## DEVELOPMENT OF A Y CHROMOSOME SPECIFIC SCREENING METHOD FOR SEXUAL ASSAULT EVIDENCE

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Forensic DNA analysis can be used to solve and link crimes. Approximately 169,000 rape cases await DNA testing<sup>1</sup> Three screening methods for male cells, acid phosphatase, prostate specific antigen p30, and microscopy, can yield false positive or false negative results. This study aims to develop a Y chromosome specific assay for detection of male DNA. The hypothesis is that development of a screening method targeting male DNA will give more accurate and sensitive results. Primers along with molecular beacons<sup>2</sup> and Lux primers<sup>3</sup> were based on male sequences derived from Y alu loci<sup>4</sup>. Replicate male control DNA samples (9948) were amplified using a range of annealing temperatures (Ta=45°C-75°C). Optimal Ta was determined to be 55°C for both loci. Sensitivity of the assay was evaluated using 9948 DNA from 0.025-5.0 ng. Sensitivity could be detected as low as 0.025ng. Parameters to detect fluorescent dyes were optimized using a flatbed laser scanner (FMBIO III plus, MiraiBio). Five different dye-probe combinations were evaluated and scanning parameters were selected based on signal to noise ratios. For example, the 488nm laser and 577/8nm emission filter were used to detect FAM labelled beacons and the 532nm laser and 605/20nm emission filter were used to detect TAMRA labelled beacons. Final experiments to determine the optimal protocols will be performed on mixtures of male and female DNA in different ratios to determine the limits of detection of male DNA. Collaborators will use the results for further testing on previously screened samples. This new assay has the potential to greatly improve the analysis of sexual assault evidence.

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<sup>1</sup> <u>http://www.dna.gov/rawmedia\_repository/287585a1\_7f44\_43ba\_972a\_8ef35cd68ea9</u>

<sup>2</sup> Tan W, Wang K, and Drake TJ (2004) Molecular beacons. Curr Opin Chem Biol 8, 547-553. (<u>http://www.molecular-beacons.org/DOWNLOAD/Tan,COCB04(8)547.PDF</u>)

<sup>3</sup> <u>https://www.invitrogen.com/content.cfm?pageID=3978</u>

<sup>4</sup> A.B. Carter, A. Salem, D.J. Hedges, C. Nguyen Keegan, B. Kimball, J.A. Walker, W.S. Watkins, L.B. Jorde, and M.A. Batzer (2003) Genome-wide Analysis of the Human Alu Yb-lineage. Human Genomics 1(3): 167-78

D.J. Hedges, J.A. Walker, P.A. Callinan, J.G. Shewale, S.K. Sinha, and M.A. Batzer (2003) Mobile Element-based Assay for Human Gender Determination. Analytical Biochemistry 312: 77-79