Validation of iPLEX-STR Multiplex PCR Kit for Single Source Samples.

Introduction

iPLEX-STR is a short tandem repeat (STR) multiplex PCR kit that interrogates 18 STR loci and the gender determination locus Amelogenin. The 18 STR loci include 13 CODIS STR loci (D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, and vWA) and 5 European standard STR loci (D1S1656, D2S441, D10S1248, D12S391, and SE33). iPLEX-STR PCR primers have been designed to generate amplification products between 75 and 420 bp and are labelled with blue, green, yellow and red fluorescent dyes. Sizing standards provided with the kit are labelled with an orange dye and contain 23 DNA fragments of 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 230, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440 and 450 bp in length.

All components of the iPLEX-STR kit, except for activator solution, are provided in lyophilised format, which allows storage of the kit at room temperature for one year without loss of sensitivity. Lyophilised PCR master mix includes primers and hot start polymerase and is pre- aliquoted into 0.2 mL PCR-reaction tubes. The reaction components are resolubilized by the addition of 5 µL of activator solution to each tube. For final PCR volume of 25 µL, up to 20 µL of DNA solution can be added per reaction. Full STR-profiles can be generated from as little as 100 pg of non-degraded genomic DNA in a full size (25 µL) PCR reaction. iPLEX-STR kit is very robust and can generate excellent results from 6 µL PCR reactions. Full STR-profiles can be generated from as little as 25 pg of purified non-degraded genomic DNA in a 6 µL PCR reaction.

iPLEX-STR allows direct amplification from unpurified TE-Chelex extracts of buccal swabs, essentially a direct PCR approach. iPLEX-STR kit contains an Internal Control that can help assess the presence of PCR inhibitors: the lyophilized master mix contains a set of blue dye labelled primers which will amplify a high molecular weight product (400 - 440 bp) whose presence and peak height correlate with the purity of the DNA extract.

iPLEX-STR kit is compatible with ABI PRISM® 310, 3100, 3130 and 3500 Genetic Analyzers. The raw data can be viewed in GeneScan Analysis Software (Applied Biosystems) and analysed with software packages like GeneMapper® *ID* Software (Applied Biosystems), GeneMarker® HID Software (Softgenetics), OSIRIS Software (Open Source Independent Review and Interpretation System by NCBI).

This study describes experiments that satisfy requirements for accuracy, precision and reproducibility when validating a new multiplex PCR kit for STR-DNA profiling of single source samples.

Materials and Methods

Samples tested and analyzed

10 DNA samples from National Institute of Standards & Technology (NIST) Standard Reference Material® 2391b, control DNA 2800M from Promega Corp., and 21 buccal swab samples from paternity casework.

iPLEX-STR Kit Components

Pre-PCR components:

12 strips of 8 x 0.2 ml reaction tubes containing lyophilised PCR primers and polymerase

Activation Solution	0.5 mL
Deionised Water	2 x 1.7 mL
Control DNA, lyophilised	1 tube

Post-PCR components:

Allelic Ladder, lyophilised	1 tube
Sizing Standard, lyophilised	1 tube

DNA Extraction from Buccal Swabs

One step DNA extraction from buccal swabs was performed using 5% TE-Chelex solution. TE-Chelex Solution was prepared as 5% w/v Chelex beads in TE^{-4} Buffer (10 mM Tris-HCl, 0.1 mM EDTA [pH 8.0]). 50 µL TE-Chelex solution was added to 0.6 mL tubes so that Chelex beads formed ~ 5 mm layer at the bottom of the tubes. Swab heads were placed in

the tubes with the TE-Chelex solution and the lids closed and secured with lid-locks. Swabs in Chelex were incubated at 100°C for 20 minutes. Following incubation, the DNA extracts were diluted 20-fold in TE^{-4} Buffer

PCR Amplification

PCR Amplification mix for 25 µL reaction:

Kit Component	**Volume per reaction**
Activator Solution	5 µL
Template DNA	up to 20 µL
Deionized Water to a final volume of	25 µL
Total Reaction Volume	**25 µL**

PCR Amplification mix for 6 µL reactions:

Kit Component	**Volume**
Activator Solution	5 µL
Deionized Water	20 µL
Master Mix Volume	**25 µL**

5 µL of the Master Mix were aliquoted into five PCR tubes and 1 µL of diluted DNA extract was added to each tube for a total reaction volume of 6 µL per PCR sample.

Thermal Cycling Protocol

94°C	98°C	59°C	72°C	94°C	59°C	72°C	90°C	59°C	72°C	68°C	4°C
3 min.	30 sec.	120 sec.	90 sec.	30 sec.	120 sec.	90 sec.	30 sec.	120 sec.	75 sec.	10 min.	hold
	4 cycles			6 cycles			20 cycles				

Electrophoresis and data analysis

Following PCR amplification, samples were run on ABI-310 and ABI-3100 *Avant* Genetic Analyzers (Applied Biosystems) and the data processed using GeneMarker® HID Software (Softgenetics).

Results and Discussion

Precision and reproducibility study

Capillary electrophoresis (CE) instruments are highly accurate and allow resolution of DNA fragments that differ by 1 bp in length. When a new multiplex PCR kit is evaluated, the allelic ladder of the kit can be used to assess precision and reproducibility of a CE instrument. Testing the allelic ladder is particularly relevant as it assesses not only the resolution of the instrument, but also confirms that alleles are correctly typed for all loci in the kit. Electrophoresis and analysis of multiple runs of allelic ladders for all capillaries of CE instrument should generate concordant results for all alleles in the allelic ladder. Particular attention should be devoted to the alleles that differ by 1bp in size. In iPLEX-STR allelic ladder such alleles are TH01 alleles 9.3 and 10, D12S391 alleles 18.3 and 19, D1S1656 alleles 14.3 and 15, 15.3 and 16, 16.3 and 17, 17.3 and 18, D2S441 alleles 11.3 and 12.

For each CE instrument, ABI 310 and ABI3100, five iPLEX-STR allelic ladder injections were analyzed. For the ABI-3100 *Avant* Genetic Analyzer with four capillaries, this translates to 20 allelic ladders that were analyzed (four capillaries x five injections). All five injections of the iPLEX-STR allelic ladder demonstrated clear resolution of the TH01 alleles 9.3 and 10, D12S391 alleles 18.3 and 19, D1S1656 alleles 14.3 and 15, 15.3 and 16, 16.3 and 17, 17.3 and 18, D2S441 alleles 11.3 and 12 on the ABI 310 and ABI 3100 CE instruments.

Concordance Studies to Assess Accuracy of iPLEX-STR DNA profiles

Accuracy of the iPLEX-STR multiplex PCR kit was assessed through a concordance study comparing STR-DNA profiles obtained with iPLEX-STR and those reported by National Institute of Standards & Technology (NIST) for 10 DNA samples from the NIST Standard Reference Material® 2391b. Standard Reference Material® 2391b DNA samples 1 through 10 were amplified in 6 µL iPLEX-STR PCR reactions and the thus obtained STR-DNA profiles were compared with those reported by NIST (1, 2). These experiments

demonstrated 100% concordance between STR-DNA profiles obtained with iPLEX-STR kit and those reported by NIST.

We also compared STR-DNA profile obtained with iPLEX-STR with those reported Promega Corporation for control DNA 2800M (3). Control DNA 2800M was amplified in a 6 µL iPLEXSTR PCR reaction and the iPLEX-STR generated STR-DNA profile was found to be 100% concordant with those reported by Promega Corporation (3).

We routinely extract DNA from buccal swabs in 5 % TE-Chelex solution and proceed with multiplex PCR amplification without further purification of these DNA extracts. In order to assessed the accuracy of iPLEX-STR DNA profiling with unpurified DNA samples extracted from buccal swabs in 5 % TE-Chelex solution, 21 buccal swabs were collected from paternity cases that were previously analyzed with Indetifiler® (Applied Biosystems) or PowerPlex® 16 (Promega Corp.) multiplex PCR kits. DNA samples from these buccal swabs were amplified using iPLEX-STR and analyzed as described in Materials and Methods. DNA-STR profiles for loci that are overlapping between iPLEXSTR and Indetifiler® / PowerPlex® 16 were found to be 100% concordant.

Sensitivity and Stability Studies

To determine sensitivity of iPLEX-STR PCR amplification kit, 400, 200, 100, and 50 pg of control DNA 9947A from Indentifiler® multiplex PCR kit were amplified in 25, 12.5 and 6.25 µL PCR reaction volumes. Two iPLEX-STR lots were used in these experiments: a 'fresh" lot (i.e., one month after manufacturing) and an 'old' lot (18 months after manufacturing). Activator solution and reaction tubes containing lyophilised PCR primers and polymerase of the older lot were kept at room temperature for 18 months prior to testing. Both kits generated full STR-DNA profile from as little as 100 pg template in 25 µL PCR reaction volume and 50 pg template in 12.5 and 6.25 µL PCR reaction volumes (see figs. 1 and 2).

Figures 1 and 2 demonstrate that allelic peak heights of samples amplified with the

lot stored for 18 months at room temperature were sufficiently robust (hundreds of RFU) such that all allele calls could be made with confidence. Because the final concentration of DNA is of course higher in the 6.25 µL PCR reaction volume, these data are the most robust.

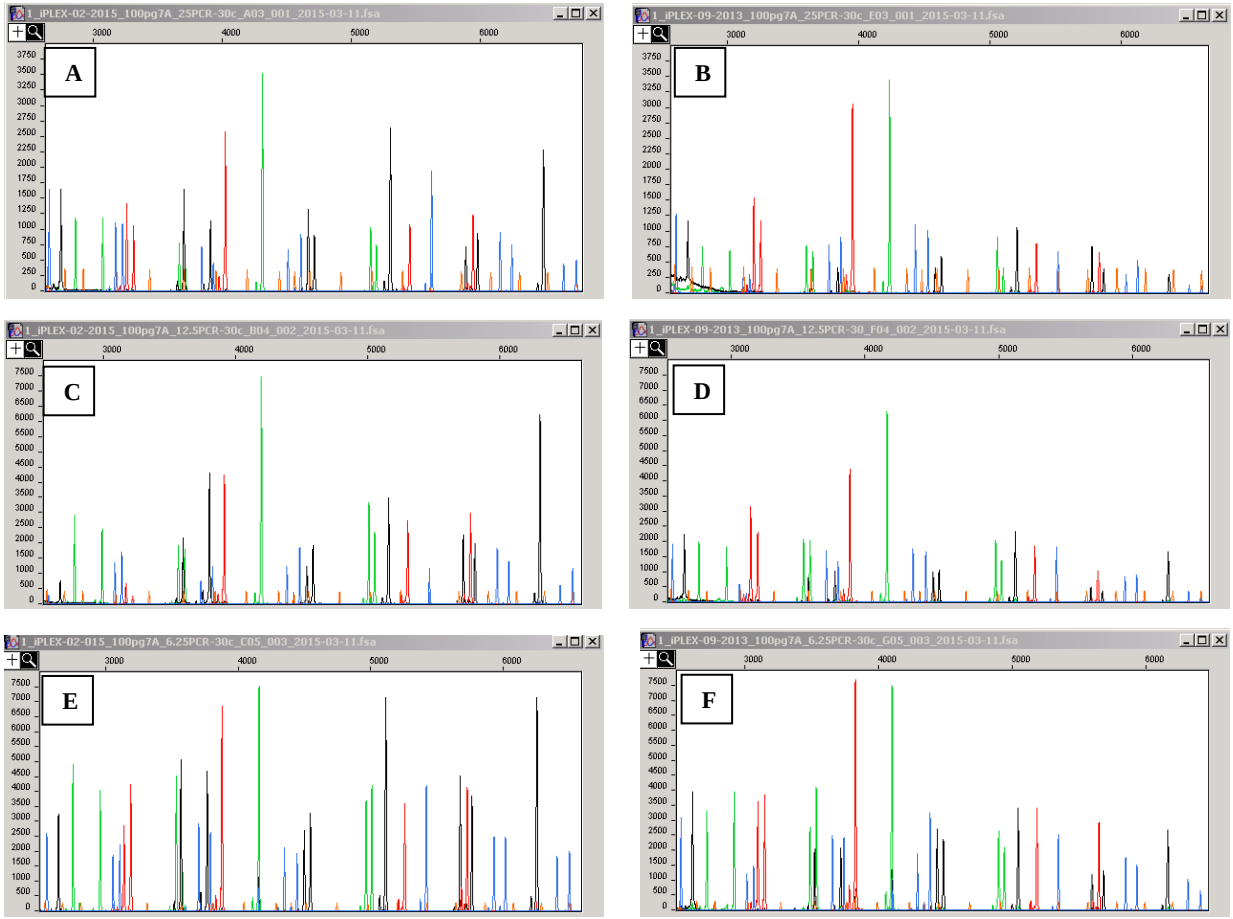

Figure 1. Stability of iPLEX-STR kit; 100 pg template DNA tested, ºRT Storage

A. 1 month-old lot, 100 pg control DNA 9947A, 25 µL PCR reaction volume.

B. 18 month-old lot, 100 pg control DNA 9947A, 25 µL PCR reaction volume.

C. 1 month-old lot, 100 pg control DNA 9947A, 12.5 µL PCR reaction volume.

D. 18 month-old lot, 100 pg control DNA 9947A, 12.5 µL PCR reaction volume.

E. 1 month-old lot, 100 pg control DNA 9947A, 6.25 µL PCR reaction volume.

F. 18 month-old lot, 100 pg control DNA 9947A, 6.25 µL PCR reaction volume.

Note vertical scale difference in panels.

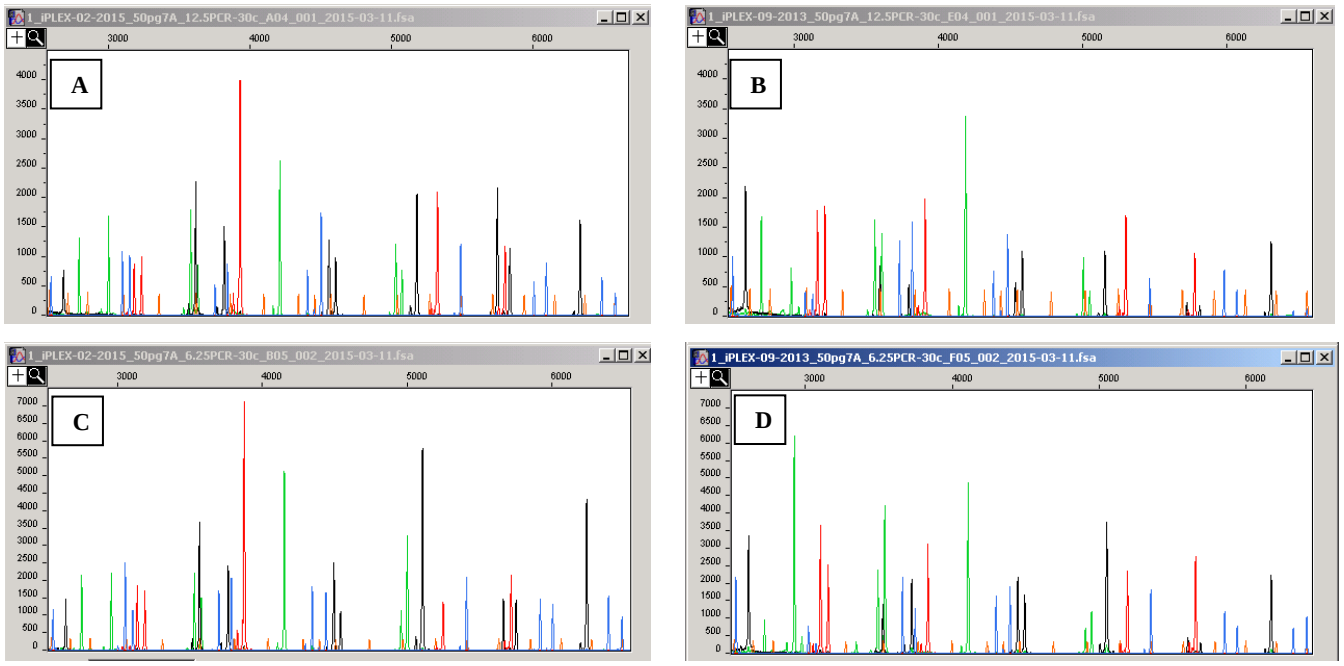

Figure 2. Stability of iPLEX-STR kit; 50 pg template DNA tested, ºRT Storage

A. 1 month-old lot, 50 pg control DNA 9947A, 12.5 µL PCR reaction volume.

B. 18 month-old lot, 50 pg control DNA 9947A, 12.5 µL PCR reaction volume.

C. 1 month-old lot, 50 pg control DNA 9947A, 6.25 µL PCR reaction volume.

D. 18 month-old lot, 50 pg control DNA 9947A, 6.25 µL PCR reaction volume.

Note vertical scale difference in panels.

Conclusion

This study demonstrates that the iPLEX-STR multiplex PCR kit can be successfully used to obtain STR-DNA profiles of single source DNA samples and that the ABI 310 and ABI 3100 *Avant* Genetic Analyzers used in this study passed validation criteria for accuracy, precision and reproducibility.

Additionally, this study demonstrates the excellent stability and sensitivity of the iPLEX-STR multiplex PCR kit as full profiles can easily be obtained from as little as 50 pg of purified template. These data also demonstrate the remarkable stability of the kit and all reagents as essentially unchanged DNA profiles were obtained after 18 months storage at room temperature.

References

1. Certificate of Analysis for PCR-Based DNA Profiling Standard,
Standard Reference Material® 2391b
National Institute of Standards & Technology. September 2008. Gaithersburg, MD

2. National Institute of Standards & Technology Update.
EDNAP and 30th ENFSI DNA WG Meeting, April 22-24, 2009 – Lisbon, Portugal
http://www.cstl.nist.gov/strbase/pub_pres/ENFSI-Apr2009-NIST-Update.pdf

3. New Control DNA for PowerPlex® Systems. Promega Corporation Web site.
https://www.promega.com/resources/profiles-in-dna/2011/new-control-dna-for-powerplex-systems/ Updated 2011. Accessed 04-17-2015.