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Title: A ROBUST UNIVERSAL METHOD FOR EXTRACTION OF GENOMIC DNA FROM BACTERIAL SPECIES

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Abstract: Background and Objectives: The intactness of DNA is the keystone of genome-based clinical investigations, where rapid molecular detection of life-threatening bacteria is largely dependent on the isolation of high-quality DNA. Various protocols have been so far developed for genomic DNA isolation from bacteria, most of which have been claimed to be reproducible with relatively good yields of high-quality DNA. Nonetheless, they are not fully applicable to various types of bacteria, their processing cost is relatively high, and some toxic reagents are used. The routine protocols for DNA extraction appear to be sensitive to species diversity, and may fail to produce high-quality DNA from different species. Such protocols remain time-consuming and tedious, thus to resolve some of these impediments, we report development of a very simple, rapid, and high throughput protocol for extracting of high-quality DNA from different bacterial species.

Methods: In so doing, 9 different bacterial species were selected based on the structural complexity of cell wall, extracellular material, economical importance and significance in clinical and biotechnological researches. In order to evaluate the quantity, quality, purity and intactness of the extracted DNA, gel electrophoresis, PCR, RAPD and restriction enzyme digestion methods were performed.

Results: Based upon our protocol, interfering phenolic compounds were removed from extraction using poly vinyl pyrrolidone (PVP) and RNA contamination was precipitated using LiCl. The UV spectrophotometric and gel electrophoresis analysis resulted in high A260/A280 ratio (>1.8) with high intactness of DNA. Quality assessment with restriction digestion showed that the isolated DNA was very pure with high quality for enzymatic reaction, in which a minimal inhibitory effect of the extraction process was observed. The PCR product yielded a clear band pattern and adequate intensity.

Conclusion: The isolated DNA from samples confirmed the accuracy of this protocol which requires no enzymatic processing and accordingly its low-cost making it an appropriate method for large-scale DNA isolation from various bacterial species in medical researches. This protocol could serve as a universal competent method for isolation of genomic DNA from a variety of microorganisms with different types of extracellular and dissimilar cellular envelopes architecture.

Keywords: Medical bacteria, genomic DNA extraction, RAPD.

Presentation: Poster