Internal Validation of GeneMapper® ID-X v.1.0.1 as an Expert System for use in a Databasing Laboratory

Rachel Keaton, BS[1]*, Natasha Pranger, FS4[2], Pamela Staton, PhD[1]

[1]Marshall University Forensic Science Program, 1401 Forensic Science Drive, Huntington, WV 25701

[2]Washington State Patrol CODIS Crime Laboratory, 2203 S. Airport Way Suite 250, Seattle, WA 98134

ABSTRACT

Expert systems such as GeneMapper® ID-X are designed to perform the duties of a DNA analyst during the profile review portion of the DNA analysis procedure. The ultimate goal of incorporating expert systems is to decrease the DNA backlog experienced by so many forensic laboratories. It is believed that GeneMapper® ID-X can effectively incorporate a laboratory's procedures and 'pass' clean, single-source DNA profiles, which can then be uploaded into the Combined DNA Index System (CODIS) without requiring a secondary analyst review. As the procedures of the Washington State Patrol currently stand, some major changes would need to be made in order for GeneMapper ID-X to have an effect on the time spent on a sample, but has the potential to reduce Washington's DNA backlog.

INTRODUCTION

The Washington State Patrol CODIS Crime Laboratory (WSPCCL) is a high throughput database lab, processing all CODIS samples for the state of Washington. In order to decrease the bottleneck effect seen during the DNA profile review process, adding automation into the WSPCCL standard operating procedure has been proposed via incorporation of expert systems. Expert systems, which are software programs designed to both generate and review DNA profiles, will decrease the time that analysts must spend manually reviewing data. The primary goal of an expert system internal validation is to ensure that any allele call made must be correctly made. Additionally, the software must be able to identify situations in which it is not qualified to make a call and requires additional instructions from a DNA analyst. Applied Biosystems GeneMapper® ID-X v.1.0.1 has been shown to be able to appropriately incorporate the standard operating procedures (SOPs) of the WSPCCL into its operating protocol, allowing it to make allele calls by the same parameters used by DNA analysts. GeneMapper® ID-X was found to not be able to appropriately identify ladders with potential migration issues, and additionally did not flag microvariants. The microvariant issue is especially problematic, since they have to be formatted in a specific way before being uploaded into CODIS. If the WSPCCL chooses to incorporate GeneMapper® ID-X into its procedure as an expert system, changes to their SOPs reflecting these concerns must be made.

MATERIALS AND METHODS

- Applied Biosystems (ABI) GeneMapper® ID-X v.1.0.1 software
- ABI GeneMapper v.3.2 software
- .fsa data generated by ABI Genetic Analyzer 3130xl
- DNA pre-extracted on Qiagen BioRobot EZ1 Workstation
- DNA pre-quantitated on ABI 7500 Real-Time PCR System
- DNA pre-amplified using ABI AmpFlSTR® Identifiler ® PCR Amplification Kit

RESULTS

Experiment 1: Establishing Parameters

The WSPCCL SOPs were incorporated into the software parameters and used to analyze a series of data. Results were observed for failures. In each case, the rationale that the software used for failing a sample was observed. Flags thrown are summarized in Table 1.

Table 1. Artifacts recognized by GeneMapper® ID-X.

Artifacts	Number of samples
PHR below 50%	263
PHR below 45%	128
Elevated stutter	58
n+4 stutter	12
Pullup	291
-A	44
+A	1
Spikes	5
Tri-alleles	5
Mixtures	0
Contamination	0
Off-ladder alleles	17
Above allelic ladder	5
Below allelic ladder	6
Missing loci	147

Experiment 2: Allelic Ladder Evaluation

Known faulty ladders were tested with the lab's SOPs in place. Figure 1A shows a ladder with known good sizing quality. Figure 1B demonstrates ladder migration.

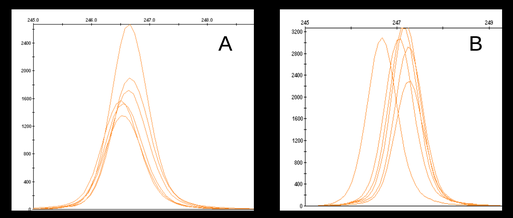

Figure 1. Allelic ladder peaks in GeneMapper® ID-X v.1.0.1.

Experiment 3: Samples Containing Artifacts

Samples that were known to fail under the laboratory's SOPs were analyzed with GeneMapper® ID-X. Figures 2 and 3 demonstrate artifacts recognized by the software.

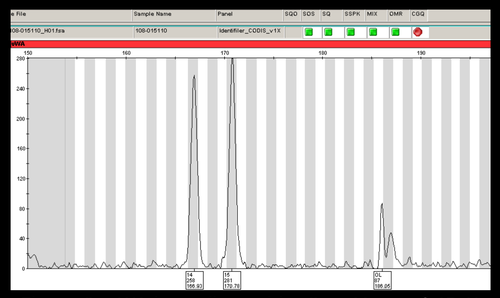

Figure 2. vWA locus flagged by GeneMapper® ID-X.

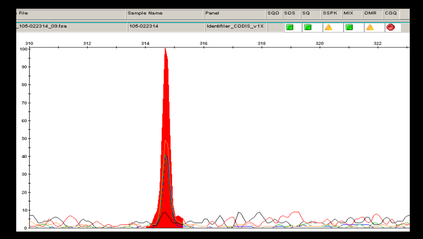

Figure 3. Spike across all color channels flagged by GeneMapper® ID-X.

Experiment 4: Comparison of Genotypes

Genotypes generated with GeneMapper® ID-X were compared with genotypes previously generated with GeneMapper v.3.2 for concordance.

CONCLUSIONS

- GeneMapper® ID-X incorporated the WSPCCL SOPs effectively
- Software was able to recognize drastic sizing and migration issues, but not to the level desired by WSPCCL
- Any aberrant peaks or anomalous data were flagged
- All genotypes generated corresponded with previously generated data
- Ladders, positive controls, negative controls and custom controls were all analyzed appropriately
- GeneMapper® ID-X effectively provided rationale behind all decisions
- History of any manual changes made was kept by the software
- Microvariants cannot be effectively incorporated to the standard required for CODIS upload, and currently must be passed by an analyst
- WSPCCL SOPs must be changed to reflect weaknesses of GeneMapper® ID-X, while still allowing it to function as an expert system

REFERENCES

1. DNA Advisory Board, "Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories," Forensic Science Communications, 2000:2(3).
2. Applied Biosystems GeneMapperâ ID-X Version 1.0/1.1: User's Guide. Accessed August 13, 2009.
3. Mulero JJ, Chang CW, Lagace RE, Wang DY, Bas JL, McMahon TP, Hennessy LK. **Development and Validation of the AmpFℓSTR® MiniFilerTM PCR Amplification Kit: A MiniSTR Multiplex for the Analysis of Degraded and/or PCR Inhibited DNA.** *Journal of Forensic Sciences* 53 (4) 838-852 (2008).
4. National DNA Index System (NDIS) Expert Systems Operational Procedures. FBI Laboratory. Created 12 June 2008, Revised 3 February 2009.
5. Short Tandem Repeat DNA Internet DataBase, created by John Butler. Accessed August 1, 2009 from: http://www.cstl.nist.gov/strbase/index.htm
6. Raymaekers M, Smets R, Maes B, Cartuyvels R. **Checklist for optimization and validation of real-time PCR assays.** *Journal of Clinical Laboratory Analysis* 23 (3) 145-151 (2009).
7. Yauk CL, Berndt ML. **Review of the Literature examining the correlation among DNA microarray technologies.** *Environmental and Molecular Mutagenesis* 48 (5), 380-394 (2007).

ACKNOWLEDGEMENTS

This project was supported by Award No. 2005-MU-BX-K020 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication/exhibition are those of the author(s) and do not necessarily reflect the views of the Department of Justice.