

**ANALYSIS OF POWERPLEX® 16 BIO USING THE
MIRAIBIO, INC. HITACHI GENETIC SYSTEMS FMBIO® II AND IIE**

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MiraiBio, Inc., Hitachi Genetic Systems, continues to offer innovative products in imaging systems. The latest release of a fluorescence scanner, the FMBIO® Iie, offers the strongest laser in the industry: 50mW vs. 20mW for the FMBIO® II. The solid-state YAG laser scanner provides unparalleled speed and resolution in imaging acquisition and analysis and provides the user a wide variety of applications, (i.e. gels, plates, and arrays).

The FMBIO® II is widely used for STR multiplex applications in casework, databasing, and paternity testing. It has been validated for use with the Promega PowerPlex® 1.1 and 2.1 by several laboratories. Additional applications include detection of arrays, SYBR (Molecular Probes) dye stained DNA gels, SYPRO dye (Molecular Probes) stained proteins gels, sequencing, and chemifluorescence.

The FMBIO® II was compared with the FMBIO® Iie using the PowerPlex® 1.1, 2.1, and the prototype of PowerPlex® 16 BIO. Collaborations in this study involved the University of North Texas, the Palm Beach County Sheriff's Office, the Promega Corporation, and MiraiBio, Inc. The average signal increase of 36%, (in OD units), on the FMBIO® Iie was similar for all three dyes used in the Promega PowerPlex® 1.1 and 2.1 systems. The FMBIO® Iie generated signals comparable to those generated with the FMBIO® II when the PMT settings of the Iie were set at 80 – 85%.

A prototype of Promega's PowerPlex® 16 BIO was also evaluated on the FMBIO® II and the FMBIO® Iie. This system uses four fluorophores. Three of the fluorophores make up the primer mix and includes Fluorescein, (Penta E, D18S51, D21S11, TH01, and D3S1358), JOE, (Penta D, CSF1PO, D16S539, D7S820, D13S317, and D5S818), and Rhodamine Red™-X®, (FGA, TPOX, D8S1179, vWA, and Amelogenin). The fourth fluorophore is Texas Red®-X and functions as the internal lane standard, (ILS). The filters used to collect the four dyes are 505nm+/-20, 577nm+/-2, 598nm+/-2, and 665nm+/-15, respectively.

The MiraiBio, Inc. Image Analysis 8.0 software was used for color separation while lookup tables for PowerPlex® 16 BIO were developed and added to the genotyping STaR Call™ 3.0 software. Color separation is facilitated by the development of a four color matrix standard, which is used to help aid in band selection. Color separation in this study was achieved directly from 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.