Short- and long-read metabarcoding of the eukaryotic rRNA operon: evaluation of primers and comparison to shotgun metagenomics sequencing

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Abstract

High-throughput sequencing for analysis of environmental microbial diversity has evolved vastly over the last decade. Currently the go-to method for microbial eukaryotes is short-read metabarcoding of variable regions of the 18S rRNA gene with <500 bp amplicons. However, there is a growing interest in long-read sequencing of amplicons covering the rRNA operon for improving taxonomic resolution. For both methods, the choice of primers is crucial. It determines if community members are covered, if they can be identified at a satisfactory taxonomic level, and if the obtained community profile is representative. Here, we designed new primers targeting 18S and 28S rRNA based on 177,934 and 21,072 database sequences, respectively. The primers were evaluated in silico along with published primers on reference sequence databases and marine metagenomics datasets. We further evaluated a subset of the primers for short- and long-read sequencing on environmental samples in vitro and compared the obtained community profile with primer-unbiased metagenomic sequencing. Of the short-read pairs, a new V6-V8 pair and the V4_Balzano pair used with a simplified PCR protocol provided good results in silico and in vitro. Fewer differences were observed between the long-read primer pairs. The long-read amplicons and ITS1 alone provided higher taxonomic resolution than V4. Together, our results represent a reference and guide for selection of robust primers for research on and environmental monitoring of microbial eukaryotes.

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