## B53 Automating the DNA Preparation and Analysis of Casework Samples

*Paraj Mandrekar, MS, Laura Flanagan, BS, Robert McLaren, PhD, and Allan Tereba, PhD*, Promega Corporation, 2800 Woods Hollow Road, Madison, WI*

After attending this presentation, the participant will understand how the various steps in DNA analysis are being automated and integrated to increase throughput and generate more reproducible results.

DNA analysis in the forensic setting frequently consists of cell separation (differential extraction), and/or sample extraction, DNA purification, human-specific quantitation, PCR amplification, DNA fragment separation, and data analysis. Automating these steps to increase throughput and control expenses will become a necessity as the demand for DNA testing increases due to new legislation, advances in trace evidence techniques, and a need to analyze backlogged nonsuspect samples. The current techniques to automate sample extraction, DNA purification, human-specific quantitation and PCR setup on a Beckman Biomek® 2000 robotic platform will be described.

Casework samples are quite varied and initial sample extractions are a significant bottleneck. While heated enzymatic digestions and centrifugation steps to increase yields are frequently necessary but are not amenable to automation, many of these processes can be performed in a 96-well format to increase efficiency and prepare the samples for robotic manipulation. The authors will demonstrate the efficient preparation of many common casework sample types using inexpensive equipment and will show how this step fits seamlessly into automated DNA purification.

The robotic purification of DNA is based on the binding of DNA to a proprietary paramagnetic particle in the presence of a strong protein denaturing solution that is effective at removing most PCR amplification inhibitors. The use of paramagnetic particles renders the DNA IQ™ System suitable for a variety of robotic platforms such as the Beckman Biomek® 2000 robotic system. A completely walk-away format has been developed, requiring about 2 hours for 88 samples. The automated system can handle different sample volumes from a variety of extracted materials, elutes DNA in a volume of 25ul to 100ul in individual tubes or 96 well plates, and makes judicious use of filtered disposable tips that are assigned to each sample well.

Following DNA purification, the robotic system can be quickly set up to perform human-specific quantitation using a unique technique based on the polymerase-catalyzed depolymerization of a probe hybridized to repeated human DNA sequences. Liberated dNTPs generated during depolymerization are used to generate ATP, which is a substrate for Luciferase, generating a light signal that is proportionate to the amount of human DNA present in the solution. Large excesses of nonhuman DNA do not interfere with the quantitation. The process requires 2 hours to process 80 samples including a one-hour incubation. The values generated from the AluQuant™ Human DNA Quantitation System, using an injecting luminometer capable of reading 96 well plates, are converted to DNA concentrations. These values can then be used to set up PCR reactions using the Biomek® 2000 robotic platform and a normalization wizard. The PCR setup is designed for accuracy and conservation of expensive reagents and provides a plate that can be placed in a thermalcycler for STR amplification. The reliability of this system will be demonstrated.

**Automation, DNA Purification, Quantitation**

 ** Presenting Author*