RAPID, ACCURATE DIGITAL DNA QUANTITATION USING THE CCDBIO 16HC

William R. Hudlow[1], Bruce Budowle[2], and Steven B. Lee[1]
[1]*MiraiBio Inc., Hitachi Genetic Systems, Alameda, CA*
[2]*Laboratory Division, FBI Academy, Quantico, VA*

Quantification of human DNA from forensic samples is achieved by hybridization of the higher primate-specific alphoid probe D17Z1 to membrane-immobilized, single-stranded human DNA and is required for analysis where possible.[3] [4] Typically, a chemiluminescent signal is captured on film and the quantity of signal is proportional to the amount of immobilized DNA. Quantification of the samples is then estimated by visual comparison to standards, assuming a linear response of signal. This and other factors may lead to variation is quantification (http://www.promega.com/geneticidproc/ussymp10proc/content/42kline.pdf). Determination of the optimal DNA template quantities for PCR may reduce undue consumption of forensic biological evidence and reagents and may avoid potential preferential amplification that may occur, for example, with some large repeat VNTR loci.

Digital imaging using a 16-bit Peltier-cooled CCD camera offers an alternate non-film based method for image acquisition with comparable sensitivity of detection (40pg in 30 minutes), a greater functional dynamic range (40ng-20pg) and an enhanced capability of data interpretation using automated software, which yields results faster than film. In addition, the data suggests that improved human DNA quantification can be obtained by not assuming a linear response of signal to known standards; but instead, should be estimated using a second order standard curve ($R^2 = 0.999$).

To assess the impact of the CCD camera imaging system on variation of quantity estimates (or the degree of reproducibility), the same blot was imaged five times within one day (so that chemiluminescent signal decay would not be a significant factor to consider), each time with a 30 minute exposure. In this report we have shown that average reproducibility in the repeated captures/analyses of replicate samples (8) on a single blot at 0.1 ng is 0.07-0.12 ng (0.01-0.02 SD/Sample), 1.0 ng is 0.92-1.03 ng (0.01-0.02 SD/Sample), 4.0 ng is 3.54-4.60 ng (0.01-0.08 SD/Sample), and 20 ng is 15.51-27.20 ng (0.15-0.31 SD/Sample). Thus, the variation due to repeat analyses of the same sample with the CCD camera is small.

Software analysis enables more effective evaluation of the data including localized background correction, setting up a grid for all standards and unknown samples, and then setting up a standard curve to quantify the unknown samples. The automated data analysis and integration for all slots/bands on a membrane occurs in a less than a minute. Furthermore, optimization of the slot blot process using commercially available slot blot kits in conjunction with new reagents (i.e., substrates) and imaged on the CCDBIO 16HC can further enhance the quantification process. Finally, a CCD camera system offers versatility for image capture of different signal sources and analysis of samples on a variety of support media, which include membranes, gel and microarrays.

[3] Waye, J.S. et al. 1989. BioTechniques 7:852-855
[4] Quality Assurance Standards for Forensic DNA Testing Laboratories STANDARD 9.3