(ULTRA)²-HIGH THROUGHPUT GENETIC ANALYSIS USING MICROFABRICATED CAPILLARY ARRAY ELECTROPHORESIS DEVICES

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Abstract
We have developed technologies enabling the next generation in high-throughput electrophoretic genotyping using our rotary confocal scanner. The capabilities of this system are illustrated by (i) a short tandem repeat (STR) DNA genotyping separation performed on a 150-mm diameter, radial 96-lane μCAE microplate in less than 8 minutes, and (ii) the parallel assay of the common H63D mutation in the human HFE gene on a 384-lane, 200-mm diameter microplate in only 7 minutes.

Keywords: Capillary Array Electrophoresis, Rotary Confocal Fluorescence Scanner, DNA Genotyping

1. Introduction
The completion of the draft sequence of the human genome heralds a revolution in our understanding of genetic complexity and variation. Higher throughput genotyping assays are now needed to identify polymorphisms and screen for affected individuals. Conventional CAE has brought us far along this path, but a paradigm shift to higher throughput microfabricated-Capillary Array Electrophoresis (μCAE) devices is now at hand. We present here approaches for ultra-high throughput, multi-color, multiplex genotyping assays on 96-lane microplates as well as massively-parallel (ultra)²-high throughput genotyping on 384-lane microplates.

High speed genotyping was first performed on μCAE devices by Woolley et al. [1] in 1997 using a 12-lane design and a linear confocal scanner, later expanded to accommodate serial loading of up to 48 samples [2]. Subsequently, a 48-lane device employing a galvanometric scanner for detection was used to analyze 96 samples [3]. Introduction of the Berkeley rotary confocal fluorescence scanner together with the 96-lane, radial μCAE design in 1999 [4] provided the paradigm shift necessary to expand to the extremely high device density and design freedom illustrated here.

2. Results and Discussion
Radial 96-lane microplates (see Fig. 1A) have been used to perform a variety of high-speed genotyping experiments such as STR, RFLP, and allele-specific PCR SNP typings of human populations [5, 6]. As an example of the versatility of the μCAE platform we present here high-speed 4-color STR typing on 150-mm diameter, 96-lane μCAE plates. Four-color energy-transfer (ET) cassette-labelled primers were used to produce the STR amplicons. Three of the four colors are used for genotyping while the fourth is reserved for a size standard. The amplicons were rapidly separated (< 8 mins) with single base resolution. The powerful capabilities of this system were demonstrated by genotyping more than 300 loci in a single run [7] on 150-mm diameter devices enabling throughputs of 1 allele every 1.5 seconds (Fig. 1B).
To further demonstrate the power of our radial confocal scanning system and the versatility of the radial format, we have designed, fabricated, and performed separations on a μCAE device with 384 radial lanes (Fig. 2). This massively parallel μCAE device was patterned on 200-mm diameter glass wafers with standard procedures. The larger substrate size allows a higher number of sample wells and longer (8.0 cm) effective separation length. A direct injection scheme is employed, allowing better than 10-bp resolution with representative DNA ladders. The 384-lane μCAE device was used to perform an RFLP analysis of the H63D (187C→G) mutation in the HFE gene. This relatively common mutation is associated with the occurrence of Hereditary Hemochromatosis (HHC), an autosomal recessive disease that causes excessive iron intake [8]. All runs on the 384-lane μCAE device were performed using...