Improved protocol for extracting genomic DNA from frozen formalin-fixed tissue resulting in high-quality whole mtDNA

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ABSTRACT. Formalin fixation and paraffin embedding is widely used for convenient and long-term storage of tumor tissue and precious sources to perform genetic studies. However, DNA fragmentation is one of the major flaws of genomic DNA isolation from formalin fixation tissues, which limits its further usage. Here, we present an improved method for isolating high-quality genomic DNA from formalin fixation tissue. We obtained high-quality genomic DNA of more than 20 kb
from samples frozen for more than 2 years. Furthermore, to verify DNA quality, the whole mitochondrial DNA (mtDNA) genomes from the normal and tumor tissue of the same patient were successfully amplified with two overlapping PCR fragments comprising more than 8379 bp in length for each fragment. In addition, the whole genomes were sequenced with a 48-well based primer panel in order to avoid potential sequencing errors from artificial recombination, which was further confirmed with an mtDNA phylogenetic strategy. Our improved DNA extraction method from formalin fixation tissue and sequencing strategy for entire mtDNA genomes will generate unambiguous sequence analysis results for clinical samples.

**Key words:** Formalin-fixed tissue; DNA isolation; Entire mtDNA genome