POWERPLEX® 16 HS

The PowerPlex® 16 HS System

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INTRODUCTION

Short tandem repeat (STR) analysis remains the foremost method for human genetic identification. STR loci consist of short, repetitive sequence elements 3–7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, readily amplified by PCR and analyzed by gel or capillary electrophoresis (5–8). STR typing is more tolerant of degraded DNA templates than other typing methods because amplification products are less than 500 bp long, much smaller than material detected using AMP-FLP (9) or VNTR (10) analysis. STR typing is also amenable to a variety of rapid DNA purification techniques that are compatible with PCR but do not provide enough DNA of appropriate quality for Southern-blot-based analyses.

Multiplex STR typing is commonly used in forensic, paternity and anthropological studies. Currently, many countries and government agencies have in place, or are working to implement, systems to effectively and efficiently create and maintain criminal DNA databases. To contribute profiles to these databases, laboratories must implement and follow strict guidelines to ensure database integrity.

As the needs of end users and requirements of government agencies change, the PowerPlex® family of STR analysis systems continues to evolve. The PowerPlex® 16 System now is available with hot-start *Taq* DNA polymerase included in a convenient and robust master mix. The addition of a DNA polymerase to a simplified set of pre-amplification reagents marks the first time the PowerPlex® 16 System has incorporated all components necessary for multiplex STR genotyping, while reducing pipetting steps.

The PowerPlex® 16 HS System[c–g] offers excellent sensitivity and resistance to common PCR inhibitors. This, coupled with the flexibility of the PowerPlex® 16 HS System, allows more interpretable data and less need for re-amplification of samples previously deemed “difficult” due to limited DNA amounts or the presence of inhibitors. In addition to improved amplification performance, current users of the PowerPlex® 16 System will notice that many key attributes remain the same, including the primer sequences. Laboratories currently using PowerPlex® 16 can apply their current injection and analysis parameters, along with panel and bin sets and spectral calibrations.

The unique master mix contained in the PowerPlex® 16 HS System makes it possible to obtain the most informative results with challenging samples. Full profiles can be generated from samples contaminated with severalfold more blood- and soil-borne inhibitors than with currently available kits.

THE POWERPLEX® 16 HS SYSTEM

The PowerPlex® 16 HS System (Cat.# DC2100, DC2101) allows co-amplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin), including Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. One primer for each of the Penta E, D18S51, D21S11, TH01 and D3S1358 loci is labeled with fluorescein (FL); one primer for each of the FGA, TPOX, D8S1179, vWA and Amelogenin loci is labeled with carboxy-tetramethylrhodamine (TMR); and one primer for each of the Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 loci is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). Amplicon size is determined by comparison with the Internal Lane Standard 600, which is labeled with carboxy-X-rhodamine (CXR). The PowerPlex® 16 HS System’s four-color chemistry allows analysis with

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the ABI PRISM® 310, 3100 and 3100-*Avant* Genetic Analyzers and Applied Biosystems 3130 and 3130*xl* Genetic Analyzers. Proper color deconvolution is obtained by matrix standardization or spectral calibration using available matrix standards (Cat.# DG4640, DG4650). Laboratories currently using most PowerPlex® chemistries (11–14) can use the same matrix or spectral files for the PowerPlex® 16 HS System.

The loci included in the PowerPlex® 16 HS System were selected because they satisfy the needs of several major standardization bodies throughout the world and include the 13 core loci selected by the Federal Bureau of Investigation for a Combined DNA Index System (CODIS) search. The PowerPlex® 16 HS System also contains two low-stutter, highly polymorphic penta-nucleotide repeat loci, Penta E and Penta D. These additional loci add significantly to the discrimination power of the system, making the PowerPlex® 16 HS System a single-amplification system with the highest power of exclusion (15), sufficient to resolve nearly all paternity disputes definitively. In addition, the extremely low level of stutter seen with Penta E and Penta D makes them ideal loci to evaluate DNA mixtures often encountered in forensic casework. Finally, the Amelogenin locus is included in the PowerPlex® 16 HS System to allow gender identification of each sample.

Everything necessary to amplify STR regions of purified genomic DNA is included in the pre-amplification components: PowerPlex® HS 5X Master Mix, PowerPlex® 16 HS 10X Primer Pair Mix and amplification-grade water. The PowerPlex® HS 5X Master Mix includes a hot-start *Taq* DNA polymerase as well as buffer, salts, $MgCl_2$, BSA and dNTPs. The PowerPlex® 16 HS 10X Primer Pair Mix contains primers identical in sequence to those in the original PowerPlex® 16 System.

The reaction volume for the PowerPlex® 16 HS System allows addition of up to 17.5 µl of template DNA, which is beneficial when working with dilute samples. Reactions are set up with fewer pipetting steps, reducing the chance of errors and contamination. Except for a shortened initial denaturation step, thermal cycling protocols also remain the same as those for the PowerPlex® 16 System. The new Internal Lane Standard 600, which includes additives that prevent formation of two artifacts (“split peak” and “n–10”) at the vWA locus (16), is included with the PowerPlex® 16 HS System. Injection parameters remain flexible to accommodate instrument variability and meet the diverse needs of the end user. The PowerPlex® 16 HS Allelic Ladder provides all of the same alleles as the PowerPlex® 16 System and uses the same panel

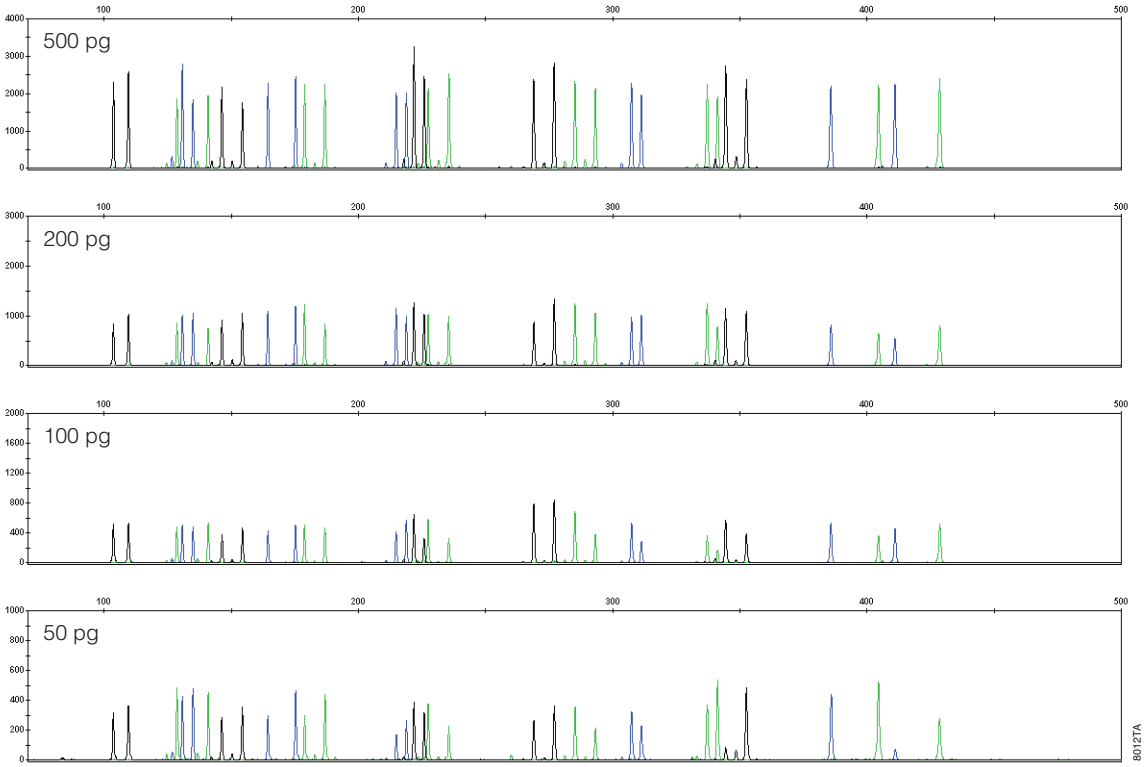

Figure 1. Sensitivity of the PowerPlex® 16 HS System. Decreasing amounts of template were amplified using the PowerPlex® 16 HS System. Each amplification was performed as directed in the *PowerPlex® 16 HS System Technical Manual* #TMD022. Once amplified, samples were separated on an Applied Biosystems 3130 Genetic Analyzer using 3 kV, 5-second injection. All alleles were called using a 50 RFU threshold.

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and bin files to help to ensure accurate allele designation. Analysis methods as well as panels and bins, which are available at: **www.promega.com/geneticidtools/panels_bins/**, are also unchanged.

POWERPLEX® 16 HS SYSTEM PERFORMANCE

The PowerPlex® 16 HS System can accommodate a range of template DNA concentrations. System balance is optimized to amplify 0.5 ng of DNA, and product specifications ensure that it will amplify as little as 100 pg of DNA. Studies performed at Promega show that full profiles can be observed with <100 pg (Figure 1). In cases with limited DNA amounts, such as touch samples, PowerPlex® 16 HS proves to be very sensitive, giving interpretable results with much less than 100 pg of DNA (Figure 1).

The unique master mix contained in the PowerPlex® 16 HS System makes it possible to obtain the most informative results with challenging samples. Full profiles can be generated from samples contaminated with severalfold more blood- and soil-borne inhibitors than with currently available kits (17,18 Figure 2, data not shown).

The hot-start *Taq* DNA polymerase included in the PowerPlex® 16 HS System maintains its sensitivity and robust activity even after reaction mixes remained at room temperature for 8 hours prior to amplification (data not shown). Thus, the PowerPlex® 16 HS System is amenable to automation and robotic plate setup, where prolonged lag times may be encountered.

Table 1. Comparing the PowerPlex® 16 HS System With the Original PowerPlex® 16 System.

Feature	PowerPlex® 16 HS System	PowerPlex® 16 System
All-in-one system	+[1]	–
Ease of use	++[2]	+
Sensitivity	++[3]	++[3]
Resistance to inhibitors	++[4]	+

[1]Includes hot-start *Taq* DNA polymerase
[2]Simplified protocol, less pipetting
[3]Both kits routinely type less than 100 pg of DNA
[4]Increased robustness with inhibitor-laden samples

CONCLUSIONS

The PowerPlex® 16 HS System offers a familiar format that incorporates all components required for multiplex STR genotyping and features a simplified protocol with fewer pipetting steps. This system offers robust performance and enough flexibility to meet the needs of a variety of end users.

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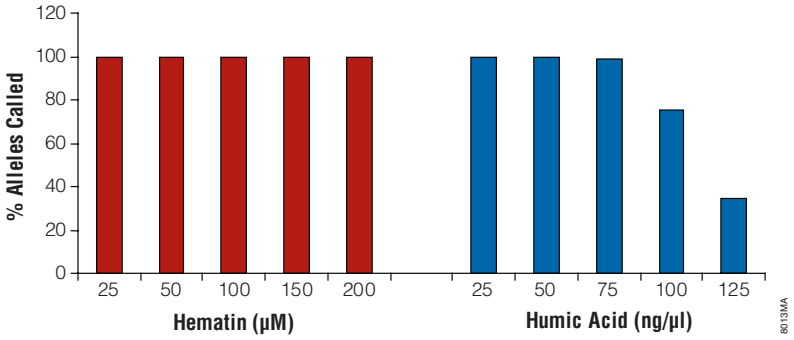

Figure 2. Robustness of the PowerPlex® 16 HS System. Common PCR inhibitors at the indicated concentrations were added to PowerPlex® 16 HS System reactions. Bars represent the percent of alleles called in the presence of hematin (red) or humic acid (blue). Replicate amplifications were set up using 500 pg of template DNA. Once amplified, samples were separated using an Applied Biosystems 3130 Genetic Analyzer and a 3 kV, 5-second injection. A fluorescence threshold of 50 RFU was used in all dye channels.