PowerPlex® 16 HS System

Now with hot start Taq in the master mix

PowerPlex® 16 HS is the most robust CODIS multiplex available.
The PowerPlex® 16 HS System is a four-color, 16-locus STR system for human identification. Building on the proven PowerPlex 16 System, this new kit is ideal for casework, database and relationship testing.
Simple to order. *Easier to use.*

*Taq* DNA polymerase in the master mix and size standard.

**Pre-amplification Components**
- PowerPlex 16 HS 10x Primer Pair Mix
- 9947A Control DNA
- Water, Amplification Grade

**Post-amplification Components**
- PowerPlex 16 HS Allelic Ladder
- Internal Lane Standard 600 (ILS 600)
Now with hot start Taq in the master mix

PowerPlex® 16 HS is the most robust CODIS multiplex available.

Introduction

Inhibitors

Sensitivity

Direct amplification

Instruments

Validation

Mixtures

Updates

www.promega.com/powerplex16HS/

Downloads

Technical Manual TMD022
GeneMapper® ID panels and bins (same as PowerPlex® 16 System)

Technical support

genetic@promega.com

Components > Ordering information

100 reactions: DC2101
400 reactions: DC2100

Custom kit sizes

Arrange through your account manager. Promega creates large-quantity, matched-lot kits to simplify your tracking and reduce packaging waste. From a few thousand reactions to tens of thousands of reactions, we can work together to define what fits best with your processes.
The PowerPlex® 16 HS System is the most discriminating CODIS multiplex

PowerPlex 16 HS System includes:
- the 13 STR loci for the Combined DNA Indexing System (CODIS)
- amelogenin and the low-stutter high-discrimination markers Penta E and Penta D

Closest Competitor Includes D2 and D19 in lieu of Pentas Penta E and Penta D are more informative than D2 and D19. This increased discrimination provides more conclusive statistics for partial matches and challenging relationship-testing cases.

**Inhibitors**

Higher success rate from challenging samples. Less sample dilution and repurification.

With a redesigned master mix, the PowerPlex® 16 HS System is a robust, one-kit solution. With 2–10+ fold increase in inhibitor tolerance over competitors, there is no need for a second system for inhibited samples. With all CODIS loci co-amplified and 8 loci ≤250bp, the PowerPlex 16 HS System is the ideal solution for casework.

- Hematin Example 1
- Hematin Example 2
- Humic Acid Example 1
- Humic Acid Example 2
- Tannic Acid Example
Inhibitor tolerance

Hematin titration (example #1): >400% higher tolerance

Amplification was performed with 500 pg of DNA using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130xl Genetic Analyzer with a 3kV, 5-second injection.
Now with hot start Taq in the master mix
PowerPlex® 16 HS is the most robust CODIS multiplex available.

Inhibitor tolerance
Hematin titration (example #2): >25% higher tolerance

Amplification was performed with 500pg of DNA using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130xl Genetic Analyzer with a 3kV, 5-second injection.
Inhibitor tolerance

Humic acid titration (example #1): >400% higher tolerance

Amplification was performed with 500pg of DNA using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130xl Genetic Analyzer with a 3kV, 5-second injection.
Inhibitor tolerance

Humic acid titration (example #2): >300% higher tolerance

Amplification was performed with 500pg of DNA using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130xl Genetic Analyzer with a 3kV, 5-second injection.
Inhibitor tolerance

Tannic acid titration: >200% higher tolerance

Amplification was performed with 500pg of DNA using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130xl Genetic Analyzer with a 3kV, 5-second injection.
Sensitivity

PowerPlex® 16 HS is sensitive.

System is quality control-tested to produce a full profile from 100pg DNA.

Amplification was performed with 50pg DNA using the GeneAmp® 9700, and 1μl of the reaction was analyzed on an Applied Biostystems 3130 Genetic Analyzer with 3kV, 5-second injection.
Sensitivity
PowerPlex® 16 HS vs. closest competitor

Amplifications were performed using the GeneAmp® 9700, and 1µl of each reaction was concurrently analyzed on an Applied Biosystems 3130x/ Genetic Analyzer with a 3kV, 5-second injection.
Direct amplification

Previously, placing blood and buccal card punches directly into STR reactions yielded poor profiles. The more robust buffer system in the PowerPlex® 16 HS System has improved performance with direct amplification.

Initial testing was performed to evaluate punch diameter size and cycle number to identify optimal conditions. A 1.2mm punch amplified for 28 cycles was shown to work well for multiple sample types.
Direct amplification with PowerPlex® 16 HS

We evaluated punch size (0.5–2.0mm) and cycle number to identify optimal conditions (data not shown). A 1.2mm punch amplified for 28 cycles worked well for multiple sample types. Samples from 11 individuals were tested in quadruplicate (STR amplifications, n = 44).

This approach was successful with multiple sample types. Laboratories should optimize injection conditions for desired signal range.

<table>
<thead>
<tr>
<th>Card</th>
<th>Sample</th>
<th>Avg. Heterozygous Peak Height (RFU)</th>
<th>Full-Profile Success Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasiCollect™ device</td>
<td>Buccal</td>
<td>2709 ± 1659</td>
<td>98%</td>
</tr>
<tr>
<td>Sampact™ device (FTA® card)</td>
<td>Blood</td>
<td>1736 ± 1009</td>
<td>100%</td>
</tr>
<tr>
<td>Protein Saver® 903 card</td>
<td>Blood</td>
<td>862 ± 516</td>
<td>100%</td>
</tr>
</tbody>
</table>
Direct amplification with PowerPlex® 16 HS

These results show that a single-amplification kit can be effective for both casework and database use.

Instrument support

**Cyclers:** Applied Biosystems GeneAmp® 9700, 9600, PerkinElmer 480

- 32-cycle protocol for highest sensitivity
- 30-cycle option (~1ng target) used by many laboratories
- Fewer cycles (~28) used for some database/paternity laboratories

**Capillary electrophoresis instruments**

- ABI PRISM® 310, 3100 and 3100-Avant Genetic Analyzers
- Applied Biosystems 3130 and 3130xl Genetic Analyzers
- **Applied Biosystems 3730 DNA Analyzer** (protocol by request)
Analysis on the Applied Biosystems 3730 Genetic Analyzer.

High throughput of the 3730 is valued for databasing and relationship testing. PowerPlex® 16 HS System 3730 protocol is available on request.
Validation

Validation has been completed and demonstrated that the PowerPlex® 16 HS System is reliable, robust and suitable for forensic use.

Developmental Validation

Philosophy

Contributors

Species Specificity

Sensitivity

Mixtures

Sizing precision

Stutter

Population Studies, Characterization of Loci & Supporting Articles

Concordance & Reproducibility

Case-type samples

Variation of cycle number

Variation of annealing temperature

Variation of primer concentration

Variation in magnesium concentration

Variation in enzyme concentration

Variation in reaction volume

Why perform developmental validation?

Manufacturers perform developmental validation studies to document that the assays are suitably robust for forensic use. These studies aim to document the following:

- Physical genetics of loci (linkage mapping)
- Population statistics (allele frequencies)
- Specificity, sensitivity and mixture studies
- Concordance with existing systems
- Robustness of assay design

Nature of the PowerPlex® 16 HS System validation

This developmental validation was community-driven by eight collaborating laboratories. The effort followed SWGDAM guidelines for developmental validation. These results have been submitted for publication and NDIS approval.

With general performance capabilities of the assay documented, the end-user laboratory can focus on proficiency and optimization.
Support publications

Population studies:

Characterization of Loci:


Original PowerPlex 16® System developmental validation, primer sequences and concordance:

Species specificity

To assess the impact of nonhuman DNA on analysis, 28 nonhuman DNAs were tested in PowerPlex® 16 HS reactions including:

1 fungus (*C. albicans*), 4 bacteria (*E. coli, E. faecalis, P. aeruginosa, S. Cervisea*), 1 bird (chicken), 10 nonprimate mammals (cat, cow, dog, dwarfhorse, goat, horse, rabbit and rat) and 12 primates (chimpanzee, gorilla, monkey and orangutan)

Some peaks smaller than amelogenin were observed in dog, cow, goat and horse. Primates displayed peaks throughout the assay range but in a pattern dissimilar to a human profile (off-ladder, partial profile, etc.). Results were similar to what has been reported for the PowerPlex 16 System.

**Contamination with forensically relevant, nonhuman DNA will not affect interpretation of PowerPlex 16 HS results.**
Sensitivity

For forensic DNA analysis, assay sensitivity is critical to detect low-concentration samples and evidence conservation. As part of developmental validation, the sensitivity of the PowerPlex® 16 HS System was verified.

Seven laboratories were provided with two single-source DNAs from which they generated a series of template concentrations by serial dilution. Six laboratories generated sensitivity data using a 32-cycle protocol, and three labs also generated data using a 30-cycle protocol. A significant amount of data was generated with \( \leq 125\text{pg} \).

Amplifications were performed using the Applied Biosystems GeneAmp® 9700, and 1µl of each reaction was analyzed on an Applied Biosystems 3130, 3130xl or an ABI PRISM® 310 Genetic Analyzer.
Sizing precision

Genotyping accuracy relies on consistent peak sizing. The Internal Lane Standard 600 (ILS 600) design incorporates the sequence of smaller fragments in each successive fragment. Consequently, the relative migration rates of the ILS 600 peaks are very consistent.

To demonstrate, 18 allelic ladder injections on the Applied Biosystems 3130x/Genetic allelic Analyzer and 17 allelic ladder injections on the ABI PRISM® 310 were analyzed. Average allele sizes showed a standard deviation of <0.07bp. This level of precision is excellent for typing microvariants and off-ladder alleles.

The average fragment size (bp) of each allele was plotted against the standard deviation observed across 18 ladders run during 18 injections on the same day on an Applied Biosystems 3130x/Genetic Analyzer.
Concordance

Consistency between STR typing kits is important when sharing offender profiles. To evaluate concordance, the United States National Institute of Standards and Technology (NIST) tested 282 samples with PowerPlex® 16 HS that were previously typed with the PowerPlex 16 System and shown to be concordant with other STR typing kits. As expected, given the primer sequences are unchanged from the original PowerPlex 16 System, concordance was demonstrated.

Reproducibility

To demonstrate reproducibility, five known samples were typed by six laboratories. All results were concordant. Additionally, two laboratories demonstrated that NIST Standard Reference Material (SRM) 2391b could be correctly typed with the PowerPlex 16 HS System.
**Case-type samples**

A key demonstration in developmental validation is the analysis of case-type samples. These re-analyses of previously typed samples illustrate the suitability of the new assay for casework application.

To demonstrate that the PowerPlex® 16 HS System is suitable for common evidence samples, three laboratories tested a total of 84 samples from 53 cases. These included mixture, single source and differentially extracted samples. Results were consistent with original findings in all cases except one where previous COfiler® data indicated a THO1 “9.3,9.3” and PowerPlex 16 HS indicated a THO1 “7,9.3”—a potential null allele. Rare null alleles are known to exist*, but search algorithms handle single-locus mismatches.

Based on these results, the PowerPlex 16 HS System can be used to accurately type casework samples.

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Variation of cycle number

Changes in PCR cycle number can produce significant changes in sensitivity and consequently provides means of significant flexibility.

To illustrate the impact of cycle number variation, two laboratories demonstrated that a reduction in cycle number can reduce signal while producing the same allele peaks (below). Additionally, two laboratories demonstrated that reduced cycle number can be used to attenuate signal from samples with excess DNA, such as washed FTA® punches (data not shown) and directly amplified punches.

Amplifications were performed using 125pg of DNA with the Applied Biosystems GeneAmp® 9700 for 28 cycles (top), 30 cycles (middle) or 32 cycles (bottom). One microliter of each reaction was concurrently analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 3-second injection.
Variation of annealing temperature

For validation, annealing temperature variation (60 ± 4°C) was examined to ensure the system’s design is robust. The data showed interpretable results 4°C below and 2°C above the recommended annealing temperature. This suggests that the assay design can tolerate minor temperature fluctuations.

Five different annealing temperatures were examined with amplifications using 500 pg of DNA and 32 cycles on the Applied Biosystems GeneAmp® 9700. One microliter of each reaction was concurrently analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection.
Validation > Variation of Primer Concentration

Variation of primer concentration

For validation, primer concentration variation was examined to ensure the system’s design is robust. The data show interpretable results with 0.75–1.5X primer concentration (with minor peak height imbalance). This suggests that the assay design can tolerate minor primer pipetting inaccuracy.

Five different primer concentrations were examined with amplifications using 500 pg of DNA and 32 cycles on the Applied Biosystems GeneAmp® 9700. One microliter of each reaction was concurrently analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection.
Variation in magnesium concentration

As a cofactor for *Taq* DNA polymerase, magnesium can impact yield and balance of a multiplex amplification. The effective concentration of magnesium can be affected by inadequate master mix thawing or mixing and chelation by sample impurities.

For validation, magnesium concentration variation was examined to ensure the system’s design is robust. The data show interpretable results with 0.75-1.5X magnesium concentration (minor peak height imbalance). This suggests that the assay design can tolerate minor fluctuation in magnesium concentration.

Five magnesium concentrations were examined with amplifications using 500 pg of DNA and 32 cycles on the Applied Biosystems GeneAmp® 9700. One microliter of each reaction was concurrently analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection.
Variation in enzyme concentration

For validation, *Taq* DNA polymerase concentration variation was examined to ensure the system’s design is robust. The data show interpretable results with 0.5–1.5X *Taq* concentration (with minimal peak height balance shifts).

This suggests that the assay includes ample enzyme to ensure robust amplification.

Five *Taq* DNA polymerase concentrations were examined with amplifications using 500 pg of DNA and 32 cycles on the Applied Biosystems GeneAmp<sup>®</sup> 9700. One microliter of each reaction was concurrently analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection.
Variation in reaction volume

Many laboratories consider reduced reaction volume as a cost-saving measure and a technique to increase signal with low input DNA quantity.

For validation, variation in reaction volume was examined to ensure the system’s design is robust. The tested reaction volumes were 6.25, 12.5, 15, 25 and 50µl reactions with 500, 250 and 100pg template per 25µl volume. Similar genotypes could be identified at all volumes. However, locus-to-locus imbalance was observed at the lowest volumes along with sister allele imbalance with low quantities of DNA input.
**Stutter**

Stutter peaks are commonly observed one repeat unit below the true allele. Documentation of locus-specific stutter rates can aid in interpretation of STR profiles.

For validation, amplification of 274 samples were analyzed for average stutter for each STR marker.
PowerPlex® 16 HS System developmental validation collaborators

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Mixtures

Mixture samples are common in forensic DNA analysis. Deconvolution of contributor profiles depends on detection of major and minor components. As part of developmental validation, the PowerPlex® 16 HS System was tested for the ability to identify minor contributor alleles from a mixture.

Six laboratories (five using 32-cycle amplification, one using 30-cycle amplification) examined two different mixture sets of known genotype, where the ratio of DNA in the sample varied from 1:19 to 19:1 (0.5 ng total DNA).

Detecting of a majority of minor contributor alleles was possible even with the most extreme mixtures.

Amplifications of two-person mixtures with 500 pg total DNA were done using the Applied Biosystems GeneAmp® 9700. One microliter of each reaction was analyzed on either an Applied Biosystems 3130, 3130xl or an ABI PRISM® 310 Genetic Analyzer.
PowerPlex® 16 HS System

Mixture example

High sensitivity allows exceptional detection of minor alleles.

Amplifications were performed using 32 cycles on the Applied Biosystems GeneAmp® 9700, and 1µL of each reaction was concurrently analyzed on an ABI PRISM® 3100-Avant™ Genetic Analyzer with 3kV, 5-second injection.