

AUTOMATION

Automated DNA Normalization and STR Multiplex Setup Methods

Paraj Mandrekar, Promega Corporation, and Tim Sherrill, Beckman Coulter, Inc.

As automation simplifies DNA purification and quantitation, bottlenecks still form at the steps of DNA normalization and assembly of STR amplification reactions. Promega and Beckman Coulter have worked together to automate DNA normalization, using DNA quantitation values to dilute each sample to a standard concentration. The Normalization Wizard, Genetic Identity Version, creates two separate methods, or sets of instructions, within the BioWorks™ software for the Beckman Coulter Biomek® 2000 Laboratory Automation Workstation. The first method performs DNA normalization, which is necessary to ensure complete DNA profiles without allele dropout or artifacts that may complicate the subsequent analysis. The second method performs PCR setup by combining the normalized DNA solutions with a multiplex STR master mix in a standard strip-tube array on the Biomek® 2000 deck. At the conclusion of both methods, the strip tubes can be capped and placed directly into a thermal cycler for amplification. These methods were designed for casework samples and operate over an 80-fold range of initial DNA concentration. The use of separate methods for normalization and amplification reaction setup is necessary to achieve an 80-fold range and retain accuracy and conservation of sample. This separation of methods also allows the user to manually add the PCR master mix to conserve this expensive reagent. Central to the design of both methods was the conservation of deck layouts to streamline the transfer of samples from current automated methods for DNA purification and quantitation on the Biomek® 2000 workstation. Additionally, the transfer steps have been optimized to reduce the risk of cross-contamination.

FROM CONCENTRATION VALUES TO NORMALIZED DNA SOLUTIONS

The Normalization Wizard uses concentration data and user inputs, such as sample volume and final DNA concentration, to generate a Biomek® 2000 method. The method uses concentration data imported from any appropriately formatted Microsoft® Excel file or the AluQuant® Calculator, Release 3.0, allowing the Normalization Wizard to be used downstream of any quantitation method. Additionally, the wizard allows the deselection of specific samples in a source plate so that empty wells and samples without detectable DNA are not processed.

ON-DECK COMBINATION OF NORMALIZED DNA AND STR MULTIPLEX

The PCR Setup Method manages the process of PCR setup on the Biomek® 2000 workstation. This method directs the transfer of a reagent cocktail in a customer-defined pattern to a strip-tube array or PCR plate for amplification. The Biomek® 2000 then thoroughly mixes and transfers DNA samples from the normalization plate to each well of reagent. All mix steps are designed to mix large volumes of samples without disturbing small volumes of dilute samples. At the conclusion of this method, the resulting tubes may be placed directly into a thermal cycler for amplification.

CONCLUSION

Promega and Beckman Coulter have automated DNA normalization and PCR setup on a Biomek® 2000 Laboratory Automation Workstation for use in the forensic laboratory. In concert with other available methods, casework samples can be processed through the steps of purification, quantitation, dilution and PCR setup with a minimum of hands-on time and effort.

Promega and Beckman Coulter have developed automated methods that use quantitation data to normalize DNA concentrations and assemble STR multiplex reactions that are ready for amplification.