TWGDAM Validation of the *GenePrint*® PowerPlex™ 1.1/Amelogenin and FFFL Systems

By Elizabeth Amiott
Promega Corporation
e-mail: genetic@promega.com

The TWGDAM (Technical Working Group on DNA Analysis Methods) validation study of the *GenePrint*® PowerPlex™1.1/ Amelogenin and FFFL Fluorescent Multiplex Systems, reported jointly by scientists from the Palm Beach County Sheriff's Office Crime Laboratory and Promega Corporation, appears in the November 1999 issue of the *Journal of Forensic Science* (JFS; reference 1*). This article presents information on validation of fluorescent STR multiplex systems for DNA typing and highlights important points of the JFS study.

Q: What is TWGDAM Validation?

TWGDAM (now known as SWGDAM) was established for the purpose of evaluating and revising recommendations of quality assurance guidelines for laboratories performing DNA testing.

Guidelines for a Quality Assurance Program for DNA Analysis were published in *Crime Laboratory Digest* in 1989 and 1991 (2,3). As DNA technology evolved, these guidelines were updated, and a revised version was published in April of 1995 (4).

The 1995 TWGDAM Quality Assurance Guidelines describe validation as follows: "Validation is the process used by the scientific community to acquire the necessary information to assess the ability of a procedure to reliably obtain a desired result, determine the conditions under which such results can be obtained and determine the limitations of the procedure. The validation process identifies the critical aspects of a procedure which must be carefully controlled and monitored." Additionally, "validation studies must have been conducted by the DNA laboratory or scientific community prior to the adoption of a procedure by the DNA laboratory."

For more information on current guidelines, see the SWGDAM (Scientific Working Group on DNA Analysis Methods) web site, **www.for-swg.org/swgdamin.htm**.

Q: What studies are included in the TWGDAM validation for the GenePrint® PowerPlex™ 1.1/ Amelogenin and FFFL Systems?

The JFS publication includes results from all of the standard validation studies described in Section 4.1.5 of the 1995 TWGDAM Guidelines. These include minimum samples studies (sensitivity), mixed samples, reproducibility, environmental and matrix studies (4).

The article also describes characteristics of the loci included in the systems such as stutter, efficiency of terminal nucleotide addition, potential for differential amplification and a comparison of results from each individual locus as a monoplex to the same locus in the appropriate multiplex configuration.

In addition, the paper includes information on what TWGDAM calls "Specific Developmental Validation" of the PCR-based multiplex systems. This includes optimal thermal cycling parameters (annealing temperature and the number of cycles in the protocol) and critical reagent concentrations (primer concentration, enzyme concentration, enzyme and buffer combinations).

Finally, the paper describes the use of allelic ladders as standards for direct comparison with samples for analysis, as well as the use of an internal lane standard for accurate base pair sizing of sample alleles and utilization of computer software to assist in interpretation of results.

Q: Are population statistics included in this publication?

Yes, the paper includes a brief description of the results of population studies for three population groups with the 12 STR loci included in these two multiplexes. A previous article by Lins *et al.* provides a more detailed description of this population study, as well as a study of inheritance involving over 300 mother-child pairs (5).

Q: What are some of the important results/highlights from the validation study?

Sensitivity: The *GenePrint*® PowerPlex™ 1.1/Amelogenin and FFFL Systems are quite sensitive, allowing detection of samples with as little as 0.2ng template DNA (Figure 1). Similar sensitivity was observed in mixed samples in which the minor DNA component could be detected at a ratio of 80:20. Mixed samples were also identified easily in nonprobative casework.

Robustness: Although the best results are obtained when using the recommended protocols and pure DNA template, the validation studies show that these systems can accommodate slight variations in cycling conditions (those resulting from differences in instrumentation and/or temperature calibration), as well as samples exposed to potentially compromising conditions. The JFS article contains detailed information on studies involving DNA obtained from fresh body fluids and stains deposited on various substrates or exposed to the environment.

Ease of use and interpretation: The *GenePrint*® PowerPlex™ 1.1/Amelogenin and FFFL Systems provide a rapid and reliable means for human identity testing because of the traditional gel-based electrophoretic separation of PCR products coupled with fluorescent excitation and detection with the Hitachi FMBIO® Fluorescence Imaging Systems. Direct comparison of sample allele migration with sequenced allelic ladders makes visual allele designation simple. The addition of the CXR-labeled Fluorescent Ladder (60–400 bases) or the Internal Lane Standard 600 as an internal lane size marker further assists interpretation by enabling the FMBIO® Analysis software to assign base pair sizes both to sample alleles and to the allelic ladders. This helps eliminate any possible confusion caused by differences in migration across the gel, eliminates the need to run allelic ladder adjacent to each sample and allows for automated genotyping using the STaR Call™ Allele Calling Software available with the Hitachi FMBIO® Fluorescence Imaging Systems.

Q: Which Taq *DNA polymerase was used for the validation studies?*

The methods and materials described in the validation of the *GenePrint*® PowerPlex™ 1.1/Amelogenin System are consistent with the protocols recommended in the Technical Manual (#TMD008) available with the multiplex system (6). Several of the studies included in the validation were performed in duplicate using AmpliTaq® DNA polymerase and Promega's STR 10X Buffer in one set and AmpliTaq Gold® DNA polymerase and Promega's Gold ST★R 10X Buffer in the second set. Results with the two enzymes were similar; however, use of AmpliTaq Gold® polymerase seems to reduce amplification artifacts that can be generated when more than the recommended 1–2ng template DNA is amplified in a reaction.

Validation of the FFFL Multiplex was performed with AmpliTaq Gold® DNA polymerase and Promega's Gold ST★R 10X Buffer as recommended in the current Technical Manual provided with the system (7). AmpliTaq Gold® DNA polymerase with the FFFL Multiplex gives significantly better amplification results than AmpliTaq® (7). For this reason, Promega now includes the Gold ST★R 10X Buffer, in addition to the 10X STR Buffer, with every *GenePrint*® FFFL System.

It is important to note that, when using AmpliTaq Gold® DNA polymerase, an 11-minute, 95°C "hot start" must be incorporated in the normal recommended cycling protocol. Also, the Gold ST★R 10X Buffer contains BSA (1,600µg/ml) as an additive and has a pH of 8.3, while the 10X STR Buffer does not contain BSA and has a pH of 9.0. The difference in pH makes the 10X STR Buffer incompatible for use with AmpliTaq Gold® DNA polymerase.

Q: Does publication of this validation study mean that the PowerPlex™ 1.1/Amelogenin and FFFL Systems are validated for use in any laboratory?

No. This publication can be used as a reference supporting the use of both systems by the scientific community but is not the only proof of validation required for laboratory accreditation. Every laboratory must be able to document internal validation studies as described by the appropriate guidelines governing quality control for that laboratory.

Q: Where can I get more information on validation?

The National Institute of Standards and Technology STRBase web site, **www.cstl.nist.gov/biotech/strbase/**, provides information on each STR locus, the DAB Guidelines and a reference list of other validation publications. Also, the official web site of the Scientific Working Group on DNA Analysis Methods, **www.for-swg.org/swgdamin.htm**, provides the current validation standards. In addition, the ASCLD-LAB Accreditation Manual, January 1994 and January 1997, provides further reference material.

REFERENCES

1. Micka, K.A. *et al.* (1999) *J. Forensic Sci.* **44**, 1243.*
2. TWGDAM (1989) *Crime Laboratory Digest* **16**, 40.
3. TWGDAM (1991) *Crime Laboratory Digest* **18**, 44.
4. TWGDAM (1995) *Crime Laboratory Digest* **22**, 21.
5. Lins, A.M. *et al.* (1998) *J. Forensic Sci* **43**, 1168.
6. GenePrint® *PowerPlex™ 1.1 System Technical Manual* #TMD008, Promega Corporation.
7. GenePrint® *Fluorescent STR Systems Technical Manual* #TMD006, Promega Corporation.

*To receive a reprint of this article, e-mail **litreq@promega.com**, and request #RP036.

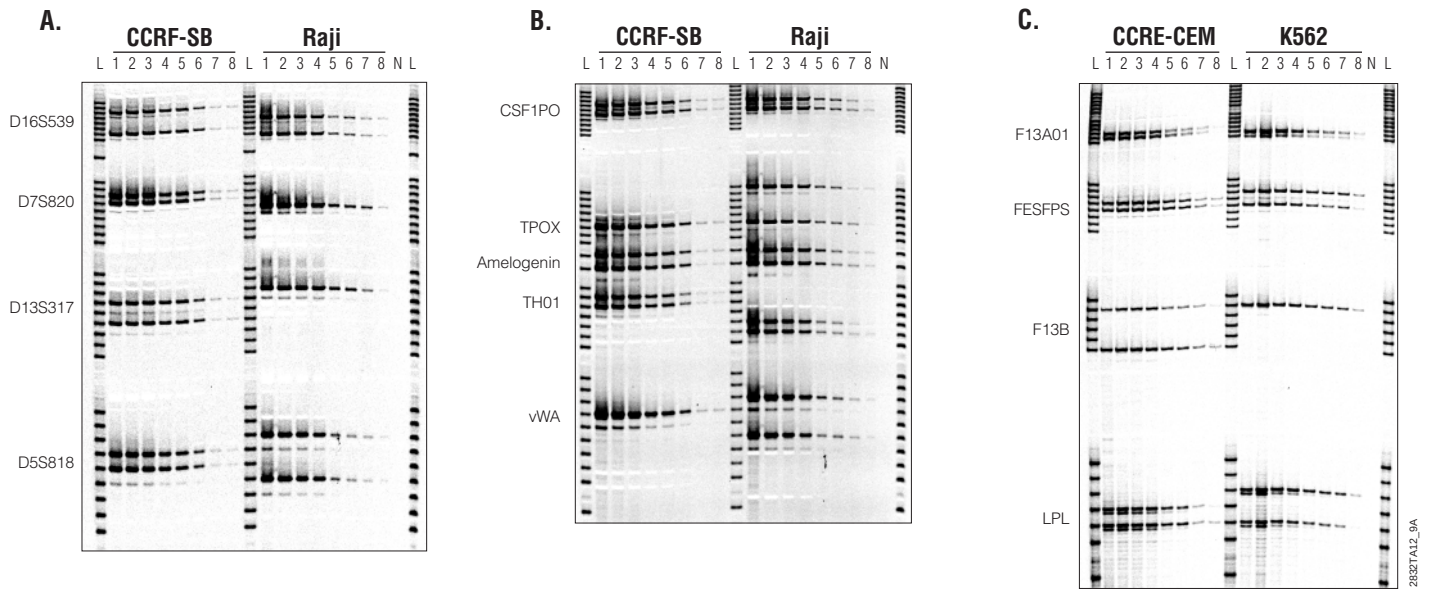

Figure 1. Sensitivity of the *GenePrint*® PowerPlex™ 1.1/Amelogenin and FFFL Systems. DNA from cell lines CCFR-SB and Raji was amplified with the PowerPlex™ 1.1/Amelogenin Systems (**Panels A and B**), and DNA from CCRE-CEM and K562 cell lines (**Panel C**) was amplified with the FFFL System using 25ng, 10ng, 5ng, 2ng, 1ng, 0.5ng, 0.2ng and 0.1ng of template. Products were separated in lanes 1–8, respectively. The lanes marked N display negative controls (no template). Lanes L of each panel include allelic ladders for the loci. All amplified products were separated by electrophoresis in 4% denaturing polyacrylamide gels and detected using the Hitachi FMBIO® Fluorescence Imaging System (Panel A, 505nm scan revealing fluorescein-labeled products of four loci; Panel B, 585nm scan revealing TMR-labeled products of five loci; Panel C, 505nm scan revealing fluorescein-labeled products from FFFL loci). *Images reprinted by kind permission of the American Society for Testing and Materials.*