Development of an Optimized DNA Extraction Protocol for High Quality DNA from Human Blood Using a Commercial DNA Extraction Kit

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DNA extraction is often the preliminary step in molecular based life sciences research. The objective of this study was to develop an optimized protocol for extraction of DNA from human blood having sufficient quantity and optimum purity that could be used in further downstream applications. DNA was extracted from blood usingFlexiGene® DNA extraction kit by QIAGEN®. Although the original protocol provided with the kit was practiced without making any modification, spectrophotometric absorbency results revealed that DNA extracted did not have sufficient purity as it especially indicated organic compound contamination. A260/A230 ratio for DNA, which needs to lie between 1.8-2.0 was detected to be between 1.3-1.4. Eventually, 70% ethanol washing to remove previously added isopropanol to pellet out DNA was repeated by doubling the volume of ethanol from 50μL mentioned in original protocol to 100μL and air-dry time to remove all ethanol used for washing impurities was increased by nearly six times to that mentioned in original protocol, from 5 minutes up to 30 minutes. As a result, A260/A230 ratio improved drastically from 1.4 to 2.2. However, protein digestion to remove all protein associates were optimum through the original protocol, which was validated by the A260/A280 ratio 1.73, very close to theideal value for DNA, 1.8. Concentration of DNA also increased from 20.8ng/μL to 234.5ng/μL by increasing the volume of 100% isopropanol from 100μL to 150μL to effectively pellet out DNA. The optimized protocol for extraction of DNA was developed accordingly by modifying the original protocol.

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