promega Application Notes

PowerPlex® Y System Validation

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Abstract

We have improved the manufacturing process for the PowerPlex® Y System to ensure lot-to-lot spectral consistency and increased ease of use. Users of the PowerPlex® Y System should see no adverse effects on amplification results. We show that the amplification results obtained with systems manufactured using the new process are comparable to, if not more sensitive than, those obtained using earlier lots manufactured with the previous process.

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® Y System[a,b]. 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 single 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 that is 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® Y System have not changed.

The *PowerPlex® Y 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® Y Allelic Ladder Mix (Part# DG750B) with the new PowerPlex® Y 10X Primer Pair Mix (Part# DK712B) and new matrix standards (Cat.# DG4640 for the ABI PRISM® 310 Genetic Analyzer and Cat.# DG4650 for ABI PRISM® 3100 and Applied Biosystems 3130 Genetic Analyzers). Experiments performed at Promega demonstrate the consistency and reproducibility of the PowerPlex® Y System with these manufacturing changes. The experiments are summarized in this Application Notes.

Materials and Methods

Human genomic DNA was isolated from buccal swabs using the DNA IQ™ Reference Sample Kit for Maxwell® 16 (Cat.# AS1040) as directed in the *DNA IQ™ Reference Sample Kit for Maxwell® 16 Technical Bulletin* #TB347 and quantified using the prototype Plexor™ HY System, which is a real-time PCR assay. Additional human genomic DNA was isolated from liquid whole blood using phenol:chloroform extraction and quantified by measuring optical density at 260nm (OD_{260}). Additional DNA templates (DNA Standard Reference Material 2395, Human Y-Chromosome DNA Profiling Standard) 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® Y 10X Primer Pair Mix. Reactions were assembled as directed in the *PowerPlex® Y System Technical Manual* #TMD018 and contained varying amounts of DNA (0.50–0.031ng), 1X PowerPlex® Y Primer Pair Mix, 2.75 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 using programmed ramps for 32 cycles (10/22) unless otherwise indicated. Amplification products were detected using the Applied Biosystems 3130 Genetic Analyzer or ABI PRISM® 3100 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

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 formulation and current inventory of PowerPlex® Y 10X Primer Pair Mixes, we assembled amplifications with decreasing amounts of DNA template (0.50–0.031ng). The sensitivity level of the new formulation was comparable to, if not more sensitive than, the current inventory (Figures 1 and 2; 3kV, 3-second injection and 3kV, 5-second injection, respectively).

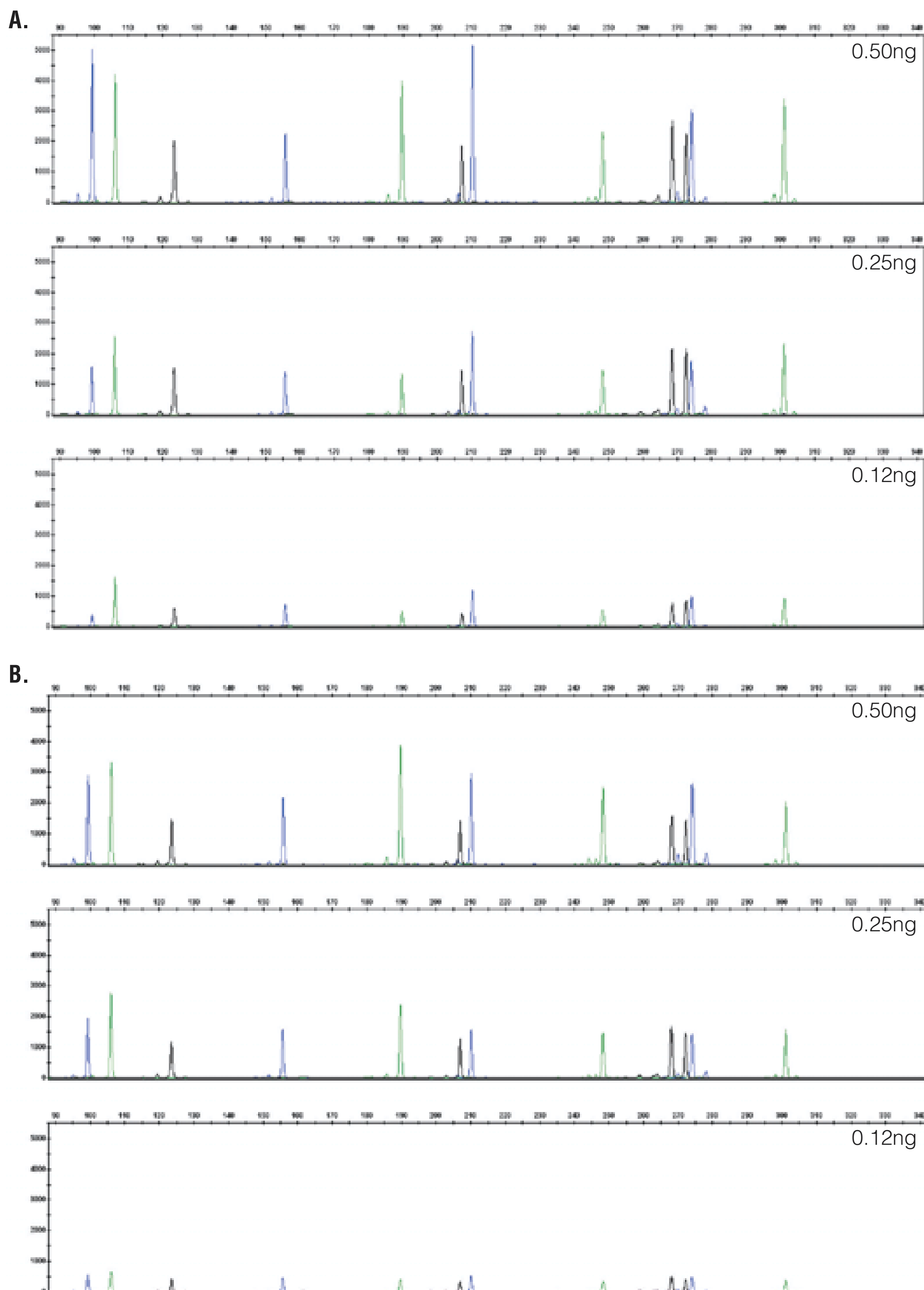

Figure 1. Representative amplifications using the PowerPlex® Y 10X Primer Pair Mix. PowerPlex® Y reactions were assembled with decreasing amounts of DNA template, as indicated, and the new formulation or current inventory of the PowerPlex® Y 10X Primer Pair Mix. The human DNA template was serially diluted 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 product was analyzed using an Applied Biosystems 3130 Genetic Analyzer, **a 3kV, 3-second injection** and a run time of 1,800 seconds. **Panel A.** The new formulation of PowerPlex® Y 10X Primer Pair Mix. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650). **Panel B.** The current inventory of PowerPlex® Y 10X Primer Pair Mix. The spectral calibration was generated using the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121).

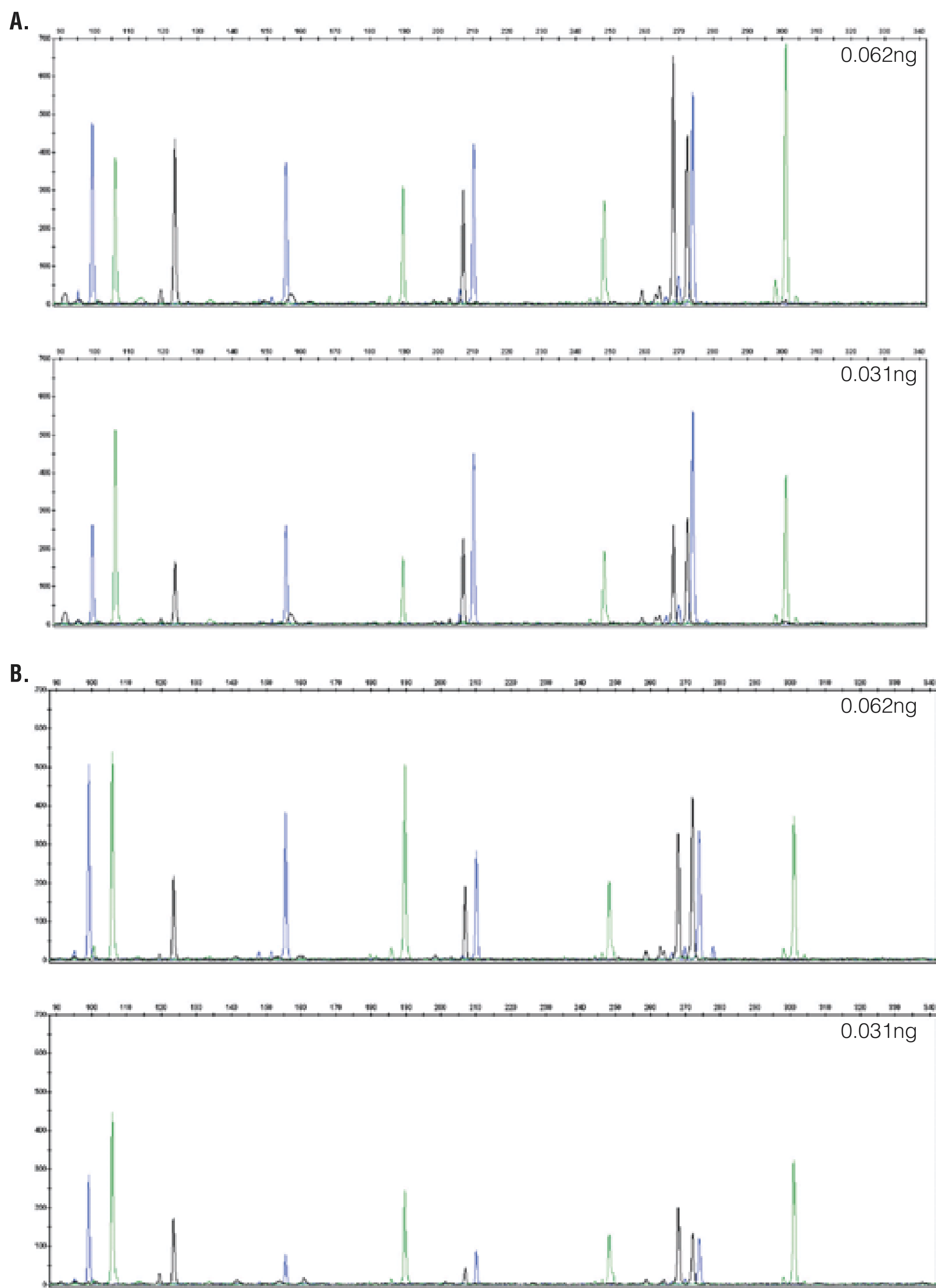

Figure 2. Representative amplifications using the PowerPlex® Y 10X Primer Pair Mix. PowerPlex® Y reactions were assembled with decreasing amounts of DNA template, as indicated, and the new formulation or current inventory of the PowerPlex® Y 10X Primer Pair Mix. The human DNA template was serially diluted 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 product was analyzed using an Applied Biosystems 3130 Genetic Analyzer, **a 3kV, 5-second injection** and a run time of 1,800 seconds. **Panel A.** The new formulation of the PowerPlex® Y 10X Primer Pair Mix. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650). **Panel B.** The current inventory of PowerPlex® Y 10X Primer Pair Mix. The spectral calibration was generated using the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121).

Male:Female Mixture

To test male:female mixtures, 0.10ng of male genomic DNA was amplified with and without 100ng of female DNA. The male:female mixtures gave full profiles with no extraneous peaks (Figure 3). In addition, the allele calls were consistent between the two amplification products. The results with the new formulation of PowerPlex® Y 10X Primer Pair Mix were comparable in the presence and absence of female DNA.

Concordance

To evaluate reproducibility, we amplified five male and one female DNA templates contained in DNA Standard Reference Material 2395, Human Y-Chromosome DNA Profiling Standards, using the new formulation of the PowerPlex® Y 10X Primer Pair Mix. Allele calls were made using the GeneScan® and Genotyper® software (ABI PRISM® 3100 Genetic Analyzer) and GeneMapper® *ID* software (Applied Biosystems 3130 Genetic Analyzer). All allele calls were consistent with those provided by NIST (data not shown).

Stutter Percentage

To compare stutter percentage, 0.50ng of six different DNA templates were amplified with the new formulation and current inventory of the PowerPlex® Y 10X Primer Pair Mix. The amplification products were run on an Applied Biosystems 3130 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 new formulation and the current inventory (Figure 4).

Thermal Cycling without Programmed Ramps

We recommend using programmed ramp times when amplifying PowerPlex® Y System reactions. However, some thermal cycler models do not allow programmed ramp times. Experiments performed at Promega provide an option for thermal cyclers that do not allow programmed ramp times. To examine the effect of a thermal cycler without ramp times, 0.50–0.031ng of three different DNA templates were amplified using an Applied Biosystems 2720 thermal cycler (32 cycles without programmed ramp times). Results were compared to those from a GeneAmp® PCR System 9700 thermal cycler using the recommended 10/22 program (32 cycles with programmed ramps). The thermal cycling profile used with the GeneAmp® PCR System 9700 is found in the *PowerPlex® Y System Technical Manual* #TMD018. The thermal cycling profile used with the Applied Biosystems 2720 thermal cycler was as follows: Initial denaturation at 95ºC for 11 minutes was followed by 32 cycles of amplification [denaturation at 94ºC for 30 seconds; annealing at 60ºC for 90 seconds; extension at 72ºC for 90 seconds], with a final extension step at 60ºC for 45 minutes. Although the balance of the new formulation using the Applied Biosystems 2720 thermal cycler without programmed ramp times was compromised, the level of sensitivity was comparable to that of the GeneAmp® PCR System 9700 thermal cycler with programmed ramp times (Figures 5 and 6).

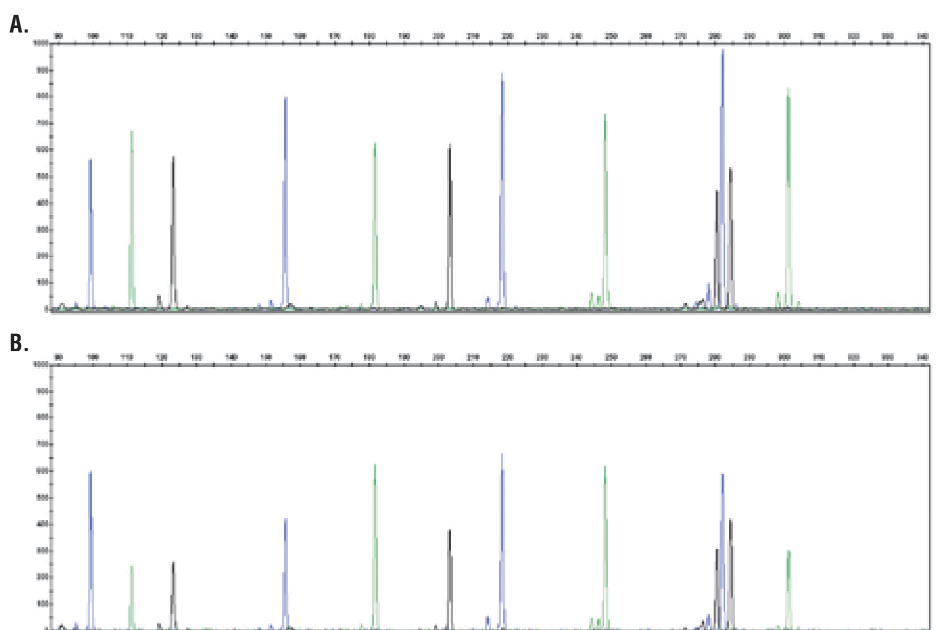

Figure 3. The effect of amplification yield in the absence and presence of female DNA. Male DNA (0.10ng) was amplified in the absence (**Panel A**) and in the presence of 100ng female DNA (**Panel B**) using the new formulation of the PowerPlex® Y 10X Primer Pair Mix. The amplification products were analyzed using an Applied Biosystems 3130 Genetic Analyzer, a 3kV, 3-second injection and a run time of 1,800 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

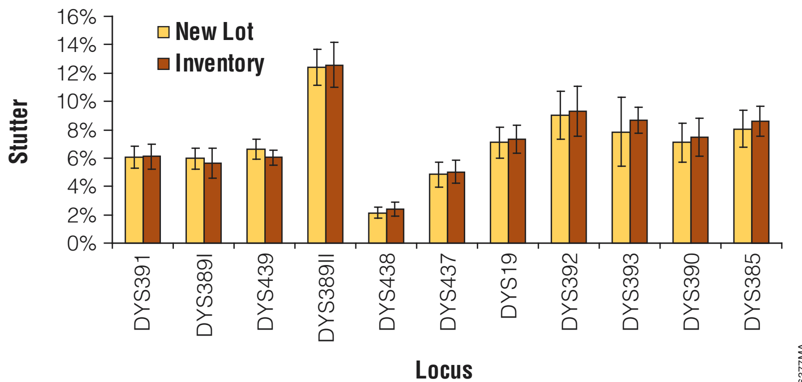

Figure 4. Stutter Percentages. The new formulation and current inventory of the PowerPlex® Y 10X Primer Pair Mix were used to amplify 0.50ng of six different DNA templates. The amplification products were run on an Applied Biosystems 3130 Genetic Analyzer using a 3kV, 3-second injection and a run time of 1,800 seconds. For the new formulation, the spectral calibration was generated using the PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650). For the current inventory, the spectral calibration was generated using the PowerPlex® Matrix Standards, 3100—Custom (Cat.# X3121).

Conclusions

We observed no adverse effects on the performance of the PowerPlex® Y System due to improvements to the PowerPlex® Y System manufacturing protocols. The performance of the new formulation was comparable to, if not more sensitive than, that of the current inventory of PowerPlex® Y 10X Primer Pair Mix, regardless of template DNA amount. The amplification results from male DNA in the presence of female DNA was comparable to that of male DNA alone, and allele calls were consistent. Although we recommend using programmed ramp times when amplifying PowerPlex® Y System reactions, initial experiments completed at Promega provide an option for use of thermal cyclers that do not allow programmed ramp times.

Editor's Note: *PowerPlex® Y Systems that include the new formulation are designated by lot numbers ending with the letter "N".*

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] The purchase of this product does not convey a license to use AmpliTaq Gold® DNA polymerase. You should purchase AmpliTaq Gold® DNA polymerase licensed for the forensic and human identity field directly from your authorized enzyme supplier.

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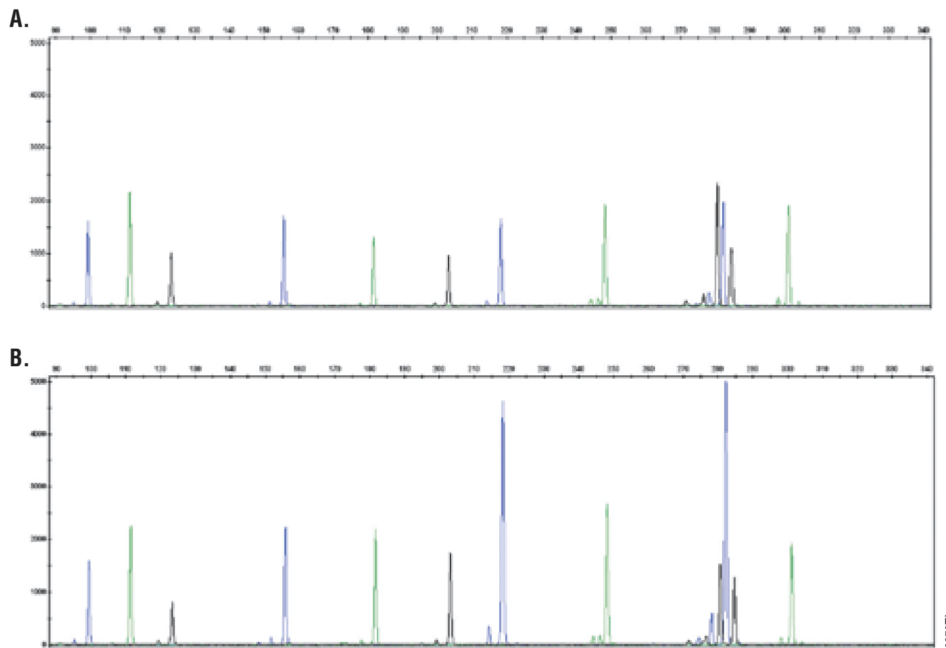

Figure 5. Representative amplifications using thermal cycling conditions with and without programmed ramp times. Human DNA (0.25ng) was amplified using the GeneAmp® PCR System 9700 and thermal cycling conditions with programmed ramp times (**Panel A**) and using the Applied Biosystems 2720 thermal cyler without programmed ramp times (**Panel B**) using the new formulation of the PowerPlex® Y 10X Primer Pair Mix. The amplification products were analyzed using an Applied Biosystems 3130 Genetic Analyzer, a 3kV, 3-second injection and a run time of 1,800 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

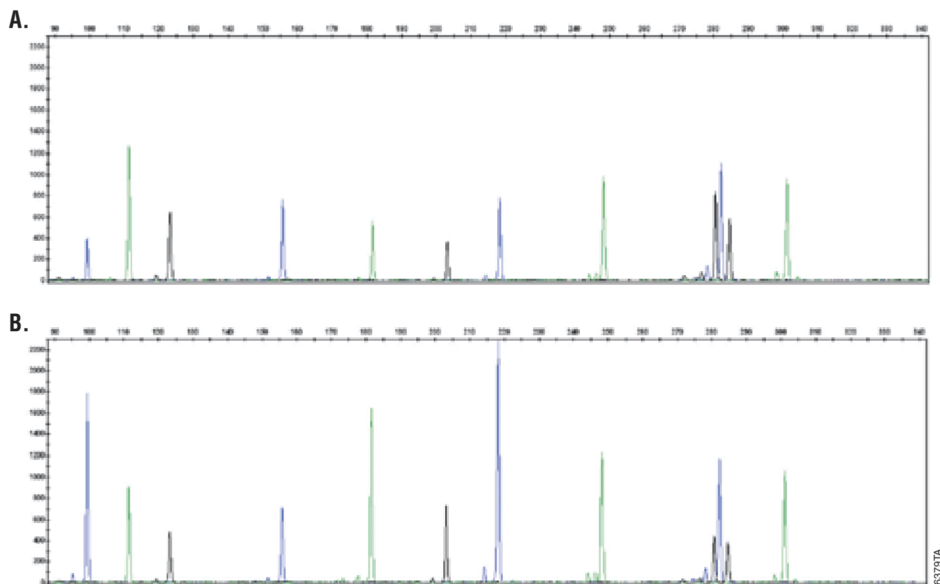

Figure 6. Representative amplifications using thermal cycling conditions with and without programmed ramp times. Human DNA (0.12ng) was amplified using the GeneAmp® PCR System 9700 and thermal cycling conditions with programmed ramp times (**Panel A**) and using the Applied Biosystems 2720 thermal cycler without programmed ramp times (**Panel B**) with the new formulation of the PowerPlex® Y 10X Primer Pair Mix. The amplification products were analyzed using an Applied Biosystems 3130 Genetic Analyzer, a 3kV, 3-second injection and a run time of 1,800 seconds. The spectral calibration was generated using the new PowerPlex® Matrix Standards, 3100/3130 (Cat.# DG4650).

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