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REFERENCE MANUAL

PowerPlex® ESI 17 Pro System Modification Validation Guide

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1. Introduction

Before implementing a specific STR analysis system for forensic DNA analysis, each laboratory needs to complete an internal validation study to establish the performance characteristics and limits of the method in their laboratory. When a substantial modification is made to a validated STR system, additional testing may be necessary to ensure that the performance characteristics of the method have not changed (1–3).

Promega has improved the PowerPlex® ESI 17 System (Cat.# DC6780, DC6781) with the release of the PowerPlex® ESI 17 Pro System[a–g] (Cat.# DC7780, DC7781), which contains a redesigned SE33 primer pair to eliminate previously seen rare artifacts and discordance, resulting in increased confidence in the results. The new system has the same PowerPlex® ESI 5X Master Mix as that provided in the PowerPlex® ESI 17 System and contains four new components as listed below.

- PowerPlex® ESI 17 Pro 10X Primer Pair Mix contains a new primer pair for SE33; primer pairs for the other 16 loci are unchanged.
- PowerPlex® ESI 17 Pro Allelic Ladder Mix has a new formulation to accommodate the new SE33 primer pair.
- CC5 Internal Lane Standard 500 Pro has a new formulation containing sacrificial hybridization oligonucleotides directed toward the new SE33 primers. These oligonucleotides prevent formation of split peaks at the SE33 locus during capillary electrophoresis. For more information on eliminating split peaks in this manner, see reference 4.
- 2800M Control DNA replaces 9947A DNA.

While a full internal validation may not be necessary, conversion from the PowerPlex® ESI 17 System to the new PowerPlex® ESI 17 Pro System requires testing to compare performance of the new kit with the original validated procedure. Laboratories should review their original validation work and determine which experiments would be affected by the changes described above. In this guide we provide suggestions for experiments that may be performed by laboratories that wish to demonstrate equivalent performance for the new PowerPlex® ESI 17 Pro System and PowerPlex® ESI 17 System by testing samples that are similar to those used in the original internal validation. In addition, the laboratory should take steps to ensure that the testing performed, including both the studies and numbers of samples tested, meets or exceeds the requirements of any governing or accrediting bodies.

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2. Reproducibility of Known and Nonprobative Samples

The objective of the reproducibility study is to demonstrate that there is no difference (discordance) between STR profiles generated with the PowerPlex® ESI 17 Pro System and STR profiles generated with the PowerPlex® ESI 17 System, with the possible exception of the SE33 locus. Rare samples with single nucleotide polymorphism (SNP) mutations had previously shown discordance at the SE33 locus between the PowerPlex® ESI 17 System and the PowerPlex® ESX 17 System or AmpF*l*STR® NGM SElect™ kit. In these samples, an X allele was designated as off-ladder or as X.1, and an X.2 allele was designated as off-ladder or as X.3 with the PowerPlex® ESI 17 System. These samples showed discordance when results obtained with the PowerPlex® ESI 17 Pro System were compared to those generated using the PowerPlex® ESI 17 System.

Testing Conditions

1. Using the PowerPlex® ESI 17 Pro System, amplify DNA from 10 to 15 known and 10 to 15 nonprobative or mock evidence samples for which STR profiles have been determined previously using the PowerPlex® ESI 17 System and your laboratory's current procedures. The amplification and electrophoresis conditions established for the PowerPlex® ESI 17 System should be appropriate for testing the PowerPlex® ESI 17 Pro System.

   Note: If previously tested samples are not available, samples can be typed with both the PowerPlex® ESI 17 Pro and PowerPlex® ESI 17 Systems concurrently. Be sure to use the PowerPlex® ESI 17 Pro Allelic Ladder to analyze the PowerPlex® ESI 17 Pro System data, and the PowerPlex® ESI 17 Allelic Ladder to analyze the PowerPlex® ESI 17 System data.

2. Determine the STR profile for each sample using the laboratory's standard analysis parameters. Verify that the correct STR profile was obtained. Pay particular attention to alleles identified at the SE33 locus. For samples that were previously discordant at the SE33 locus between the PowerPlex® ESI 17 System and the PowerPlex® ESX 17 System or AmpF*l*STR® NGM SElect™ kit, confirm that they are now concordant between the PowerPlex® ESI 17 Pro System and the PowerPlex® ESX 17 System or AmpF*l*STR® NGM SElect™ kit. Samples that are discordant at the SE33 locus when amplified with the PowerPlex® ESI 17 System and PowerPlex® ESI 17 Pro System may be observed due to the change in primers for that locus. The alleles identified at the SE33 locus using the PowerPlex® ESI 17 Pro System can be verified by amplifying the sample with the PowerPlex® ESX 17 System.

3. Optional Testing for Sensitivity

The objective of this study is to determine the quantity of template DNA below which amplification is not expected to yield a DNA profile. Comparing the results obtained with the PowerPlex® ESI 17 Pro System to those obtained with the PowerPlex® ESI 17 System will demonstrate whether there is a difference in sensitivity between the two systems.

Testing Conditions

1. For each of two to five known DNA samples, prepare dilutions of 1ng/µl, 0.5ng/µl, 0.25ng/µl, 0.125ng/µl, 0.063ng/µl and 0.032ng/µl. Alternatively, recreate the experiment performed to test the sensitivity of the PowerPlex® ESI 17 System in the laboratory's original validation.
2. Amplify the samples using both the PowerPlex® ESI 17 Pro and PowerPlex® ESI 17 Systems. The amplification and electrophoresis conditions established for the PowerPlex® ESI 17 System should be appropriate for testing the PowerPlex® ESI 17 Pro System.
3. Analyze the amplified samples using the laboratory's standard analysis parameters, and determine the quantity of template at which you no longer generate STR results for each of the STR systems. Compare the results for the PowerPlex® ESI 17 Pro and PowerPlex® ESI 17 Systems, and determine whether there is a difference in sensitivity between the systems.

4. References

1. ILAC-G19:2002 Guidelines for Forensic Science Laboratories. This can be viewed online at: **www.ilac.org/documents/g19_2002.pdf**
2. Revised validation guidelines. Scientific Working Group on DNA Analysis Methods (SWGDAM) (2004) *Forensic Science Communications* **6(3)**. This can be viewed online at: **www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/standards/2004_03_standards02.htm#matmod**
3. Validation and implementation of (new) methods. ENFSI Standing Committee for Quality and Competence (2006). This can be viewed online at **www.enfsi.eu/page.php?uid=92**
4. McLaren, B. *et al.* (2008) A solution for the split peak and n–10 artifacts at the vWA locus in PowerPlex® 16 and PowerPlex® ES Systems. *Profiles in DNA* **11(2)**, 13–15.

5. Related Products

Product	Size	Cat.#
PowerPlex® ESX 16 System	100 reactions	DC6711
	400 reactions	DC6710
PowerPlex® ESX 17 System	100 reactions	DC6721
	400 reactions	DC6720
PowerPlex® ESI 16 System	100 reactions	DC6771
	400 reactions	DC6770
PowerPlex® ESI 17 Pro System	100 reactions	DC7781
	400 reactions	DC7780

*Not For Medical Diagnostic Use.

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[a]U.S. Pat. Nos. 5,843,660, 6,479,235, 6,221,598 and 7,008,771, Australian Pat. No. 724531, Canadian Pat. Nos. 2,118,048 and 2,251,793 and other patents and patents pending.

[b]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153 and other patents and patents pending.

[c]STR loci are the subject of U.S. Pat. No. RE 37,984, German Pat. No. DE 38 34 636 C2 and other patents issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, e.V., Germany.

[d]Licensed under U.S. Pat. Nos. 5,338,671 and 5,587,287 and corresponding patents in other countries. For Research Use Only. Not for use in diagnostic procedures.

[e]Allele sequences for one or more of the loci vWA, FGA, D8S1179, D21S11 and D18S51 in allelic ladder mixtures is licensed under U.S. Pat. Nos. 7,087,380, 7,645,580, Australia Pat. No. 2003200444 and corresponding patent claims outside the US.

[f]This product or portions thereof is manufactured and sold under license from GE Healthcare under Australia Pat. No. 692230, Austria Pat. No. E236994, Belgium Pat. No. 0743987, Canada Pat. No. 2231475, EP Pat. Nos. 0743987 and 0851867, France Pat. Nos. 0743987 and 0851867, Germany Pat. Nos. 19581489, 69530286.8 and 0851867, Italy Pat. Nos. 0743987 and 0851867, Japan Pat. No. 3066984, Liechtenstein Pat. Nos. 0743987 and 0851867, Netherlands Pat. Nos. 0743987 and 0851867, Spain Pat. Nos. 2197193 and 2173310, Sweden Pat. Nos. 0743987 and 0851867, Switzerland Pat. Nos. 0743987 and 0851867, United Kingdom Pat. Nos. 0743987 and 0851867, U.S. Pat. Nos. 5,654,419, 5,688,648, 5,869,255, 6,177,247, 5,707,804, 6,028,190, 6,544,744, 7,015,000 and 5,728,528 and other pending and foreign patent applications.

[g]TMR-ET, CXR-ET and CC5 dyes are proprietary.

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AmpF*l*STR is a registered trademark of LifeTechnologies. NGM SElect is a trademark of Life Technologies, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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