GENETIC ANALYSIS OF SNPs USING THE LUMINEX 100 AND MASTERPLEX™ GT FROM MIRAIBIO INC.

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MiraiBio Inc. has recently introduced an automated liquid bead array system the Luminex 100. This system is capable of conducting multiplex analysis of up to 100 different tests in a single tube. Using the XYP platform auto sampler, it is possible to process 96 samples for 100 different SNPs in less than 1 hour. This technology is extremely flexible and has already been used to conduct both DNA and protein assays. Advanced genetic analysis software has also been developed, tested and commercialized: Masterplex™ QT for proteins and Masterplex™ GT for SNPs.

There are several different methods for analyzing SNPs (Ahmadian A and J Lundeberg. "A brief History of Genetic Variation Analysis." Biotechniques. 2002. 32:1122-1137). In forensics, SNPs have been used in HLA DQ alpha and Polymarker tests for casework. This method consists of direct hybridization of PCR products hybridized to allele specific probes immobilized on a membrane (reverse dot blot technology). Although this technology is robust, the current method is manual (with some capability of robotic processing of 24 samples at a time) and is limited to a small number of SNPs.

The probes can be immobilized on any surface that can be coated including microarrays. This method dramatically enhances the multiplexing capabilities since tens of thousands of probes can be arrayed and interrogated. However, solid phase hybridization of these allele specific probes is both costly ($200-$500 per sample) and due to low levels of non-specific cross hybridization often requires conducting more than one array per sample for confidence.

Primer extension technology has been used for SNP multiplexing in several approaches. These include liquid bead arrays on the Luminex 100 (Taylor *et al.* 2001. Biotechniques 30:661-666, 668-669.) and primer extension in the presence of fluor-tagged dideoxyNTPs followed by capillary electrophoresis or PAGE. The number of SNP loci that can be simultaneously extended and resolved in a single electrophoresis run limits this latter technology. Other technologies that have been used include MALDITOF-mass spectrometry, denaturing HPLC and fluorescence resonance energy transfer based detection methods (Lareu *et al*. Forensic Sci Int 118:163-168). These methods although effective have one or more of the following limitations: limited speed (resolution/speed considerations), limited multiplexing (FRET methods are limited to very low numbers of SNPs per well based on the availability of fluorescent tags that can be multiplexed), reproducibility of the assays (as mentioned above for microarrays) and/or cost of instrumentation/tests.

The main advantages of the liquid bead array detection technology coupled with a universal bead array are 1) the capability of multiplexing up to 100 different SNPs in a single tube using the same capture sequences (on universal bead arrays) so that the capture hybridization of the 100 SNPs can be conducted isothermally with low cross hybridization, 2) the technology is rapid and automated (96 samples in less than 1 hour for 100 SNPs) and 3) low instrumentation cost and cost per SNP since 100 SNPs can be multiplexed in the same tube.

Several different methods have been developed for SNP typing on the Luminex 100 including direct coupling of allele specific oligos (ASOs) to beads followed by hybridization of PCR products containing the SNP of interest and tag-primer extension or allele-specific extension followed by hybridization to bead arrays with universal anti-tags. Comparisons from internal and external laboratory testing using these and other methods will be presented. Automated genotyping of SNPs and QC of individual beads (SNPs), bead multiplexes, reagents and instrument runs will be demonstrated on different types of SNP data using Masterplex™ GT.