A141 Application Studies on Direct Quantitation, Direct PCR, and mtDNA Sequencing Without DNA Purification From Saliva Spotted Paper

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After attending this presentation, attendees will have information about direct PCR.

This presentation wll impact the forensic science communty by discussing direct PCR without DNA isolation.

Buccal cell DNA concentrations were directly quantified in this study by real time PCR without a prior DNA purification step. For this purpose, a newly developed direct q-PCR mixture was used. A total of 86 saliva-spots were collected and divided into three groups (A, B, and C). The "A" group samples were collected regardless of tooth brushing, but the "B" and "C" groups were collected before and after tooth brushing respectively from the same individuals. The quantification of each sample was performed three times and followed by calculation of their average concentration. The result showed that about 84.9% of quantified saliva spot had a DNA concentration range of 0.041 to 1.0 ng/µL. The average DNA concentration values from 89% of "B" group samples were higher than those from corresponding "C" samples. Moreover, "B" group samples showed higher PCR success rates than those of "C". These results suggest that the PCR buffer can amplify saliva directly, regardless of possible PCR inhibitors in saliva. It is thought that if a sufficient amount of saliva is spotted on saliva spotted paper at the sampling stage, PCR reaction can be successfully performed without a prior DNA quantification procedure. Additionally, mtDNA templates were directly amplified using the same direct PCR buffer, followed by sequence analysis with a cycle sequencing kit. In general, the purified DNA template is required for an amplification of a hypervariable region in routine mtDNA sequencing. It was possible to directly amplify ~ 1 kb mtDNA template using direct PCR buffer system from blood, saliva, and hair roots followed by sequence analysis with a terminator cycle sequencing kit. The resulting HV1 and HV2 sequence data showed good resolution without particular noise peaks and whole sequence concordance with data from a routine method. In the case of hair roots, pre-lysis with DTT and proteinase K is necessary, but needs no further purification step.

In conclusion, streamlined work flow through the studied PCR buffer system appears to be suitable for fast forensic DNA analysis or analysis for criminal DNA databases.

Direct Quantitation, Direct PCR, mtDNA Sequencing

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