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21840 | Optimization of DNA Extraction Methods for Metagenomic Analysis in Surface Water Samples

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Background & Aim: Metagenomics is vital for studying microbial communities, shedding light on ecosystem dynamics and biodiversity. However, microbial DNA extraction is a challenge, namely when dealing with environmental samples. We aimed to optimize DNA extraction from water and sediment samples of different rivers used for drinking water production to obtain high-quality DNA for metagenomics analysis. **Methods:** The tested samples included 28 water and 29 sediments from 6 rivers of North of Portugal (10-months/2022-2023). At the same day of sampling, water (450-900 ml) was filtered through a 0.22µm nitrocellulose membrane (450 ml per membrane). The sediments varied in the type of texture and water in turbidity. Membranes and sediments were stored at -80ºC before DNA extraction using the DNeasy PowerSoil kit (Qiagen) with standard or modified conditions (e.g. increasing sample amount-SA or temperatures adaptation-TA and time extension-TE in lysis steps). The aim was to obtain a yield of >20ng/µl (>1000ng) and a DNA/protein 260/280 ratio of 1.6-2.5 (NanoDrop spectrophotometer) required for sequencing. PCR of 16S rRNA gene was done to confirm DNA extraction in test samples and in negative controls to exclude contaminations. **Results:** Standard protocol conducted to DNA concentrations of 28-40 ng/µl (260/280>1,6/>1000ng) for only 2 water and 2 muddy sediments, with all other samples having concentration of 0-10 ng/µl. With protocol modifications required DNA concentrations and quality was obtained with TE+TA (n=26 of water; n=23 of muddy sediments; 21-85ng/µL) and TE+TA+SA (n=4 of sandy sediments; 25-140ng/µL). The DNA of 23 samples, including of 19 under protocol modifications, were sent for outsourced sequencing services, all passing quality control. Negative controls did not amplify the 16S RNA gene. **Conclusions**: Unique characteristics of environmental samples may influence

the amount and quality of microbial DNA extraction for metagenomics, which can be easily overcome by few modifications in commercial kits protocols.

Keywords: Metagenomics, Environmental Samples, Dna Extraction.

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