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Development and validation of mitochondrial DNA based approach for rapid identification of environmental chemical exposed victims

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Abstract

The rising toll of mortality due to different accidental or occupational environmental exposures necessitates early identification of exposed victims for their appropriate therapeutic intervention. However, this is mainly restricted by the lack of precise biomarkers and effective detection methodologies. Since, mitochondria are the prime target of different environmental exposures, these sub-cellular organelles offer possibilities of getting utilized for developing effective exposure associated strategies. The presence of unique inheritance pattern, rapid evolutionary rate, low recombination rate, higher copy number and resistance to degradation make mtDNA an important and indispensable tool for such studies. Therefore, we aim to design an mtDNA based molecular approach for rapid identification of the environmental chemical exposure associated victims. In order to analyze whole genome sequence of mtDNA, particular attention was devoted for primer selection from established libraries to adjust the melting temperature of all pairs. The specificity of selected primers was checked through BLAST analysis to avoid nDNA co-amplification. Total DNA was extracted from both human peripheral blood and lymphocytes and quantified for mtDNA amplification. Results of our PCR analysis showed clear amplification of whole ~16000 bp of mtDNA without co-amplification of NUMTs. Gel pictures showed the presence of a clear band of all the 9 fragments which correlated with their respective size. For absolute quantification real-time PCR analysis using primers for mt-ND1 (NADH dehydrogenase, subunit 1) gene (ND1-F, 50 CCCTAAAACCCGCCACATCT 30; ND1-R, 50 GAGCGATGGTGAGAGCTAAGGT 30) in mtDNA and other primer pair for the amplification of nuclear gene human (β-actin) was further performed. The ratio of mtDNA copy number to nDNA value was determined to identify absolute mtDNA copies (Mean 0.69 ± 0.05). After ascertaining the empty DNA copy numbers, the amplified products were purified for downstream mtDNA sequencing studies. The analysis was performed in collaboration with SciGenom Labs by using ABI 3730 XI equipped with 30 cm capillary array to facilitate the mtDNA fragment analysis. Currently, the investigations are ongoing and the obtained results may help to design novel mtDNA based fluorescence resonance energy transfer hybridization (FRET) - real time PCR assay for rapid identification of environmental chemical exposed victims.

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