Genotyping by Arrayed Primer Extension (APEX) Using the BioOdyssey™ Calligrapher™ MiniArrayer

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Introduction

With the completion of the Human Genome Project, the use of molecular technologies for the study of human genetic variation has increased considerably. The identification and study of single nucleotide polymorphisms (SNPs) promise to increase our understanding of how genetic factors contribute to disease. Genotyping based on SNPs is increasingly used in both human genetic studies, and in pharmacogenomics to elucidate the genetic basis of differing responses to therapeutics.

For SNP detection, laboratories need specific, reliable, high-throughput methods that can be easily automated. One strategy to reduce cost and increase throughput is SNP detection using DNA microarrays. Microarray-based genotyping allows simultaneous testing of multiple SNPs from a single human DNA sample (Wang et al. 1998). In our laboratory, we use a genotyping method that combines DNA microarrays with APEX technology (Syvanen et al. 1990, Ugozzoli et al. 1992, Kurg et al. 2000, Tebbutt et al. 2004).

APEX is a genotyping method involving hybridization of sample DNA to specific oligonucleotide primers followed by single-nucleotide extension. The first step requires amplification of target DNA sequences containing SNP(s) using PCR. Subsequently, fragmented PCR products are hybridized to SNP-specific primers immobilized on the microarray slide via their 5' ends. The oligonucleotides are designed to be complementary to the SNP loci of interest, with the final 3' nucleotide immediately adjacent to the polymorphic base. After primer hybridization, the SNP-specific extension reaction is performed using differentially labeled fluorescent dideoxynucleotide triphosphate (ddNTP) terminators and a thermostable DNA polymerase. Finally, microarray slides are scanned, and the spot intensity data obtained using softWoRx microarray image analysis software (Applied Precision, LLC) are used for genotype analysis by SNP Chart software (Tebbutt et al. 2005).

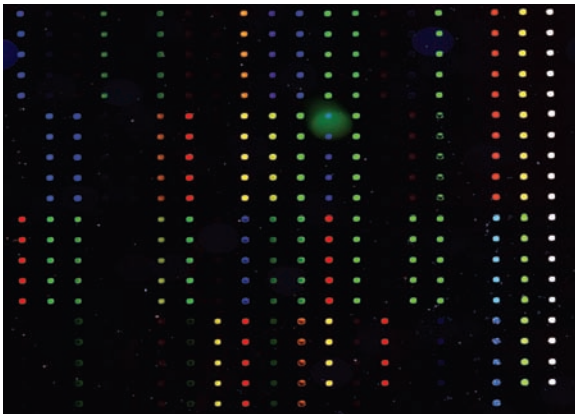
Methods

Seven SNP loci (rs1382938, rs1417269, rs1451613, rs1467372, rs1484729, rs1506508, and rs1932819) from three human DNA samples obtained from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository at the Coriell Institute for Medical Research (**http://coriell.umdnj.edu/**) and one negative control were amplified using PCR with dUTP and dTTP at a 1:4 ratio using HotStarTaq DNA polymerase (QIAGEN). PCR products were then precipitated and fragmented using uracil N-glycosylase, and fragmented DNA and untreated sample were analyzed by electrophoresis on agarose gels.

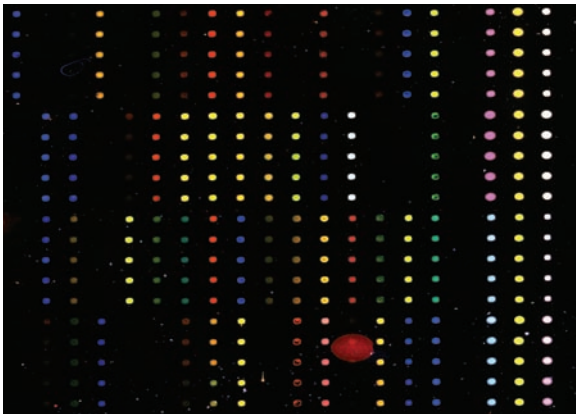
APEX and allele-specific APEX oligonucleotide primers for the detection of the seven SNP loci, as well as control APEX primers, were printed onto CodeLink slides (Amersham Biosciences) using the BioOdyssey Calligrapher miniarrayer. Primers were at 50 pmol/µl in 150 mM sodium phosphate print buffer (pH 8.5) and were printed to specific grid positions on the microarray slides according to the manufacturer's recommended protocols. The 5' end of each oligonucleotide probe was amino-modified, allowing its covalent attachment to the slide. Array quality was checked by staining with fluorescent SYBR Green II.

APEX reactions were performed on the arrayed slides as described previously (Tebbutt et al. 2004), and the slides were subsequently scanned in the arrayWoRx biochip reader (Applied Precision, LLC). Array experiments were performed in triplicate for each sample. Gridding and segmentation analysis were performed on four individual gray-scale TIFF images to create the false-color blended images shown in Figure 1. Spot intensity data for each of the four fluorescent channels (A, C, G, T) were imported into SNP Chart software, where genotype calling was performed (Tebbutt et al. 2005).

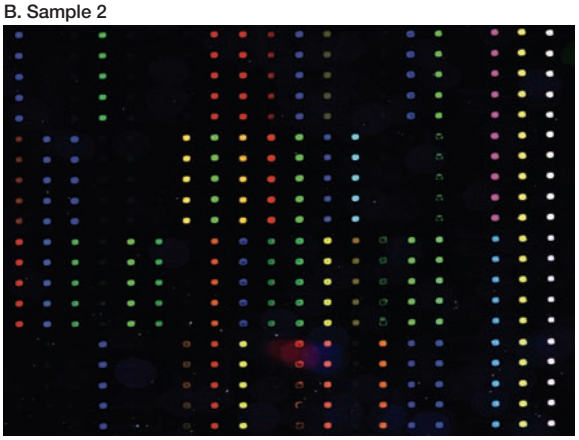
A. Sample 1



C. Sample 3



B. Sample 2



D. Negative PCR control sample

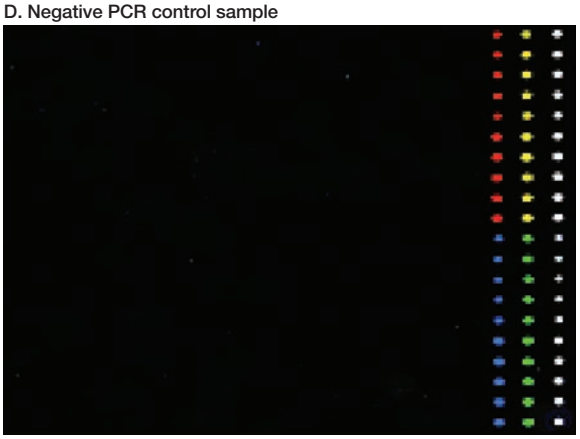


Fig. 1. False-color images of selected arrays from APEX genotyping results of Coriell samples. A–C, samples 1, 2, and 3, respectively. Colored spots indicate APEX or allele-specific primers that have been extended by a single base, with color specificity indicating which base has been incorporated. The final 3 columns in each panel show positive-control APEX probes. • = A; • = C; • = G; • = T. **D**, false-color image of a negative PCR control from APEX; only positive controls generated a signal.

Results and Discussion

The genotyping results obtained for the three samples were compared to previously validated genotypes. Of the total 21 possible genotypes (7 SNPs for each of 3 Coriell samples), all 21 were called correctly, an overall accuracy of 100%.

Figure 1 shows blended false-color images of selected arrays for each of the three human DNA samples obtained from Coriell (Figure 1A–C) and a negative control (Figure 1D).

Conclusions

This study demonstrates the successful use of the BioOdyssey Calligrapher miniarrayer for microarray-based genotyping by APEX. The arrayed primers are precisely placed by the miniarrayer, allowing easy downstream data analysis.

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