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ESTABLISHMENT OF A PROTOCOL FOR GENOMIC DNA EXTRACTION FROM HUMAN SALIVA

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Extraction of sufficient quantities of high-quality DNA is a prerequisite for genetic research and clinical diagnostic studies. Obtaining patient compliance for invasive and painful sample collection procedures is difficult. Saliva is a non-invasive source of DNA; however, the lack of standardized protocols that do not rely on overnight incubations or expensive kits has greatly limited its usage. Hence, the present study aimed to set up a protocol for isolating high-quality genomic DNA from saliva collected from 21 healthy volunteers belonging to two age groups. Unstimulated whole saliva was collected from the participants and subjected to a modified phenol-chloroform DNA extraction method. The effects of the time of sample collection, storage conditions, and sample volume on the quality of extracted DNA were determined by repeating the protocol under the respective non-standard conditions. Each DNA extraction was followed by DNA purity and concentration estimation, agarose gel electrophoresis, and PCR amplification using primers specific for the common periodontal pathogen, *Porphyromonas gingivalis.* The proposed protocol was able to produce DNA of mean purity values >1.700 and mean concentrations >100 μg mL^{-1}. Moreover, PCR amplification revealed the absence of *P. gingivalis* in all samples. The statistical analysis using SAS 9.00 revealed that sample collection 15 min after a meal significantly reduced the quality of extracted DNA. In contrast, saliva storage for 48 h at -20 ℃ before DNA extraction produced no such difference ($P < 0.05$). The novel protocol also produced high-quality DNA from a minimum of 0.1 mL of saliva. Overall, this optimized protocol allows the successful economic isolation of high-quality genomic DNA from human saliva using readily available reagents and laboratory conditions, suitable for diagnostics and large-scale population-based studies.

Keywords: Non-invasive, PCR amplification, Phenol-chloroform method, *Porphyromonas gingivalis*