Multiplexed Genotyping on the Universal Array using Ligation

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Introduction

Single nucleotide polymorphisms (SNPs) are single base changes occurring at a specific position in genomic DNA. Occurring approximately once in every 1000 bases, SNPs are the most frequent DNA sequence variations found in the human genome. Analysis of these genetic polymorphisms offers the potential for identifying disease-causing genes and establishing markers for individualized medicines. With the near completion of the human genome sequence, accurate, high-throughput, and cost-effective methods for SNP genotyping of populations will be required. This technical bulletin describes the application of a subset of the Tm100 Universal Sequence Set in combination with ligation chemistry for multiplexed SNP genotyping on the Luminex LabMAP™ platform.

Ligation Chemistry

Ligase-mediated genotyping offers a simple approach to analyze SNPs. Oligonucleotide ligation assay (OLA) [1] is one of the most well established examples of these methods. The assay uses a pair of allele-specific oligonucleotide probes (one specific for the wild-type allele and the other specific for the mutant allele) and a common reporter probe. The terminal 3' base of the allele-specific probes is positioned at the polymorphic base of the target DNA, and is immediately adjacent to the 5' end of the common probe. The common probe has a 5' phosphate molecule and a 3' reporter. The gene fragment containing the polymorphic site is amplified by PCR (see **Figure 1**) and incubated with these probes. In the presence of DNA ligase, ligation of the common reporter probe to the allele-specific probe(s) occurs only when there is a perfect match between the wild-type (wt) or the mutant (mut) probe and the PCR-derived target DNA.

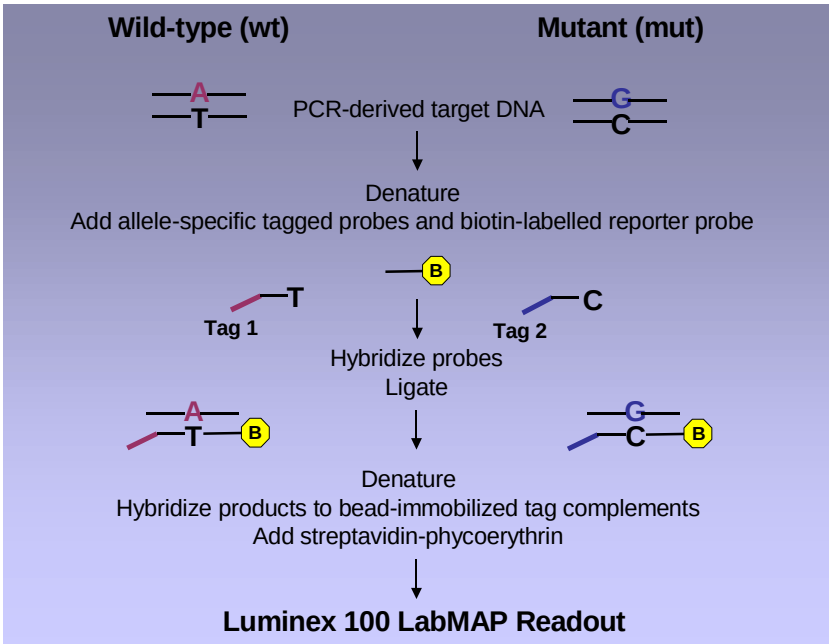

Figure 1: *Schematic of SNP genotyping using the Tm/Luminex Universal Array and ligation*

In the application described here, each allele-specific probe has a unique 24-mer tag from the Universal Sequence Set incorporated onto its 5' end. Biotin is used as the label on the 3' end of the common reporter probes. The biotin-labeled products are captured through hybridization of their tag sequence to tag complements that are covalently coupled to spectrally addressable polystyrene microspheres from Luminex Corp. (Austin, TX). The captured, biotin-labeled products are detected by the addition of a streptavidin-conjugated phycoerythrin (SA-PE). Microsphere and SA-PE fluorescence are measured using the Luminex 100 system.

Experimental Approach

To demonstrate the application of the Tm100 Universal Sequence Set to multiplexed SNP genotyping using ligation, a subset of 20 tag sequences was selected from among the 100 available tags. The DNA targets used in the ligation reaction were PCR-derived products of wt and mut alleles for each of the 10 selected genes/SNPs. Twenty allele-specific probes and 10 biotin-labeled common probes were designed to interrogate the SNPs in these DNA targets. Each allele-specific probe was synthesized with one of the tag

sequences on its 5' end. Every ligation reaction mixture contained all 20 allele-specific probes, 10 biotin-labeled common probes, and thermostable DNA ligase. The 20 DNA targets were included in ligation reactions individually (i.e., 20 separate tubes) and also as a pooled mixture in one tube. Following the ligation reactions, hybridization of the tagged products was carried out in the presence of all 20 tag complements (each coupled to a spectrally distinct Luminex bead population). To test the accuracy of the assay, an internal blind study was carried out. Five samples containing various combinations of the 20 possible alleles were independently prepared and analyzed.

Results

Each of the 20 alleles individually present in the pooled mixtures of all allele-specific probes could be accurately detected with the Tm/Luminex Universal Array platform (see **Figure 2**). Using the ligation chemistry to distinguish the SNPs, increased non-specific signal was observed in some cases. Since the non-specific signal was seen primarily between the wt and mut allele pairs of certain SNPs, it appears to be predominately caused by misligation of the allele-specific and reporter probes by the ligase enzyme. Nonetheless, specific signals from the perfect matches could be unambiguously detected above the background signal. When the 20 DNA targets were pooled, all of the 20 alleles could be confirmed in a single multiplex reaction. No compromise in the accuracy of identification was observed between the multiplexed and uniplexed target results (data not shown).

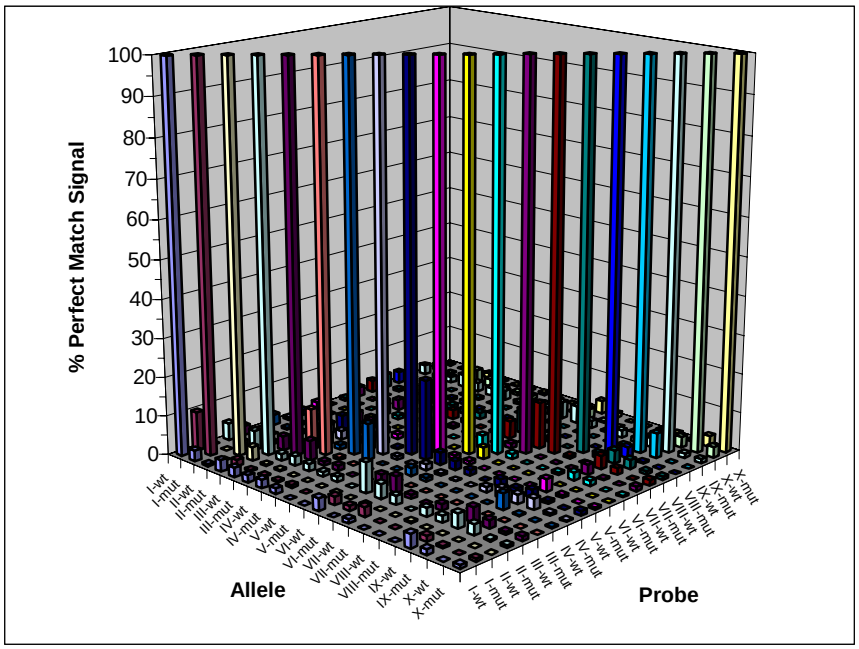

Figure 2. *Specificity of SNP detection achieved using the Tm/Luminex Universal Array and ligation*

In the blind study, all the SNPs present in the five samples were identified with 100% accuracy using the Tm/Luminex Universal Array platform and ligation. The results for one of the samples (containing nine out of the ten SNPs) are illustrated in **Figure 3**. For each SNP, the allelic ratio values were calculated by dividing the net median fluorescence intensity (MFI) for one of the alleles (wt or mut) by the sum of the net MFI for both alleles. Based on repeated results generated in-house, samples with a wt or mut allelic ratio > 0.75 for a given SNP were assigned as

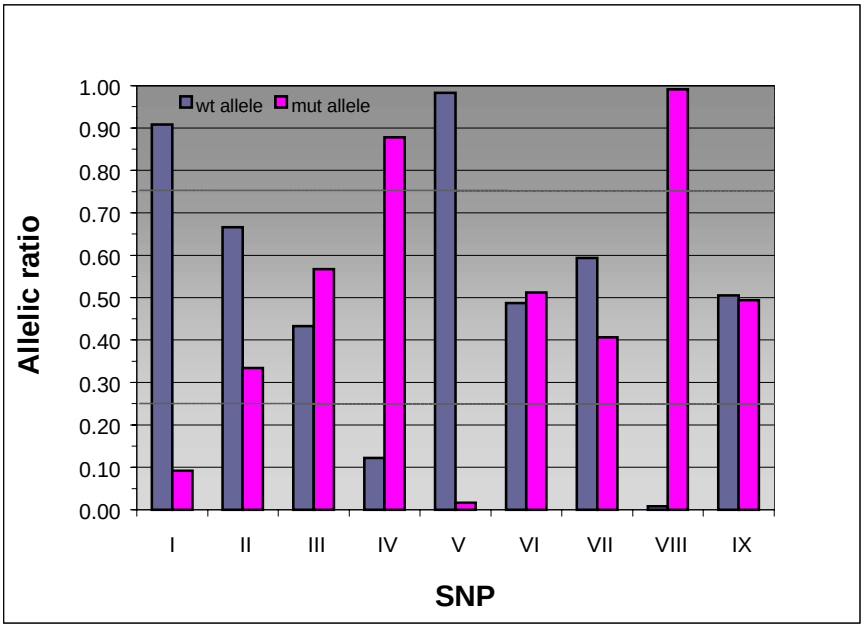

Figure 3. *Identification of SNPs present in a sample prepared as part of a blind study.*

homozygous wt or homozygous mut, respectively. Samples with wt and mut allelic ratios both ranging from 0.25 – 0.75 were assigned as heterozygous. The sample shown in Figure 3 was homozygous wt for SNPs I and V, heterozygous for SNPs II, III, VI, VII and IX, and homozygous mut for SNPs IV and VIII.

Conclusions

Our results demonstrate that the Tm/Luminex Universal Array is a highly specific and flexible platform that can be applied to multiplexed SNP genotyping. The ease of adapting existing technologies including OLA and ASPE (see Technical Bulletin-ASPE) to the Tm/Luminex Universal Array has been clearly demonstrated. Any combination from the set of 100 Tm Universal Sequences (see Technical Bulletin-401) and 100 Luminex microspheres may be selected for the analysis of multiple DNA-based targets (e.g., SNPs) in a single reaction vessel. The Tm Universal Sequence Set permits a single optimized set of sequences to be used repeatedly for analyzing multiple sets of SNPs. This provides a powerful alternative to the custom arrays that usually require multiple, target-specific sequence design and hybridization optimization steps for each new application. The outstanding flexibility and multiplexing capabilities of the Tm/Luminex Universal Array platform will prove essential as additional SNPs become available and the demands for high-throughput and cost-effectiveness increase.

Business Development Inquiries

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1. OLA is a proprietary technology of Applied Biosystems (Applera Corp.).

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