DEVELOPMENT AND VALIDATION OF A SIX-LOCUS Y-CHROMOSOME STR MULTIPLEX SYSTEM FOR HUMAN IDENTITY APPLICATIONS

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LEARNING OBJECTIVES: This presentation will familiarize the participants with the development of a multiplex Y-chromosome STR genotyping system, and its utility in paternity and forensic applications.

In recent years, short tandem repeat (STR) sequences have become the markers of choice for human identification. Human Y-chromosome STR markers have recently gained importance and publicity due to their ability to establish paternal relationship. They also have important applications as additional markers in forensic casework. While the STR markers contained in the commercially available genotyping kits are more than adequate to uniquely type a given sample, there are instances where unequivocal results are difficult to obtain using these markers. A good example of this type of a situation would be mixed blood samples of two or more individuals of which at least one of the individuals is a male. In this case, should the male be the suspect, it would be difficult to unambiguously determine the genotype of the male sample from the mixed sample using the conventional STR markers that are available to the forensic community.

Although the PCR conditions for these markers are well established, there have been limited efforts to develop single-tube multiplexed assay utilizing more than 3-4 Y-specific STRs. In most forensic casework, the availability of evidence sample is the limiting factor and therefore, a multiplexed system is extremely important.

Y-PLEX^{TM} 6, a multiplexed Y-chromosome STR system established by ReliaGene Technologies, Inc., provides analysis of six Y-chromosome loci namely DYS393, DYS19, DYS389, DYS390, DYS391, and DYS385. The primers sets are labeled with FAM and TAMERA so that the amplified products can be analyzed by using 310 Genetic Analyzer, 377 Gene Sequencer, or Hitachi FMBIO®.

We report the development of a multiplex genotyping assay that utilizes six Y-specific tetranucleotide STR markers. Details of the common alleles from U.S. population groups, multiplexing strategy utilized and problems encountered in optimizing the system will be presented in detail. Results of some preliminary validation according to DAB guidelines including creating a population database will also be presented.