ROBUST MULTIPLEX AMPLIFICATION OF 18 Y-CHROMOSOME STR LOCI

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Several studies and common experience indicate that males commit the majority of violent crimes. For example the Bureau of Justice Statistics reports that males commit about 80% of all violent crimes and 95% of sexual offenses in the United States. Although autosomal short tandem repeat markers, the current loci of choice for the forensic analysis of biological evidence, are normally fully capable of adequately discriminating between unrelated individuals, there are several circumstances in which
Y chromosome polymorphisms could be a useful addition to the forensic scientist's armamentarium.

It is possible, and in certain circumstances advantageous, to make use of the unique biology of the Y chromosome for forensic purposes. Firstly, Y-chromosome specific systems may prove invaluable for the identification of the genetic profile of the male component in mixed male/female specimens in those instances in which the female portion is present in overwhelming quantities relative to the male. This could be due to the deposition of semen by an azoospermic or oligospermic male, to cases of oral sodomy where only trace amounts of male buccal epithelial cells may be present, or due to the normal degradative and sample loss processes that occur with the passage of time. Additionally, Y systems could be used to determine the number of semen donors in cases of multiple perpetrator rape.

A third reason for employing Y chromosome polymorphisms would be in criminal paternity analysis or disaster victim identification. The haplotype of a missing individual may be determined by typing a male relative such as a son, brother, father, nephew or uncle. Fourthly, the ability to specifically detect a male profile could obviate the need for the time consuming and often times inefficient differential extraction procedure for the separation of sperm and non-sperm fractions. Finally, male specific systems may provide additional statistical discrimination for autosomal makers in mixture or relative cases.

Although more than thirty potentially useful STR loci have been described on the Y chromosome, a more limited number have been appropriately evaluated for forensic use. Our aim in this work has been to extend the set of Y chromosome loci available for operational use and incorporate them into multiplex PCR assays using a standard analytical platform, thus enabling transfer to operational laboratories. We have developed two multiplex systems (MPI and MPII) that allow for the robust co-amplification of 18 Y-STRs and their detection-using laser induced fluorescence subsequent to separation by capillary electrophoresis. Loci include DYS19, DYS385 (a)&(b), DYS388, DYS389I & II, DYS390, DYS391, DYS392, DYS393, DYS425, DYS434, DYS437, DYS438, DYS439, Y-GATA-C4, Y-GATA-A7.1, Y-GATA-H4.

The development of male specific systems presents certain unique problems. Owing to its evolutionary history, the Y-chromosome is not only home to a variety of primary structure duplication, but it also retains a certain level of sequence homology with the X-chromosome. Since this creates the possibility of the appearance of X chromosome artifacts, it is essential to ensure that any amplified alleles truly arise from the male component. We have, therefore, optimized our multiplexes such that no confounding artifacts are obtained when the female: male DNA ratio is 100:1.

We will present data to demonstrate that the two multiplex systems are robust over a wide range of primer, magnesium and DNA polymerase concentrations and under a variety of cycling conditions. Complete male haplotypes can be obtained with as little as 100-250 pg of template DNA. Results of our mixture studies involving varying ratios of male: female and male: male DNA will be presented. Importantly, it is not necessary with our multiplex systems to employ a differential extraction strategy to obtain a male haplotype (or haplotypes in the case of multiple male donors) in cases of sexual assault.