Abstracts

WS1

Utilisation of SNPs in forensic science. What are the advantages and what are the challenges?

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In recent years a considerable industry has developed to develop SNP technology in the field of medical diagnostics. However the requirements for forensic science are quite different. Approximately 50 SNPs are needed to give a discrimination power that is equivalent to short tandem repeat (STR) multiplexes that are currently in use today.

Bearing in mind that the quantity of DNA available for analysis may be sub-nanogram, one of the biggest challenges is to develop multiplexed reactions that comprise such a large number of loci, yet are balanced and robust enough for stringent forensic applications.

Finding SNPs to assess is not a problem because there are now more than 1 million that can be accessed on public databases, the most noteable being the SNP consortium Ltd (http://snp.cshl.org/).

We have developed a method that enables rapid evaluation of multiplexes that can be used on a variety of platforms including gel electrophoresis and microfabricated arrays. In the first instance gel electrophoresis may be the first method of choice until it becomes clear which platforms will become widely utilised.

Whether SNPs will replace STRs as the primary method of choice is a matter of conjecture at present. Nevertheless it is worth examining the criteria for change. The potential of massive throughput may result in significant cost advantages. In addition, SNPs are better able to analyse highly degraded DNA because the distance between primer binding sites can be designed to be very short.

Current national DNA databases are STR based and any transition to replace them with SNPs is likely to be problematical because of incompatibility. SNPs will have a place in the identification of Y chromosomal and mitochondrial polymorphisms and perhaps they are best viewed initially as supplementary to existing methods rather than a complete replacement.

WS2

SNP Genotyping using Megaplex PCR Amplification and Linear Probe Arrays

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There has been some interest expressed in having a highly discriminating single nucleotide polymorphism (SNP)-based assay for forensic applications. Because SNPs have only 2 alleles compared to 5 or more alleles in STR loci, many more SNPs (~50) are required to provide a significant level of discrimination between individuals. Therefore, to be of most value to the forensics community, 50 SNPs should be co-amplified from a few nanograms of DNA and the genotyping results should be accurate and quantifiable. Most of the available SNP genotyping assays are designed to amplify 1–10 SNPs per PCR amplification using over 10 ng DNA for each reaction. Consequently, 5–50 amplification reactions per sample would need to be run to achieve a level of discrimination appropriate for human identification applications. As the number of required amplification reactions increases, so do the chances of sample mix-up and contamination. The cost and set-up time of the assay are also greatly increased when more PCR amplifications have to be performed per sample. Perhaps of greatest importance to the forensics community is the need to consume a total of 50 or more nanograms of DNA per sample to obtain genotypes at all of the SNPs.

We have been developing SNP-based genotyping assays for the cardiovascular and inflammatory diseases research communities. At present, we routinely co-amplify 50–55 SNPs in a single reaction and perform two hybridizations to obtain genotypes. Under current assay conditions, accurate results can be obtained from as little as 5 ng and from as much as 200 ng DNA. Quantitation of DNA is not essential for the SNP assay, a feature that makes it very attractive to the research and diagnostic communities. In our assay, SNP detection is performed using an array of SSO probes immobilized in a line format on a strip of nylon membrane. Each strip can accommodate 58 probes and, using new instruments, up to 48 strips can be processed at a time. The hybridization instrument is being modified so that an image of the strips can be generated and transferred for analysis without removing the strips from the trays. By choosing a different panel of SNPs and further optimizing the megaplex PCR conditions, this assay could be valuable for human identification applications.

WS3

Matching odds with no database, and other SNP tales

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Single nucleotide polymorphisms (SNP) have the potential to be equally discriminating as loci of high polymorphism such as VNTR-RFLP or MVR systems – you just need more of them. This is obviously true for forensic identification, and it is also true for more complex problems such as deciding sibship or obtaining evidence from mixed stains.

How many weak systems are necessary to equal the discriminating potential of a strong system depends on several things. First, it depends on the problem: For forensic identity, one RFLP is worth about 2 STR's or 6 SNP's; whereas for paternity testing the corresponding ratio is 1:3:12, and for a typical mixed stain situation, 1:4:27. SNP's are relatively clumsy (but not hopeless) for mixed stain cases. Second, the tradeoff ratio varies depending on whether the relationship being investigated is true or false. The nature of the tradeoffs will be discussed, and results presented.

Finally, it will be shown how a large panel of SNP's can be effective for forensic identification even when no population data is available for the relevant population. The ability to squeeze out information despite a database of size zero somewhat flies in the face of common wisdom on recommended database size, but it is possible and it can be a distinct advantage for the odd case involving an unusual human population, or a non-human population.

WS4

Multiple Platforms for SNP Analysis

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WS5

High throughput multiplex assays for mutation detection using a Flow Sorter Fluoranalyzer.

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The study of genetic variations can be used to explain and/or diagnose many diseases, to study variation in drug response, to establish the origin of biological material and the relatedness between individuals. Currently, the most popular method used consists in the use of a DNA amplification step followed by analysis for the presence of the expected product(s) by the use of fluorescent detection and/or size fractionation, sometimes performed after hybridization to probe(s).
Some of the major challenges facing laboratories performing this type of assay is to find a technology platform that would allow for the simultaneous testing of large number of different loci or a variety of alleles. This is mostly due to the laborious nature of existing processes and/or the high cost of many of the existing assays. In addition, it is difficult to find a method and instrumentation that can efficiently handle both single and large number of samples.
Luminex Corporation (Austin TX) has developed a technology that allows for the parallel assay of up to 100 different probes. The bioassay consist of a collection of up to 100 different fluorescently colored beads or microspheres and a flow sorter fluoroanalyzer (Luminex^{100}). The surface of the beads has been modified to allow for the covalent attachment of oligonucleotide probes or proteins. The Luminex^{100} instrument contains a laser to classify the beads as they flow in front of a detector and a second laser to measure the amount of labeled product (reporter dye) bound to each bead.
One type of multiplex assay developed in our laboratory allows for simultaneous detection of the large number of different mutations used to identify the alleles for the human HLA-DRB1 locus. Each allele contains several polymorphic regions and hundreds of alleles have been identified for this locus. For this assay, each color microsphere (up to 100 different colors) was conjugated to a different sequence probe and the different bead-probes were combined into a single mixture. The range of mutations identified by the different probes used in this assay varies from single nucleotide polymorphisms to multiple nucleotide changes. The combined microspheres were hybridized to fluorescently labeled amplified DNA corresponding to Exon 2 of DRB. After hybridization the sample was analyzed in a Luminex^{100} equipped with an XY platform that allows for the automated analysis of up to 96 samples. The median fluorescence intensity of the labeled DNA captured by each bead-probe was used to determine whether the amplified DNA contained sequences complementary to the different probes. This information was used, with the aid of specially designed software, to determine the possible alleles present in a sample.
Another type of multiplex assay, with broad range of genetic applications including paternity and forensic testing, consists in the analysis of single nucleotide polymorphism (SNP). In contrast with most of the methods used for SNP detection, that examine only one or two alleles at a time in individual loci, the use of the Luminex^{100} may allow for the simultaneous analysis of up to 50 biallelic loci. To perform this type of assay, each bead class was conjugated to a different probe and the different bead classes mixed together. The SNP loci were amplified individually, or in a few multiplex reactions. The amplified products were combined with the beads into a single hybridization reaction, and after incubation, were analyzed in the Luminex^{100}. The results generated by the instrument were transferred to a genotyping software for analysis. These examples serve to illustrate the possibility of using the Luminex^{100} instrument and the fluorescent microspheres to develop a variety of simple and efficient probe based genotyping assays.

WS6

The Applied Biosystems technology portfolio for high-throughput genotyping and SNP screening

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Since completion of the human genome project an increased effort has focused on identification, validation and screening of populations for potentially informative Single Nucleotide Polymorphism (SNP) markers.

Applied Biosystems has been instrumental in development and commercialisation of a choice of technologies, both reagent and instrument systems, suited for both SNP validation and screening.

Current SNP Validation technologies include an electrophoresis based solution (the SNaPshot Assay) and MALDI-TOF MS (the Pinpoint Assay). In addition to these choices, and ideally suited for SNP screening applications from low to ultra high throughput, the use of the dual probe 5' Nuclease assay utilising TaqMan probes and the Applied Biosystems ABI PRISM 7900HT provides an additional solution that is both scalable and cost effective up to 100s of thousands of SNPs per day.

These technologies will be reviewed and compared during the presentation.

WS7

Real-time PCR probe systems – New Developments in Scorpion Primers

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There are a number of real-time PCR probe systems currently available. These include TaqMan probes, molecular beacons, hybridisation probes and Scorpion primers. We have continued to develop Scorpion primers to simplify their design and synthesis and to improve their performance. The original Scorpion primer contains the probe as a hairpin loop structure, which is attached to a PCR primer by a PCR stopper. The fluorophore and quencher are held in close proximity by the hairpin. After extension of the primer during PCR, the probe and its target are within the same strand of DNA and hybridisation is intra-molecular. This makes Scorpion primers fast and efficient. We have simplified the design of Scorpion primers by replacing the stem sequence and quencher with a separate quencher oligonucleotide. In the Duplex Scorpion format the quenching is inter-molecular and signal generation is intra-molecular. This is an ideal combination, giving much improved signal intensity compared with the original format.

WS8

Electronic Based Systems for DNA Diagnostic, Pharmacogenomic, and Human Identification Applications

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Nanogen is developing electronic based systems for single nucleotide polymorphism (SNP), gene expression and short tandem repeat (STR) analysis, for applications in pharmacogenomic research, DNA diagnostics, and human identification. A variety of active micro-electronic array (100 and 400 test sites) and other electronic based hybridization devices are being designed for these applications. Nanogen's Molecular Biology Workstation with its NanoChip™ microelectronic array provides the end-user with "make your own chip" capabilities. This system is designed to carryout SNP, point mutation, and STR analysis for genotyping, patient stratification and forensic applications. New applications for this system will include gene expression and "on chip" amplification, utilizing strand displacement amplfication (SDA). Other new technology will include an integrated microelectronic "sample to answer" system for point of use applications. This system will contain integrated components for electronic based sample preparation, cell lysis, DNA amplification, and multiplex hybridization analysis.

WS 9

Strategies for SNP Genotyping by Mass Spectrometry

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The identification and characterization of single nucleotide polymorphisms (SNPs) has become an important goal in genomic studies due to their association with many genetic diseases. With an estimated abundance of one SNP in every 100–300 bp of the human genome, typing of these genetic markers demands analytical methods capable for accurate measurements with reasonable sample throughput. Due to the short time period (ns-range) required for the detection of the molecular masses of DNA molecules <40 bp, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a promising technique for high-throughput analysis of SNPs. The general strategy of the most promising approaches for SNP detection by MALDI-TOF MS is to generate SNP-containing products through the extension of a primer annealed to the 5'-end upstream of the target polymorphic site. Dependent on the presence of dideoxynucleotides (ddNTP) the primers are extended only by a single ddNTP or by 1–5 desoxynucleotides (dNTP) before a terminating ddNTP is incorporated. The resulting products are identified through the accurate measurement of their molecular masses which is detected directly without the need for a specific labeling of the analytes. Since the resolution of MALDI-TOF MS is capable to determine mass changes involved in single base substitutions in a mass range between 3–40 bp, the analysis of a multiplex set of relevant SNP-products is possible. Recent developments in sample preparation and data interpretation allow for a fully automated process for the analysis and characterization of SNPs. The efficiency of MALDI-TOF MS-based typing of SNPs will be presented for different primer extension assays.

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WS10

The use of SNP-typing in Forensic Medicine

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WS11

DNA Microarrays for SNP Typing

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We are developing DNA microarrays for SNP typing in forensics using the Affymetrix 417 microarrayer and the microarray scanner Affymetrix 418. Two different types of microarray are being developed. One for paternity testing which includes 100 autosomal SNPs and another for Y chromosome SNP typing. The advantages of using SNPs include the low mutation rates (in paternity testing), the sensitivity of Y chromosome SNP typing in mix stains when the male component is in a very low proportion and the possibility of using high-throughput methods for typing.

A total of 32 Y chromosome SNPs were selected including the most interesting ones in Western European populations. The SNPs selected include some classical ones and some recently reported Y SNPs (Underhill et al., 2000).

Criminal casework (for Y or mt SNP typing) and paternity testing need separate consideration. The type of slides for each application, hybridization strategies and typing strategies will be shown and discussed. The advantages and disadvantages of the methods used to increase typing accuracy are discussed as well as the PCR multiplex strategies which must necessarily be combined with microarrays technology, at least in criminal casework applications.

Underhill PA et al.
Y chromosome sequence variation and the history of human populations. Nat Genet. 26(3):358–61 (2000)