NEW LABELING AND MICROFLUIDIC GENETIC ANALYSIS TECHNOLOGIES

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The human genome project spawned a variety of new technologies including Energy Transfer (ET) fluorescent dye labels[1] and capillary array electrophoresis[2] that facilitated the early completion of the project. These advanced labeling and separation technologies together with recently developed microfabricated genetic analysis systems will also be valuable for advancing the field of human identification. In particular, the recent development of ET-cassettes[3] makes it convenient to apply these advanced labeling technologies to STR analysis as well as polymorphism identification and typing. We recently introduced the method called Polymorphism Ratio Sequencing (PRS)[4] that exploits these novel labeling strategies to focus on the identification and/or typing of genetic differences; the utility of PRS is demonstrated through complete and rapid mitochondrial resequencing. In addition, microfabricated microfluidic genetic analysis systems have evolved to the point where it is routine to perform a wide variety of high-speed capillary electrophoretic (CE) analyses. Microfabrication also permits the production of very high density electrophoretic analysis devices that provide unprecedented analysis throughput. Radial capillary array electrophoresis microplates coupled with a novel rotary confocal scanning system have been developed that can analyze 96 or more genotyping and sequencing samples in parallel in minutes.[5] Recent work has extended these systems to genotyping on 384 lane devices.[6] The full exploitation of these high-speed and high-throughput analysis capabilities will require the integration of large numbers of channels of arbitrary design and length on a wafer and the integration of very low volume nanoliter sample amplification and manipulation on the chip as well.[7-9] The development of such integrated technology will facilitate the fabrication of microfluidic microprocessors that could be used in point-of-care clinical and genetic analyzers, and in integrated microfluidic sequencing chips. Progress and prospects for fully integrated microfluidic DNA sequencing and genotyping systems with the capability of dramatically reducing analysis cost and improving reliability in high-throughput and point-of-analysis applications will be presented.[9]

1. Ju, J., Glazer, A. N. and Mathies, R. A. Energy Transfer Primers: A New Fluorescence Labeling Paradigm for DNA Sequencing and Analysis, Nature Medicine 2, 246-249 (1996).
2. Kheterpal, I. and Mathies, R. A. Capillary Array Electrophoresis DNA Sequencing, Analytical Chemistry, 71, 31A-37A (1999).
3. Medintz, I. L., Berti, L. and Mathies, R. A. Novel Energy Transfer Fluorescence Labeling Cassette, BioTechniques 32, 270 (2002).
4. Blazej, R. G., Paegel, B. M. and Mathies, R. A. Polymorphism Ratio Sequencing: A New Approach for Single Nucleotide Polymorphism Discovery and Genotyping, Genome Research, 13, 287-293 (2003).
5. Paegel, B. M., Emrich, C. A., Wedemayer, G., Scherer, J. R. and Mathies, R. A. High-Throughput DNA Sequencing in a 96-Lane Microfabricated Electrophoresis Device, Proc. Natl. Acad. Sci. U.S.A. 99, 574-579 (2002).
6. Emrich, C. A., Tian, H., Medintz, I. and Mathies, R. A. Microfabricated 384-Lane Capillary Array Electrophoresis Bioanalyzer for Ultra High-Throughput Genetic Analysis, Analytical Chemistry, 74, 5076-5083 (2002).
7. Lagally, E. T., Emrich, C. A. and Mathies, R. A. Fully Integrated PCR-Capillary Electrophoresis Microsystem for DNA Analysis. Lab-on-a-Chip, 1 (2) 102-107 (2001).
8. Paegel, B. M., Yeung, S. H. I. and Mathies, R. A. Microchip Bioprocessor for Integrated Nanovolume Sample Purification and DNA Sequencing, Anal. Chem., 74, 5092-5098 (2002).
9. Paegel, B. M., Blazej, R. G., and Mathies, R. A. Microfluidic Devices for DNA Sequencing Sample Preparation and Electrophoresis Analysis, Current Opinions in Biotechnology, 14, 42-50 (2003).