

Hypothesis

- Nanopore sequencing should produce more accurate data on Viperfish gut microbiota than Illumina sequencing via 16S rRNA identification.

Viperfish

- Found in the marine waters of tropical areas (Gibbs et al., 1984).
- Bathypelagic fish that can live at 2800 meters below the ocean surface (Butler et al.,2001).
- Habitats have limited light, low temperatures, high pressure, and low nutrition levels (Collins et al., 2021). Its diet consists of crustaceans and bony fish (Gibbs et al., 1984).

16S rRNA gene

- The 16S ribosomal RNA gene is a small subunit commonly used in sequencing to identify bacteria (Clarridge et al., 2004).

Nanopore vs. Illumina

- A third-generation sequencing method, Nanopore can process long DNA strands and generate long reads (Santos et al., 2020).
- A mature second-generation sequencing method, Illumina can generate high accuracy short reads (Johny et al., 2018).
- Illumina 16S rRNA sequencing often utilizes the MiSeq sequencer and focuses on the V3 region, while Nanopore can sequence the entire 16S gene.

Methodology

Sample Collection

- Viperfish were collected at 44°39'07.2"N 126°03'00.0"W, about 650 m below ocean surface.
- The intestine and stomach of the Viperfish were dissected and frozen at -80°C.

DNA Extraction

- Total DNA was isolated from each of the dissected sample with DNeasy Blood and Tissue kit.
- DNA quality and quantity were checked with Nanodrop.

Library Preparation

- Nanopore 16S Barcoding Kit 24 V14 was used to amplify the 16S rRNA gene.

Nanopore Sequencing

- The prepared libraries were sequenced using MinION Mk1B.

Sequence Data Analysis

- Sequencing data were analyzed with EPI2ME (Version 3.7.3)
- Sequence alignment and species identification workflow: Fastq 16S (Version 2023.04.21-1804452)

Results

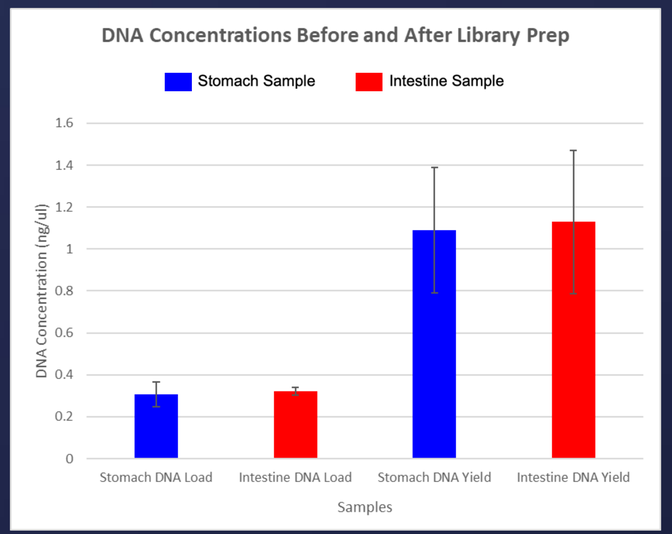

Figure 1. On average, the library preparation yield from intestine DNA samples are similar to that of stomach.

Figure 2. The number of reads generated over time. Passed reads have quality scores equal to or above 8, while failed reads quality scores are below 8.

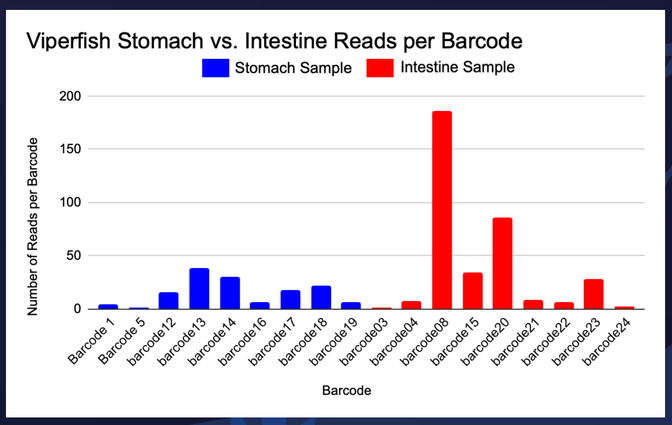

Figure 3. The number of reads from intestine DNA samples are overall higher than those of the stomach samples.

Discussions/Conclusions

Unexpectedly, the quality of our Nanopore sequencing data is lower than that of Illumina sequencing data reported in the similar study by Johny et al.

- The number of reads is also lower. A total of 66.8 k reads were generated in our experiment (Figure 2), while Johny et al. reported 902.9 k.
- The library prep yields were lower than expected (Figure 1), which likely resulted in the lower number of reads.
- Fish DNA should be the majority of the total extracted DNA, and probably lowered PCR efficiency during library prep.
- The inhibited PCR may have caused an abundance of PCR artifacts, resulting in the overall low quality score.

Although the library prep yields are similar between stomach and intestine samples (Figure 1), the intestine samples generally have higher number of passed reads than the stomach samples (Figure 3).

- Fish generally have the more bacterial growth in their intestines than stomach (Apun et al., 2010). The libraries from stomach samples may contain more of the PCR artifacts.

We conclude that reducing the amount of fish DNA is key to improve future trials, and Illumina sequencing should be performed in parallel to Nanopore for quality checking.

Study Limitations & Future Directions

- Sequences of human and bacteria often living in humans were detected from the samples.
- Better sterile technique should be practiced to avoid contamination in the future.

- The study by Johny et al. extracted bacterial DNA from fish gut contents, via QIAamp DNAstool minikit.
- Extracting DNA without lysing the full intestine should significantly reduce the amount of fish DNA and improve PCR efficiency and quality during library prep.

References

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