Development of a Real-Time Multiplex Assay for Total Human DNA and Male DNA Quantitation Using Alu and Y-specific Repeats

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Determining the sex of the individual who was the donor of a stain or determination of the amount of male DNA relative to female DNA in a sample is often critical in forensic analysis. The first example is important where the victim (often female) has been attacked by a suspect (often male). In these situations, it is forensically important to find the blood not "belonging to the owner" on the item of evidence (blood from the female on the male's clothing or vice versa). A method that could quickly identify those stains as male or female could allow the examiner the chance to be selective in the stains that are analyzed further by STR analysis and hence save time and money by limiting the number of stains that require further analysis. The second example includes analysis of sexual assault cases. Often seminal fluid stain extracts contain female DNA in addition to the DNA arising from the seminal fluid. These mixtures can range from a small contribution of female DNA to a very high amount relative to the male DNA present. Under those circumstances where female DNA contributes significantly to the total DNA present in the sample, the determination of the total human DNA present may not yield definitive information to determine if a male profile could be obtained. Some of the serological testing done prior to DNA extraction to determine the presence of semen (prostatic antigen, P30) are very sensitive and can detect extremely low levels of semen; however, these tests are often not good predictors of the success of STR typing from a particular sample. A method that could determine the amount of male DNA present in a sample could allow the forensic scientist to decide if a sample has ample male DNA for further analysis. The STR analysis of semen stains takes considerable time and effort and could be streamlined by eliminating stains early in the DNA analysis process not meriting further attention. Knowing the amount or percentage of male DNA from a vaginal swab or differential extraction will indicate the probability of obtaining a full STR profile or whether Y STRs will be necessary. By multiplexing quantitation with sex typing, the decision about what stain should be further analyzed is made simultaneously with the quantitation of the stain and hence no further work is required than what must be done prior to STR analysis. We have previously developed several real-time PCR methods to quantitate human DNA in forensic samples using the human Alu sequence (Nicklas and Buel, J Forensic Sci 2003; 48:936-44; Nicklas and Buel, J Forensic Sci 2005). Because this sequence is multicopy, assays using this sequence are much more sensitive (down to 0.5pg) than assays developed by others using a single copy gene (down to ~25pg). We have developed a multiplex total human and male DNA quantitation assay using the human Alu sequence and a human Y chromosomal repeat. This assay uses TaqMan MGB probes with FAM and VIC dyes for the two amplified sequences. The assay has been optimized for probe/primer concentration, mastermix and PCR conditions. The assay is reproducible and consistent from male to male across ethnic groups. The male DNA assay has a >500,000 fold difference in detecting male vs female DNA. This assay is much more sensitive than the current commercially available assay (0.5pg vs ~25pg). This assay is currently being validated for forensic casework.