Promega

applicationnotes

PowerPlex®16 versus Identifiler® Systems

Sensitivity and Effects of Inhibitors

By Robert McLaren, Promega Corporation

Abstract

Analysis of DNA recovered from a casework environment can be fraught with difficulties, including DNA degradation, limiting amounts of DNA and the presence of PCR inhibitors. Overcoming these challenges requires the use of a sensitive STR amplification system that is relatively insensitive to the inhibitory effects of DNA contaminants common in casework samples. We examined the relative sensitivities of the single-amplification PowerPlex® 16 System and AmpFlSTR® Identifiler® PCR amplification kit and determined the effect of common PCR inhibitors on the ability of these kits to generate full profiles. The PowerPlex® 16 System was clearly more sensitive than the Identifiler® kit when amplifying picogram amounts of DNA and demonstrably less sensitive to PCR inhibitors.

Introduction

The PowerPlex® 16 System[a–d] and Identifiler® PCR amplification kit have been used by laboratories for many years to type single-source samples. The nature of these samples is such that DNA is typically not limited and PCR inhibitors are seldom an issue. However, as labs move to a single-amplification system for casework it becomes important to understand how these kits function in a situation where DNA may be limited and the DNA that is available may be contaminated with amplification inhibitors. To gain an understanding of how these kits compare in a casework environment, we examined the relative sensitivities of both kits to detect trace amounts of DNA and determined the effect that PCR inhibitors have on the ability of these kits to generate full profiles.

Materials and Methods

Single-source human genomic DNA was isolated from liquid human blood using the Tissue and Hair Extraction Kit (for use with DNA IQ™) (Cat.# DC6740) and DNA IQ™ System (Cat.# DC6700). Briefly, 20µl of liquid blood was incubated with 80µl of Incubation Buffer/Proteinase K Solution (prepared as per *Tissue and Hair Extraction Kit (for use with DNA IQ™) Technical Bulletin* #TB307) at 56°C for 1 hour. DNA was extracted using the Tecan Freedom EVO® 100 liquid handler and DNA IQ™ System following the protocol for aqueous samples (1). DNA concentration was determined using the PicoGreen® dsDNA quantitation reagent (Invitrogen). A twofold dilution series of DNA from 0.20ng/µl to 0.00312ng/µl was generated, and 10µl of each diluted DNA was amplified in triplicate in a 25µl reaction using the PowerPlex® 16 System (Cat.# DC6530) or AmpFlSTR® Identifiler® kit (Applied Biosystems Part# 4365489). PowerPlex® 16 amplifications were carried out using the GeneAmp® PCR System 9700 for 32 cycles (10/22 cycling) as per the *PowerPlex® 16 System Technical Manual* #TMD012. AmpFlSTR® Identifiler® amplifications were carried out using the GeneAmp® PCR System 9700 for 28 cycles as per the *AmpFlSTR® Identifiler® PCR Amplification Kit User's Manual*. Amplification products were detected using the Applied Biosystems 3130 Genetic Analyzer. Electrokinetic injection was carried out at 3kV for 5 seconds for both kits. Data were analyzed using GeneMapper® *ID* software, version 3.2, with a 50RFU analysis threshold and locus-specific stutter filtering.

For the inhibitor study, hematin, humic acid or a black denim extract was used as the inhibitor. A 1mM hematin stock was made in 0.025N NaOH, and dilutions of this stock were made in TE^{-4} buffer (final concentration in the amplification reactions was 1.0µM, 2.0µM, 5.0µM, 10µM, 20µM or 40µM). A 5mg/ml stock of humic acid was made in water, and dilutions of this stock were made in TE^{-4} buffer (the amount per 25µl amplification reaction was 100ng, 150ng, 175ng, 200ng, 225ng, 250ng or 300ng). To make a black denim extract, one 5 × 5mm square swatch of black denim was extracted using the ReadyAmp™ Genomic DNA Purification System (Cat.# A7710), a premade, easy-to-use slurry of Chelex® 100 resin, as described in the *ReadyAmp™ Genomic DNA Purification System Technical Bulletin* #TB190. The final volume of black denim extract was 200µl.

Sensitivity

Full profiles were obtained from 2ng down to 0.25ng of human genomic DNA using both the PowerPlex® 16 System and Identifiler® kit. However, the PowerPlex® 16 System generated full profiles with as little as 62.5pg of template DNA (Figure 1) and partial profiles with 31.25pg. The Identifiler® kit only yielded partial profiles at 62.5pg of DNA and did not yield any peaks above 50RFU in two out of the three replicates at 31.25pg of DNA; the third replicate had only one allele at the D19S433 locus with a peak height of 67RFU. For callable peaks above 50RFU with 62.5pg of DNA, the peak height range observed with the PowerPlex® 16 System was 76–669RFU, whereas that with Identifiler® was 52–85RFU, highlighting the strength of the PowerPlex® 16 System to amplify small amounts of DNA.

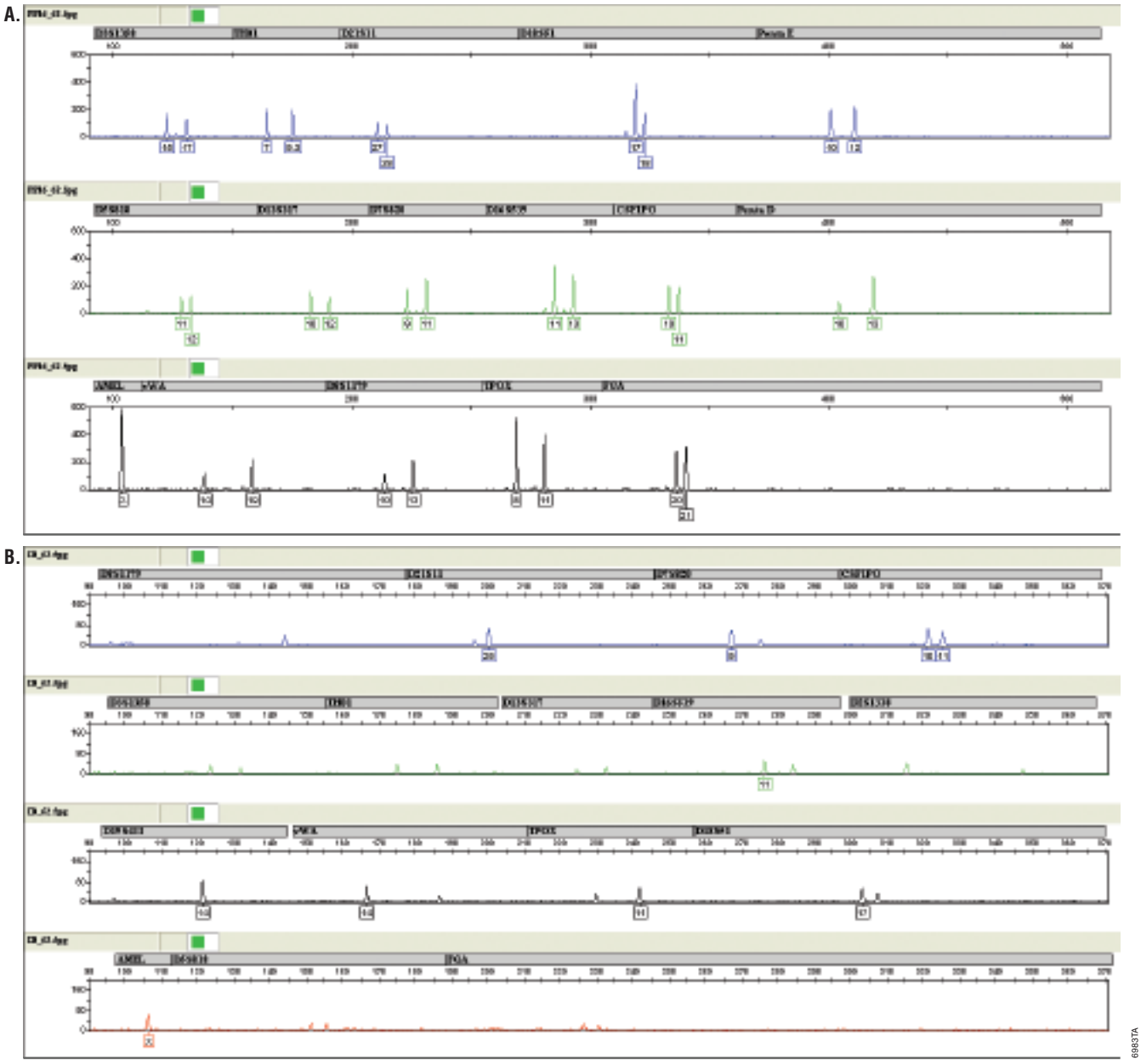

Figure 1. Representative amplifications of 62.5pg of DNA using the PowerPlex® 16 System and Identifiler® kit. Amplifications of 62.5pg of human genomic DNA were performed using the PowerPlex® 16 System (**Panel A**) and Identifiler® kit (**Panel B**) on the GeneAmp® PCR System 9700, and 1µl of each amplification was analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection.

A reduced peak height ratio was seen at 62.5pg of DNA at some loci (Figure 1). However, this is expected when amplifying at this low level of input DNA due to sampling error (stochastic effects). The same loci did not reproducibly show the same reduced peak height ratio in the triplicate amplifications, confirming this was a stochastic effect (Figure 2).

Inhibitors

Amplifications were performed with 1ng of human genomic DNA in 25µl PowerPlex® 16 or Identifiler® amplification reactions with increasing amounts of various PCR inhibitors. Both the PowerPlex® 16 System and Identifiler® kit were able to generate full profiles with up to 10µM hematin. However at 20µM hematin, the Identifiler® kit yielded callable

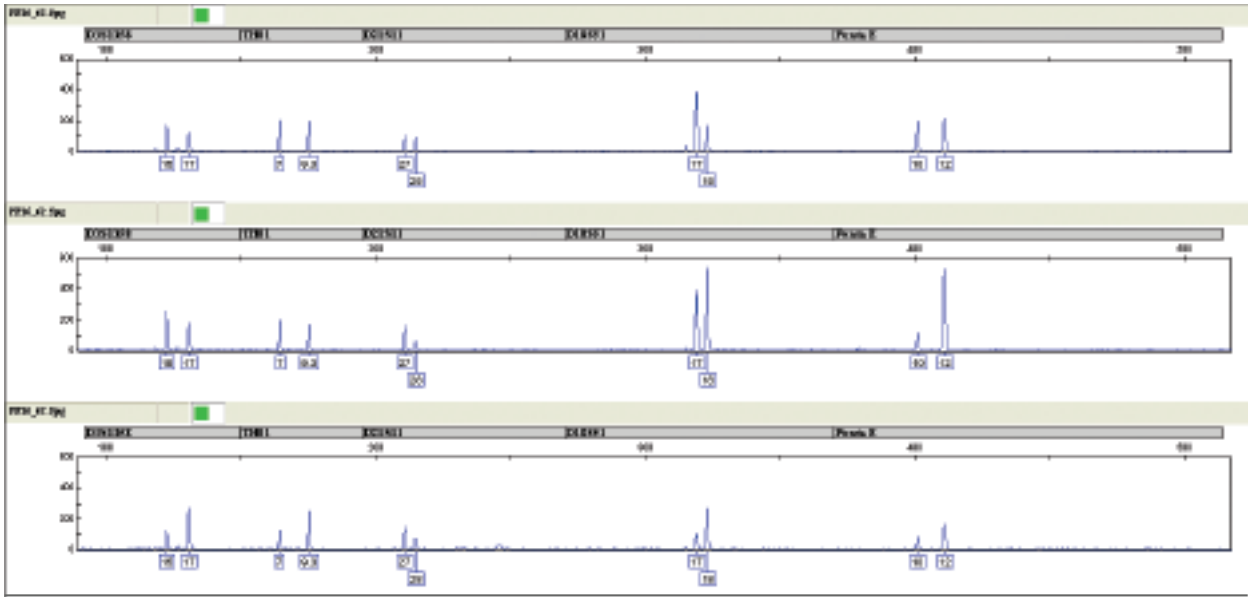

Figure 2. Stochastic variation in peak height ratio between triplicate amplifications. The fluorescein channel (blue) from triplicate amplifications of 62.5pg of human genomic DNA using the PowerPlex® 16 System is shown. As 62.5pg of DNA corresponds to roughly 10 cells, this amount of DNA is in the range where sampling error (stochastic effects) becomes an issue. Thus, sister allele balance with this amount of DNA can fluctuate significantly. Note the variation in peak height ratios between replicates at all five loci in this dye channel.

peaks at only D8S1179 and amelogenin (Figure 3, Panel B), whereas the PowerPlex® 16 System still allowed calls at 13 autosomal loci (Figure 3, Panel A) with dropout occurring at D18S11, Penta E and amelogenin. Peak heights observed with PowerPlex® 16 in the presence of 20µM hematin were considerably higher than those at D8S1179 and amelogenin with the Identifiler® kit. Control amplifications with 0.005N NaOH, the equivalent NaOH concentration of the 20µM hematin amplification, did not show any inhibition of the PowerPlex® 16 System or Identifiler® kit (data not shown).

Full profiles were detected in the presence of up to 250ng of humic acid per 25µl amplification reaction with PowerPlex® 16 but only up to 200ng for the Identifiler® kit. At 300ng of humic acid only the Penta D and Penta E loci dropped out with PowerPlex® 16, whereas the D7S820, CSF1PO, D13S317, D16S539, D2S1338, D18S51 and FGA loci dropped out with Identifiler® (Figure 4).

As some labs still extract DNA from casework stains with Chelex® resin, we decided to examine the effect of dye carryover from black denim, a common casework substrate, on amplification. Chelex® extractions were performed on a 25mm^2 section of black denim as described in Materials and Methods, and various volumes were spiked into 25µl amplification reactions with 1ng of human genomic DNA. In the presence of 1µl of black denim extract a full profile was obtained with PowerPlex® 16, whereas with Identifiler® one allele at D18S51 dropped out. However, the remaining alleles at the other loci had significantly reduced peak heights. With 2µl of extract, PowerPlex® 16 had callable alleles at 10 autosomal loci, whereas Identifiler® had callable alleles above the 50RFU threshold at only amelogenin. All autosomal loci had dropped out (Figure 5).

One interesting observation from the inhibition experiments was which PowerPlex® 16 loci tended to drop out first with increasing concentrations of PCR inhibitors. In addition to the larger loci, which one might expect to be more sensitive to inhibitors (e.g., Penta D, Penta E and D18S51), one of the first loci to drop out was amelogenin (Panel A of Figures 3, 4 and 5). Amelogenin is one of the smallest loci in the multiplex yet appears as sensitive to PCR inhibitors as larger loci such as the Penta loci. This observation has been confirmed with several other inhibitors (data not shown). Dropout of amelogenin may be useful as a guide to indicate the presence of PCR inhibitors in a sample as opposed to DNA degradation, as one would not expect amelogenin to drop out in a clean (i.e., devoid of inhibitors) degraded DNA sample.

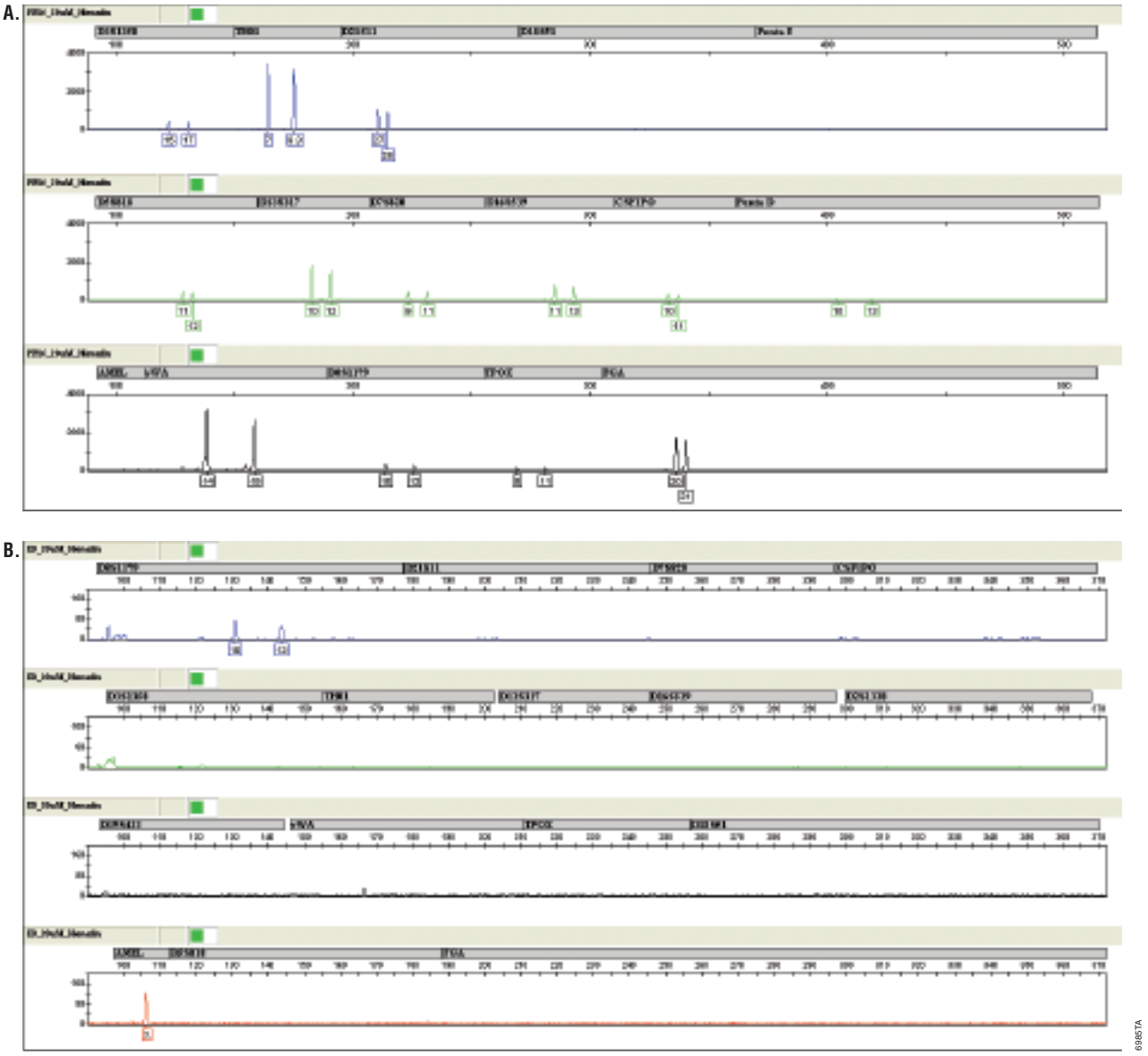

Figure 3. Effect of 20µM hematin on amplification of 1ng of human genomic DNA using the PowerPlex® 16 System and Identifiler® kit. Amplifications of 1ng of human genomic DNA were performed using the PowerPlex® 16 System (**Panel A**) and Identifiler® kit (**Panel B**) and the GeneAmp® PCR System 9700 in the presence of 20µM hematin, and 1µl of each amplification was analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection. Note the scale of the Y axis for the PowerPlex® 16 System is 4,000RFU, whereas that for the Identifiler® kit is 200RFU.

Conclusions

When amplified following the manufacturer's recommended cycling conditions and analyzed side by side on the same Applied Biosystems 3130 Genetic Analyzer, the PowerPlex® 16 System clearly demonstrated greater sensitivity than Identifiler® in generating profiles from trace amounts of DNA. This was due to the higher peak heights that were generated with the PowerPlex® 16 System compared to Identifiler® using the same mass of template DNA. In addition, the PowerPlex® 16 System was demonstrably less sensitive to the effects of PCR inhibitors, which enables more DNA-typing information to be obtained from impure template samples.

The combination of superior sensitivity and relative robustness in the presence of PCR inhibitors makes the PowerPlex® 16 System an excellent choice for a single-amplification system for casework. Moreover, dropout of amelogenin may be a useful tool to indicate when a sample is contaminated with PCR inhibitors and in need of further purification.

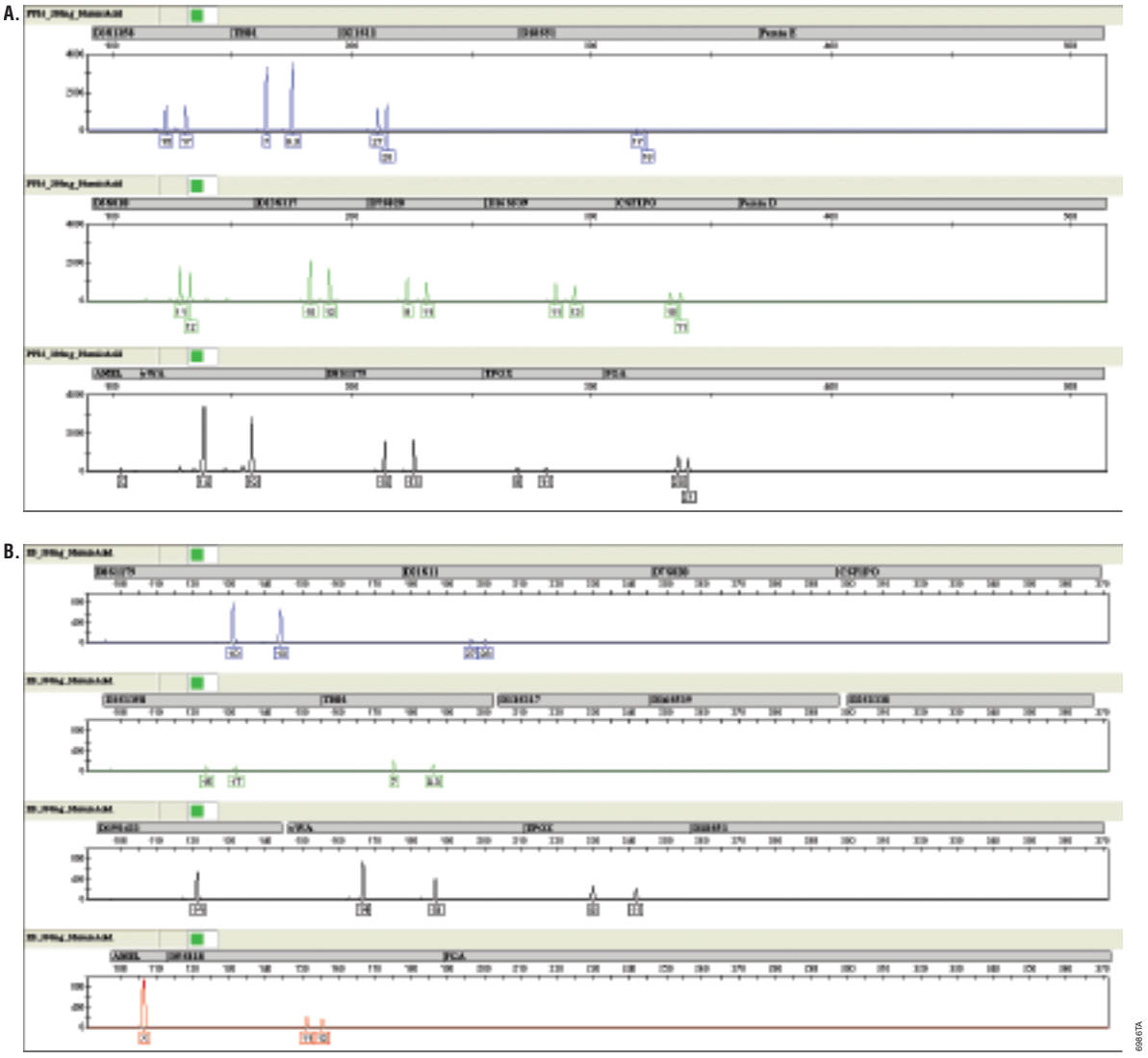

Figure 4. Effect of 300ng humic acid on amplification of 1ng of human genomic DNA using the PowerPlex® 16 System and Identifiler® kit. Amplifications of 1ng of human genomic DNA were performed using the PowerPlex® 16 System (**Panel A**) and Identifiler® kit (**Panel B**) on the GeneAmp® PCR System 9700 in the presence of 300ng humic acid, and 1µl of each amplification was analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection. Note the scale of the Y axis for PowerPlex® 16 System is 4,000RFU, whereas that for the Identifiler® kit is 1,000RFU.

Reference

1. Cowan, C. (2006) The DNA IQ™ System on the Tecan Freedom EVO® 100. *Profiles in DNA* **9** (1), 8–10.

Ordering Information

Product	Size	Cat.#
PowerPlex® 16 System*	100 reactions	DC6531
	400 reactions	DC6530
DNA IQ™ System**	100 reactions	DC6701
	400 reactions	DC6700
Tissue and Hair Extraction System (for use with DNA IQ™)**	100 reactions	DC6740

*Not for Medical Diagnostic Use.

**For Laboratory Use.

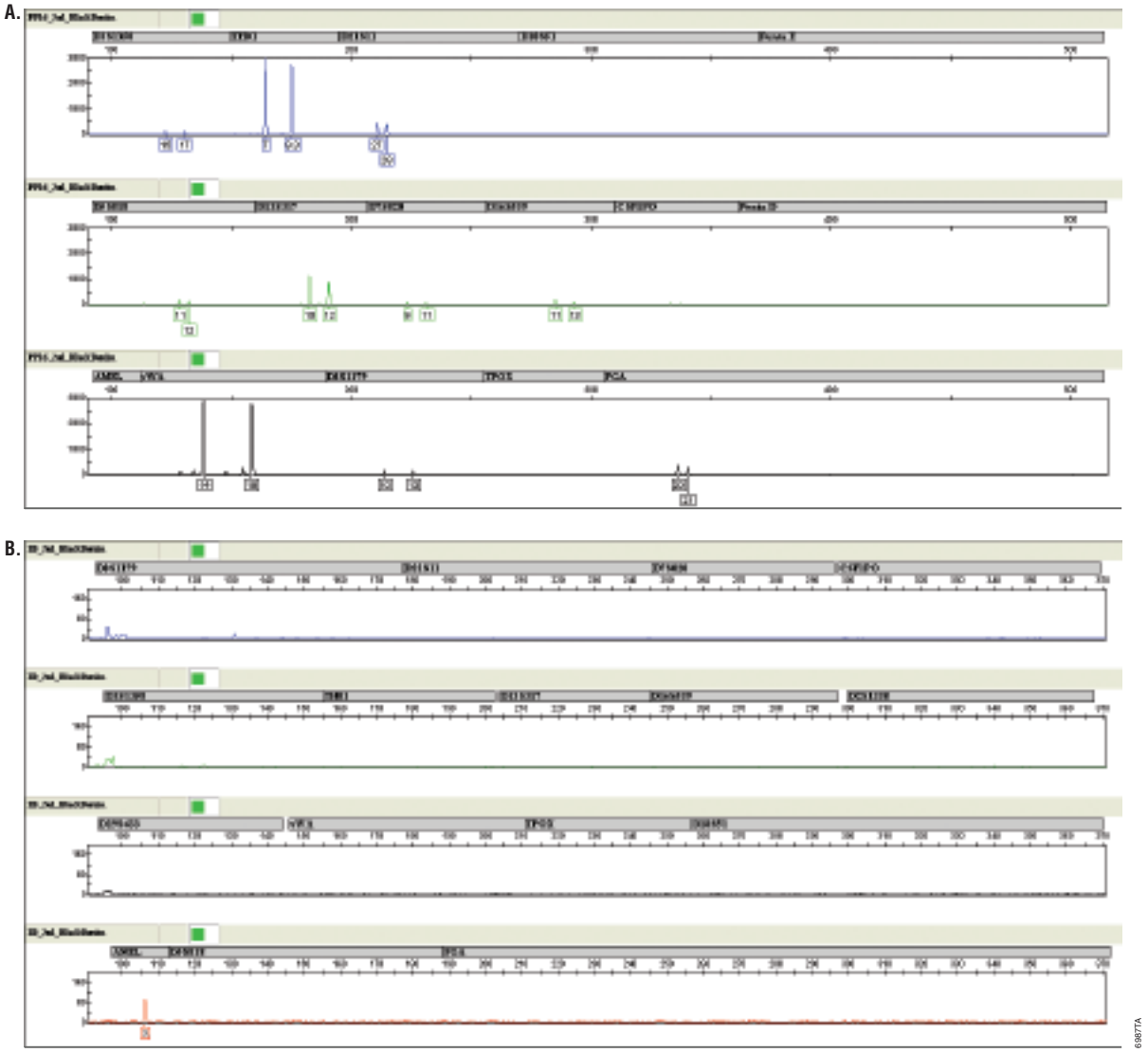

Figure 5. Effect of 2µl of black denim extract on amplification of 1ng of human genomic DNA with the PowerPlex® 16 System and Identifiler® kit. Amplifications of 1ng of human genomic DNA were performed using the PowerPlex® 16 System (**Panel A**) and Identifiler® kit (**Panel B**) and a GeneAmp® PCR System 9700 in the presence of 2µl of black denim extract, and 1µl of each amplification was analyzed on an Applied Biosystems 3130 Genetic Analyzer with a 3kV, 5-second injection. Note the scale of the Y axis for PowerPlex® 16 System is 3,000RFU, whereas that for the Identifiler® kit is 200RFU.

[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 and patents pending.

[d] 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.

PowerPlex is a registered trademark of Promega Corporation. DNA IQ and ReadyAmp are trademarks of Promega Corporation.

AmpFlSTR, GeneMapper and Identifiler are registered trademarks of Applera Corporation. AmpliTaq Gold and GeneAmp are registered trademarks of Roche Molecular Systems, Inc. Chelex is a registered trademark of Bio-Rad Laboratories, Inc. Freedom EVO is a registered trademark of Tecan AG Corporation. PicoGreen is a registered trademark of Molecular Probes, Inc.

Promega Corporation • 2800 Woods Hollow Road • Madison, WI 53711-5399 USA • Telephone 608-274-4330 • Fax 608-277-2601
www.promega.com
©2007 Promega Corporation. All Rights Reserved.
Prices and specifications subject to change without prior notice.
Printed in USA. REV.12/07
16108-AN-GI
Part #AN156