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A MULTIPLEXED SYSTEM FOR QUANTIFICATION OF Y-DNA AND TOTAL HUMAN DNA

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Accurate quantification of DNA in forensic samples is essential for defining input DNA needed for obtaining good STR profiles. Simultaneous quantification of male specific DNA and total human DNA in a sample is useful in sexual assault cases. In this presentation we 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 assembled 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 excess 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.