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Validation of a universal DNA extraction method for human and microbiAL DNA analysis

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ABSTRACT

The study of microbiomes has enormous potential for forensic science because microorganisms are ubiquitous and particular communities of microbes are often associated with specific processes or environments. With recent advances in microbiome science, new opportunities exist for microbiome technologies in forensic science (PMI estimation, location of clandestine graves, soil analysis and personal identification). Before a new technology is accepted by the forensic science, it requires an initial validation phase.

The aim of our study was to evaluate if the DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega) is suitable for microbial DNA extraction, without modifications. Ten bacterial strains were selected and subjected to the GenElute Bacterial Genomic DNA extraction protocol (Sigma-Aldrich) and to the DNA IQ™ Casework Pro Kit for Maxwell® 16 protocol. Extracted DNA was quantified and submitted to NGS analysis on an Ion S5 NGS System. Data were analyzed using the Ion Reporter Software metagenomics workflow. Our work has shown that it is possible to purify both microbial and human DNA using the Promega kit, thus making it possible to analyze both human and microbial DNA from a single trace, a pivotal factor in forensics where the quantities of biological material available are usually very limited.

1. Introduction

The forensic potential of microorganisms is becoming increasingly apparent as a consequence of advances in molecular sciences and genomics [1]. Next to their abundance, the composition of microbial communities associated with humans is very diverse at different body sites. NGS technologies have increased exponentially the amount of sequencing data available and their high sensitivity allowed to investigate 16S rRNA gene for detecting previously unknown, rare and often non-cultivable microbiome members. Accurate profiling of microbial communities associated with different body sites requires DNA extraction methods that provides DNA of adequate quality and quantity as well as sufficient coverage of the original bacterial community [2].

In this study, we evaluated if the forensic extraction method used for human biological traces is able to extract also microbial DNA. Two different commercial kits, GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) and DNA IQ™ Casework Pro Kit for Maxwell® 16 (Promega) were compared. The sampled body sites were mouth, noise and ear.

In addition, the performance of the two methods was investigated analyzing two mock samples, one from each extraction protocol, and several dilutions of the mock samples.

2. Material studied, methods, techniques

Ten different bacterial species, 6 Gram-positive (*Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecium, Bacillus thuringiensis, Listeria monocytogenes, Mycobacterium abscessus*) and 4 Gram-negative (*Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*), were selected, plated onto blood agar base supplemented with 5% defibrinated horse blood and incubated at 37 °C for 24 h (72 h for *M. abscessus*). A single colony was inoculated in 5 mL of brain heart infusion broth and incubated at 37 °C for 24 h (72 h for *M. abscessus*). Each culture was split into two aliquots and DNA was extracted with two different kits: GenElute Bacterial Genomic DNA Kit and DNA IQ™ Casework Pro Kit for Maxwell® 16, following the manufacturer's instructions. Each extraction was performed in triplicate. Extracted DNA was quantified by NanoDrop.

2 µl of DNA extracted from each bacterial culture were mixed in

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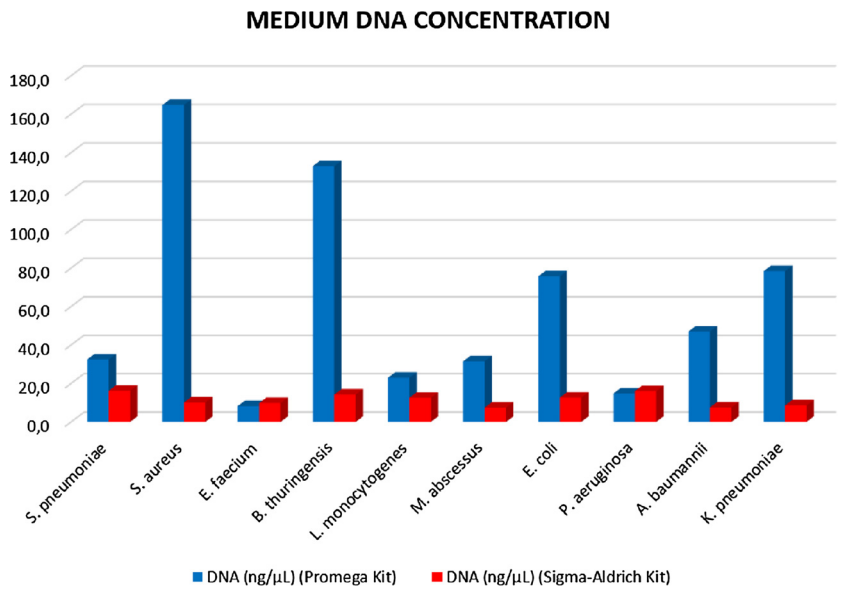

Fig. 1. Comparison between DNA concentrations obtained from different bacterial species using two different extraction methods.

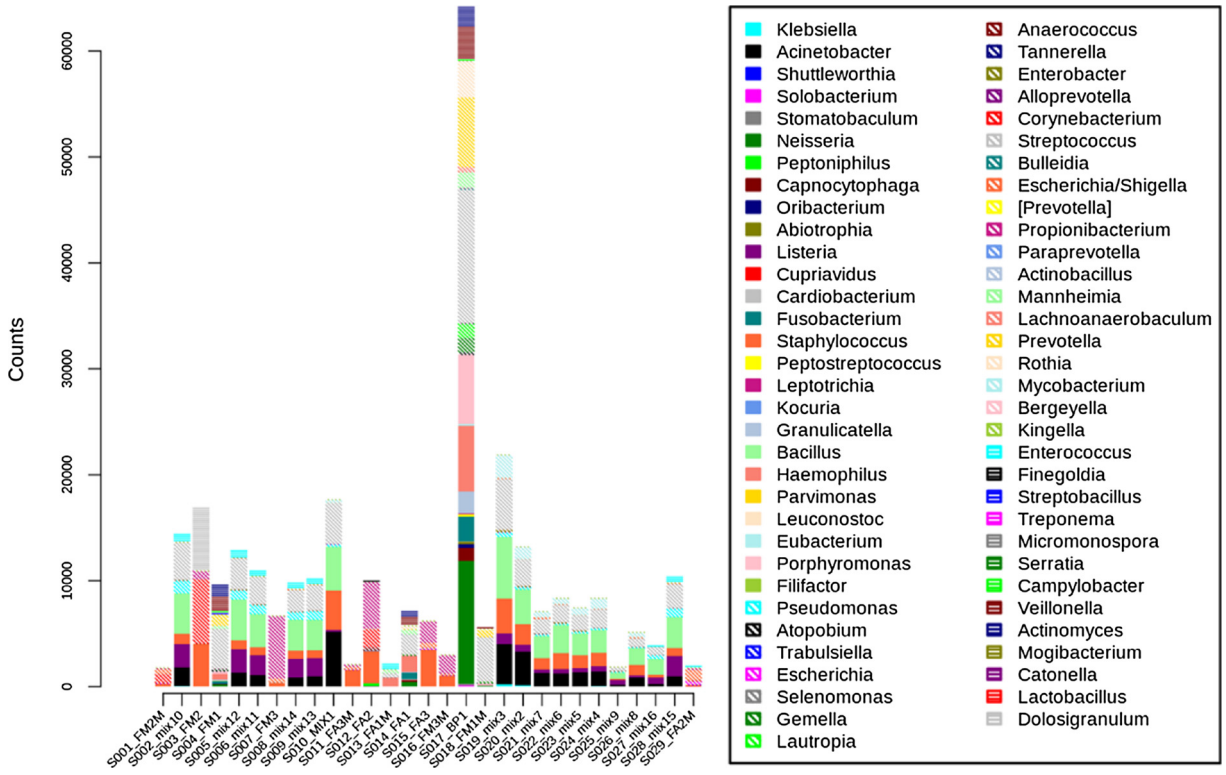

Fig. 2. OTU composition for the analyzed samples. OTU cluster are defined by a 97% identity threshold of the 16S gene sequences to distinguish bacteria at the genus level.

order to set up mock community samples (MIX1 and MIX9). Mock community samples were then diluted 1:5 (MIX2 and MIX10), 1:10 (MIX3 and MIX11), 1:20 (MIX4 and MIX12), 1:30 (MIX5 and MIX13), 1:40 (MIX6 and MIX14), 1:50 (MIX7 and MIX15) and 1:100 (MIX8 and MIX16). Total DNA quantity in the mock community samples were similar. For Mix 1 to 8 Promega DNA extraction protocol was used, while MIX 9 to 16 were subjected to Sigma-Aldrich protocol.

Buccal swabs (BP1, FA1, FA1M, FM1, FM1M) were collected in duplicate from three people, nasal (FA2, FA2M, FM2, FM2M) and ear (FA2, FA2M, FM2, FM2M) swabs were collected in duplicate from two people and submitted to the two extraction protocols. Sample with alphanumeric code ending with "M" were submitted to GenElute Bacterial Genomic DNA extraction protocol.

Library preparation was performed according to the Ion 16S™ Metagenomics Kit protocol. The barcoded libraries for each sample were pooled into a single library in equal molar quantities and submitted to template preparation and enrichment by Ion Chef™ System and then sequenced on Ion Gene Studio S5™ system (Thermofischer Scientific), on an Ion 520 chip. Data was processed by the Torrent Suite version 5.10 and data were analyzed using the Ion Reporter Software v5.10, metagenomics workflow.

3. Results

Both extraction methods yielded DNA in suitable concentration and quality for sequencing on Ion Gene Studio S5.

DNA IQ™ Casework Pro Kit for Maxwell® 16 performed much better than GenElute Bacterial Genomic DNA Kit for all the bacteria species, except *E. faecium* and *P. aeruginosa* where DNA concentrations were similar (Fig. 1).

Fig. 2 shows bacterial community structure at genus level in the analyzed samples. In the mock community samples all the expected microbial families were identified and no differences in bacterial community pattern were observed in diluted samples for both extraction methods. Biological samples showed the expected bacterial community for the sampled body sites, even if a certain degree of interpersonal variability was observed. Samples extracted with DNA IQ™ Casework Pro Kit for Maxwell® 16 showed a higher number of reads than the corresponding samples extracted with GenElute Bacterial Genomic DNA Kit.

4. Discussion

In this study, we have evaluated two different DNA extraction methods from mock community samples and biological samples collected on different body sites. Both extraction methods yielded DNA in suitable concentration and quality for sequencing on Ion Gene Studio S5, but DNA IQ™ Casework Pro Kit for Maxwell® 16 performed much better than GenElute Bacterial Genomic DNA Kit. Furthermore, the hands-on time was significant shorter for the semi-automatic Promega protocol.

In the forensic field, samples to be analyzed often contains small amount of biological material, thus it is of paramount importance to extract as much information as possible from them and in a short timeframe. So a DNA extraction protocol suitable for both human and microbial DNA is very useful.

5. Conclusion

Our work demonstrated that DNA IQ™ Casework Pro Kit for Maxwell® 16 was able to extract microbial DNA suitable for microbiome analysis without modifications and performed much better than the GenElute Bacterial Genomic DNA Kit. Using the Promega kit is very useful for a forensic lab performing human DNA and microbiome analysis on biological samples because the extracted DNA can be subjected to both applications.

Declaration of Competing Interest

None.

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