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Conference Abstract

Optimization and Application of direct PCR in community metabarcoding

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Abstract

Direct PCR allows the amplification of DNA from animal or plant tissue samples without the need for DNA extraction and purification steps. For this procedure, dry tissue is homogenized, dissolved in water and subsequently amplified, thus, its successful application largely depends on the absence of PCR inhibitors. Although this method has been successfully applied in barcoding approaches of invertebrates, it has not yet been attempted in metabarcoding approaches. We used nonbiting midges (Diptera: Chironomidae) to test if amplicons produced by direct PCR could be used for next-generation sequencing. To access whether direct PCR is applicable for a variety of chironomid species, we tested 236 adult specimens randomly selected from emergence traps of an artificial pond mesocosm. We used ground tissue, corresponding to 0.1% of the specimens' biomass, and a direct PCR protocol following Wong et al. (2014) for amplification. In total, 98 % of the samples were successfully amplified and we found a diverse community comprised of 20 different genera. In order to compare direct PCR and ´traditional´ DNA isolation-PCR, we created mock communities (14 species) and used both approaches for the amplification of a 421 bp COI fragment. After a second PCR for indexing and adapter ligation, samples were sequenced on an Illumina sequencer. We found only slightly lower recovery rates for mock communities with the direct PCR approach compared to traditional protocols. These recovery rates were further improved for both methods when an equal biomass (ca. 0.006 mg) of chironomid specimens was used. With our approach, it was possible to detect species which constituted only 1% of the entire biomass of a sample. Generally, direct PCR did not have a large effect on sequence

read abundance. However, read abundance varied strongly between species. We are currently investigating whether this was caused by primer bias or an artifact of differently degraded tissue. This study is a proof of principle that the amplicons produced by direct PCR can be used for next-generation sequencing, with possible applications for future biomonitoring projects and portable laboratory technologies. We are currently using this technique to monitor a large-scale chironomid community experiment (artificial pond mesocosm facility) covering weekly samples taken over two summer half-years.

Keywords

Direct PCR, community metabarcoding, Chironomidae

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