B100 A Multiplexed System for Quantification of Total Human DNA and Human Male DNA

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After attending this presentation, attendees will learn about a methodology for simultaneous quantitation of human DNA and human male DNA in forensic biological samples in single PCR reaction using real-time PCR technology.

This presentation will impact the forensic community and/or humanity by demonstrating a real time assay for simultaneous quantitation of human male and total human DNA in biological samples.

Quantification of human DNA in forensic samples is essential for defining input DNA needed for obtaining interpretable STR profiles. Simultaneous quantification of human male DNA and total human DNA in an evidence sample e.g. sexual assault is desirable to reduce the consumption of evidence sample. In this presentation the authors will discuss the performance of a prototype multiplex reaction that amplifies the Y-specific SRY region, the RNA component of RNase P (H1 RNA) and an internal positive control (IPC).

A multiplex assay was designed that amplifies SRY (FAM™ dye- labeled probe), RNase P (VIC® dye-labeled probe), and an IPC (NED™ dye-labeled probe). The multiplex was optimized *in silico* to avoid interactions between the oligonucleotides and minimize formation of primer-dimers. This was confirmed by laboratory testing. The RNaseP and SRY assays were human specific with minimal cross-reactivity to DNA from other species. A control male DNA was used for the generation of standard curves for both assays. The primer and probe concentrations were optimized to ensure that the Y-DNA was detected and quantified accurately in the presence of a large quantity of female DNA. Performance data, including precision, accuracy, and reproducibility, will be discussed. Application to different sample matrices (blood, semen, saliva, vaginal swabs etc.) will be presented.

DNA Quantitation, Real Time PCR, DNA Analysis

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