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Genotyping for single nucleotide polymorphism using a multiplex detection assay

M. Osada*, M. D'Ambrose, I. Balazs

Lifecodes Corporation, 550 West Avenue, Stamford, CT 06902, USA

Abstract

The objective of this work is to demonstrate the feasibility of using a multifunctional flow sorter fluoroanalyzer to perform a simple and rapid high throughput assays for Single Nucleotide Polymorphism (SNP) genotyping. Using this system, we have been able to demonstrate the feasibility of performing highly specific detection and discrimination of multiple bi-allelic SNPs in a single reaction.

Keywords: Single nucleotide polymorphism (SNP); High throughput analysis; Fluoroanalyzer; PCR; Genotyping

1. Introduction

Over the last few years, great efforts have been made to identify sequence variation in the human genome [1]. Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation and, to date, over 1.6 million SNPs have been identified and localized by the International SNP Map Working Group [2]. On average, one SNP can be found every 1000 bp throughout the human genome [3]. Compared to other forms of genetic variation, SNPs have relatively low mutation rates [4]. SNPs have been shown to have applications in a variety of genetic studies [5]. Numerous methods and instrumentation to perform SNP genotyping have been described; however, very few can be used for the simultaneous detection of a large number of SNPs in an efficient and cost-effective manner.

The Luminex 100™ (Luminex, Austin, TX), used in these SNP genotyping studies, can simultaneously detect up to 100 different analytes. The components of this system are a flow sorter fluoroanalyzer instrument and up to 100 uniquely labeled fluorescent microspheres (LabMAP™). The instrument contains two lasers, one to identify each microsphere class

* Corresponding author. Tel.: +1-203-328-9522; fax: +1-203-328-9599.
E-mail address: mosoda@orchid.com (M. Osada).

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and the second to quantify the amount of fluorescently tagged reporter molecules bound to it. Microspheres can be chemically coupled with DNA. For example, for the detection of different DNA sequences, each microsphere class can be coupled with a different sequence-specific oligonucleotide and hybridized to a mixture of labeled DNA sequences.

2. Material and methods

2.1. SNP loci

Sequence information for the SNP loci used in this study was obtained from the SNP Consortium and NCBI dbSNP databases.

2.2. PCR amplification

DNA samples from unrelated individuals were isolated form liquid blood or oral swabs. The amplification mixtures contained 5 ng of genomic DNA, appropriate amounts of biotin-labeled primer mixture, 0.2 mM dNTPs, PCR buffer and Platinum Taq DNA polymerase (Life Technologies) in a final volume of 25 μl.

2.3. Conjugation of probes

Oligonucleotides representing each SNP allele were covalently attached to different color-coded LabMAP™ microspheres (Luminex) according to the manufacturer's protocol.

2.4. Hybridization

Biotin-labeled PCR products were hybridized with the mixture of microspheres-probe conjugates at 47 °C for 30 min followed by 56 °C for 10 min. After hybridization, streptavidin R-phycoerythrin (Molecular Probes, OR) was added and incubated at 47 °C for 5 min. Samples were diluted with sheath fluid and analyzed using a Luminex-100™.

2.5. Data analysis

The median fluorescent intensity (MFI) of phycoerythrin in each bead type was used to determine the presence or absence of each SNP sequence in the amplified sample. Following background subtraction, the MFI of the two possible alleles of a locus were normalized by dividing the MFI of each allele by the sum of the MFI of both alleles.

3. Results

The objective of this study was to examine the feasibility of performing multiplex SNP detection using the Luminex platform. First, we examined the ability of the Luminex assay to discriminate the alleles in individual SNP loci. Second, we tested the ability of this

system to resolve a mixture of two or more loci. Fig. 1 shows the results obtained for two loci, TSC-A and GC-1. These loci were individually amplified and hybridized to bead-probe conjugates that contained specific oligonucleotide probes for each allele for both loci. The allelic ratio of the homozygous samples was 5- to 10-fold higher for positive alleles than negative alleles, while the ratio of alleles in heterozygous samples was close to 1. Each assay was repeated three times to determine reproducibility (Fig. 1). The accuracy and sensitivity of the assay were confirmed by comparing genotypes obtained with the Luminex assay with those generated by sequence-specific priming. No significant cross-reactivity was observed between the probes for these two loci. The equivalent results were obtained when both loci were co-amplified and detected in a single reaction.

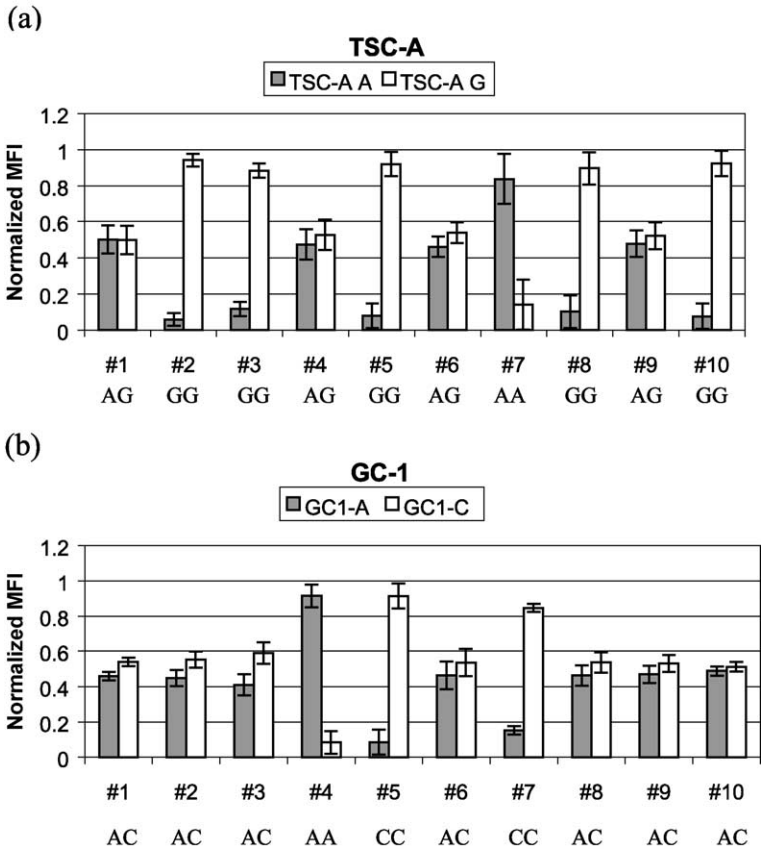

Fig. 1. Genotyping of TSC-A and GC1 loci in 10 Caucasian samples PCR products generated from allele-specific amplifications of TSC-A (a) and GC1 (b) were hybridized with a bead-probe mixture containing allele-specific oligonucleotide probes for both SNP loci. For each locus, gray and white bars indicate two alleles. The normalized MFI values are shown in the *Y*-axis. The genotypes obtained for 10 different samples are indicated in the *X*-axis. Each data represents the average of three independent experiments and the error bars represent the standard deviations.

To further increase the efficiency of the assay, we performed a multiplex amplification of 10 SNP loci. All amplified products were assayed in a single reaction tube containing a mixture of 20 different probes, one for each allele for all 10 SNP loci. The results of these multiplexed assays were compared with genotypes of each locus amplified and assayed individually. There was complete concordance between these two types of results (data not shown).

4. Discussion

Using the multiplexed assay system described above, we have demonstrated the possibilities of simultaneously analyzing a large number of SNPs in a single reaction. In addition, this instrument has the XY platform capabilities to automatically analyze up to 96 samples. The protocol utilized for these assays does not require a post-PCR purification step and the entire typing process, including DNA isolation, can be completed in less than 5 h. The fluorescent microsphere-based SNP genotyping assay may be used as a sensitive and reliable high throughput method to significantly reduce the reagent and labor costs associated with SNP genotyping.

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