

Automated DNA Normalization and Typing PCR Setup Using Forensic B&E Casework Samples

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Forensic casework samples typically cover a variety of biological sources and substrates and, when purified, display broad concentration ranges. These samples frequently contain substances that can inhibit PCR assays. Normalization of these DNA casework templates is extremely important for successful downstream DNA typing. A MultiPROBE® II Forensic Workstation from PerkinElmer Life and Analytical Sciences was used to develop a fully automated DNA normalization and typing PCR setup process. Casework DNA samples were first purified using a modified Promega DNA IQ® extraction protocol. A single WinPREP® protocol was then used to perform the following steps: (I) forensic DNA normalization and dilution, and (II) PCR setup for STR-based typing using the ABI AmpFLSTR® Profiler Plus® Amplification Kit. The MultiPROBE II-automated robotic system has the following features:

- Creates a dilution work list using a standard Excel DNA concentration data file generated from a real time thermal cycler.
- Divides DNA samples, prepared from a variety of casework sample types, into dilution groups based on their DNA concentration range (40 pg/ul to 367 ng/uL).
- Dilutes widely varying amounts of DNA to a target concentration of 1 ng per PCR reaction in 96-well format in a 15 uL reaction volume.
- Correctly calculates required volumes of DNA sample and diluent, mixing them in a dilution plate with an appropriate mix volume, also calculated from the work sheet.
- Automatically creates the appropriate volume of PCR Master Mix from kit reagents for N normalized samples and aliquots it into a PCR plate.
- Increases throughput at least 10 fold compared to manual processing.
- Appropriate for mixed casework samples due to wide range of DNA concentrations encountered. Can also be used for reference and database forensic samples.

We will present experimental details, liquid handling performance data and analysis results showing that the MultiPROBE II robotic system successfully carries out all steps needed for a walk-away forensic DNA normalization and PCR setup

protocol. The WinPREP template is amenable for use with any commercial forensic STR and Y-STR analysis kit.