

## Amplicon sequencing using the portable Oxford Nanopore MinION for fish DNA reference database: a fast, reliable and promising approach

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Tanzania hosts fish diversity in the coral reefs and seagrasses that are threatened by overfishing, calling a need for effective and rapid monitoring. Application of molecular techniques enables biodiversity assessment through environmental DNA applications. Unfortunately, in developing countries there are incomplete reference databases and no readily available sequencing services. We used the portable Oxford Nanopore's MinION Sequencing device to analyse 190 fishes from Tanzania (with reference photographs). In addition to the standard COI based identification, we employed a novel approach to amplify two variable regions of the 12S gene in one PCR product covering the fragments recommended for eDNA studies.

We successfully amplified about 670bp of the 12S region ("miFish" and "teleo") across a broad range of fish taxa and sequenced at the ZMT- Biolab. Amplification success of this novel 12S fragment was higher as in COI. For the 655bp COI fragment we obtained 131 high quality barcodes of 665bp (+24 low quality barcodes) giving 117 exact BLAST matches within the OTU threshold (identity  $\geq$  99%, query Coverage  $\geq$  80%) and 14 close matches (94-98% identity) by BLAST searches. Close matches were from Perciformes, Beloniformes and Clupeiformes, indicating species lacking reference sequences. For 12S we gained 171 valid barcodes with length 661-741bp (mean 670bp). Only 58% of the barcodes revealed  $>99\%$  identity, 67 close match (90-99% identity), 4 weak match ( $<90\%$  identity), highlighting the importance of generating a regional reference database in advance of eDNA metabarcoding.

Using the protocol from DNA extraction to sequence data can take up to two days, and with further experience one day can be possible. In conclusion, the portable and affordable MinION Sequencing provided comparable results to the traditional sanger sequencing method. These promising results suggest that the device can be adapted by managers to rapidly uncover biodiversity for protection and sustainability.

### Keywords

MinION; Barcoding; Sequencing; Gene; Database