

INTEGRATION OF DNA PURIFICATION, QUANTITATION AND STR ANALYSIS

Allan Tereba, Benjamin Krenke, Michelle Mandrekar, Paraj Mandrekar, Nadine Nassif, Dawn Rabbach, John Shultz and Cynthia Sprecher
Promega Corporation, Madison, WI



DNA analysis is a lengthy process consisting of sample acquisition, stain extraction, DNA purification, quantitation, amplification, detection and data analysis. As the demand for testing increases there is a growing necessity to provide an integrated approach to sample analysis. We will describe automation friendly technologies for several of these steps that have been specifically designed to work together.

We developed the DNA IQ™ System, a new stain extraction and DNA purification approach that is rapid, easy to use, amenable to a wide variety of sample types and efficient with very small samples. The DNA IQ™ System provides a 45-minute extraction procedure that is compatible with most solid supports. DNA is then purified using a proprietary magnetic particle. The DNA IQ™ System removes virtually all PCR amplification inhibitors including dyes found in blue or black denim, soil and black leather while quantitating total DNA for database samples. Both the sperm and epithelial fractions generated from rape kits can be input directly into the purification process. To further simplify this process, the DNA IQ™ System has been adapted to work on the Beckman Biomek® 2000 robotic system in a completely walk-away format.

To quantitate human-specific DNA, we developed the AluQuant™ System, a unique technology based on the polymerase catalyzed depolymerization of a probe hybridized to repeated human DNA sequences. The dNTPs released during depolymerization are used to generate ATP which is a substrate for Luciferase. This generates a light signal that is proportionate to the amount of human DNA present in the solution. The technique requires no gels and yields discrete values for the quantitation. The process requires two hours including a one-hour incubation. Like the DNA IQ™ System, the AluQuant™ System works on the Beckman Biomek® 2000.

We previously developed PowerPlex® 16, a single amplification system that amplifies the CODIS thirteen core short tandem repeat (STR) loci, the amelogenin sex determinant locus, and two pentanucleotide STR repeat loci. This system was optimized for the ABI PRISM® 310 Genetic Analyzer and ABI PRISM® 377 DNA Sequencer. To increase the efficiency of the fragment separation process, a matrix was developed that allows PowerPlex® 16 to be run on an ABI PRISM® 3100 Genetic Analyzer. Consistent with our modular approach, we will continue to integrate the various steps in the analysis process and develop automated approaches.