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Application Notes

PowerPlex® 16 System Validation

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Abstract

We have improved the manufacturing process for the PowerPlex® 16 System to ensure lot-to-lot spectral consistency and increased ease of use. We show that the amplification results obtained with systems manufactured using the new process are comparable to those obtained using earlier lots manufactured using previous protocols. Because the performance with both PowerPlex® 16 formulations is comparable, users of the PowerPlex® 16 System should see no adverse effects on amplification results.

Introduction

The Promega Genetic Identity team is committed to continuously improving the quality of our products. We would like to inform you of improvements to the manufacturing processes for the PowerPlex® 16 System[a,b,c]. Previously, the fluorescent molecules used to synthesize the dye-labeled primers for the PowerPlex® Systems were a mixture of isomers. We modified our primer synthesis protocols to use pure dye isomers. This process change allows improved spectral calibration performance and lot-to-lot consistency.

We also instituted a process improvement for the JOE-labeled primers in the PowerPlex® 16 System to use the same linker as that used in the PowerPlex® Y System. This change reduces the number of matrix standards required to use the PowerPlex® Systems and increases convenience for the end user. The chemistry modifications did not necessitate any changes in the PowerTyper™ Macros or in the panel and bin files for the GeneMapper® *ID* software. No other changes have been made. Importantly, the primer sequences for the PowerPlex® 16 System have not changed.

The *PowerPlex® 16 System Technical Manual* and the Technical Bulletins for the PowerPlex® Matrix Standards have been updated to reflect these changes and to highlight the importance of using the new PowerPlex® 16 Allelic Ladder Mix (Part# DG600B) with the new PowerPlex® 16 10X Primer Pair Mix (Part# DK711B) and new matrix standards (Cat.# DG4640 [ABI PRISM® 310 Genetic Analyzer], or DG4650 [ABI PRISM® 3100 and Applied Biosystems 3130 Genetic Analyzers]). Experiments performed at Promega demonstrate the consistency and reproducibility of the PowerPlex® 16 System with these manufacturing changes. The experiments are summarized in this Application Note.

Materials and Methods

Human genomic DNA was isolated from liquid whole blood using the DNA IQ™ System (Cat.# DC6701) as directed in the *DNA IQ™ System—Database Protocol* #TB297 or by phenol:chloroform extraction. DNA was quantified using PicoGreen® dsDNA quantitation reagent (Molecular Probes) or by measuring optical density (O.D.). Eight additional DNA templates (DNA Standard Reference Material 2391b, PCR-Based DNA Profiling Standard) and 9947A and 9948 DNA were obtained from the National Institute of Standards and Technology (NIST). All amplifications were performed in parallel reactions using the new formulation or current inventory of the PowerPlex® 16 10X Primer Pair Mix. Reactions were assembled as directed in the *PowerPlex® 16 System Technical Manual* #TMD012 and contained varying amounts of DNA (0.05–2.0ng), 1X PowerPlex® 16 Primer Pair Mix, 4 units of AmpliTaq Gold® DNA polymerase, and Gold ST★R 1X Buffer containing 1.5mM $MgCl_2$. DNA templates were diluted in water. Amplifications were carried out using the GeneAmp® PCR System 9700 for 32 cycles (10/22) unless otherwise indicated. Amplification products were detected using the ABI PRISM® 310 or 3100 Genetic Analyzer or Applied Biosystems 3130 Genetic Analyzer. Data were analyzed using the GeneMapper® *ID* software, version 3.2, with Promega panel and bins sets, version 3.2.0, or the GeneScan® and Genotyper® software with the PowerTyper™ Macros.

Bleedthrough

The JOE-labeled primers of the PowerPlex® 16 System now have the same linker as that used in the PowerPlex® Y System. Thus, no separate JOE A or JOE B matrix standards are necessary since the spectral performance will be the same regardless of the system used.

As a result of these modifications, bleedthrough between dye channels is 4% or less, allowing higher sample peak heights. However, as with previous lots of the PowerPlex® Systems, there may be some bleedthrough from CXR into TMR if the peak heights of the Internal Lane Standard 600 (ILS 600) fragments are above 1,000RFU. We recommend adjusting the amount of ILS 600 to ensure that peak heights are below 1,000RFU.

Sensitivity

To compare the sensitivity of amplifications using the new and current inventory of PowerPlex® 16 10X Primer Pair Mixes, we assembled amplifications with decreasing amounts of DNA template (2.0–0.05ng). The sensitivity level was comparable for both formulations (Figures 1 and 2).

Stability

To examine stability, the PowerPlex® 16 10X Primer Pair Mix, PowerPlex® 16 Allelic Ladder Mix and ILS 600 were subjected to 15 freeze-thaws. After 5, 10 and 15 freeze-thaws, aliquots of the primers were taken and used in PowerPlex® 16 amplifications. The amplification products, allelic ladder mix and ILS 600 were analyzed

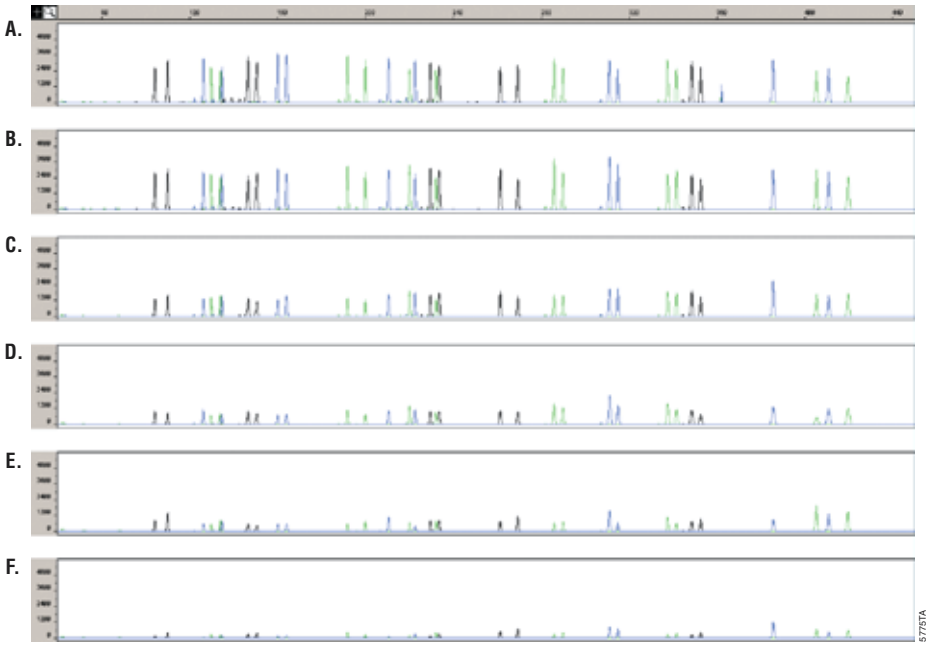

Figure 1. Representative amplifications using the current formulation of the PowerPlex® 16 10X Primer Pair Mix. PowerPlex® 16 reactions were assembled with decreasing amounts of DNA template (**Panel A.** 2.0ng, **Panel B.** 1.0ng, **Panel C.** 0.5ng, **Panel D.** 0.2ng, **Panel E.** 0.1ng, **Panel F.** 0.05ng) and the current formulation of the PowerPlex® 16 10X Primer Pair Mix. The human DNA template was serially diluted in water, and 2.5µl of the dilution was added to each reaction. Amplifications were performed using the GeneAmp® PCR System 9700, and 1.0µl of amplification products was analyzed using an ABI PRISM® 3100 Genetic Analyzer, a 3kV, 11-second injection and a run time of 2,000 seconds. The spectral calibration was generated using the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121).

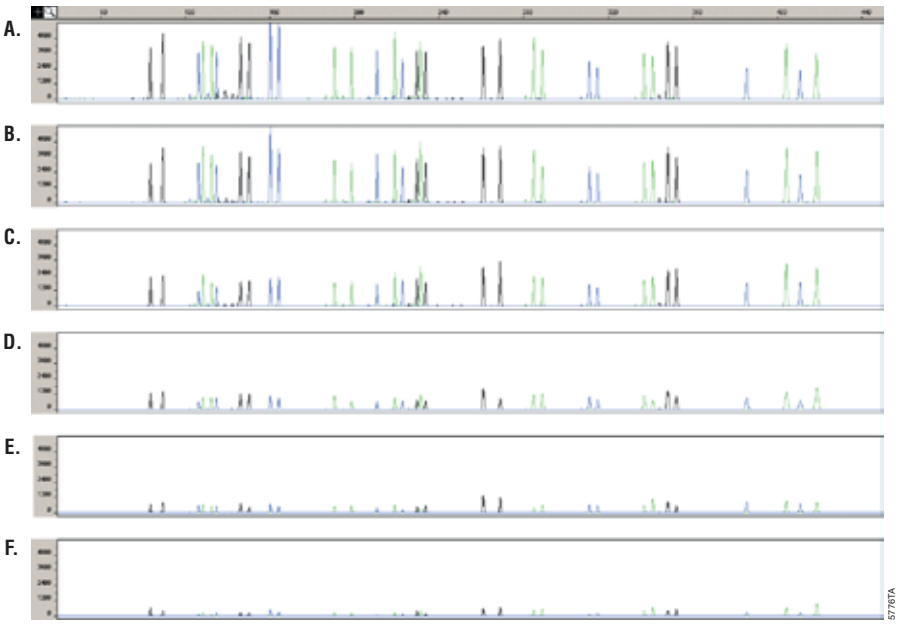

Figure 2. Representative amplifications using the new formulation of the PowerPlex® 16 10X Primer Pair Mix. PowerPlex® 16 reactions were assembled with decreasing amounts of DNA template (**Panel A.** 2.0ng, **Panel B.** 1.0ng, **Panel C.** 0.5ng, **Panel D.** 0.2ng, **Panel E.** 0.1ng, **Panel F.** 0.05ng) and the new formulation of the PowerPlex® 16 10X Primer Pair Mix. The human DNA template was serially diluted in water, and 2.5µl of the serial dilution was added to each reaction. Amplifications were performed using the GeneAmp® PCR System 9700, and 1.0µl of amplification products was analyzed using an ABI PRISM® 3100 Genetic Analyzer, a 3kV, 11-second injection and a run time of 2,000 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

using an ABI PRISM® 3100 Genetic Analyzer. We observed no significant performance differences for the PowerPlex® 16 10X Primer Pair Mix (Figure 3). No degradation was detected for the PowerPlex® 16 Allelic Ladder Mix and ILS 600 (data not shown).

We then exposed the primer pair mix to full-spectrum light for 24 hours at room temperature and vortexed the primers for 5 minutes prior to use in a PowerPlex® 16 amplification. We observed no decrease in peak heights due to vortexing or exposure to light (Figure 4).

Concordance

To compare reproducibility, we amplified eight DNA templates contained in DNA Standard Reference Material 2391b, PCR-based DNA Profiling Standards, and 9947A and 9948 DNA using the new PowerPlex® 16 10X Primer Pair Mix. Allele calls were made using the GeneScan® and Genotyper® software (ABI PRISM® 310 Genetic Analyzer), GeneMapper® *ID* software (ABI PRISM® 3100 Genetic Analyzer) or FSS-i^{3TM} Expert Systems Software (Applied Biosystems 3130 Genetic Analyzer). All allele calls were consistent with those provided by NIST (data not shown).

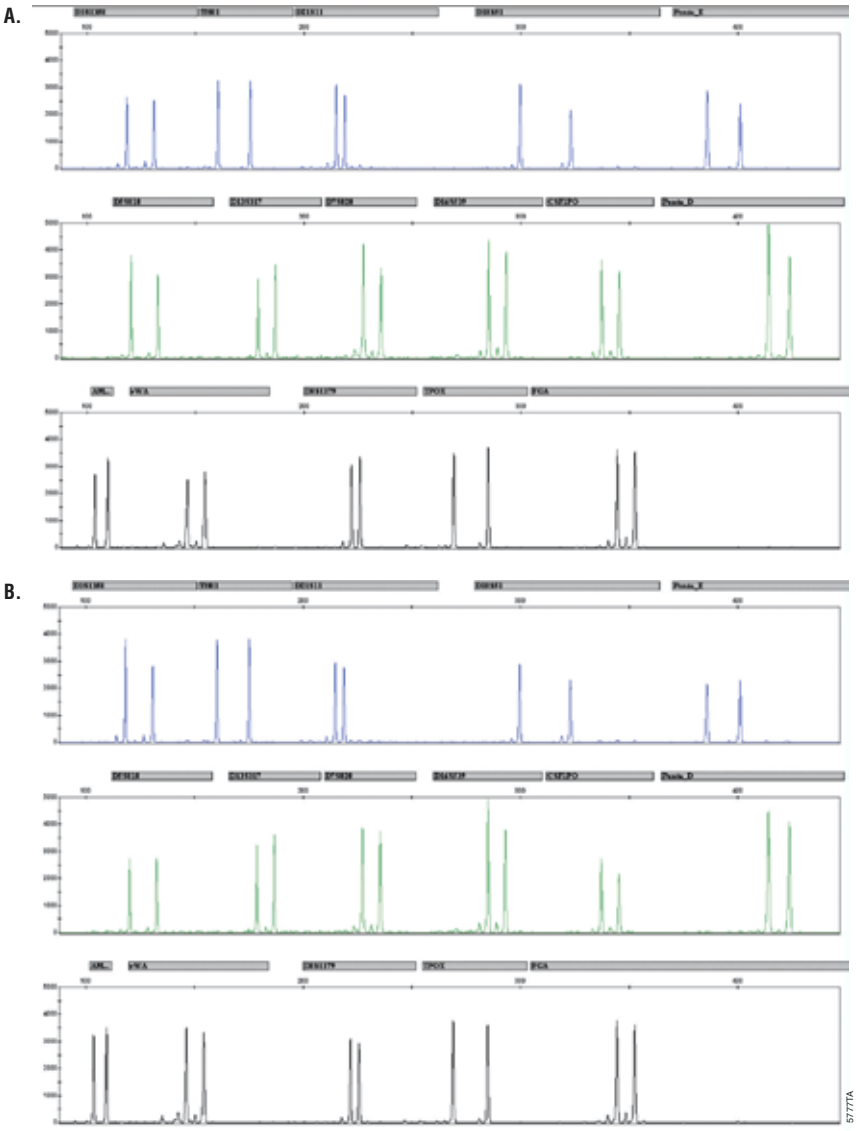

Figure 3. Stability of the new PowerPlex® 16 10X Primer Pair Mix. The PowerPlex® 16 10X Primer Pair Mix was subjected to 15 freeze-thaws. Prior to freeze-thawing (**Panel A**) and after 15 freeze-thaws (**Panel B**), the primers were used in a standard 25µl PowerPlex® 16 reaction. The amplification products were analyzed using an ABI PRISM® 3100 Genetic Analyzer, a 3kV, 11-second injection and a run time of 2,000 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

Stutter Percentage

To compare stutter percentage, 0.5ng and 1.0ng of three different DNA templates were amplified with new and current inventory of the PowerPlex® 16 10X Primer Pair Mix. The amplification products were run on an ABI PRISM® 3100 Genetic Analyzer and analyzed using the GeneMapper® *ID* software, and the results were compared. No detectable differences in stutter peak percentages were seen between the current inventory and new primer lots, except for the Penta E locus. This stutter percentage was determined to be less than 1% with the new formulation (Figure 5).

Conclusions

The improvements to the PowerPlex® 16 System manufacturing protocols had no effect on the performance of the PowerPlex® 16 System. The new and current formulations of the PowerPlex® 16 10X Primer Pair Mix performed comparably in all amplifications, regardless of the amount of template DNA, vortexing, exposure to light and number of freeze-thaws performed.

Editor's Note: PowerPlex® 16 Systems that include the new formulation of the PowerPlex® 16 10X Primer Pair Mix are designated by lot numbers ending with the letter "N".

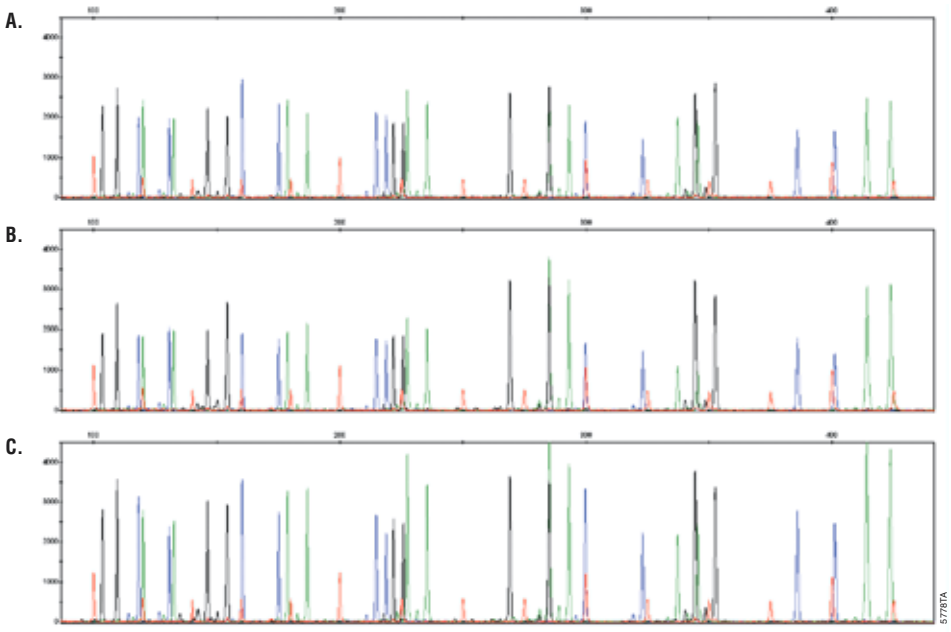

Figure 4. Stability of the new PowerPlex® 16 10X Primer Pair Mix. The PowerPlex® 16 10X Primer Pair Mix was untreated (**Panel A**), exposed to full-spectrum light in an amber tube for 24 hours (**Panel B**) or exposed to full-spectrum light in an amber tube for 24 hours and vortexed at high speed for 5 minutes prior to use (**Panel C**). The primer pair mixes were used in PowerPlex® 16 amplifications, and the amplification products were analyzed using an ABI PRISM® 3100 Genetic Analyzer, a 3kV, 11-second injection and a run time of 2,000 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

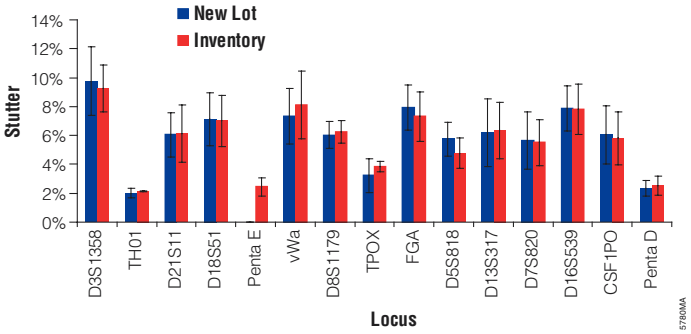

Figure 5. Stutter percentages. The new formulation and current inventory of the PowerPlex® 16 10X Primer Pair Mix were used to amplify 0.5ng and 1.0ng of three different DNA templates. The amplification products were run on an ABI PRISM® 3100 Genetic Analyzer using a 3kV, 11-second injection and a run time of 2,000 seconds. For the new formulation, the spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650). For the current inventory primers, the spectral calibration was generated using the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121).

Ordering Information

Product	Size	Cat.#
PowerPlex® Matrix Standards, 310	(50µl each dye)	DG4640
PowerPlex® Matrix Standards, 3100/3130	(25µl each dye)	DG4650
PowerPlex® 16 System	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® Y System	50 reactions	DC6761
	200 reactions	DC6760
PowerPlex® ES System	100 reactions	DC6731
	400 reactions	DC6730
PowerPlex® 1.2 System	100 reactions	DC6101
PowerPlex® 16 BIO System	100 reactions	DC6541
	400 reactions	DC6540
PowerPlex® 1.1 System	100 reactions	DC6091
	400 reactions	DC6090
PowerPlex® 2.1 System	100 reactions	DC6471
	400 reactions	DC6470

Not for Medical Diagnostic Use.

[a] 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. The development and use of STR loci are covered by U.S. Pat. No. 5,364,759, Australian Pat. No. 670231 and other pending patents assigned to Baylor College of Medicine, Houston, Texas.

Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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

[c] U.S. Pat. Nos. 6,238,863 and 6,767,703 and other patents pending.

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

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Printed in USA 06/06
13658-AN-PU
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