B5 Development of a Y Alu DNA Screening Assay Using Y Alu Derived Sequence for Detection on the FMBIO III Plus

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After attending this presentation, attendees will learn the use of Y alu derived primers used to detect male DNA.

This presentation will impact the forensic community by discussing the development of an accurate and rapid Y chromosome specific screening test for male DNA. This new assay has the potential to greatly improve time of sexual assault screening and may alleviate the backlog of evidence samples. The forensic science community in the United States has more than 150,000 backlog mixture samples presently. However, present screening methods are tedious and may lead to false positives or false negatives.

Forensic DNA has become an important tool for solving crime. Approximately 169,000 rape case samples await testing. The currently utilized screening tests are tedious and may lead to false positive or false negative results. The goal of this study is to develop an accurate, rapid, Y chromosome specific screening test for male DNA. The chosen target for the male DNA primers are Y Alu derived sequences, STYa and Y 90.[1]

Two different strategies for detection of amplicons have been tested: (1) molecular beacons,[2] and (2) amplifluor primers.[3] Scanning parameters have been determined for the fluorescent dye FAM used in detection of the primers and PCR products. Preliminary results indicate FAM detection is optimal with an excitation by a 488nm laser and a 532/8 nm band pass filter with a 515nm long pass blocking filter at a focal depth 1.5mm, and a photomultiplier tube sensitivity setting of 45% on a fluorescent scanner (FMBIO III plus, MiraiBio Inc. Alemeda, CA).

Parameters for amplification and detection using replicate positive male DNA samples and female DNA were determined. Control male DNA was amplified using Amplifluor primers. When target is present the Amplifluor primer hybridizes and unfolds separating the reporter dye from the quencher. Amplification and detection were performed in a single step in Microamp PCR 8 tube strip Reaction Tubes (PN 801- 0580- Applied Biosystems, Foster City, CA). After amplification, the 8 tube strips were placed into a 96 well clear bottom plate (NUNC 96 Microwell Optical Bottom Plate (#164588) to hold them upright and directly scanned on the FMBIO. Using this strategy, detection of male DNA from 5ng down to 125 pg was achieved. Optimization of primer concentration, sensitivity of input DNA down to 25 pg and results using different ratios of male and female mixtures will also be presented.

Collaborators will use the results for further testing on previously screened samples. This new assay has the potential to greatly improve time of sexual assault screening and may alleviate the backlog evidence samples.

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References:

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Forensic Screening, Y Alu Assay, FMBIO III

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