

promega Application Notes

Amplifications Using the PowerPlex® 16 System and a 10X Primer Pair Mix Stored in TE⁻⁴ Buffer or in Water Yield Comparable Results

Abstract

Promega is changing the solution used to prepare the 10X Primer Pair Mix in the PowerPlex® 16 System from water to TE⁻⁴ buffer. We show that the amplification results with the 10X Primer Pair Mix prepared in TE⁻⁴ buffer are comparable to those with the 10X Primer Pair Mix prepared in water, even when we varied reaction parameters (the amount of DNA template, volume of TE⁻⁴ buffer, the thermal cycler used, amount of AmpliTaq Gold® DNA polymerase used and primer concentrations). Because the performance with both primer pair formulations is comparable, users of the PowerPlex® 16 System should see no adverse effects on amplification results.

Introduction

DNA is traditionally stored in water or a buffered solution, such as TE⁻⁴ (10mM Tris-HCl, 0.1mM EDTA [pH 8.0]). DNA stored in TE⁻⁴ buffer is more stable due to the buffering capacity of Tris and the presence of EDTA (1). For this reason, the 10X Primer Pair Mix for the PowerPlex® Y System^(a) (Cat.# DC6761) is already prepared using TE⁻⁴ buffer. Also, Promega is changing the solution used to resuspend the PowerPlex® 16 10X Primer Pair Mix from water to TE⁻⁴ buffer. In this study, we demonstrate that there are no discernible differences in amplification results using PowerPlex® 10X Primer Pair Mixes prepared in water or in TE⁻⁴ buffer. We also show that amplification with the PowerPlex® 16 10X Primer Pair Mix prepared in TE⁻⁴ buffer is as sensitive as the primer pair mix prepared in water.

Materials and Methods

Human genomic DNA was isolated from buccal swabs using the DNA IQ™ System^(b) (Cat.# DC6701), as directed in the *DNA IQ™ System—Database Protocol #TB296*. DNA was quantified by PicoGreen® dsDNA Quantitation Reagent (Molecular Probes). Nine additional DNA templates (DNA Standard Reference Material 2391b, PCR-based DNA Profiling Standard) were obtained from the National Institute of Standards and Technology (NIST), including 9947A. All amplifications were performed in parallel reactions using the PowerPlex® 16 System^(a,c,d) (Cat.# DC6531) and a PowerPlex® 16 10X Primer Pair Mix prepared in water or in TE⁻⁴ buffer. The two primer pair mixes had identical primer sequences and were manufactured using identical protocols. Reactions were assembled as directed in the *PowerPlex® 16 System Technical Manual #TMD012* and contained 1ng of DNA, 1X primer, 1X AmpliTaq Gold®

DNA polymerase and 1.5mM MgCl₂, unless otherwise indicated. DNA templates were diluted in 19.2µl of TE⁻⁴ buffer, unless otherwise indicated. Amplifications were carried out using the Perkin-Elmer GeneAmp® PCR System 9600, unless otherwise indicated, for 32 cycles (10/22 cycling). Amplification products were detected using the ABI PRISM® 310 or 3100 Genetic Analyzer and the GeneScan® analysis software. Sample files were imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro.

Variations in Amplification Reaction Conditions

Primers stored in TE⁻⁴ buffer are more stable than primers stored in water (data not shown), but we wanted to be sure that the use of TE⁻⁴ buffer to resuspend the primers did not affect amplification results. We varied the amplification conditions and compared the results obtained with the two primer pair formulations to determine if there were any effects due to the new formulation.

We examined the effect of variations in magnesium concentration on amplifications performed with both formulations of the primer pair mixes. We prepared Gold ST★R 10X Buffer without MgCl₂ (500mM KCl, 100mM Tris-HCl [pH 8.3 at 25°C], 1% Triton® X-100, 2mM each dNTP and 1.6mg/ml BSA) and added MgCl₂ to the reactions separately to a final concentration of 1mM, 1.25mM, 1.5mM, 1.75mM or 2mM. For both primer formulations, the reactions produced similar results (Figure 1). The best balance between loci was achieved with 1.5mM MgCl₂. Increasing MgCl₂ concentration increased yield of only the smallest loci. The locus-to-locus balance was affected at 1.25mM MgCl₂, and allele drop-out was observed with 1mM MgCl₂ with both formulations of primer pairs. Overall, the amplification results were comparable.

We then determined the effect of differences in primer concentration by assembling parallel reactions with 0.5X, 0.75X, 1X, 1.5X or 2X primer concentration. With both formulations of primer pairs, as primer concentration increased, small loci were preferentially amplified, and as primer concentration decreased, yield decreased for small loci and increased for large loci. Again, the results were comparable with both formulations of primer pairs (data not shown). We then varied the amount of AmpliTaq Gold® DNA polymerase per reaction from 0.5X to 4X. No drop-out of alleles was observed with reduced polymerase amounts, but lower yield was

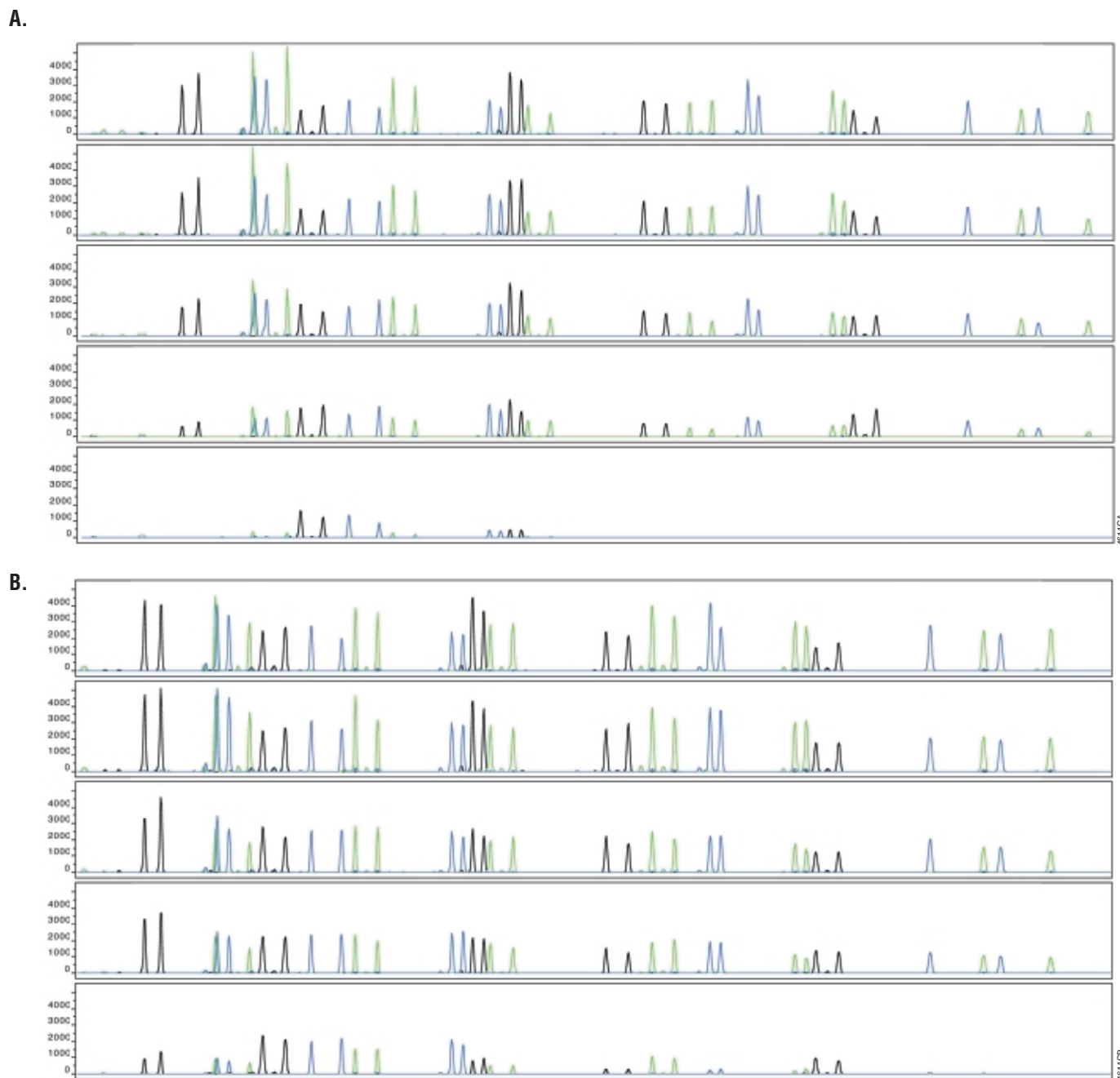


Figure 1. Magnesium titration. PowerPlex® 16 System reactions were assembled in parallel using the old formulation (**Panels A**) and new formulation (**Panels B**) of the 10X Primer Pair Mix. Amplifications were performed in the presence of 2mM, 1.75mM, 1.5mM, 1.25mM or 1mM $MgCl_2$. Data are shown in order of decreasing magnesium concentrations from top to bottom panels.

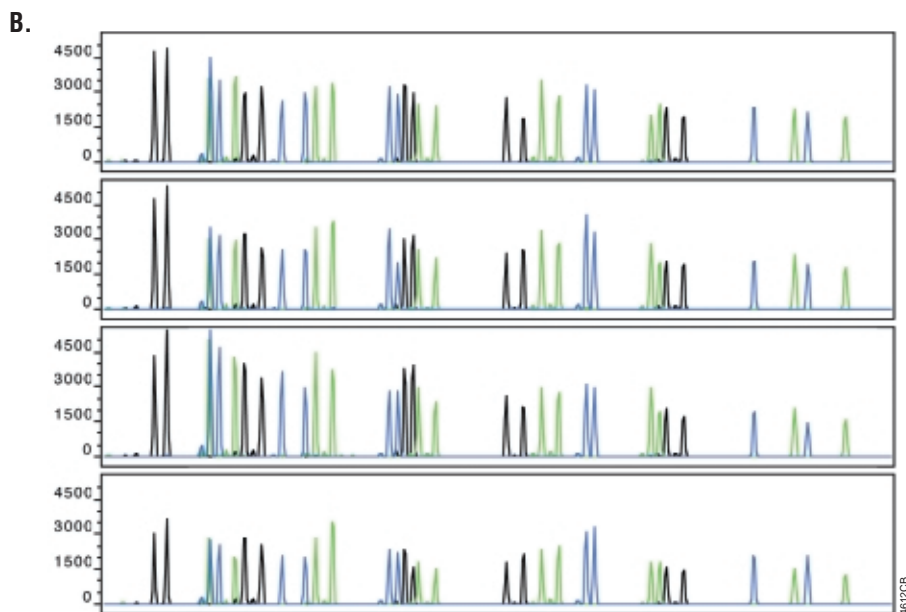
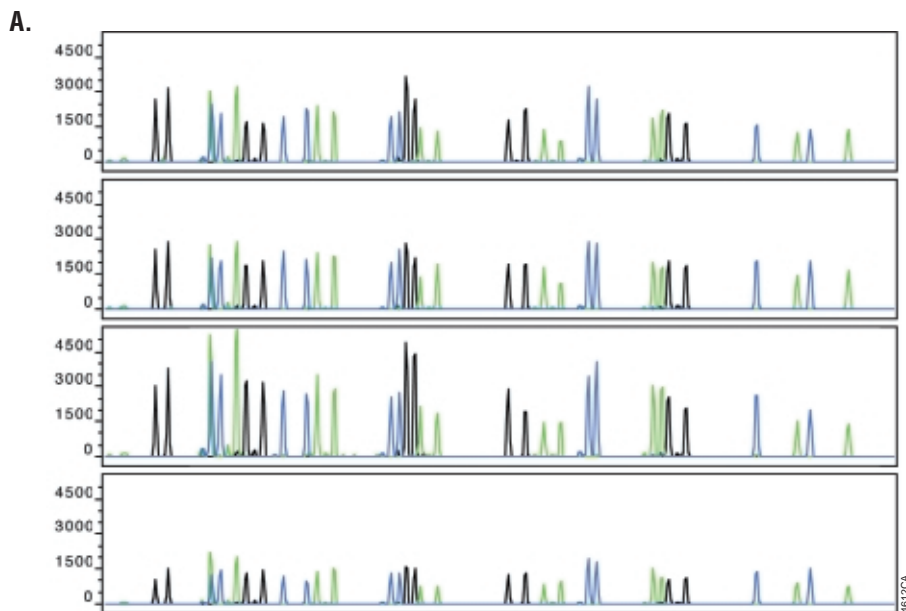


Figure 2. Effects of different thermal cyclers. PowerPlex® 16 System reactions were assembled in parallel using the old formulation (**Panels A**) and new formulation (**Panels B**) of the 10X Primer Pair Mix. Amplifications were performed using the Perkin-Elmer GeneAmp® PCR System 9600 (top panels), GeneAmp® PCR System 9700 with the silver block (second panels), GeneAmp® PCR System 2400 (third panels) and the Model 480 thermal cycler (bottom panels) following the recommended cycling parameters for each instrument, as directed in the *PowerPlex® 16 System Technical Manual #TMD012*.

observed in larger loci with both formulations of primer pairs. We observed the best balance of amplified loci at 1X AmpliTaq Gold® concentration. Peak heights increased at the higher polymerase concentrations with both formulations of primer pairs. We saw no differences in the results generated with the two formulations of primer pairs (data not shown).

Comparison of Thermal Cyclers

To ensure that the primers gave similar results on all of the recommended thermal cyclers, we assembled parallel reactions with the two primer pair formulations and used the Perkin-Elmer GeneAmp® PCR System 9600, GeneAmp® PCR System 9700 (with the silver block), GeneAmp® PCR System 2400 and the thermal

cycler Model 480 to amplify the DNA. We observed no consistent performance differences between the results generated on the same instrument with the two primer pair formulations (Figure 2).

Testing of NIST Standard Samples

We amplified 1ng of each NIST DNA standard to ensure that the new formulation of the PowerPlex® 16 10X Primer Pair gave allele calls that matched the known genotype of these DNA standards. Both formulations of the primer pairs provided the correct genotype for each of the NIST standards (data not shown).

Sensitivity

To compare the sensitivity of amplifications with the old and new primer pair mixes, we assembled amplifications with decreasing amounts of DNA templates (2 to 0.2ng). We diluted the indicated amount of DNA in the maximum volume (19.2 μ l) of TE⁻⁴ buffer or water and amplified using both primer pair formulations. Amplifications of DNA diluted in TE⁻⁴ buffer are shown in Figure 3, Panels A and B. We observed no consistent

performance differences with both primer pair formulations. The same is true for amplifications of DNA diluted in water (compare Panels C and D in Figure 3). For DNA template diluted in water or in TE⁻⁴ buffer, the new formulation is as sensitive as the old formulation.

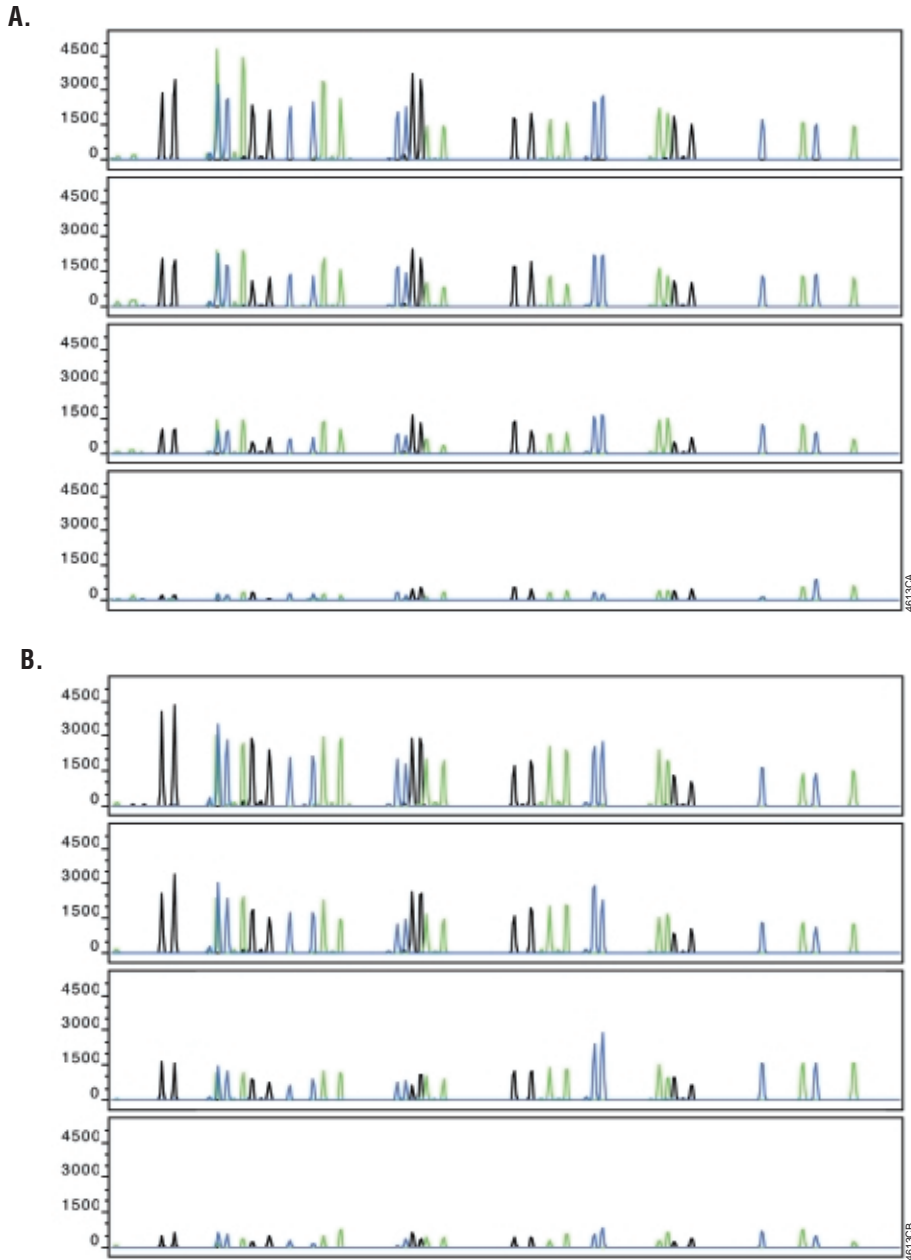
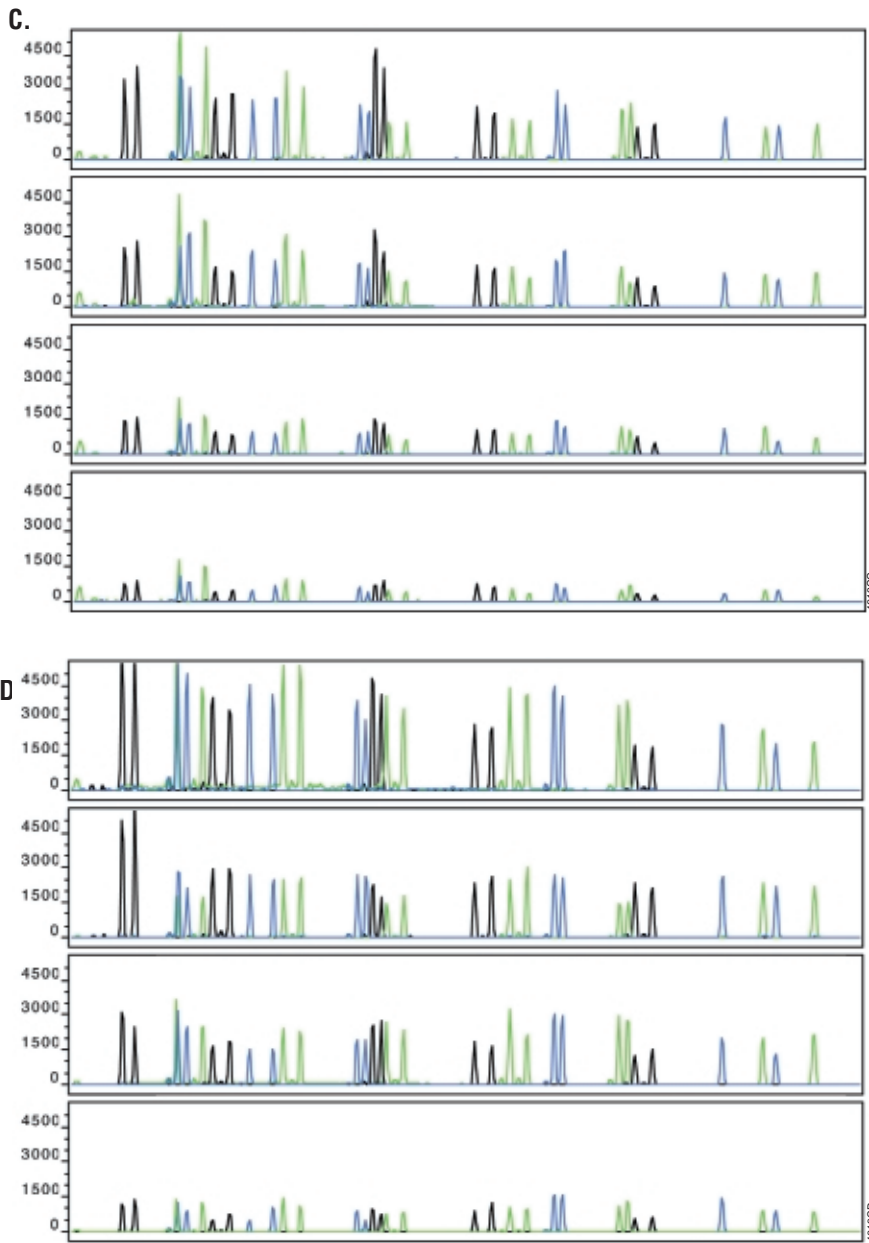


Figure 3. Sensitivity of old and new primer pair formulations. PowerPlex® 16 System reactions were assembled using the old formulation (**Panels A and C**) and new formulation (**Panels B and D**) of the 10X Primer Pair Mix. The desired amount of DNA template was diluted in 19.2 μ l of TE⁻⁴ buffer (**Panels A and B**) or 19.2 μ l of water (**Panels C and D**). Amplifications were performed with 2ng, 1ng, 0.5ng and 0.2ng of DNA. Data are shown in order of decreasing DNA amount from top to bottom panels.



Robustness

When comparing the old and new primer pair formulations, we found that the presence of the maximum volume of TE⁻⁴ buffer had no effect on the success of the amplification, even when reaction conditions were varied from the optimal conditions. Compare Figure 1 Panel A to Panel B [magnesium titration] and Figure 2 Panel A to Panel B [thermal cyclers] and compare Figure 3 Panels A and B to Figure 3 Panels C and D [amounts of DNA template]. This was also true for reactions in which the AmpliTaq Gold[®] DNA polymerase and primer concentrations were varied (data not shown). The fact that the amplification results were comparable with the old and new primer pair

mixes, even when the DNA template was diluted in the maximum possible volume of TE⁻⁴ buffer (19.2µl), indicates that the new formulation of primers is as robust as the old formulation.

While the addition of up to 19.2µl of TE⁻⁴ buffer had no effect, this may not be true of DNA stored in TE buffer (10mM Tris-HCl, 1.0mM EDTA [pH 8.0]). The higher concentration of EDTA in TE buffer will chelate more of the magnesium in the reaction, which can result in suboptimal amplification. If the template DNA is stored in TE buffer rather than TE⁻⁴ buffer, the volume of the DNA sample added should not exceed 20% of the final reaction volume.

Conclusions

The PowerPlex® 16 10X Primer Pair Mixes prepared in water and in TE⁻⁴ buffer performed comparably in all amplifications, regardless of the amount of template DNA, the thermal cycler used and the primer, magnesium and AmpliTaq Gold® DNA polymerase concentrations. The addition of 2.5µl TE⁻⁴ buffer used to resuspend the primers also had no effect on the results; in fact, the amplification results were not affected even if the maximum possible volume of TE⁻⁴ buffer (19.2µl) was added to the reaction. Amplification results were comparable for both primer pair formulations. Because the performance is comparable, we feel that laboratories should not need to revalidate the PowerPlex® 16 System.

The change in the PowerPlex® 16 10X Primer Pair Mix will become effective with primer pair lot number 18384801; this lot and all newer lots will use TE⁻⁴ buffer to resuspend the primer pairs. Future lots of 10X Primer Pair Mixes for the PowerPlex® ES System^(a), beginning with lot number 18474101, and the PowerPlex® 16 BIO System^(a,c,d,e), beginning with lot number 18637401, will be manufactured with TE⁻⁴ buffer rather than water. We also plan to change the solution used to resuspend the 10X Primer Pair Mixes in the PowerPlex® 1.1^(a,f), 1.2^(a,f), and 2.1^(a,c,f) Systems in the future. As demonstrated here, this change should not affect amplification results. This change is not necessary for the PowerPlex® Y System because TE⁻⁴ buffer is already used to prepare the PowerPlex® Y 10X Primer Pair Mix. We will continue to generate more real time stability data, and if the product shelf life is extended, the expiration dates will be changed accordingly.

Ordering Information

Product	Size	Cat. #
PowerPlex® 16 System ^(a,c,d)	100 reactions	DC6531
	400 reactions	DC6530
PowerPlex® Y System ^(a)	50 reactions	DC6761
	200 reactions	DC6760
PowerPlex® 16 BIO System ^(a,c,d,e)	100 reactions	DC6541
	400 reactions	DC6540
PowerPlex® ES System ^(a)	100 reactions	DC6731
	400 reactions	DC6730
PowerPlex® 1.1 System ^(a,f)	100 reactions	DC6091
	400 reactions	DC6090
PowerPlex® 1.2 System ^(a,f)	100 reactions	DC6101
PowerPlex® 2.1 System ^(a,c,f)	100 reactions	DC6471
	400 reactions	DC6470

Not for Medical Diagnostic Use.

Reference

1. Guidet, F. and Langridge, P. (1992) *Meth. Enzymol.* **216**, 3–12.

- ^(a) Use of Promega's STR Systems requires performance of the polymerase chain reaction (PCR), which is a patented process. Purchase of Promega's STR Systems does not include or provide a license with respect to PCR. Users of Promega's STR Systems may, therefore, be required to obtain a license with respect to the use of PCR. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.
- ^(b) U.S. Pat. Nos. 6,027,945, 6,368,800 and 6,673,631, Australian Pat. No. 732756 and other patents and patents pending.
- ^(c) U.S. Pat. No. 6,238,863, Australian Pat. No. 758639 and other patents pending.
- ^(d) U.S. Pat. Nos. 5,843,660, 6,479,235 and 6,221,598, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048 and other patents pending.
- ^(e) PowerPlex® 16 BIO System incorporates dye conjugates made with the Rhodamine Red™-X and Texas Red®-X fluorescent reactive dyes, which are licensed from Molecular Probes, Inc., under U.S. Pat. Nos. 5,798,276 and 5,846,737 for DNA analysis. Rhodamine Red is a trademark of Molecular Probes, Inc., and Texas Red is a registered trademark of Molecular Probes, Inc.
- ^(f) U.S. Pat. Nos. 5,843,660 and 6,221,598, Australian Pat. No. 724531, Canadian Pat. No. 2,118,048 and other patents pending.

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