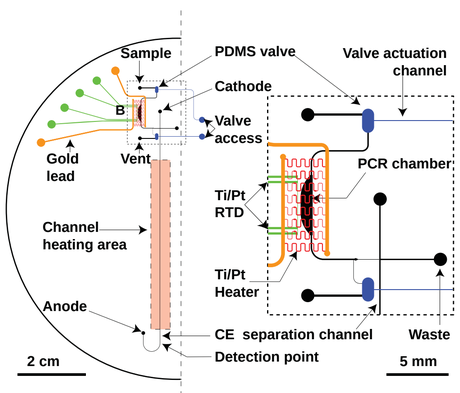
INTEGRATED PORTABLE MICROCHIP SYSTEM FOR RAPID FORENSIC SHORT TANDEM REPEAT TYPING

Peng Liu,† Stephanie H. I. Yeung,† Tae Seok Seo, Kyoung-Jin Shin,* Nathaniel Beyor,† James R. Scherer and Richard A. Mathies

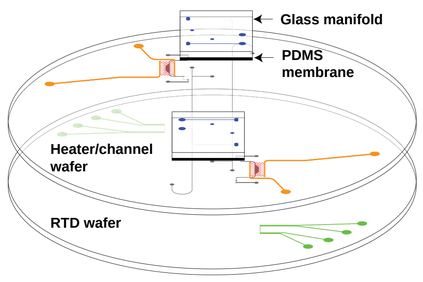
Department of Chemistry, University of California, Berkeley, CA 94720
† UCSF/UCB Joint Graduate Group in Bioengineering, University of California, Berkeley, CA 94720
*** Department of Forensic Medicine, Yonsei University Medical College, Seoul 120-752, Korea**

Microfabrication technology, or so-called micro-total analysis system, has attracted increasing attentions due to its ability to integrate multiple conventional molecular biology processes at microliter to nanoliter scale in a single device with high-throughput, high-sensitivity and low-sample consumption.[1,2,3] Here we present a portable forensic analysis system consisting of a microfluidic device for amplification and separation of STR fragments together with an instrument that contains all the necessary optical and electronic components for chip operation. We have explored the forensic applications of this portable system by performing 4-plex mini-Y chromosome STR typing consisting of a sex-typing marker, amelogenin, and three Y-STR loci (DYS390, DYS393 and DYS439). Limit of detection analysis showed that all the amplicons can be detected down to 20 copies of male standard DNA. We also evaluated the performance of the system in resolving male alleles in the presence of female DNA. A balanced profile can be obtained for all four loci even at a ratio of 1:10. To demonstrate the capabilities of the PCR-CE system to perform real-world forensic analysis, we also successfully typed human bone and oral swab extracts from case evidence previously processed and analyzed by the Palm Beach County Sheriff's Office.[4] Our demonstration of successful STR analyses performed on this portable PCR-CE system validates the concept of point-of-analysis DNA typing in forensic caseworks or at a security check, where rapid on-site screening or identification is demanded.

PCR-CE Microchip



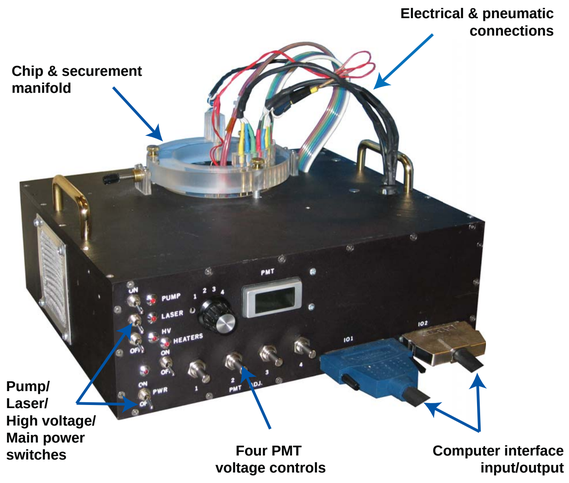
Mask design for the PCR-CE microchip



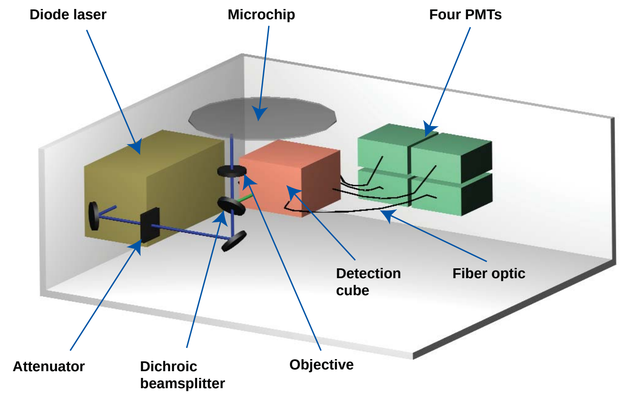
Exploded view of the assembly of the PCR-CE microchip

- 4-inch chip with four-layer structure
- Microfabricated RTDs and heaters for thermal cycling (12 °C/s for heating and 5 °C/s for cooling)
- 160-nL PCR reactor, 7-cm CE separation channel
- Total PCR time ~1 hr., CE separation time 8 min
- Primers labeled with energy-transfer dyes

Integrated Portable Instrument



Photograph of the portable PCR-CE system

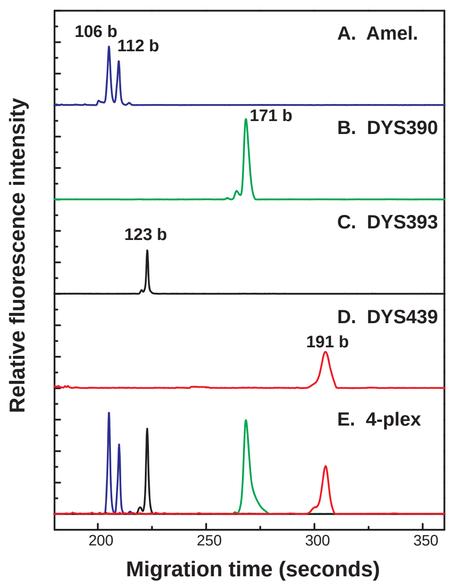


The schematic of four-color fluorescence detection system of the instrument

- Dimensions 10 x 12 x 4 inches
- Integrated 488 nm laser excitation and four-color confocal fluorescence detection system
- Pneumatic actuation system for on-chip valves and pumps
- Electronics for heaters and temperature sensors
- Integrated high voltage power supplies for electrophoresis

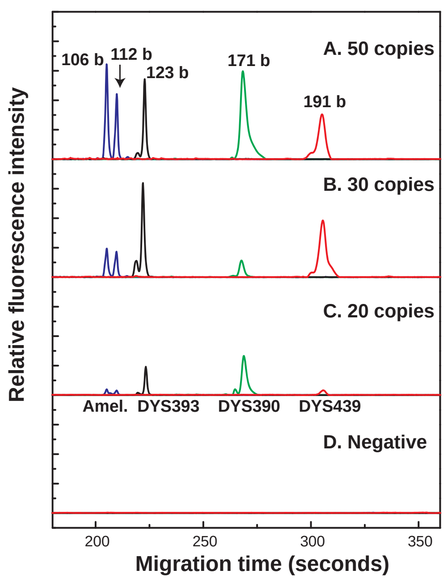
4-plex mini-Y chromosome STR typing

1. Monoplex and Mutiplex STR Amplifications



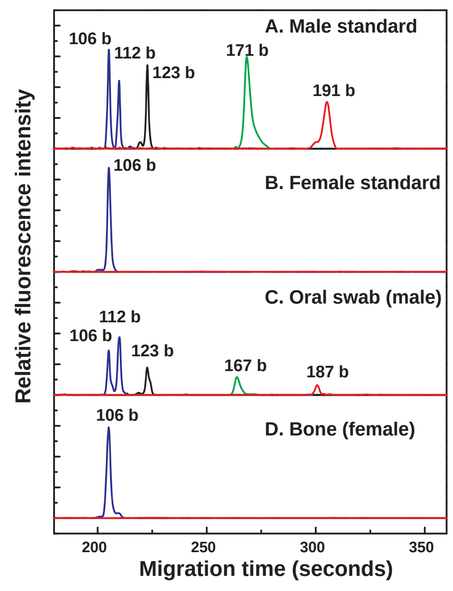
- Analysis of Amelogenin, DYS390, DYS393 and DYS439
- Monoplex from 20 copies of male standard DNA with 32 PCR cycles
- Multiplex from 50 copies of male standard DNA with 35 PCR cycles

2. Limit of Detection Analysis



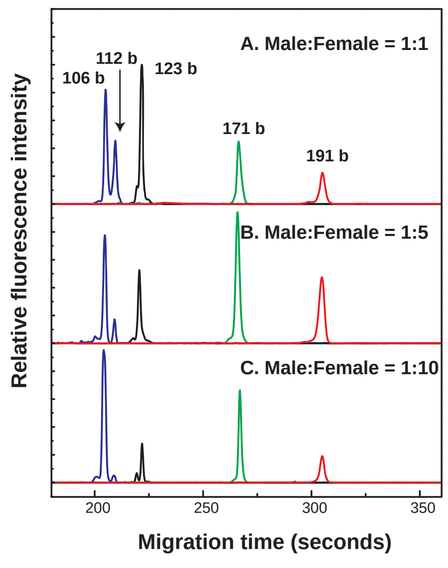
- Analysis of male standard DNA dilution series
- 35 PCR cycles followed by direct CE separation
- 20 Copy sensitivity with 5X multiplex PCR capability

3. Analysis of Samples from Forensic Casework



- Sex determination from human genomic DNA
- Multiplex amplifications of DNA extracted from oral swab and human bone samples
- Multiplex from 50 copies of male standard DNA with 35 PCR cycles

4. Male DNA Analysis in Female DNA Background



- Amplification from male and female standard DNA mixture with constant male DNA amount of 50 copies
- Successful amplification at male-to-female ratio of 1:10
- Information of male-to-female DNA ratio obtained from peak area ratio of Y to X in Amelogenin

Field Test

- All necessary equipments packed in a box of 2.5 x 1.5 x 1.5 ft.
- 4-plex mini-Y STR typing performed outdoors
- No interference from environment

References:

1. Yeung, S. H. I., Greenspoon, S.A., McGuckian, A.B., Crouse, C.A., Emrich, C.A., Ban, J. and Mathies, R.A. Rapid and high-throughput forensic short tandem repeat typing using a 96-lane microfabricated capillary array electrophoresis microdevice. J. Forensic. Sci., in press (2006).

2. Lagally, E. T., Emrich, C. A. & Mathies, R. A. Fully integrated PCR-capillary electrophoresis microsystem for DNA analysis. Lab-on-a-Chip 1, 102-107 (2001).

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4. Liu, P., Seo, T.S., Beyor, N. Shin, K.J., Scherer, J.R. & Mathies, R. A. Integrated portable PCR-CE microsystem for rapid forensic STR analysis. In preparation (2006).

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College of Chemistry
University of California, Berkeley

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