INTERLABORATORY STUDIES ON MULTIPLEXED mt DNA HV AND Y CHROMOSOME SNP TYPING USING AN AUTOMATED LIQUID BEAD ARRAY SYSTEM

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One of the newest technologies available for scoring DNA polymorphisms is an automated liquid bead array system. This system, Luminex 100™, is capable of conducting multiplex analysis of up to 100 different tests in a single tube. Using the XYP platform autosampler, it is possible to process 96 samples for 100 different alleles in less than 1 hour.

This technology is extremely flexible and has already been used to conduct both DNA and protein assays.

Several different types of kits are either already commercially available or soon to be released. Kits for analysis of the mitochondrial hypervariable regions I and II and Y chromosome SNPs have been developed in laboratories at Invitrogen Corp. The Luminex 100 and XYP have been introduced by MiraiBio, Inc.

The forensic utility of mtDNA and Y chromosome markers are well established. MtDNA HVI and HVII regions have been used by forensic laboratories on degraded minute samples such as bone and hair samples. Due to the high copy number of mtDNA it has been used to assist in forensic casework when the sample is limited and/or degraded. In addition, since mtDNA is maternally inherited, a reference sample can be acquired from the maternal lineage for comparative purposes even in the absence of a reference standard from the suspect or victim. Y chromosome markers have also been useful in forensic DNA casework. Y chromosome specific markers may provide a useful screen on sexual assault evidence to eliminate potential donors before conducting a differential extraction. Furthermore, they will also be useful in paternity cases.

Liquid arrays of allele-specific oligonucleotides for mtDNA HV 1 and HV2 and Y chromosome polymorphic sites were coupled to fluorescently tagged latex microspheres. Each oligonucleotide was associated with a particular "color" microsphere. DNA samples were amplified by PCR with primers labeled with a fluorescent reporter dye. The labeled amplification products were hybridized to the virtual array of oligonucleotides on microspheres, and then analyzed directly, without washing, in the Luminex 100 flow system. This instrument automatically samples each well in a 96 well microtitre plate and then computes the median fluorescent intensity of reporter dye associated with each color classification of microsphere, and hence with each allele-specific oligonucleotide.

In order to evaluate the performance of the Luminex 100™ system and the mtDNA and Y SNP kits, MiraiBio Inc. and Invitrogen initiated a developmental validation study. A haplotyping consortium was formed consisting of laboratories from, forensic, industrial and academic communities. Participants received 3 days of training in use of the instrument and SNP kits. Studies were initially conducted on known amplified samples. Five to six well-characterized samples were amplified, pooled and then

split so that each participant would start with an equal set of 5-6 amplified samples. This was done to minimize variability in results due to small difference in the efficiency of amplification which would likely occur between laboratories. Each participant laboratory independently ran 5-6 samples in triplicate for both mtDNA and Y SNP kits on the Luminex 100™ with the XYP platform. Panels of both mtDNA for HV1 and HV2 as well as Y chromosome SNPs plus the amelogenin locus were used to successfully genotype samples. Haplotyping results on a subset of these samples using the Luminex system were corroborated by subsequent sequencing. Additional characterized samples were analyzed independently at several sites. The availability of more mtDNA and Y polymorphic sites will allow the expansion of these panels to further increase their power of discrimination.

Results from these and other mtDNA and Y SNP liquid bead array studies will be presented, as well as software tools for analysis of the results.

Iannone, MA. *et al*. 2000. Cytometry 39:131-140, Carlson, D. 2000.Microsphere assays for Y-chromosome SNPs. 11th International Symposium on Human Identification. Promega Abstracts, Carlson, D. *et al*. 2001. Screening Mitochondrial DNA Polymorphisms with Liquid Arrays. Proc. American Academy of Forensic Sciences.Vol VII:77: Cytokines- Carson, RT. *et al*. 1999. J. Immunological Methods. 227:41-52.