AN ANALYSIS OF SINGLE AND MULTICOPY METHODS FOR DNA ANALYSIS BY REAL-TIME POLYMERASE CHAIN REACTION

Heather E. LaSalle[1], George Duncan[1], Bruce McCord[2]

[1]Broward Sheriff's Office, Ft. Lauderdale, Florida,
[2] Florida International University, Dept: Chemistry and Biochemistry, Miami, Florida

The goal of this paper was to examine and compare two different commercially available approaches to the determination of the relative quantities of autosomal and Y DNA using real-time PCR. Quantifiler, utilizes a Taqman assay with single copy probes for both autosomal human and Y quantification. The other method, Plexor HY utilizes a primer quenching assay with multicopy probes for its quantification of autosomal and human DNA. To test these approaches we have utilized a set of 3 different NIST human DNA quantification standards to examine the precision, accuracy and sensitivity of the real time PCR assays. We also examined data from both systems utilizing casework samples. The results show that both systems produced linear estimates for DNA quantity over a broad range of input DNA. However we did observe some odd effects when comparing the 3 different NIST standards which we attributed to issues with sequence variations in the different standards. Overall, the single copy approach provided better accuracy while the multicopy approach produced better sensitivity. Thus the choice of which system to use should depend upon the goals of the user.