HIGH THROUGHPUT GENOTYPING OF THE THIRTEEN CORE CODIS LOCI USING HITACHI'S FMBIO® ANALYSIS 8.0 AND STAR CALLÔ 3.0 SOFTWARE

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ABSTRACT

Short tandem repeats (STRs) have become routinely used in the testing of paternity and forensic DNA samples. STRs offer a number of benefits over traditional DNA testing, which include the ability to co-amplify multiple loci in a single tube, also known as multiplexing. While a number of multiplex kits are commercially available, none of these simultaneously co-amplify the 13 loci (D5S818, D13S317, D7S820, D16S539, CSF1PO, TPOX, TH01, vWA, FGA, D21S11, D8S1179, D18S51, and D3S1358) required for uploading the generated profiles into the national Combined DNA Index System (CODIS) databank. Using the Promega PowerPlex™ 1.1 and 2.1 systems in conjunction with Hitachi's FMBIO® Analysis 8.0 and STaR Call™ software, a merged file, which contains all 13 CODIS loci, can be generated. The ease of operation, new functions of the FMBIO® Analysis 8.0 and STaR Call™ 3.0 software will be discussed in this presentation.

SUMMARY

The Hitachi FMBIO® II is a laser induced fluorescent scanner, which allows the separation of the electrophoresis and scanning steps resulting in a high throughput system. When commercially available precast gels, such as Hitachi's R^3 gels, are utilized, throughput can be further increased. Using the PowerPlex 1.1 and 2.1 kits the 13 core CODIS loci are amplified and then genotyped using Hitachi's FMBIO® Analysis and StaR Call™ Software. Improvements to the FMBIO® Analysis Software allow the assignment of base pair values to be generated in approximately one-half the time of previous versions.

New features included in FMBIO® Analysis 8.0:

- An improved color separation algorithm, which incorporates bands and background areas to achieve color separation
- A new color separation method, which allows the bands in any sample lane to be used to create a color separation matrix
- The ability to save parameters, these include:
    Gray level adjustment values
    4x4 color separation matrices, which allow four color multiplexing
    Lane templates including a new lane fitting function with numbered lanes
    Displayed information
- A preferences page, which allows the saved gray level values, color separation matrices, lane templates, autoband parameters and displayed information to be used repeatedly

Once base pair values have been assigned to the detected bands, allele calls can be made using the STaR Call™ 3.0 software. The STaR Call™ program is a Microsoft Excel Macro-sheet, which is both easy to use and rapidly generates allele calls.

Improvements to the STaR Call™ 3.0 Software include:

- A streamlined Import/Evaluation process
- Automatic merging of multiple DAT files into a single worksheet containing all 13 core CODIS Loci

- Highlighted alleles, in the merged files, for the overlapping loci (TPOX, TH01 and vWA) when the results from the PowerPlex 1.1 & 2.1 kits are not in agreement

In conclusion, when the PowerPlex 1.1 & 2.1 kits are used in conjunction with the Hitachi FMBIO® Analysis 8.0 & STaR Call™ 3.0 software, a merged file containing all 13 CODIS core loci can be rapidly generated, therefore making the FMBIO/PowerPlex combination an excellent choice for high throughput genotyping in forensic and paternity testing laboratories.