DEVELOPMENTAL VALIDATION OF PROMEGA PLEXOR HY SYSTEM FOR THE QUANTIFICATION OF AUTOSOMAL AND MALE HUMAN DNA USING THE ROCHE LIGHTCYCLER 480

Kevin R. Condel and M. AL. Salih
DNA Reference Lab, San Antonio, TX

Promega's Plexor HY kit was recently designed and released for the purpose of a concurrent quantification of autosomal and male DNA from a single human sample. The Roche lightcyler 480 is also a recent newcomer to the real time PCR world with some advanced multiple processing capabilities. To this end, we are reporting on comprehensive developmental and in-house validation studies utilizing Plexor HY in combination with the Roche lightcycler 480 real- time PCR instrumentation to quantify DNA in forensic samples. Following calibration to NIST standards and traditional DNA quantification methods, the kit and the instrumentation were both tested extensively for reproducibility, sensitivity, human specificity and the ability to perform in the presence of PCR inhibitors and severely degraded DNA. A variety of mixtures were also tested to determine the system limits for the detection of male DNA in the presence of significantly increased levels of female DNA. Further, the system was also validated to determine its suitability for forensic casework analysis by using forensic simulated cases, various types of non-probative case samples and proficiency case samples. The Promega Plexor HY system performs exceptionally well except for its ability to occasionally over-estimate DNA concentrations due to the presence of multiple copy targets. In contrast, the presence of these multiple copy targets appears to guard well against all false negative DNA quantifications. Furthermore, the Plexor HY kit proved to be very specific to the quantification of both autosomal and Y-chromosome human DNA and can safely detect as little as 6.7 picograms using 2 ul input sample per assay. This detection level is found to be more than 37 times below the level required for full genotyping. In general, the combination of Promega Plexor HY system and the Roche Lightcycler 480 provided results that are comparable to or exceeding DNA quantification technologies that are currently in use.