



B145 Automated PCR Setup for Casework Samples as Part of a Total Automated System

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After attending this presentation, the participant will have an understanding about a new automated PCR setup system designed for casework samples that is flexible, customer friendly and integrated with automated DNA purification and quantitation modules.

This presentation will impact the forensic community by demonstrating streamlined casework processing to alleviate the backlog cases inundating the community.

Automated workstations have proven their utility for processing sample backlogs for offender databases. These systems can be optimized for a single sample type and do not have to be efficient. The varied sample quality and amount encountered in casework presents additional challenges in automated sample processing. Additionally, contamination concerns restrict the flexibility of robotic systems and require extensive validation. This presentation will discuss advances in developing an automated PCR setup system that works over a 50 fold range of initial DNA concentration. In addition, improvements for automated DNA extraction and human-specific quantitation using the same robotic instrument as PCR setup, the Beckman Coulter Biomek® 2000 Workstation, will be described. Developed initially as independent modules, these three steps can be integrated with minimal hands-on time. This modular approach provides quicker access to automation and minimizes cost.

Automated PCR setup has been in use for many years. However, due to the wide range of DNA concentrations associated with casework samples, few laboratories have successfully automated this tedious and time-consuming process. We modified the Beckman Coulter Normalization Wizard developed for the Biomek® 2000 Workstation so the program can dilute the DNA to a customer defined concentration starting with DNA concentrations between 0 and 5ng/μl. The initial DNA concentration values are imported from a modified AluQuant® Calculator. The user then selects the final concentration and volume parameters. The system will flag samples that are too dilute or concentrated and allow the user to exclude individual wells. This process conserves on expensive amplification reagents. After the DNA has been diluted to the desired concentration a set amount of this DNA and PCR master mix are added to a PCR plate and mixed. The user then caps the plate and places it in a thermal cycler. Master mix can also be added manually if desired.

We have integrated this PCR setup module with DNA purification and quantitation on the same robotic platform to maximize its usefulness and are continuing to gain experience on new sample types. To provide the maximum recovery and flexibility, samples are currently preprocessed manually to remove biological material from solid supports. In most cases, this involves incubation of the support in DNA IQ™ Lysis Buffer, followed by centrifugation through a spin basket. Samples containing very small amounts of DNA are incubated in a Proteinase K solution while samples containing sperm and epithelial cells are treated using the standard differential extraction procedure. Once the samples have been extracted from the solid support they are transferred to a Biomek® 2000 Workstation for hands off purification using the DNA IQ™ System. Recent modifications to this automation program reduce processing time and adjust for environmental factors, such as low humidity.

Human-specific quantitation is required for casework samples to ensure the amplification of an appropriate amount of DNA. The AluQuant® Human DNA-Specific Quantitation System was developed to allow an automated approach to this step. Using solution-based hybridization of a highly repeated human specific sequence, the method is sensitive, provides numerical results, and does not rely on amplification of the sample DNA. Recent improvements to the automated process allow the use of 4μl of sample DNA and improve sensitivity. In addition, the DNA concentrations calculated in the AluQuant® Calculator are automatically formatted for easy importation into the Normalization Wizard program.

While not yet a “black box” sample analysis system, the current setup provides a flexible system that automates several time consuming processing steps on one robotic platform. Hands-on time between the different programs is minimized and primarily involves replenishing the deck with labware and reagents.

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