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Abstract

Determination of DNA polymorphisms from formalin-fixed tissues using whole genome amplification method

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Biopsies and surgically resected tissues are fixed by formalin for histological diagnosis as a major clinical practice. On the other hand, these specimens also are important materials for some biological analyses. However, the DNA obtained from formalin-fixed material is severely degraded, and polymerase chain reaction (PCR) amplification using such material is very limited. In this study, we examined the use of formalin-fixed tissues for analyses DNA polymorphisms based on whole genome amplification. The specimens were collected from remained tissues of autopsy in our laboratory that fixed by 10% formalin solution from one to ten years. The DNA extraction was carried out by phenol/chloroform method from the formalin-fixed samples composed of heart and liver (each for three cases). One minisatellite (D1S80), three microsatellite STR polymorphisms (vWA, TH01 and CSF1PO) were investigated. The majority of the DNA isolated from the tissues had molecular weights lower then 350 base pair (bp), and no DNA patterns could amplify using those templates. In order to amplify more DNA polymorphic markers from the formalin-fixed tissues, whole genome amplification technique was performed. After amplification by degenerate oligonucleotide-primed PCR (DOP-PCR), the products contained DNA with increased molecular weight up to one-kilo bp. The vWA and TH01 loci were typed using the DOP-PCR products by 5 years in all of tissues. Furthermore, we purified large molecular size fragments from the DOP-PCR products by agarose electrophoresis, and then successfully amplified the STR patterns for 10 years old tissues in vWA and TH01 loci, and for 7 years old in CSF1PO locus. But no D1S80 locus was successfully detected even used of only fixed one-year tissues