DEVELOPMENT OF A HIGH THROUGHPUT SYSTEM FOR DETECTION OF HUMAN MALE DNA IN FORENSIC SAMPLES

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Screening of sexual assault evidence samples for the presence of sperm or semen is generally the first step in forensic DNA analysis. Currently used methods include detection of seminal protein p30, acid phosphatase and microscopic examination for presence of sperm. These screening methods provide, at times, false positive and negative results, and/or low throughput. Further, these screening tools are not targeted to detect the presence of male DNA; some types of evidence samples containing tissue or body fluids other than semen such as saliva stains, bite marks, and fingernail scrapings cannot be processed. Thus, there is a need for a sensitive, reliable and high throughput screening method for the detection of male DNA in forensic samples without requiring differential extraction of the female cells.

We have developed a novel screening system, Y-Detect, for the detection of male DNA in all types of forensic samples. The method is based on PCR amplification of an *Alu* insertion fixed within the Y chromosome. The *Alu* family of interspersed repeats is the most successful of the mobile genetic elements within primate genomes, having amplified to a copy number of greater than 1,000,000 per haploid genome. *Alu* repeats are unique nuclear markers that are ideally suited for human identity testing. Individual *Alu* repeats are approximately 300 bp in length and are thought to be derived from the 7SL RNA gene. Y-Detect is a two-plex PCR system that achieves amplification of human male and Avian DNA. Simultaneous amplification of Avian DNA enables monitoring for the presence of possible PCR inhibitors in each tube. The primers for human and Avian DNA are labeled with FAM and JOE generating fragments of 156 and 202 bp, respectively, that are separated on 310 or 3100 Genetic Analyzers. The developmental validation studies were performed according to the DNA Advisory Board's (DAB) Quality Assurance Standards. Using the Y-Detect assay, it is possible to detect as little as 10 pg of male DNA. Our protocol is designed to consume less than 10% of the evidence sample. Individual assays can be performed using a 96 well format to facilitate high-throughput screening. The Y-Detect assay is a sensitive, valid and robust multiplex system for the screening of biological samples for the presence of human male DNA.