Accepted Manuscript

Title: Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards

Author: Kathryn Oostdik Kristy Lenz Jeffrey Nye Kristin Schelling Donald Yet Scott Bruski Joshua Strong Clint Buchanan Joel Sutton Jessica Linner Nicole Frazier Hays Young Learden Matthies Amber Sage Jeff Hahn Regina Wells Natasha Williams Monica Price Jody Koehler Melisa Staples Katie L. Swango Carolyn Hill Karen Oyerly Wendy Duke Lesley Katzilierakis Martin G. Ensenberger Jeanne M. Bourdeau Cynthia J. Sprecher Benjamin Krenke Douglas R. Storts

PII: S1872-4973(14)00089-1
DOI: http://dx.doi.org/doi:10.1016/j.fsigen.2014.04.013
Reference: FSIGEN 1148

To appear in: *Forensic Science International: Genetics*

Received date: 25-3-2013
Revised date: 4-4-2014
Accepted date: 28-4-2014

Please cite this article as: K. Oostdik, K. Lenz, J. Nye, K. Schelling, D. Yet, S. Bruski, J. Strong, C. Buchanan, J. Sutton, J. Linner, N. Frazier, H. Young, L. Matthies, A. Sage, J. Hahn, R. Wells, N. Williams, M. Price, J. Koehler, M. Staples, K.L. Swango, C. Hill, K. Oyerly, W. Duke, L. Katzilierakis, M.G. Ensenberger, J.M. Bourdeau, C.J. Sprecher, B. Krenke, D.R. Storts, Developmental Validation of the PowerPlex*regd* Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards, *Forensic Science International: Genetics* (2014), http://dx.doi.org/10.1016/j.fsigen.2014.04.013

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

1 **Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference**
2 **Samples: A 24-locus Multiplex for New Database Standards.**

3 Kathryn Oostdik[1], Kristy Lenz[1], Jeffrey Nye[2], Kristin Schelling[2], Donald Yet[2], Scott Bruski[2], Joshua Strong[2],
4 Clint Buchanan[3], Joel Sutton[3], Jessica Linner[3], Nicole Frazier[3], Hays Young[4], Learden Matthies[5], Amber
5 Sage[5], Jeff Hahn[6], Regina Wells[7], Natasha Williams[8], Monica Price[8], Jody Koehler[9], Melisa Staples[10], Katie
6 L. Swango[10], Carolyn Hill[11], Karen Oyerly[12], Wendy Duke[12], Lesley Katzilierakis[12], Martin G. Ensenberger[1],
7 Jeanne M. Bourdeau[1], Cynthia J. Sprecher[1], Benjamin Krenke[1], and Douglas R. Storts[1]

8 [1]Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711

9 katie.oostdik@promega.com, kristy.lenz@promega.com, martin.ensenberger@promega.com,
10 jeanne.bourdeau@promega.com, benjamin.krenke@promega.com, cindy.sprecher@promega.com,
11 doug.storts@promega.com

12 [2]Michigan State Police, 333 S. Grand Ave. P.O. Box 30634, Lansing, MI 48909

13 NyeJ1@michigan.gov, schellingk@michigan.gov ,yetd@michigan.gov, bruskis@michigan.gov,
14 strongj@michigan.gov

15 [3]United States Army Criminal Investigation Laboratory, 4930 North 31st Street, Forest Park, GA 30297

16 This article does not constitute endorsement of any product by the U.S. Army Criminal Investigation
17 Laboratory, the Department of the Army, or the Department of Defense.

18 clint.buchanan1@us.army.mil, joel.d.sutton2.civ@mail.mil, jessica.k.linner.civ@mail.mil,
19 nicole.r.frazier.civ@mail.mil

20 [4]Arkansas State Crime Laboratory, 3 Natural Resources Drive, Little Rock, AR 72205

21 hays.young@crimelab.arkansas.gov

22 [5]Los Angeles County Sheriff's Department, 1800 Paseo Rancho Castilla, Los Angeles, CA 90032

23 LKMatthi@lasd.org , ARSage@lasd.org

24 [6]Kansas Bureau of Investigation, Forensic Laboratory Division, 1620 SW Tyler St. Topeka, KS 66612

25 jeff.hahn@kbi.state.ks.us

26 [7]Kentucky State Police, 100 Sower Blvd, Frankfort, KY 40601

27 regina.wells@ky.gov

28 [8]Washington State Patrol, Crime Laboratory Division, 2203 Airport Way S., Seattle, WA 98134-2028

29 Natasha.williams@wsp.wa.gov, monica.price@wsp.wa.gov

Accepted Manuscript

ACCEPTED MANUSCRIPT

30 [9]Texas Dept of Public Safety, Crime Laboratory, 5805 N Lamar Blvd., Austin, TX 78752

31 jody.koehler@dps.texas.gov

32 [10]New Hampshire State Police, Forensic Laboratory, 33 Hazen Drive, Concord, NH 03305

33 melisa.staples@dos.nh.gov, katie.swango@dos.nh.gov

34 [11]National Institute of Standards and Technology, Biochemical Science Division, 100 Bureau Drive,
35 Gaithersburg, MA 20899

36 becky.hill@nist.gov,

37 [12]Oklahoma State Bureau of Investigation, 800 East 2nd Street, Edmond , OK 73034

38 karen.oyerly@osbi.ok.gov, wendy.duke@osbi.ok.gov, Lesley.katzilierakis@osbi.ok.gov

39

40 Corresponding author:

41 Kathryn Oostdik

42 Katie.oostdik@promega.com

43 1-800-356-9526 ext 1207 (USA)

44 2800 Woods Hollow Road

45 Madison, WI 53711

46 USA

47 **Abstract: The original CODIS database based on 13 core STR loci has been overwhelmingly successful**
48 **for matching suspects with evidence. Yet there remain situations that argue for inclusion of more loci**
49 **and increased discrimination. The PowerPlex® Fusion System allows simultaneous amplification of**
50 **the following loci: Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317, Penta E, D16S539,**
51 **D18S51, D2S1338, CSF1PO, Penta D, TH01, vWA, D21S11, D7S820, D5S818, TPOX, DYS391, D8S1179,**
52 **D12S391, D19S433, FGA, and D22S1045. The comprehensive list of loci amplified by the system**
53 **generates a profile compatible with databases based on either the expanded CODIS or European**
54 **Standard Set (ESS) requirements. Developmental validation testing followed SWGDAM guidelines and**
55 **demonstrated the quality and robustness of the PowerPlex® Fusion System across a number of**
56 **variables. Consistent and high-quality results were compiled using data from twelve separate forensic**
57 **and research laboratories. The results verify that the PowerPlex® Fusion System is a robust and**
58 **reliable STR-typing multiplex suitable for human identification.**

59 **Keywords: forensic science, short tandem repeat, validation, PowerPlex(R)**

60

Accepted Manuscript

ACCEPTED MANUSCRIPT

60 **Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference**
61 **Samples: A 24-locus Multiplex for New Database Standards.**

62

63 **Introduction**
64 The discrimination power of STR technology is derived from the combination of allele calls at multiple
65 loci. By combining several independent loci, scientists can identify individuals precisely and with
66 significant supporting probabilities. The current US database, which is based on the CODIS 13 core STR
67 loci, has been overwhelmingly successful for matching suspects with evidence. Yet there remain
68 situations that argue for inclusion of more loci and increased discrimination. Additional loci would aid in
69 missing persons cases and distinguish family members in closely related communities. Furthermore,
70 with expanded locus overlap between multiple databases, global cooperation and data exchange would
71 be facilitated. Both the European and US forensic communities have taken steps towards these goals
72 with adoption of the European Standard Set (ESS) [1,2] and proposal of the expanded CODIS core loci
73 [3,4].
74
75 The PowerPlex® Fusion System allows simultaneous amplification of the loci: Amelogenin, D3S1358,
76 D1S1656, D2S441, D10S1248, D13S317, and Penta E labeled in fluorescein; D16S539, D18S51, D2S1338,
77 CSF1PO, and Penta D labeled in JOE; TH01, vWA, D21S11, D7S820, D5S818, TPOX, and DYS391 labeled in
78 TMR-ET; D8S1179, D12S391, D19S433, FGA, and D22S1045 labeled in CXR-ET. The system incorporates
79 the expanded CODIS--required loci plus the optional markers, Penta E, Penta D, D22S1045, and TPOX,
80 and addresses the updated ESS requirements [Supplemental Table 1]. Profiles generated using the
81 PowerPlex® Fusion System are compatible with databases founded on either CODIS or ESS
82 requirements. Based on current 5-dye technology, the system is compatible with the Applied
83 Biosystems® 3130 and 3500 Series Genetic Analyzer capillary electrophoresis instruments and does not
84 require upgrades to existing collection and analysis software versions. The PowerPlex® Fusion System
85 was developed to amplify both extracted DNA and DNA from solid support materials such as FTA® cards,
86 swabs, and nonFTA punches without compromising performance. This approach allows one system to
87 be used for both casework and database activities.
88
89 Developmental validation was performed to demonstrate the quality and robustness of the PowerPlex®
90 Fusion System across a number of variables. SWGDAM 2004 guidelines [5] were followed, although the
91 results also meet the 2012 guidelines [6]. Consistent and high-quality results were compiled using data
92 from twelve separate forensic and research laboratories including the Michigan State Police casework
93 and CODIS units, US Army Criminal Investigation Laboratory casework and database units, Arkansas
94 State Crime Laboratory, Kansas Bureau of Investigation, Texas Department of Public Safety, Kentucky
95 State Police, New Hampshire State Police, Washington State Patrol Crime Laboratory Division, Los
96 Angeles County Sheriff's Department, Oklahoma State Bureau of Investigation, National Institute of
97 Standards and Technology (NIST), and Promega Corporation. Studies evaluated robustness and
98 performance with case-type samples, sensitivity samples, inhibitors, mixtures, and several PCR
99 conditions. The conclusions reported here support the fact that the PowerPlex® Fusion System is
100 suitable for human identification in casework and database applications.
101

102 **Materials and Methods:**
103 Samples
104 Extracted DNA and solid support materials including FTA® card punches (GE Healthcare), cotton swabs,
105 and nonFTA Bode Buccal DNA Collectors™ (Bode Technology Group) were evaluated. Each laboratory
106 examined its own extracted DNA samples for the majority of the studies. However, the sensitivity,

Accepted Manuscript

ACCEPTED MANUSCRIPT

107 mixture, and reproducibility DNA samples were prepared by a single site and distributed. DNA was
108 purified by organic extraction for all studies except case-type samples, which were extracted using EZ1®
109 reagents and instrumentation (Qiagen) in addition to organic extraction. Unless otherwise noted, 500pg
110 of extracted DNA was amplified.
111
112 Laboratories evaluated buccal Indicating FTA® cards or Bode Buccal DNA Collectors™ from their own
113 collections for all studies outside of the reproducibility studies. For the reproducibility studies, single
114 donors collected a series of cotton swabs, Indicating FTA® cards, or Bode Buccal Collectors™ for
115 distribution to multiple sites. Single 1.2mm punches from buccal Indicating FTA® cards were added
116 directly to reactions. Swabs and nonFTA punches required a pretreatment step prior to addition into
117 amplification reactions. Buccal cotton swab samples were incubated in SwabSolution™ Reagent
118 (Promega Corporation) as directed in the technical manual [7], and 2µl of the resulting lysate was added
119 as the template to amplification reactions. Single 1.2mm nonFTA punches from Bode Buccal DNA
120 Collectors™ were treated with PunchSolution™ Reagent (Promega Corporation) as described in the
121 technical manual [8]. Once treated, PCR amplification mix was added to the well and amplification
122 performed.
123
124 The species cross–reactivity study was performed using a number of commercially available non-human
125 DNAs. Ten nanograms of each domestic animal or microbial species was amplified in duplicate. Species
126 samples included chicken, pig, mouse, bovine, cat, dog, rabbit, deer, horse, *Escherichia coli,*
127 *Enterococcus faecalis, Lactobacillus acidophilis, Streptococcus mutans, Staphylococcus epidermidis,*
128 *Micrococcus luteus, Fusobacterium nucleatum, Streptococcus salivarius, Streptococcus mitis,*
129 *Acinetobacter lwoffi, Pseudomonas aeruginosa, Candida albicans,* and *Saccharomyces cerevisiae*. Three
130 primate species, chimpanzee (male; Coriell Institute), macaque (male; Coriell Institute), and gorilla
131 (gender unknown; privately obtained), were evaluated using 500pg. The sensitivity study utilized two
132 DNA dilution series provided to all test sites. Test quantities included 500pg, 200pg, 100pg, and 50pg.
133 An inhibitor study evaluated hematin (Sigma-Aldrich), humic acid (Sigma-Aldrich), tannic acid (Sigma-
134 Aldrich), and EDTA (Sigma-Aldrich) titrations. Each inhibitor study site prepared its own extracted DNA,
135 inhibitor stocks and dilutions. Two mixture series, one male-male and one male-female, were prepared
136 and distributed. Mixture ratios included 0:1, 1:19, 1:9, 1:5, 1:2, 1:1, 2:1, 5:1, 9:1, 19:1, and 1:0 for each
137 series. The total template quantity was 500pg per reaction. Concordance was performed with
138 extracted DNA from 652 unrelated individuals from Caucasian, Hispanic, African-American, and Asian-
139 American ethnic groups. Reaction volume studies used 1.2mm punches of blood on Indicating FTA®
140 cards, in addition to buccal Indicating FTA® cards described previously.
141
142 DNA Amplification
143 Amplification reactions were performed at 25µl volumes on a GeneAmp® PCR System 9700 thermal
144 cycler using a 96-well silver or gold block and max ramp rates as described in the PowerPlex® Fusion
145 System Technical Manual [9], unless otherwise noted. The thermal cycling method for extracted DNA
146 samples was: 96°C for 1 minute; 30 cycles of 94°C for 10 seconds, 59°C for 1 minute, and 72°C for 30
147 seconds, followed by a 60°C final extension for 10 minutes and a 4°C soak. The cycle number and final
148 extension hold time was modified for solid support materials due to the substantial increase in template
149 amount with these materials. FTA® card punches were amplified for 27 cycles, swab lysates were
150 amplified for 27 or 25 cycles, and treated nonFTA punches were amplified for 25 or 26 cycles. All
151 amplification reactions with solid support substrates utilized a 20 minute final extension. Further cycle
152 number optimization was evaluated in a cycle number study. Within that study extracted DNA samples
153 were amplified for 29, 30, and 31 cycles, FTA® card punches for 26, 27, and 28 cycles, and treated
154 nonFTA punches for 25, 26, and 27 cycles. A reduced reaction volume study compared Bode Buccal DNA

ACCEPTED MANUSCRIPT

155 Collector™ punch amplification reactions in the presence or absence of AmpSolution™ Reagent
156 (Promega Corporation). Reactions with AmpSolution™ Reagent were assembled as described in the
157 Amplification Setup for AmpSolution™–Dependent PowerPlex® Systems section in the PunchSolution™
158 Kit Technical Manual [8]. Briefly, 5µl of water was replaced with 5µl of AmpSolution™ Reagent per
159 reaction.
160
161 Amplification Analysis
162 For sample detection 1µl of amplification product or allelic ladder was combined with 1µl of CC5 Internal
163 Lane Standard (Promega Corporation) and 10µl of HiDi™ Formamide (Life Technologies™). Samples
164 were denatured at 95°C and snap-cooled on ice for 3 minutes. Sample detection was performed using
165 the Applied Biosystems® 3130 and 3500 Series Genetic Analyzers and an Applied Biosystems® 3730 DNA
166 Analyzer (Life Technologies™). Spectral resolution for all three instruments was established on the G5
167 dye set using the PowerPlex® 5-Dye Matrix Standards, 3100/3130 (Promega Corporation). The 3130 and
168 3500 Series Genetic Analyzers were run using POP-4®polymer (Life Technologies™). However, the 3730
169 DNA Analyzer was run using POP-7™ polymer (Life Technologies™). All capillary electrophoresis
170 instruments used a 36-cm array. Injections on the 3130 Series Genetic Analyzer were performed at 3kV
171 for 5s, except a 1.5kV 5s injection was used in the reaction volume and cycle number studies to reduce
172 signal saturation. Additionally, an initial concordance study was performed using 1kV 3s injections and a
173 confirmatory concordance study used 2kV 5s injections. Injections on the 3500 Series Genetic Analyzer
174 were performed at 1.2kV for 10s or 24s. The stutter study, however, was conducted using a 1.2kV 18s
175 or 1.2kV 12s injection. The 3730 DNA Analyzer used a 3kV 5s injection.
176
177 Data analysis was performed using GeneMapper® *ID* Software version 3.2 or GeneMapper® *ID-X*
178 Software version 1.2 (Life Technologies™) with the PowerPlex® Fusion panel, bin, and stutter files
179 version 1.0. The minimum analytical threshold varied with instrumentation and test site. Validation
180 sites used previously validated minimum thresholds which were based on internal peak height
181 preferences and instrument performance. Thresholds from each validation site were preserved,
182 especially with sensitivity and mixture tests, to normalize the peak height preferences between sites. By
183 using analysis methods specific to individual data sets, the collective results are more consistent
184 between sites and more reflective of typical laboratory performance. In general, data collected on the
185 3500 Series Genetic Analyzer utilized a 175 RFU threshold, and the 3730 DNA Analyzer used a 100 RFU
186 threshold. The minimum threshold with the 3130 Series Genetic Analyzer varied from 50 to 175 RFU.
187 Any departures from these thresholds are listed below.
188
189 The species study used a 50 RFU threshold with 3130*xl* Genetic Analyzer data. The sensitivity study
190 employed a 50, 75, or 100 RFU threshold with the 3130 Series Genetic Analyzer data, a 50 RFU threshold
191 with the 3500 Genetic Analyzer data, and a 100 RFU threshold with the 3730 DNA Analyzer data. The
192 inhibitor study data from 3130 Series Genetic Analyzers was analyzed with a 50 RFU or 150 RFU
193 threshold. The reaction volume study used a 50 RFU threshold with 3130 Series Genetic Analyzer data.
194 Reproducibility testing employed 50, 150, or 175 RFU thresholds with 3130 Series Genetic Analyzer data.
195 Analysis of case-type samples used a threshold of 75 RFU with the 3130 Series Genetic Analyzer data
196 and 175 RFU with the 3500 Series Genetic Analyzer data. Mixture analysis utilized 50, 75, or 100 RFU
197 thresholds with the 3130 Series Genetic Analyzers data. The concordance studies used a 50 RFU
198 threshold with the 3130 Series Genetic Analyzer data.
199
200 **Results:**
201
202 Species Cross–Reactivity

Accepted Manuscript

ACCEPTED MANUSCRIPT

203 Cross-reactivity with environmental microbial species or other non-human species should be minimal to
204 ensure human data is not obscured. Multiple macro- and microorganism species DNAs were amplified
205 with the PowerPlex® Fusion System to demonstrate low cross-reactivity with non-human species. Ten
206 nanograms of each domestic animal or microbial species was amplified in duplicate for 30 cycles.
207 Species samples included chicken, pig, mouse, bovine, cat, dog, rabbit, deer, horse, *E. coli, E. faecalis, L.*
208 *acidophilis, S. mutans, S epidermidis, M. luteus, F. nucleatum, S. salivarius, S. mitis, A. lwoffi, P.*
209 *aeruginosa, C. albicans,* and *S. cerevisiae*. Three non-human primate species, chimpanzee (male),
210 macaque (male), and gorilla (gender unknown), were evaluated using 500pg.
211
212 No amplification products were detected with most domestic species or any of the microbial species
213 tested. Minimal peaks were observed with 10ng of chicken, pig, and mouse DNA, and those peaks were
214 located between panels or called off-ladder. Chicken DNA generated a peak in the JOE channel at
215 approximately 216 bases between the D18S51 and D2S338 panels. Pig DNA produced a peak in the JOE
216 channel at approximately 365 bases between the CSF1PO and Penta D panels. Lastly, mouse DNA
217 generated an off-ladder peak at approximately 180 bases in the fluorescein channel at D1S1656 [Figure
218 1]. As expected due to the genetic similarities between humans and other primates, the three non-
219 human primate samples generated multiple on and off-ladder peaks, although there were clearly
220 distinct from human profiles (data not shown).
221
222 Sensitivity Studies
223 To evaluate performance across a range of DNA quantities, five sites tested two extracted DNA dilution
224 series. Final quantities of 500pg, 200pg, 100pg, and 50pg were amplified in triplicate for 30 cycles.
225 Further data analysis was performed to assess the inter-allelic peak height ratios by dividing the
226 minimum heterozygous allele peak height by the maximum heterozygous allele peak height. Sample
227 detection was performed on 3130 and 3500 Series Genetic Analyzers and a 3730 DNA Analyzer.
228 Individual laboratory analysis thresholds were preserved to normalize peak height preferences and
229 instrument noise at each site.
230
231 DNA quantities of 100pg and greater allowed assignment of 100% of the expected alleles [Figure 2]. At
232 50pg, the percent alleles called dropped slightly to 97.2%. Drop out did not occur regularly at a
233 particular locus, but sporadically amongst loci. Similar sensitivity was observed on the 3130 and 3500
234 Series Genetic Analyzers and a 3730 DNA Analyzer.
235
236 Average peak height ratios were greater than 70% at all DNA quantities over 50pg, and equal to 70%
237 using 50pg [Figure 2]. A decrease in locus peak height ratio was seen with decreasing DNA quantity, as
238 seen with other STR systems (data not shown). The 3130 and 3500 Series Genetic Analyzers and the
239 3730 DNA Analyzer gave equivalent ratios.
240
241 Stability and Inhibitor Studies
242 Environmental inhibitors can compound the issue of obtaining profiles from low–level samples by
243 affecting amplification performance. Typical environmental and purification-related PCR-inhibitors,
244 hematin, humic acid, tannic acid, and EDTA, were titrated into PowerPlex® Fusion reactions containing
245 extracted DNA or FTA® card punches. Two validation sites evaluated performance using 3130 Series
246 Genetic Analyzers with a 3kV 5s injection.
247
248 Full, concordant profiles were obtained with hematin concentrations ≤1000µM using extracted DNA at
249 Site 1 and ≤500µM using extracted DNA or an FTA® card punch at Site 2 [Supplementary Figure 1]. With
250 humic acid, full profiles were generated with ≤200ng/µl using extracted DNA and ≤100ng/µl using FTA®

Accepted Manuscript

ACCEPTED MANUSCRIPT

251 card punches [Supplementary Figure 2]. Full profiles were generated with 100ng/µl to 300ng/µl tannic
252 acid using extracted DNA depending on test site and ≤300ng/µl using an FTA® card punch
253 [Supplementary Figure 3]. Lastly, full profiles were obtained with ≤0.4mM EDTA using either extracted
254 DNA or an FTA® card punch [Supplementary Figure 4]. Slight differences in inhibitory concentrations
255 were observed between sites. The results are likely due to variation in the creation and dilution of the
256 inhibitory compounds separately at each validation site. Because the compounds necessary for room-
257 temperature storage can cause PCR inhibition, reactions with FTA® card punches often generated partial
258 profiles at lower inhibitor concentrations than reactions with extracted DNA. However, in the EDTA
259 titration study reactions with FTA® card punches generated significantly more allele calls than reactions
260 with extracted DNA. Reactions with FTA® card punches commonly had higher peak heights than
261 reactions with extracted DNA, allowing more alleles to be called.
262
263 Reproducibility
264 A shared genotype database relies on consistent results between laboratories to generate matches.
265 Several materials were evaluated to demonstrate genotyping reproducibility and reliability. Five sites
266 evaluated panels of extracted DNA, buccal Indicating FTA® cards, buccal cotton swabs, and nonFTA Bode
267 Buccal DNA Collectors™ with three replicates for each sample. Samples were detected using 3130 and
268 3500 Series Genetic Analyzers or a 3730 DNA Analyzer.
269
270 Five sites evaluated the NIST SRM2391c PCR –Based DNA Profiling Standard samples A-D. Complete and
271 concordant profiles were gathered at each of the five test sites for all samples (n=72), except with
272 sample D. Sample D was a mixture sample with four alleles at D12S391: 18.3, 19, 22, and 23. All alleles
273 were consistently called except the 19 allele. Although the 19 allele resolved as a distinct shoulder on
274 the 18.3 allele peak, neither the GeneMapper® *ID* nor the GeneMapper® *ID-X* software called the minor
275 contributor 19 allele[Figure 3]. Similar resolution was seen across all replicates on the 3130 and 3500
276 Series Genetic Analyzers and a 3730 DNA Analyzer, and can be expected with closely-spaced minor
277 contributor alleles.
278
279 Complete and concordant profiles were gathered from multiple solid support substrates. All five buccal
280 cotton swab samples gave full and concordant profiles from both test sites (n=45). A complete and
281 concordant profile was seen for four buccal Indicating FTA® card samples and SRM2391c sample F (cells
282 spotted onto an FTA® card) at each of four test sites (n=70). Five nonFTA punches from four Bode
283 Buccal DNA Collectors™ and the SRM2391c sample E (cells spotted onto S&S 903 paper) gave full and
284 concordant profiles (n=54). Two of the sample sources, one FTA® card and one Bode Buccal DNA
285 Collector™, produced low peak heights at each evaluation site, presumably due to poor cell transfer
286 onto the surface or low shedding of buccal cells from the donor. Any partial profile samples were fully
287 concordant at all amplified loci.
288
289 Artifacts specific to the migration of PowerPlex® Fusion System amplification products on POP-7™
290 polymer were observed. Artifacts were labeled by the GeneMapper® *ID* Software, version 3.2, at
291 approximately 88 bases in the fluorescein channel and approximately 90 bases in the JOE channel. All
292 samples except allelic ladder contained the artifacts, including negative controls. Artifacts may be
293 reduced by performing sample electrophoresis immediately after amplification. These artifacts were
294 not observed on POP-4® polymer and are noted in the technical manual [9].
295
296 Case-type samples
297 Forensic casework samples represent a wide variety of sample quantity, background contaminants, and
298 biological sample types. Four validation sites evaluated a total of 76 case-type samples from their own

ACCEPTED MANUSCRIPT

299 collections [Table 1]. Samples were extracted from a variety of sources by organic and EZ1® extraction
300 methods. Detection was performed on either an Applied Biosystems® 3130 or 3500 Series Genetic
301 Analyzer, and data was analyzed with GeneMapper® ID-X software.
302

Sample type	Extraction	Full Profile	n
Blood from liquid sample, FTA® card, or Whatman™ paper	EZ1® , Organic	21	23
Cigarette butts	EZ1®	2	3
Buccal swab	EZ1®	7	10
Differential extractions (epithelial and sperm fractions from 2 samples)	EZ1®	4	4
Touch samples	EZ1®	4	12
Blood with soil	EZ1®	0	1
Case-type mixtures (<300pg input, multiple donors)	EZ1®	0	4
Leather with blood stain	EZ1®	0	1
Hair root	Organic	9	9
Saliva	Organic	7	9

303 Table 1. Case-type samples evaluated with the PowerPlex® Fusion System. Four validation sites
304 evaluated performance with 76 samples from their own collections. Complete, expected profiles were
305 collected with at least one sample within each category, except blood with soil, leather, and mixture
306 samples, which generated partial, expected profiles.
307
308 Full and concordant profiles were collected at each site from multiple sample types including blood,
309 cigarette butts, buccal swabs, differential extracts, touch samples, mixtures, hair, and saliva. Little to no
310 allelic drop out was observed when 500pg of DNA was amplified, and several samples with less than
311 200pg yielded full profiles. When partial profiles were generated, significant genotype information was
312 generally collected. Although clear amplification inhibition was observed in a reaction with 76pg of DNA
313 extracted from leather, information from 14 loci was retrieved [Supplemental Figure 5]. Amplification
314 was seen with all touch samples, and as expected, several contributors were detected. Samples known
315 to have multiple contributors produced allele calls consistent with the contributor profiles. Although no
316 single contributor profile was complete, three of four mixed samples produced significant profile
317 information with at least one allele at all autosomal loci using ≤210pg total template DNA [Supplemental
318 Figure 6]. In these partial profile case-type samples, allelic drop out occurred with the largest loci, TPOX,
319 D22S1045, DYS391, and Penta E, which are either less informative or not required by databases. Full or
320 significant partial profile information was successfully collected with typical case-type samples using a
321 range of template amounts.
322
323 Mixtures
324 To evaluate mixture detection performance, two mixture series were created and distributed, one male-
325 male and one female-male, at the ratios: 1:0, 19:1, 9:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:9, 1:19, 0:1. Five sites
326 amplified a total quantity of 500pg of DNA for 30 cycles. Alleles unique to the minor contributor were
327 counted and presented as a percentage of the total number of unique alleles expected (percent unique
328 alleles called).
329
330 Multiple contributors were detected with all mixture ratios at all five test sites. An average of 88% of
331 unique minor contributor alleles were detected in 1:9 mixture ratios and an average of 55% were
332 detected in 1:19 mixture ratios [Supplemental Figure 7]. The minor donor contribution in these samples
333 was 50pg and 25pg, respectively. Similar results were gathered with Applied Biosystems® 3130 and
334 3500 Series Genetic Analyzers. As the mixture ratio increased, the average number of alleles detected
335 decreased. These results are comparable to what has been reported with smaller, 16- and 17-locus

ACCEPTED MANUSCRIPT

336 multiplexes [10, 11], indicating that the addition of loci has not compromised performance for mixture
337 analysis.
338
339 Concordance
340 The primer sequences contained within the PowerPlex® Fusion System are highly conserved from
341 previously released systems such as the PowerPlex® ESI, 18D, and 21 Systems. These primer sequences
342 have demonstrated a high level of concordance during product use and concordance testing in previous
343 validation studies on the original CODIS 13 loci plus D2S1338, D19S433, Penta E and Penta D [12,13], and
344 the new ENSFI loci [10,11,14]. To further demonstrate concordance as a complete system, the National
345 Institute for Standards and Technology (NIST) performed an initial concordance study comparing
346 genotypes from 652 unrelated individuals using a pre-release PowerPlex® Fusion System to
347 commercially available PowerPlex® 16 HS and PowerPlex® 21 Systems and further compared to
348 AmpFLSTR® NGM™, Identifiler™, YFiler™, Profiler®, Minifiler™ and Sinofiler™ PCR Amplification Kits (Life
349 Technologies™), and Investigator® ESSplex Plus and IDplex Plus systems (Qiagen). At its commercial
350 release a minor change was made to the D16S539 primers. A confirmatory concordance study was
351 performed using a subset of 182 African-American samples. Samples were detected using an Applied
352 Biosystems® 3130 Series Genetic Analyzer with a 1kV 3s injection for the original sample set and 2kv 5s
353 injection for the confirmatory sample set.
354
355 Three discordant calls out of 39,198 alleles tested were observed at amelogenin, D7S820, and
356 D22S1045. No discordances were observed at D16S539 with the updated primers. One discordant
357 sample generated Y, Y results at amelogenin with the PowerPlex® Fusion System and all other systems
358 except Investigator® ESSplex Plus and IDplex Plus. In the second sample, sequencing confirmed 8 and 11
359 alleles at D7S820. The 8, 11 genotype was generated using the PowerPlex® 16 and Minifiler™ systems.
360 However, the PowerPlex® Fusion, Profiler®, Sinofiler™, and PowerPlex® 21 systems produced an 8, 9.3
361 genotype. A deletion is suspected between the primer binding sites of the two sets of systems. Finally,
362 a previously unknown discordance was observed at D22S1045. Well balanced 14, 17 alleles were
363 amplified using the PowerPlex® ESI and ESX Systems. In contrast, amplification using the PowerPlex®
364 Fusion System yielded a severely imbalanced 14 allele. The PowerPlex® Fusion System is suitable for
365 comparison with previously gathered profiles from multiple systems, as the observed discordances were
366 rare and unique.
367
368 Precision
369 Allele calls rely on similar migration between the sample and allelic ladder standard. Therefore,
370 migration and sizing precision must be consistent and within the bin window for accurate allele calls. To
371 demonstrate precision, allelic ladders were detected at five sites on Applied Biosystems® 3130 and 3500
372 Series Genetic Analyzers and an Applied Biosystems® 3730 DNA Analyzer. This study addressed typical
373 sources of variability such as differences between capillaries and injections. Standard deviations in
374 sizing were calculated for each allele.
375
376 The maximum standard deviation of an allele was 0.1 bases on the 3130*xl* and 3500*xL* Genetic Analyzers
377 [Figure 4, Supplemental Figure 8]. Comparable results were gathered between the four sites with all
378 genetic analyzers tested. The 3130 Genetic Analyzer and 3730 DNA Analyzer generated more variability
379 than the other instruments [Supplemental Figure 9]. The maximum standard deviation of any allele was
380 0.16 bases, observed at FGA with the largest alleles (44.2-50.2), on both instruments. The 0.5-base bin
381 window set by the bin file is greater than three standards deviations of either 0.1 or 0.16 bases, the
382 largest sizing variations observed. Sizing variability increased with locus and allele size. Those loci with

Accepted Manuscript

ACCEPTED MANUSCRIPT

383 the largest sizes; FGA, Penta D, DYS391, TPOX, and Penta E, had alleles with the greatest standard
384 deviations.
385
386 Stutter
387 Amplification of repeat sequences by DNA polymerases often produces slippage products one or more
388 repeat units shorter or larger than the true sequence length [15, 16]. Because the level of stutter
389 products as a percentage of the full–length allele products remains roughly constant, filters can be
390 constructed to remove allele calls on stutter position peaks below that stutter percentage. To calculate
391 the average observed stutter for each locus, 116 unrelated genomic DNAs were amplified with the
392 PowerPlex® Fusion System for 30 cycles. Samples were detected using an Applied Biosystems® 3500xL
393 Genetic Analyzer using a 1.2kV 18s or 1.2kV 12s injection. A peak height ratio of the stutter peak height
394 to the allele peak height was calculated. To ensure accurate calculation of the true stutter ratio, allele
395 peak heights greater than 30,000 RFU and less than 175 RFU were removed from the data set. Stutter
396 peaks that resided between two true alleles two repeats apart (e.g., 8, 10) were removed as well. Peaks
397 in this position are often inflated due to the additive effect of minus and plus stutter peaks migrating at
398 the same size.
399
400 The stutter filter for the GeneMapper® *ID* and *ID-X* files is set as the mean stutter ratio at each locus plus
401 three standard deviations. The GeneMapper® ID-X stutter file includes filters for plus stutter for the
402 trinucleotide repeat locus D22S1045 and the n–2 peak seen with D1S1656. The highest stutter
403 percentages were seen with D12S391 and D1S1656, and the stutter ratio increased with increasing
404 repeat number. The stutter data and summary are presented in Supplemental Table 2 and 3.
405
406 Reaction Volume
407 Laboratories commonly reduce reaction volume for cost-saving purposes. Although recent STR system
408 improvements have allowed the use of a variety of solid support substrates containing inhibitory
409 chemicals, amplification reactions using these materials with reduced reaction volumes can be
410 negatively affected. Results with reduced reaction volumes of 12.5µl and 6.25µl were compared with
411 results with standard 25µl reaction volumes using extracted DNA, 2µl cotton swab lysate, FTA® card
412 punches, and nonFTA punches. A further study compared results with one and two punches of buccal
413 and blood FTA® card in 25µl, 12.5µl, and 6.25µl reaction volumes. Samples were detected using Applied
414 Biosystems® 3130 Series Genetic Analyzers with a 3kV 5s injection.
415
416 Full profiles were generated for all extracted DNA and swab lysates at 25µl, 12.5µl, and 6.25µl reaction
417 volumes [Figure 5]. Little variability was observed. Extracted DNA and swab lysates are homogenous
418 and therefore sampling did not contribute to variability.
419
420 Successful amplification was achieved using one buccal FTA® card punch in 25µl and 12.5µl reaction
421 volumes [Figure 5]. Over 90% of the alleles were called at volumes 12.5µl and greater. Reaction
422 volumes of 6.25µl showed a significant decrease in allele calls and a sharp rise in reaction failures.
423 Sampling variability was observed with this substrate.
424
425 In a more comprehensive examination of FTA® card samples, successful amplification was observed in
426 12.5µl reactions using one blood FTA® card punch. Two punches from either buccal or blood FTA® cards
427 in 12.5µl reactions regularly allowed successful amplification; however, allele drop out and amplification
428 failures increased compared to reactions using one punch [Supplemental Figure 10]. With 6.25µl
429 reaction volumes less than 50% of the expected alleles were called with one or two punches of buccal or
430 blood FTA® cards. Amplification was unreliable and several complete failures were seen.

Accepted Manuscript

ACCEPTED MANUSCRIPT

431
432 In initial testing PunchSolution™-treated nonFTA punches demonstrated a clear reduction in percent
433 alleles called in 12.5µl reactions, and no amplification was observed with 6.25µl reaction volumes. The
434 lytic chemicals in the PunchSolution™ Reagent presumably overwhelmed the reactions with significantly
435 reduced reaction volumes. Further testing was performed with AmpSolution™ Reagent to improve
436 amplification of nonFTA punches in reduced volume reactions. PunchSolution™-treated nonFTA
437 punches were amplified in the presence and absence of AmpSolution™ Reagent at a reaction volume of
438 25µl, 12.5µl, or 6.25µl. The percentage of alleles called was significantly increased at 12.5µl and 6.25µl
439 reaction volumes in the presence of AmpSolution™ Reagent compared to reactions amplified without
440 AmpSolution™ Reagent [Figure 6].
441
442 Cycle Number
443 The amount of amplifiable DNA on solid support materials can vary widely, and therefore results can
444 benefit from cycle number optimization. Three sites examined extracted DNA, FTA® card punches, or
445 nonFTA punches from their own collections with varying cycle numbers. Extracted DNA was evaluated
446 using 29, 30, and 31 cycles; FTA® card punches using 26, 27, and 28 cycles; and nonFTA punches using
447 25, 26, and 27 cycles. Samples were detected using Applied Biosystems® 3130 and 3500 Series Genetic
448 Analyzers using 3kV 5s and 1.2kV 24s injections, respectively. A 1.5kV 5s injection on an Applied
449 Biosystems® 3130 Series Genetic Analyzer was used with one donor to reduce signal saturation.
450
451 Full profiles were detected for extracted DNA and nonFTA punches at all cycle numbers tested. FTA®
452 card punches generated full profiles at both 27 and 28 cycles. At the lowest cycle number tested, 26
453 cycles, 98% of alleles were called; eleven of the twelve FTA® card punches yielded full profiles, while one
454 yielded only a partial profile. This sample gave exceptionally low signals compared to the other two
455 replicates from the same donor's FTA® card. With all substrates, peak heights rose steadily with each
456 additional cycle, as expected, and signals were often saturated at the highest cycle number tested.
457 Signal strength with increasing cycle number using solid support materials was highly variable but
458 collectively resulted in signal increases similar to extracted DNA. Robust amplification was observed
459 using cycle numbers lower than suggested at multiple sites, demonstrating the recommended cycle
460 numbers can accommodate a range of material sources.
461
462 When following the recommended template quantity and cycle numbers, artifacts in D18S51 at 214
463 bases, TH01 at 76 bases, and D12S391 at 176–180 bases commonly remain under the minimum
464 threshold. Increased sample signal, particularly at high cycle number, directly correlated with an
465 increase in the incidence of called artifacts and artifact peak height.
466
467 Annealing Temperature
468 Departures from the optimal annealing temperature can reduce yields or generate artifacts which can
469 affect data interpretation. Annealing temperatures 2°C above and below the recommended annealing
470 temperature of 59°C were evaluated by amplifying extracted DNA and FTA® card punches. Samples
471 were detected using an Applied Biosystems® 3130 Series Genetic Analyzer with a 3kV 5s injection.
472
473 Full profiles were observed for extracted DNA and FTA® card punches at all temperatures tested: 57°C,
474 59°C, and 61°C. A slight increase in artifacts was observed at 57°C, two degrees below the
475 recommended annealing temperature. An off-ladder artifact in D18S51 at 214 bases and an artifact in
476 D12S391 at 180 bases were observed only in extracted DNA samples [Supplemental Figure 11]. These
477 artifacts were below the 50 RFU minimum analytical threshold at 59°C but, at 57°C, increased slightly to
478 rise above the threshold.

Accepted Manuscript

ACCEPTED MANUSCRIPT

479
480 Magnesium Titration
481 Extensive master mix optimization was performed during development to achieve robust amplification
482 without the introduction of nonspecific artifacts. However, a number of inhibitors and common
483 template storage buffers can affect the available magnesium within a reaction. To determine the effect
484 of magnesium concentration on sample results, extracted DNA, cotton swab lysate, FTA® card punches,
485 and nonFTA punches were tested at magnesium concentrations 20% above and below the commercial
486 formulation. Detection was performed using an Applied Biosystems® 3130 Series Genetic Analyzer with
487 a 3kV 5s injection.
488
489 Full profiles were generated at ±20% magnesium concentrations for extracted DNA and swab lysates.
490 Full profiles were observed with FTA® card punches using 1X and +20% magnesium concentrations and
491 with PunchSolution™-treated nonFTA samples using 1X and –20% magnesium concentrations
492 [Supplemental Table 4]. In reactions with FTA® card punches and decreased magnesium, 99% of alleles
493 were called. The D22S1045 alleles dropped out in one of the six FTA® card punch replicates. In the
494 nonFTA punch reactions with a +20% magnesium concentration, 99% of alleles were called, with one of
495 the six replicates yielding low peak heights compared to the other replicates which caused the DYS391
496 allele to drop out.
497
498 Minimal artifacts were observed with increased magnesium concentration. Reactions with swab lysates
499 and nonFTA punches showed no additional artifacts with increased magnesium. Extracted DNA and one
500 of two FTA® card donors produced a low-level artifact in D12S381 at 180 bases in the +20% samples that
501 was not present in the 1X magnesium reactions. FTA® card punches from two donors generated a low-
502 level off-ladder artifact in D18S51 at 185 bases that was observed with increased magnesium (data not
503 shown).
504
505 Primer Titration
506 To determine the effect of primer concentration changes on the PowerPlex® Fusion System results,
507 extracted DNA and FTA® card punches were evaluated with primer concentrations 25% above and
508 below the recommended concentration. Samples were detected using an Applied Biosystems® 3130
509 Series Genetic Analyzer with a 3kV 5s injection.
510
511 Full profiles were generated with both extracted DNA and FTA® card punches at all primer
512 concentrations tested. Little impact was seen on peak heights with variation in primer concentration,
513 and no discrete artifact peaks developed. However, a 25% increase in primer concentration created
514 more minus A product in reactions with extracted DNA than reactions with the recommended primer
515 concentration. This effect was not as pronounced using FTA® card punches.
516
517 **Conclusions:**
518 The PowerPlex® Fusion System was developed for human identification STR analysis of casework and
519 reference samples using extracted DNA and solid support substrates. Following SWGDAM and NDIS
520 validation guidelines, twelve forensic and research laboratories demonstrated strong performance
521 throughout validation testing for the PowerPlex® Fusion System. Minimal cross-reactivity, low-level
522 sensitivity and mixture detection, precise and accurate allele calls, and robust performance with
523 casework samples and in the presence of inhibitors were observed. Strong amplification and minimal
524 artifacts were generated under several suboptimal PCR conditions. Multiple DNA sources encompassing
525 extracted DNA, FTA® card punches, swabs, and nonFTA punches produced full and concordant profiles.
526 These validation results verify that the PowerPlex® Fusion System is a robust and reliable STR-typing

Accepted Manuscript

ACCEPTED MANUSCRIPT

527 multiplex suitable for human identification.

Accepted Manuscript

ACCEPTED MANUSCRIPT

References:

[1] Council of the European Union, Draft Council Resolution on the exchange of DNA analysis results, The Public Register of Council Documents, Brussels, 15870/09 ENFOPOL 287 CRIMORG 170 (2009) 1–7. http://register.consilium.europa.eu/pdf/en/09/st15/st15870.en09.pdf Accessed February 19, 2013.

[2] L.A. Welch, P. Gill, C. Phillips, R. Ansell, N. Morling, W. Parson, J.U. Palo, I. Bastisch, European Network of Forensic Science Institutes (ENFSI): Evaluation of new commercial STR multiplexes that include the European Standard Set (ESS) of markers, Forensic Sci. Int. Genet. 6 (2012) 819–826.

[3] D.R. Hares, Expanding the CODIS core loci in the United States, Forensic Sci. Int. Genet. 6 (2012) e52–e54.

[4] D.R. Hares, Addendum to expanding the CODIS core loci in the United States. Forensic Sci. Int. Genet. 6 (2012) e135.

[5] Scientific Working Group on DNA Analysis Methods (SWGDAM), Revised Validation Guidelines, Forensic Sci. Commun. 6 (2004). http://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/july2004/index.htm/standards/2004_03_standards02.htm Accessed September 15, 2012.

[6] Scientific Working Group on DNA Analysis Methods (SWGDAM), Validation Guidelines for DNA Analysis Methods. http://swgdam.org/SWGDAM_Validation_Guidelines_APPROVED_Dec_2012.pdf Accessed February 19, 2013.

[7] Promega Corporation, SwabSolution™ Kit Technical Manual, TMD037, Revision 6/12. http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Manuals/101/SwabSolution%20Kit%20Protocol.pdf

[8] Promega Corporation, PunchSolution Kit Technical Manual, TMD038, Revision 5/12. http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Manuals/101/PunchSolution%20Kit%20Protocol.pdf

[9] Promega Corporation, PowerPlex® Fusion System Technical Manual, TMD039, Revision 10/12. http://www.promega.com/~/media/Files/Resources/Protocols/Technical%20Manuals/101/PowerPlex%20Fusion%20System%20Protocol.pdf

[10] V.C. Tucker, A.J. Hopwood, C. J. Sprecher, R.S. McLaren, D.R. Rabbach, M.G. Ensenberger, J.M. Thompson, D.R. Storts, Developmental validation of the PowerPlex® ESX 16 and PowerPlex® ESX 17 Systems, Forensic Sci. Int. Genet. 6 (2012) 124–131.

[11] V.C. Tucker, A.J. Hopwood, C. J. Sprecher, R.S. McLaren, D.R. Rabbach, M.G. Ensenberger, J.M. Thompson, D.R. Storts, Developmental validation of the PowerPlex® ESI 16 and PowerPlex® ESI 17 Systems: STR multiplexes for the new European standard, Forensic Sci. Int. Genet. 5 (2011) 436–448.

[12] M.G. Ensenberger, J. Thompson, C.R. Hill, K. Homick, V. Kearney, K.A. Mayntz-Press, P. Mazur, A. McGuckian, J. Myers, K. Raley, S.G. Raley, R. Rothove, J. Wilson, D. Wieczorek, P.M. Fulmer, D.R. Storts, B.E. Krenke. Developmental validation of the PowerPlex® 16 HS System: an improved 16-locus fluorescent STR multiplex. Forensic Sci Int Genet. 4 (2010) 257-64.

[13] K. Oostdik, J. French, D. Yet, B. Smalling, C. Nolde, P.M. Vallone, E.L. Butts, C.R. Hill, M.C. Kline, T. Rinta, A.M. Gerow, S.R. Allen, C.K. Huber, J. Teske, B.E. Krenke, M.G. Ensenberger, P.M. Fulmer, C. Sprecher. Developmental validation of the PowerPlex®18D System, a rapid STR multiplex for analysis of reference samples. Forensic Sci Int Genet. 7 (2013) 129-35.

[14] C.R. Hill, D.L. Duewer, M.C. Kline, C.J. Sprecher, R.S. McLaren, D.R. Rabbach, B.E. Krenke, M.G. Ensenberger, P.M. Fulmer, D.R. Storts, J.M. Butler. Concordance and population studies along

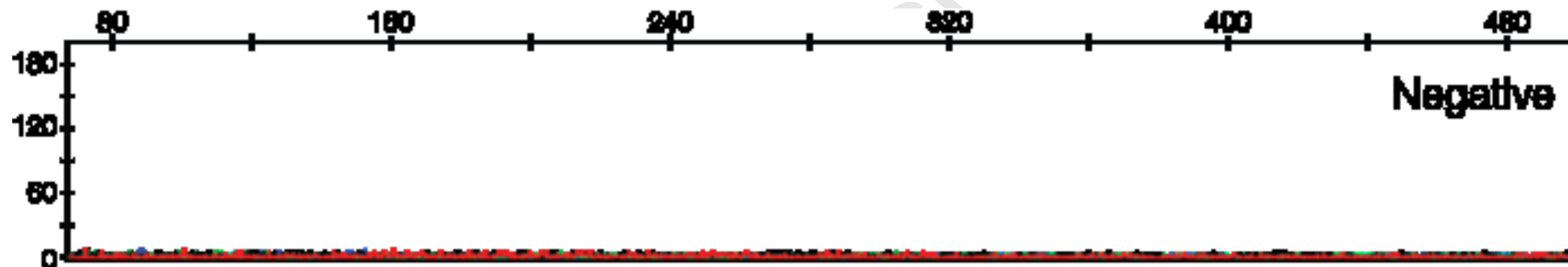
Accepted Manuscript

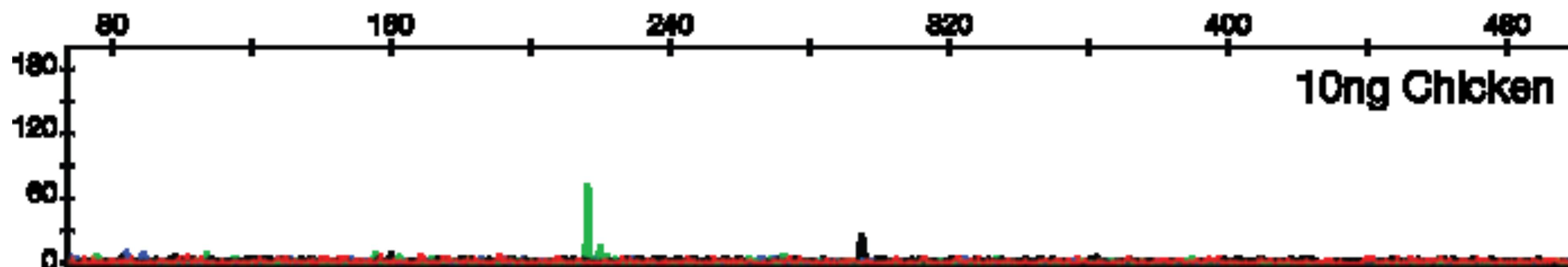
ACCEPTED MANUSCRIPT

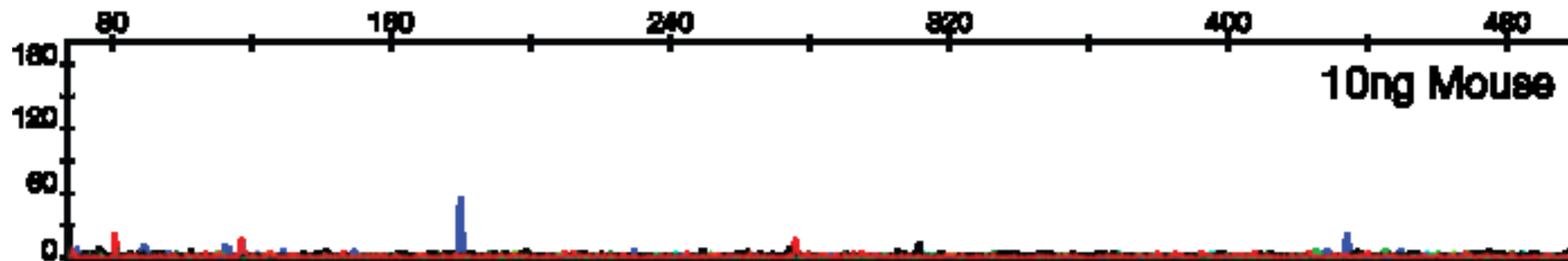
576 with stutter and peak height ratio analysis for the PowerPlex ® ESX 17 and ESI 17 Systems.
577 Forensic Sci Int Genet. 5 (2011) 269-75.
578 [15] C. Schlötterer, D. Tautz, Slippage synthesis of simple sequence DNA, Nucleic Acids Res. 20 (1992)
579 211–215.
580 [16] P.S. Walsh, N.J. Fildes, R. Reynolds, Sequence analysis and characterization of stutter products at
581 the tetranucleotide repeat locus vWA, Nucleic Acids Res. 24 (1996) 2807–2812.
582

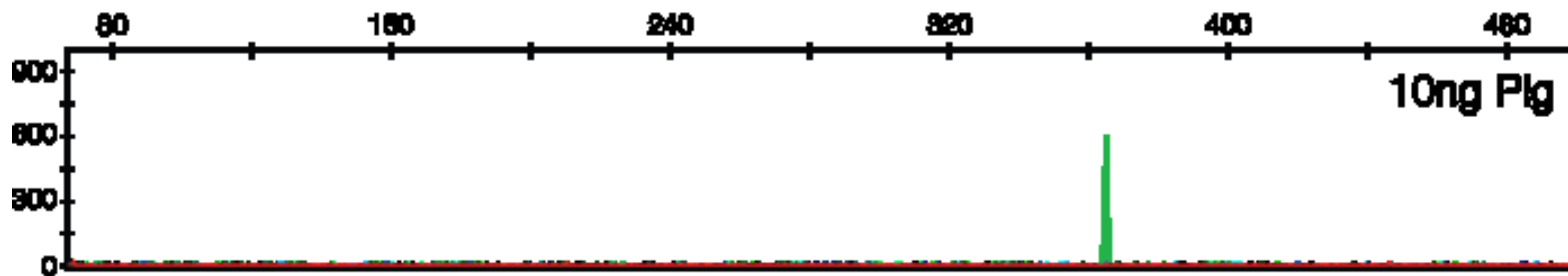
Accepted Manuscript

Fusion Fig 1 Species 11439MA.tiff

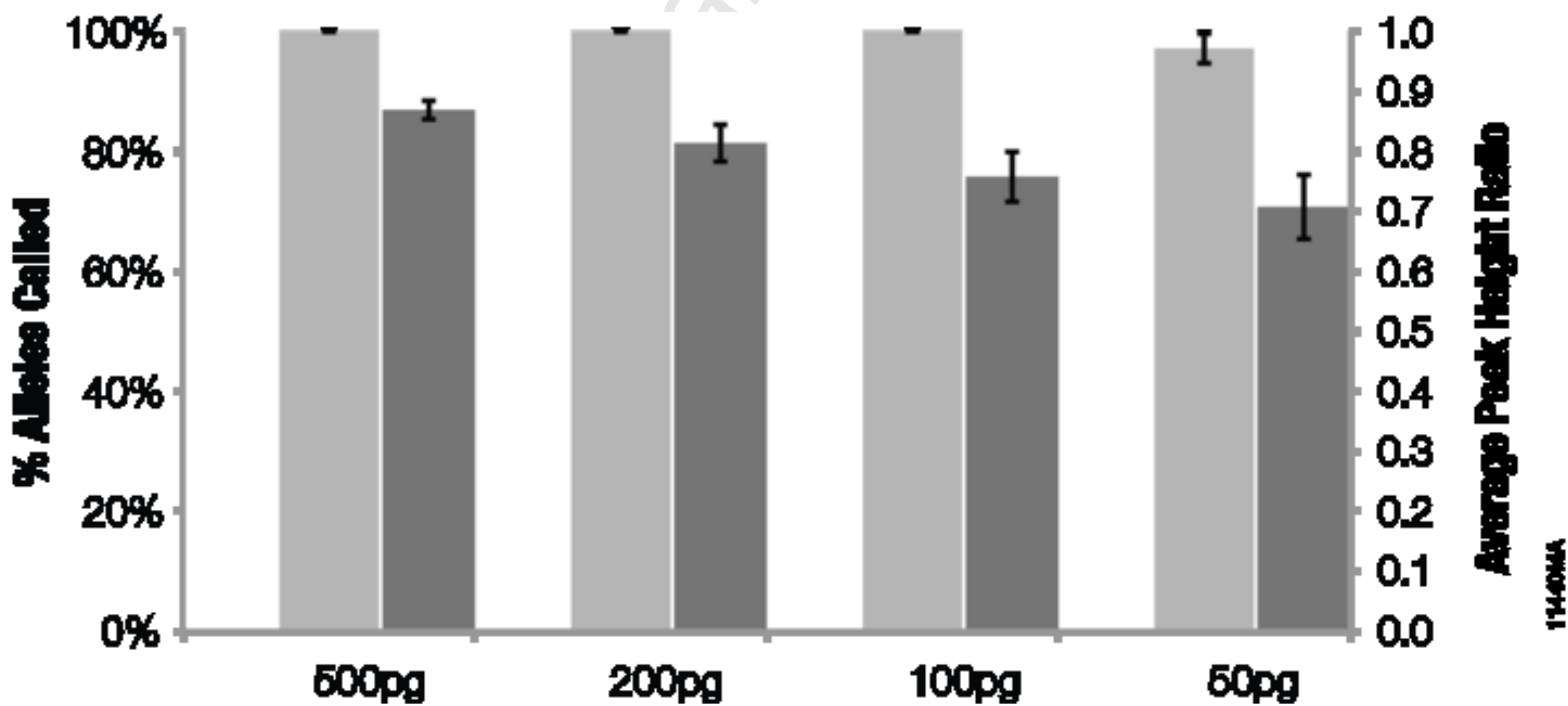


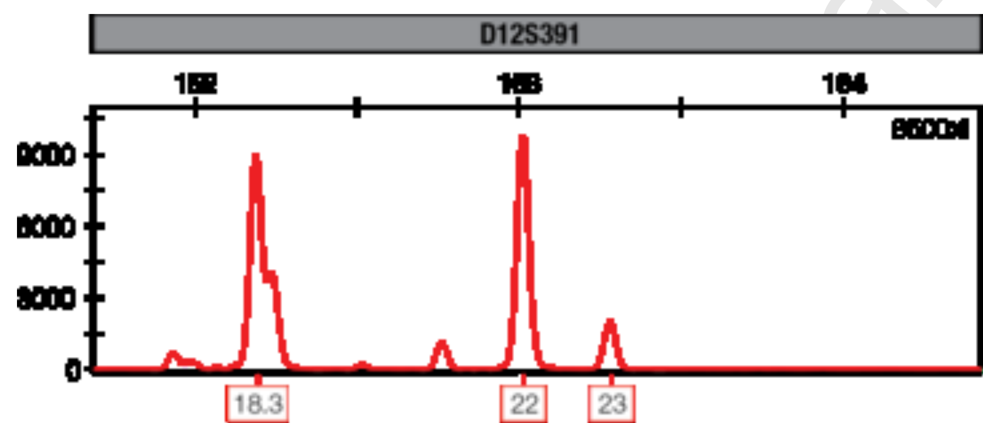


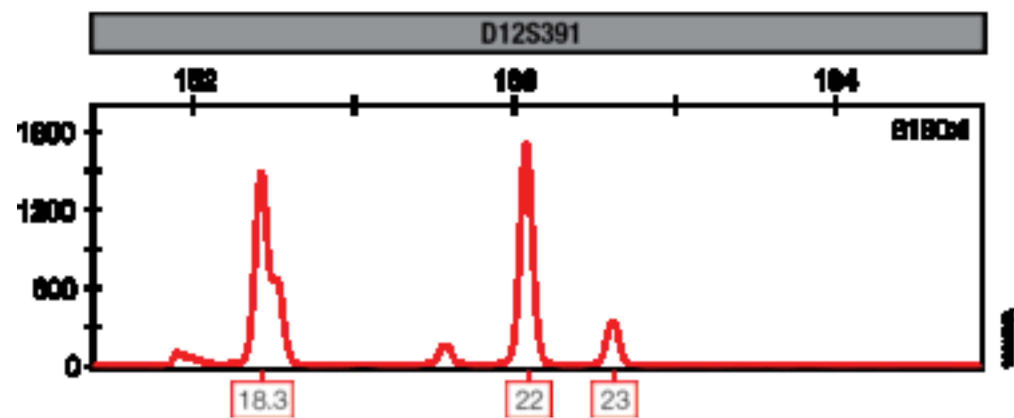




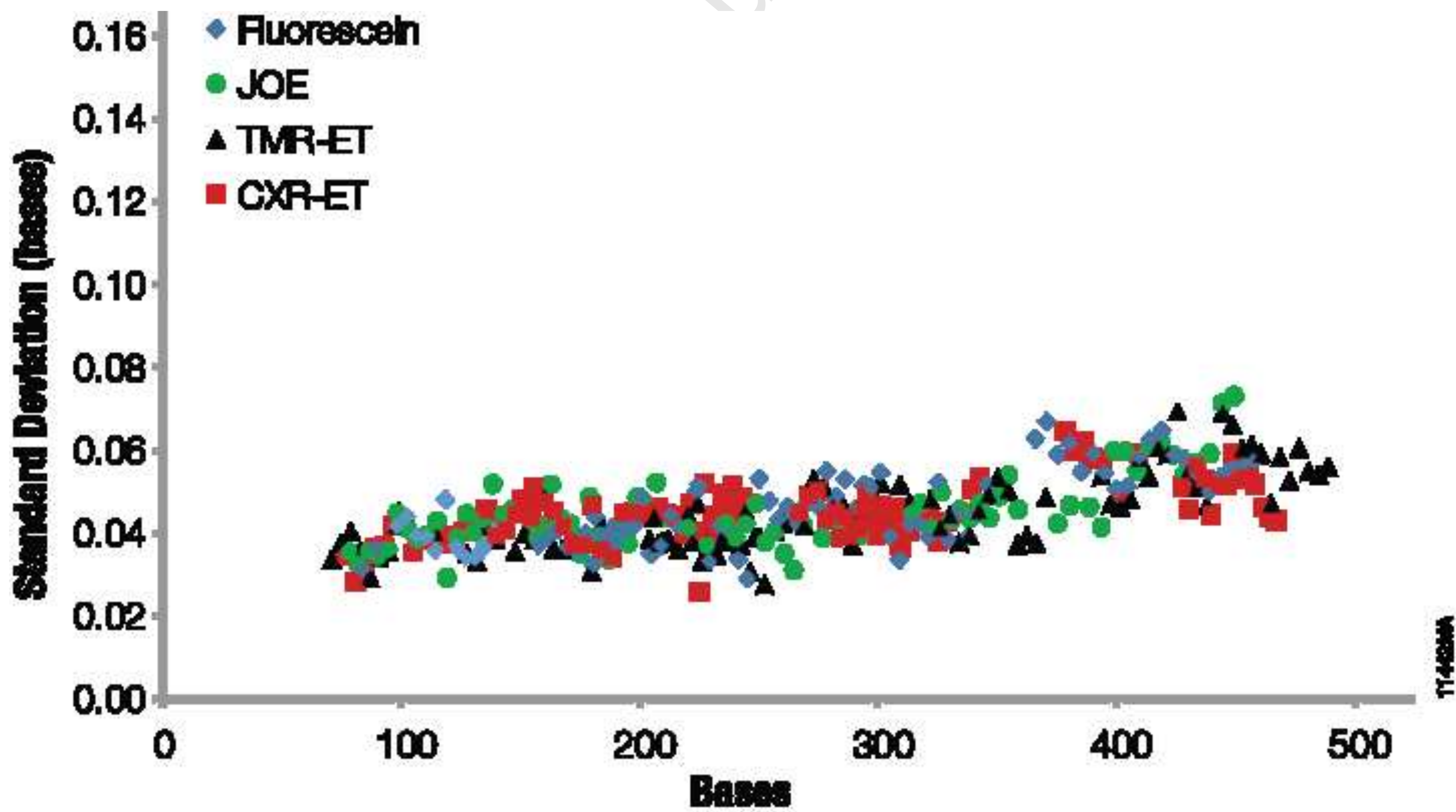
Fusion Fig 2 Sensitivity 11440MA.tiff




D12S391
18.3
22
23

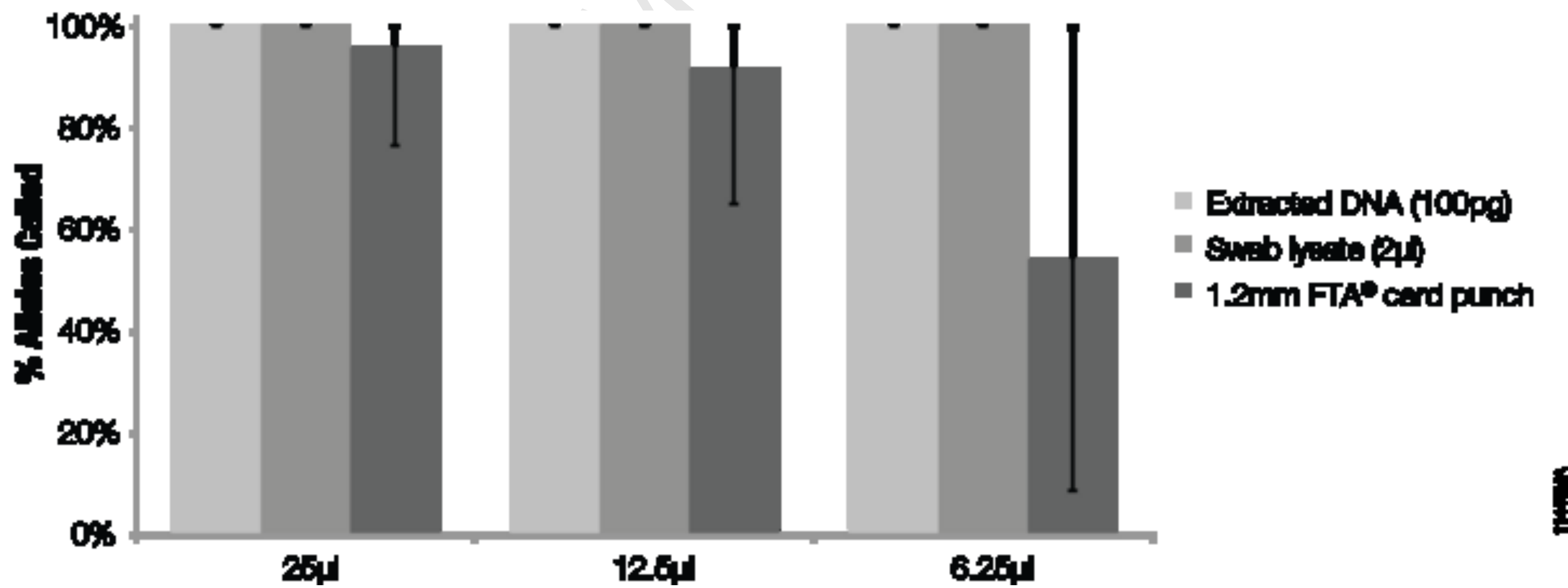

D12S391
18.3
22
23

Fusion Fig 4 Precision 3500xl 11442MA.tiff

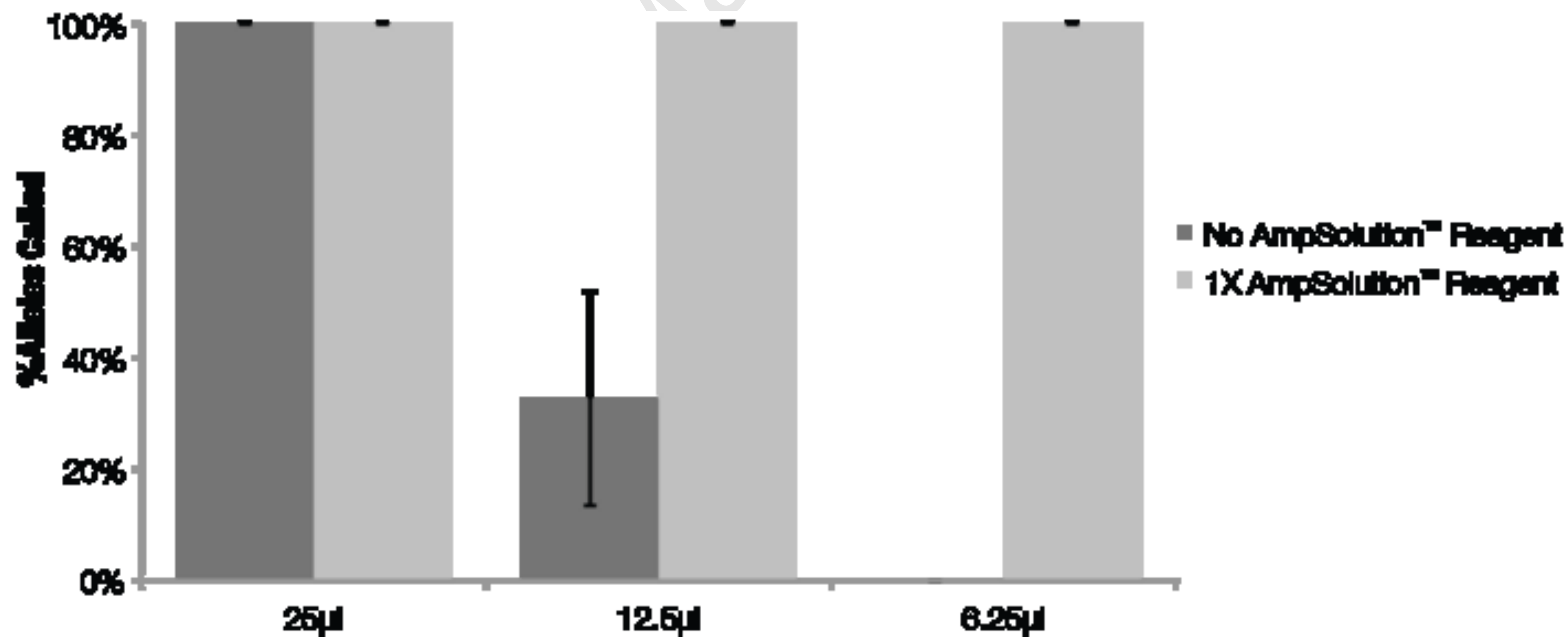


Fusion Fig 5 Rxn Vol 11443MA.tiff

Manuscript



Fusion Fig 6 AmpSoln nonFTA 11444MA.tiff



ACCEPTED MANUSCRIPT

Developmental Validation of the PowerPlex® Fusion System for Analysis of Casework and Reference Samples: A 24-locus Multiplex for New Database Standards.

Figure legends:

Figure 1. Electropherograms showing detectable peaks with the indicated species using the PowerPlex® Fusion System. Peaks were detected with 10ng of chicken, mouse, or pig DNA. Samples were detected using an Applied Biosystems® 3130*xl* Genetic Analyzer with a 3kV 5s injection. Peak amplitude threshold was 50 RFU. (n=2)

Figure 2. Percent alleles called (left-hand axis; light gray) and average sample peak height ratio (right-hand axis; dark gray) over a DNA titration from 500pg to 50pg. Samples were detected using an Applied Biosystems® 3130 and 3500 Series Genetic Analyzers using 3kV 5s or 1.2kV 10s injections, respectively, and a 3730 DNA Analyzer using a 3kV 5s injection. Error bars represent standard deviation. (n=36)

Figure 3. Electropherograms showing D12S391 allele 18.3 with the allele 19 shoulder. Samples were detected using the Applied Biosystems® 3500*xL* and the 3130*xl* Genetic Analyzers with a 1.2kV 24s or 3kV 5s injection, respectively. Similar resolution was observed on the Applied Biosystems® 3730 DNA Analyzer.

Figure 4. Size standard deviation for each allele within the PowerPlex® Fusion Allelic Ladder Mix. One microliter of allelic ladder was detected using a Applied Biosystems® 3500*xL* Genetic Analyzer at Promega Corporation using a 1.2kV 24s injection. Similar results were obtained at two other sites. (n=48)

Figure 5. Percent alleles called using extracted DNA or pretreated solid support materials with reduced-volume reactions. Samples were detected using Applied Biosystems® 3130*xl* Genetic Analyzers with a 3kV 5s injection. Error bars represent standard deviation. (Extracted DNA, n=3; Swab lysate, n=4; FTA® card punch, n=27)

Figure 6. Percent alleles called using nonFTA punches as the template source in reduced-volume reactions. Reactions were amplified in the presence and absence of AmpSolution™ Reagent. (n=6)

Accepted Manuscript

ACCEPTED MANUSCRIPT

Highlights

- Developmental validation results from twelve forensic and research laboratories are presented for the PowerPlex® Fusion System.
- Results using both extracted DNA and solid support materialsare presented.
- Low-level sensitivity and mixture detectionand robust performance with case-type samples and inhibitors were observed.
- Strong amplification and minimal artifacts were generated under several suboptimal PCR conditions.

Accepted Manuscript