A SINGLE ASSAY FOR HUMAN-SPECIFIC QUANTIFICATION OF LESS THAN ONE PICOGRAM DNA AND DETECTION OF THE PRESENCE OF PCR INHIBITORS IN FORENSIC SAMPLES

Maura T. Costello and James W. Schumm
Bode Technology Group; 7364 Steel Mill Rd. Springfield, VA

We describe the development, validation, and application of a duplex real-time PCR assay for human-specific quantification of DNA samples containing as little as 0.5 pg/µl of DNA. The assay simultaneously detects PCR inhibitors within the sample. It is important to include human-specific quantification of DNA in casework sample analysis to insure successful DNA amplification and profiling.

Much recent research has focused on the use of real-time quantitative PCR to achieve this goal. This approach is less labor intensive, less time consuming, more accurate, and lends itself to automation better than previous methods such as slot-blot hybridization (1). Our work builds on that described by Nicklas and Buel (2), Richard *et al*. (3), and the commercially available Quantifiler™ Kit (Applied Biosystems, Foster City, CA). We have combined the sensitivity and human specificity of Alu-based real-time quantification with the presence of an internal positive control allowing detection of PCR inhibitors in the sample. Alu sequences are short, repeated elements that are interspersed throughout the primate genome in upwards of 500,000 copies. We selected the Yb8 subfamily of Alu genes because of its sequence specificity to higher primates (4). Using this target, we developed primers and a fluorogenic probe for a quantitative real-time PCR assay (5).

The assay also contains an internal positive control (IPC) system that is multiplexed with the Alu quantification system, consisting of a fixed quantity of non-human DNA template added to each reaction well, and a second set of primers and fluorogenic probe specific for the non-human template. The combination of human DNA quantity data from the Alu system and DNA quality data from the IPC system provides the analyst with substantial information to aid in deciding dilution or concentration schemes prior to STR amplification, thereby significantly reducing the number of samples that need to be re-evaluated following initial profiling. Validation work indicates the assay is accurate and precise in the range of 50 ng/µl to 0.5 pg/µl. Thus less than one human genome equivalent can be detected accurately. Species specificity tests indicate the assay is at least 5000 times more specific for higher primate DNA than any other species tested. The IPC system is very sensitive to inhibition observed with addition of hematin, indigo, or humic acid. The assay has been successful with a variety of non-probative sample types. The features of this assay will allow us to apply it very effectively to evaluation of touch evidence samples.

With so little sample available in these situations, it is critical to make the right decision to use more (with limiting amounts) or less (with inhibitors present) extracted DNA in the first profiling test.

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