The FSS-i3™ software helps DNA laboratories quickly and accurately obtain allele calls and assess data quality with minimal manual review by an analyst.

FSS-i3™ Expert Systems

By Martin Bill1 and Curtis Knox2

1The Forensic Science Service®, United Kingdom, and 2Promega Corporation

With the advent of automation and other solutions to reduce backlogs in sample processing from extraction to amplification and typing, data review has now become a bottleneck that laboratories need to address. Software-directed allele calls do not address data quality; therefore, at least a short manual review of the data for each sample is necessary. An expert systems software package not only provides a second set of independent allele calls but also gives the analyst the confidence to skip examination of sample data that the software has judged to be of high quality. This allows analysts to more efficiently spend their time concentrating on “problem” samples.

The Forensic Science Service® (FSS) software business unit has recently announced the launch of a suite of expert systems software branded as FSS-i3™ (FSS i-cubed). This software helps DNA laboratories quickly and accurately obtain allele calls and assess data quality with minimal manual review by an analyst.

The FSS-i3™ expert systems software comprises a suite of programs: i-STRess, i-STReam and i-ntegrity. i-STRess is the core DNA interpretation tool and interprets raw data to produce DNA profiles by applying fully configurable rules and filters. i-STReam produces best-fit analysis of two-person mixed DNA profiles using peak heights or peak area data and allele designations determined by i-STRess. i-ntegrity provides a full within-batch comparison that highlights potential contamination events.

Some of the main features of the FSS-i3™ software include:

- Interfaces with existing commercial software
- Works with any STR multiplex
- Uses user-customizable input/output files
- Allows user-configurable rule sets
- Interprets single-source DNA profiles
- Interprets two-person, mixed DNA profiles
- Checks for potential "within-batch" sample-to-sample contamination

I-STRess

i-STRess is the primary component of the software. After basic data (base pair size, peak height and peak area) are generated and saved to a text file, this file can be imported to the i-STRess software. The software evaluates all ladders in the batch, gives each a pass or fail status and then uses the passing ladders to create a consensus ladder. This consensus ladder is used to make sample allele calls. After allele calls are made, an extensive set of rules, which can be optimized by the user to fit individual interpretation protocols, is applied. These rules can be activated, deactivated or programmed to fire but not necessarily be brought to the analyst’s attention. Samples that trigger rule firing are displayed and easily reviewed by an analyst (Figure 1). Upon review, the analyst has the option to edit allele calls. All edits are kept in a log file, which is automatically saved.
I-STREAM
i-STReam is the first of two unique modules contained in the software. This module evaluates two-person mixtures and produces a best-fit major profile. This aids the reporting analyst in mixture deconvolution and unbiased interpretation. The basis for the mathematical calculations used by this software has been previously published (1). A least-squares method is used to estimate the approximate ratios of the two DNA donors present in the mixture, and a list of all possible candidate genotypes is generated. Each candidate genotype is evaluated by using the determined mixture ratio and user-defined settings, which can be optimized to fit laboratory interpretation protocols. The software then identifies the combinations of two DNA donors that best fit the data for each locus. In cases where more than one profile combination fits the scenario presented by the data, these alternatives are also included by the software (Figure 2).

I-NTEGRITY
i-NTEGRITY constitutes the second unique feature of the FSS-I™ expert systems software. This module checks for potential sample-to-sample contamination within a batch by comparing all alleles called in a sample to the alleles in every other sample in the batch. The user can configure the level at which the software flags potential contamination events (e.g., 16 or more matching alleles between two samples) and can also break down potential contamination by category—major to major, major to minor or minor to minor component. A summary of the evaluation is automatically generated and saved by the software, and this summary is easily printed or archived should a question about contamination arise at a later date (Figure 3).

USER OPTIMIZATION
The FSS-I™ expert systems software includes more than 20 rules settings, which the user can optimize to achieve data analysis that closely approximates the interpretation guidelines in the individual laboratory. RFU threshold cutoffs, flags for low heterozygote or homozygote RFU levels, flags for imbalanced sister allele peaks, and recognition of spectral pull-up peaks are just a few examples. Many rules can also be configured to use peak height or peak area, depending on the user’s preference. The “Rules Settings” window allows the user to activate or deactivate rules, configure individual settings within each rule, and apply rule changes to an individual batch or make them default settings.
SOFTWARE VALIDATION
The FSS-i3™ expert systems software has been developed over time by the FSS and refined by casework analysts. Upon developing the software package, a large validation project was undertaken. Data from approximately 20,000 convicted offender samples and 50,000 crime-stain samples were analyzed using the software and compared to previous allele calls. Minor discrepancies were noted for only 0.03% of all samples, and all of these samples triggered rule firings that flagged the sample for further review by the user. This resulted in all discrepancies being resolved and no errors in calls made by the software. The results of the validation studies will be published in the future.

CONCLUSIONS
The FSS-i3™ expert systems software presents many benefits to forensic, databasing and paternity laboratories. These benefits include:

• Improved consistency by employing a set of objective rules for interpreting DNA profiles
• Significant reduction in the time required to interpret DNA data
• Simplified interpretation of mixed DNA profiles
• Increased rate of loading DNA profiles to databases

For further information about the software, please contact Dr. Chris Maguire at The Forensic Science Service® at: i_cubed@fss.pnn.police.uk

EDITOR’S NOTE: The Forensic Science Service® and Promega Corporation are working toward an agreement that would name Promega as the exclusive distributor and provider of technical support for the FSS-i3™ expert systems software in the United States. Promega is committed to deliver the highest quality training and service in the forensic community.

REFERENCE