

## INTER-LABORATORY STUDIES ON THE POWERPLEX<sup>®</sup> Y USING THE FMBIO<sup>®</sup> III PLUS

**Steven B. Lee<sup>1,6</sup>, Xavier Aranda<sup>2</sup>, Dennis Yip<sup>2</sup> and Arthur Eisenberg<sup>2</sup>,  
Cecelia A. Crouse<sup>3</sup>, Amy McGuckian<sup>3</sup>, Tateo Nagai<sup>1</sup>, Marco Calavetta<sup>1</sup>, Meihua  
Chu<sup>1</sup>, Takeshi Shimizu<sup>4</sup> and Stephen Rodgers<sup>1,5</sup>**

<sup>1</sup>MiraiBio Inc, Alameda, CA 94502, <sup>2</sup>University of North Texas, Fort Worth, TX 76107, <sup>3</sup>Palm Beach County Sheriff's Office, West Palm Beach, FL 33406, <sup>4</sup>Hitachi Software Engineering Co., Ltd. Life Science Research Center, 1-1-43, Suehiro-cho, Tsurumi-ku, Yokohama, 230-0045 Japan, <sup>5</sup>VA Department of Forensic Science, Richmond, VA 23219, <sup>6</sup>Forensic Science Program, Justice Studies Department, One Washington Square, San Jose State University, San Jose, CA 95192

The FMBIO<sup>®</sup> III plus provides imaging acquisition and analysis with unparalleled speed, sensitivity and resolution for a wide variety of applications, (i.e. gels, plates, and arrays) and detection of a wider fluorescent dye range than that of the FMBIO<sup>®</sup> II and II e. The FMBIO<sup>®</sup> III plus has three solid-state lasers: a 20mW, 488nm, a 10mW, 635nm and the strongest laser in the industry: a 50mW, 532nm, YAG laser. Other features include new analogue broad range photo multiplier tubes (300-800nm) with 60,000 hours of lifetime, wide focal depth range from 3.0mm down to 4.0mm up (increments of 0.05mm), and a new auto focus feature that assists in finding the signal maximum to set the optimal focal point.

The FMBIO<sup>®</sup> III plus is a relatively new instrument compared to the FMBIO<sup>®</sup> II and II e that are widely used in casework, data banking, and paternity STR multiplex testing. MiraiBio and Promega have established collaborations with the University of North Texas Health Sciences Center, Virginia Department of Forensic Services and the Palm Beach County Sheriff's Office to evaluate the performance of the FMBIO<sup>®</sup> III plus using Promega's PowerPlex<sup>®</sup> Y STR kits.

Y chromosome specific STR testing is a powerful tool for forensics (Krenke et al. 2005. For. Sci Intl. 148:1-14). PowerPlex<sup>®</sup> Y uses four fluorescent dyes: carboxy-tetramethyl rhodamine (TMR), carboxy-x-rhodamine (CXR), fluorescein (FL) and 6-carboxy-4', 5' dichloro-2', 7' - dimethoxy-fluorescein, (JOE). Three of these are used to label the primers for Y STR loci. They include: TMR for DYS385, DYS390 and DYS393, Fluorescein for DYS389II, DYS439, DYS389I, DYS391, and JOE for DYS392, DYS19, DYS437, and DYS438. CXR is reserved for the Internal Lane Standard (ILS).

In order to optimize fluorescent color multiplexing on the new FMBIO III<sup>®</sup> plus, testing and validation of a combination of band pass and blocking filters has been completed and the FMBIO<sup>®</sup> III plus was utilized to detect the PowerPlex<sup>®</sup> Y STR multiplex. Complete color separation was achieved using the following optimized filter sets. The filters used to collect the four dyes are TMR : 598nm/8nm & 550 LP block, Fluorescein : 520nm/40nm with a 515 LP block, CXR : 650nm/15nm with a 570 LP block and JOE : 555nm/20nm with a 550 LP block.

Image Analysis 3.0.0.26 software was used for color separation. Color separation in this study was achieved directly from the raw data without going through gray scale adjustments. All parameters for band calling, gray scale adjustment, and color separation can be saved and reloaded as standards for subsequent analysis. Lookup tables for the PowerPlex<sup>®</sup> Y are being developed and are being tested and added to the genotyping STaR Call 3.0 software. Sensitivity of detection for all loci was down to 0.125ng using 32 cycles and intra-gel precision of sizing for 31 ladders on 7 gels was within +/- 0.4 bp for all loci. A series of mixture studies were used to verify the lower detection level of the FMBIO<sup>®</sup> III plus down to approximately 100pg for the minor contributor.