

Summary

Quantification of human DNA in forensic samples is an important step during STR profiling because the STR genotyping is sensitive to the quantity of DNA used in the PCR reaction. This study focuses on the importance of quantification in the entire process of genetic analysis. Two real time PCR platforms (Roche LightCycler480 System and ABI 7900 RT PCR) were used to compare two commercial kits in terms of DNA quantification. It was found out that accuracy of absolute quantification values in commercial quantification kits is strongly dependent on the construction of calibration curve. Especially low template DNA samples were used to assess whether Quantifiler™ or Plexor® HY System can determine a minimum quantification value (cut off value) below which STR profiles would consistently fail to be detected. The usage of Plexor® HY System enabled to determine the cut off quantification value more exactly probably due to different molecular background and chemistry used in this kit. Reliability and other issues connected with cut off value are discussed. In order to better understand the relationship between the quantity of DNA and the number of detectable loci series the dilution experiment with standard DNA007 was done. Quantitative and qualitative consequences of input DNA amount in evaluation of DNA profiles performed by different amplification kits are considered.

Keywords: DNA quantification; real-time PCR; low level DNA; validation; cut off quantification value