

**OPTIMIZATION OF RECOVERY AND ANALYSIS OF TOUCH DNA
FROM SPENT CARTRIDGE CASINGS**

By

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ABSTRACT

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Firearms, particularly pistols, are commonly used in violent crimes, though the actual weapon used is rarely recovered. Nevertheless, spent cartridge casings ejected during shooting are often left at the scene and recovered by law enforcement. These casings may contain DNA deposited by the loader of the firearm, who could potentially be identified using short tandem repeat (STR) analysis. However, DNA recovered from spent casings is often degraded and present in low copy numbers. Owing to this, crime laboratories have had limited STR typing success from casings, thus, it is essential that methods for DNA recovery and analysis be optimized. Multiple variables, such as swabbing or soaking casings, pre-treatment of soaking vessels, shaking casings during soaking, pre-digestion incubation of soaked samples, and the duration of digestion with concurrent shaking were examined, with the goal of optimizing methods to improve DNA yields. Volunteers loaded cartridges into the magazine of a pistol, cartridges were fired, casings were collected, DNAs were recovered and extracted with one of five optimized methods (double swab or soak with an organic extraction, double swab or soak with a silica-based extraction, or single swab with a non-binding DNA extraction), DNAs were quantified, and amplified with AmpF ℓ STR[®] MiniFiler[™] and/or PowerPlex[®] Fusion. Comparisons of DNA yields and STR profiles demonstrated double swabbing with organic extraction and amplification with Fusion generated significantly more DNA and alleles consistent with the loader. Ultimately, optimization of protocols for DNA recovery and analysis from spent cartridge casings generated a significant increase in loader STR data.

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TABLE OF CONTENTS

LIST OF TABLES	vii
LIST OF FIGURES	xvii
INTRODUCTION	1
Composition of a Cartridge and Ejection of Cartridge Casings	1
Class Characteristics: Identifying a Type of Firearm	3
Individual Characteristics: Identifying a Specific Firearm	3
Fingerprints: Identifying the Loader of a Firearm	5
Touch DNA: Identifying the Loader of a Firearm	7
Techniques for DNA Extraction	8
Real-Time PCR: Targeting Loci for DNA Quantification	10
STR Analysis: Identifying Individuals	13
Previous Studies on DNA Recovered from Spent Casings	17
Goals of This Study	20
MATERIALS AND METHODS.....	22
Methods for Cell Recovery	22
Swabbing Cartridge Casings	22
Soaking Cartridge Casings	23
Methods for DNA Extraction.....	24
Organic Extraction.....	24
QIAamp® DNA Investigator Extraction.....	25
Fingerprint DNA Finder® Extraction.....	25
DNA Quantification via Real-Time PCR Analysis	25
STR Amplification.....	27
Capillary Electrophoresis.....	28
Optimization of Cell Recovery and DNA Extraction	29
Decontamination of Transfer Pipette Bulbs	29
Pre-digestion Treatments Investigated Within the Soaking Method.....	30
Digestion Optimization.....	31
Comparison of Optimized Cell Recovery and DNA Extraction Methods.....	31
Obtaining Ammunition and Testing for Foreign DNA on Live Cartridges	31
Loading Cartridges	32
Collection of Spent Cartridge Casings	32
Optimized Method for Soaking Cartridge Casings	34
Comparison of DNA Yields	34
Comparison of STR Profiles.....	35
RESULTS	37
Optimization of Cell Recovery and DNA Extraction Methods	37
Decontamination of Transfer Pipette Bulbs	37
Pre-treatment of Transfer Pipette Bulbs with Yeast rRNA	38

Shaking Casings During Soak Period.....	39
Pre-digestion Incubation at 85°C.....	40
Optimal Digestion Time	41
Shaking Swabs During Digestion.....	42
Comparisons of Optimized Cell Recovery and DNA Extraction Methods	43
Comparisons of DNA Yields.....	43
Comparison of MiniFiler™ and Fusion STR Profiles	45
Comparisons of Individual and Consensus Fusion STR Profiles.....	48
Degradation of DNA Recovered from Spent Cartridge Casings.....	54
DISCUSSION	56
APPENDICES	72
APPENDIX A. ASSIGNMENT OF CELL RECOVERY AND DNA EXTRACTION METHODS TO SPENT CARTRIDGE CASINGS.....	73
APPENDIX B. DNA QUANTITIES FROM SPENT CASINGS ASSAYED WITH OPTIMIZED CELL RECOVERY AND DNA EXTRACTION METHODS···	84
APPENDIX C. COMPARISON OF AMFℓSTR® MINIFILER™ STR PROFILES AND POWERPLEX® FUSION STR PROFILES	98
APPENDIX D. ANALYSIS OF LOADER AND NON-LOADER ALLELES IN STR PROFILES AMPLIFIED WITH AMPFℓSTR® MINIFILER™ AND POWERPLEX® FUSION	121
APPENDIX E. POWERPLEX® FUSION STR PROFILES·	149
APPENDIX F. CONSENSUS POWERPLEX® FUSION STR PROFILES	208
REFERENCES	236

LIST OF TABLES

Table 1. Comparison of the AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ and the PowerPlex $^{\text{®}}$ Fusion (Life Technologies, 2014; Promega, 2014).	16
Table 2. Primer, probe, and IPC template sequences for rtPCR. HEX and 6FAM fluorescent dyes were on the 5' end of the <i>Alu</i> and IPC probes, respectively. BHQ1 and Iowa Black $^{\text{®}}$ FQ (IABkFQ) are quenchers on the 3' end of the <i>Alu</i> and IPC probes, respectively.	26
Table 3. PCR Amplification of extracted DNA with AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ and PowerPlex $^{\text{®}}$ Fusion.....	28
Table 4. Run parameters for capillary electrophoresis on an AB 3500 genetic analyzer with AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ and PowerPlex $^{\text{®}}$ Fusion amplified products.	29
Table 5. Examination of foreign DNA on the inner portion of transfer pipette bulbs.	30
Table 6. Spent cartridge casing collection events. Letters (A, B, and C) indicate a different firearm/magazine.	33
Table 7. DNA quantities recovered from treated and non-treated bulbs. A higher average DNA yield (1.18 pg) was recovered from transfer pipette bulbs subjected to various treatments than the untreated bulb (0.41 pg). DNA yields were calculated based on 28 μ L retention.	37
Table 8. Mann-Whitney pairwise comparisons (2-tailed) of DNA quantities retrieved with the optimized cell recovery and DNA extraction methods. (Bold = significantly greater DNA yields)	44
Table 9. Descriptive statistics of profiles amplified with MiniFiler $^{\text{™}}$ and Fusion (bold). The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp $^{\text{®}}$ extraction; D = soak + QIAamp $^{\text{®}}$ extraction; E = FDF $^{\text{®}}$ extraction	46
Table 10. Mann-Whitney pairwise comparisons (2-tailed) examining the number of loader and non-loader alleles present in MiniFiler $^{\text{™}}$ and Fusion profiles generated with the optimized methods: A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp $^{\text{®}}$ extraction; D = soak + QIAamp $^{\text{®}}$ extraction; E = FDF $^{\text{®}}$ extraction	47
Table 11. RMP comparison of MiniFiler $^{\text{™}}$ and Fusion profiles of casing 34.4. Single alleles at a given locus were considered homozygous and frequency calculations for D13 and FGA in the MiniFiler $^{\text{™}}$ profile were calculated by adding the frequencies of all allele combinations.	48

Table 12. Shapiro Wilk test for normality on the percentages of loaders' profiles processed with the optimized cell recovery and DNA extraction methods and amplified using Fusion.	49
Table 13. Mann-Whitney pairwise comparisons (2-tailed) of the percentages of loaders' profiles processed with the optimized cell recovery and DNA extraction methods and amplified using Fusion. (Bold = significantly greater percentages of loaders' profiles).....	49
Table 14. Descriptive statistics of individual and consensus profiles of DNAs amplified with Fusion. The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp® extraction; D = soak + QIAamp® extraction; E = FDF® extraction. Consensus profiles of methods A and B were generated per volunteer using the three individual profiles from casings (Collections 2 and 3), in which organic extractions were performed with either double swabbing or soaking.....	51
Table 15. The degree of linear correlation between the DNA yields and the amount of loader alleles amplified in Fusion profiles. The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp® extraction; D = soak + QIAamp® extraction; E = FDF® extraction.	52
Table 16. Example of a consensus profile where non-loader alleles were rare in individual profiles and consequently excluded in the consensus, yet some alleles (<i>e.g.</i> , 16.3 and 17.3 at D1, 29 at D21, and 13 at D5) present in Profile 33-7A were consistent with the loader but not included in the consensus profile. Refer to Appendix E for explanation of table symbols.	53
Table 17. Example of a consensus profile where multiple non-loader alleles were represented in the consensus profile. Refer to Appendix E for explanation of table symbols.	54
Table A1. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 1.	73
Table A2. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 2.	76
Table A3. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 3.	82
Table B1. DNA quantities recovered from spent cartridge casings using a double swab technique (Sweet <i>et al.</i> , 1997) and organic extraction.	84
Table B2. DNA quantities recovered from spent cartridge casings using a soaking technique and organic extraction.....	87

Table B3. DNA quantities recovered from spent cartridge casings using a double swab technique (Sweet <i>et al.</i> , 1997) and QIAamp® DNA Investigator extraction.....	90
Table B4. DNA quantities recovered from spent cartridge casings using a soaking technique and QIAamp® DNA Investigator extraction.....	93
Table B5. DNA quantities recovered from spent cartridge casings using a single swab technique and FDF® extraction.....	96
Table C1. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer CC during Collection 1.	98
Table C2. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer Q during Collection 1.....	99
Table C3. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer LL during Collection 1.....	100
Table C4. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer YY during Collection 1.....	101
Table C5. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer RR during Collection 1.	102
Table C6. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer U during Collection 2.....	103
Table C7. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer MM during Collection 2.....	104
Table C8. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer S during Collection 2.	105
Table C9. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer V during Collection 2.....	107
Table C10. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer L during Collection 2.	110
Table C11. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer T during Collection 2.	114

Table C12. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.....	115
Table C13. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer N during Collection 2.....	116
Table C14. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer B during Collection 2.	117
Table C15. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.	118
Table C16. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.....	119
Table C17. Alleles amplified with AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion from spent cartridge casings loaded by volunteer II during Collection 2.	120
Table D1. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a double swab technique (Sweet <i>et al.</i> , 1997) and organic extraction.	121
Table D2. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a soaking technique and organic extraction. DNA extract 3-5A is the only sample extracted with an organic extraction and amplified with PowerPlex® Fusion that does not have allelic data due to high levels of contamination.	126
Table D3. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a double swab technique (Sweet <i>et al.</i> , 1997) and QIAamp® DNA Investigator extraction.	130
Table D4. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a soaking technique and QIAamp® DNA Investigator extraction.....	134
Table D5. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a single swab and FDF® extraction.	138
Table D6. Summary of alleles recovered in consensus and individual STR profiles generated from DNA extracts retrieved via a double swab technique (Sweet <i>et al.</i> , 1997) and organic extraction. Consensus profiles are presented first (Con. = Consensus) and the next three casing identifiers are the individual profiles.	141
Table D7. Summary of alleles recovered in consensus and individual STR profiles generated from DNA extracts retrieved via a soaking technique and organic extraction. Consensus profiles	

are presented first (Con. = Consensus) and the next three casing identifiers are the individual profiles.	145
Table E1. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer CC and collected individually during Collection 1.	150
Table E2. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer CC and collected in triplicate during Collection 1.	151
Table E3. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Q and collected individually during Collection 1.	152
Table E4. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer LL and collected individually during Collection 1.	153
Table E5. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer YY and collected individually during Collection 1.	154
Table E6. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer RR and collected individually during Collection 1.	155
Table E7. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer RR and collected in triplicate during Collection 1.	156
Table E8. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer M and collected individually during Collection 1.	157
Table E9. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer GG and collected individually during Collection 1.	158
Table E10. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer U during Collection 2.	159
Table E11. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer MM during Collection 2.	160
Table E12. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer S during Collection 2.	161
Table E13. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer VV during Collection 2.	163

Table E14. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer V during Collection 2.	164
Table E15. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer HH during Collection 2.....	166
Table E16. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer L during Collection 2.....	167
Table E17. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer OO during Collection 2.....	169
Table E18. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer T during Collection 2.....	170
Table E19. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.....	171
Table E20. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer N during Collection 2.	173
Table E21. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer B during Collection 2.....	175
Table E22. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer D during Collection 2.	177
Table E23. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.....	178
Table E24. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer SS during Collection 2.....	180
Table E25. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.	181
Table E26. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer II during Collection 2.....	182
Table E27. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.....	183
Table E28. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.....	184

Table E29. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer QQ during Collection 3.....	185
Table E30. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.	186
Table E31. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.	187
Table E32. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.	188
Table E33. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.	189
Table E34. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.....	190
Table E35. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.....	191
Table E36. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.....	192
Table E37. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.....	193
Table E38. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer KK during Collection 3.....	194
Table E39. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.....	196
Table E40. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.	197
Table E41. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.....	198

Table E42. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.	199
Table E43. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.....	200
Table E44. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.....	201
Table E45. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.	202
Table E46. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.	204
Table E47. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.....	206
Table E48. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.....	207
Table F1. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer U during Collection 2.....	209
Table F2. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer MM during Collection 2. Casings 3-5A was excluded from analysis due to contamination, consequently it was not used in construction of the consensus STR profile.....	210
Table F3. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer S during Collection 2.	211
Table F4. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer VV during Collection 2.....	212
Table F5. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer V during Collection 2.....	213
Table F6. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer HH during Collection 2.....	214

Table F7. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer L during Collection 2.	215
Table F8. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer OO during Collection 2.	216
Table F9. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer T during Collection 2.	217
Table F10. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.	218
Table F11. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer N during Collection 2.	219
Table F12. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer B during Collection 2.	220
Table F13. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer D during Collection 2.	221
Table F14. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.	222
Table F15. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer SS during Collection 2.	223
Table F16. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.	224
Table F17. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer II during Collection 2.	225
Table F18. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.	226
Table F19. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer QQ during Collection 3.	227
Table F20. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.	228

Table F21. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.	229
Table F22. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.	230
Table F23. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer KK during Collection 3.	231
Table F24. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.	232
Table F25. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.	233
Table F26. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.	234
Table F27. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.	235

LIST OF FIGURES

Figure 1. Anatomy of a live cartridge. Taken from Guns & Ammo Info, 2014. http://www.gunsandammo.info/ammo/ammo-101	2
Figure 2. Extraction and ejection of a spent cartridge casing, followed by the subsequent input of a new round in the chamber of the pistol. Taken from Ruger Forum, 2014.....	2
Figure 3. The anatomy of a pistol chamber, viewed through the ejection port, identifying various tools that generate marks on cartridge casings. Taken from Thompson, 2010.....	5
Figure 4. Diagram of the Polymerase Chain Reaction. Taken from Butler, 2005.....	10
Figure 5. IPC Amplification Plot: The x-axis of the graph represents the cycle number, while the y-axis is the amount of fluorescence. The cycle threshold (C_t) value is the cycle number in which the sample passes the threshold line and this value is used to determine the starting concentration of DNA. In this example, one sample was inhibited and did not cross the threshold. The IPC for all other reactions had a C_t value of approximately 25, indicating successful amplification and no PCR inhibition.	12
Figure 6. Diagram of a short tandem repeat (STR) allele at the TH01 locus. In this example, there are nine repeat units (TCAT) between the flanking regions, so the individual has a 9 allele. The second allele at TH01 comes from their other parent, and may contain the same or a different number of repeat units. Taken from Butler, 2005.....	14
Figure 7. An example of stochastic sampling effects. When a small number of DNA templates are available from the start, there is a chance that some alleles will be amplified more than the others, resulting in imbalanced allele peak height. Taken from Krane, 2007.	15
Figure 8. Common STR artifacts as a result of stochastic sampling effects and low copy, degraded DNA. Taken from Butler and Hill, 2010.	16
Figure 9. Example of a casing soaking in 700 μ L of digestion/tissue lysis buffer.	24
Figure 10. Average DNA yields from yeast rRNA pre-treated (organic = 108.99 pg and QIAamp [®] = 21.54 pg) and not pre-treated transfer pipette bulbs (organic = 108.06 pg and QIAamp [®] = 17.75 pg) prior to the soak period (n = 2 per extraction method). Given these results, bulbs were not pre-treated in subsequent experiments.	38
Figure 11. Average DNA yields from casings that were shaken at 900 rpm (organic = 54.11 pg and QIAamp [®] = 13.64 pg) and casings that were stationary (organic = 377.52 pg and QIAamp [®] = 13.64 pg) prior to the soak period (n = 2 per extraction method). Given these results, casings were not pre-treated in subsequent experiments.	38

= 25.51 pg) for the 30 min soak period (n = 4 per extraction method). Given these results, casings were not shaken in subsequent experiments. 39

Figure 12. Average DNA yields from samples that were subjected to a pre-digestion incubation (organic = 144.59 pg and QIAamp® = 26.45 pg) and samples that were not (organic = 59.91 pg and QIAamp® = 18.06 pg) (n = 2 per extraction method). Based on these results, soaked samples were incubated for 10 min at 85°C in subsequent experiments..... 40

Figure 13. Average DNA yields recovered from samples digested for 1 h (double swab + organic = 43.09 pg; soak + organic = 85.38 pg; double swab + QIAamp® = 5.5 pg; soak + QIAamp® = 0.93 pg; FDF® = 84.9 pg) or digested overnight (double swab + organic = 38.59 pg; soak + organic = 41.69 pg; double swab + QIAamp® = 1.83 pg; soak + QIAamp® = 3.39 pg; FDF® = 19.2 pg) or digested for 30 min (FDF® = 34.8 pg). Owing to these results, 1 h digestion was performed in subsequent experiments. 42

Figure 14. Average DNA quantities recovered from swabs that were either shaken (organic = 95.37 pg and QIAamp® = 22.68 pg) or stationary (organic = 59.91 pg and QIAamp® = 18.06 pg) during digestion (n = 2 per extraction method). Given these results, cell/DNA digestions included shaking in subsequent experiments. 43

Figure 15. Median DNA quantities recovered using optimized cell recovery and DNA extraction methods. Median DNA yields from organic extractions (double swab = 25.32 pg and soak = 14.95 pg) were significantly higher than the median DNA yields from QIAamp® extractions (double swab = 3.81 pg and soak = 1.18 pg) and the median DNA yield from FDF® extractions (0.20 pg). 45

Figure 16. Median percentages of loaders' profiles recovered using optimized cell recovery and DNA extraction methods followed by amplification with Fusion. Median percentages of loaders' profiles from organic extractions (double swab = 25.8% [n = 90] and soak = 18.2% [n = 89]) were higher than loaders' profiles from QIAamp extractions (double swab = 4.8% [n = 56] and soak = 6.7% [n = 36]). The median percentage of loaders' profiles from FDF® extractions was 0.0% (n = 14). 50

Figure 17. Frequency of consistent loader alleles amplified at each locus, illustrating preferential amplification of shorter amplicons. The loci are arranged according to their amplicon sizes (short to long) for each dye channel. With the exception of D13 (9.7%) and D7 (9.9%), the loci containing smaller amplicons had higher frequencies of amplification. Frequencies of the smallest locus in each channel: Amel = 47.5%, D16 = 36.3%, THO1 = 44.6%, D8 = 41.5%. Frequencies of the largest locus in each channel: Penta E = 10.4%, Penta D = 7.5%, DYS391 = 8.6%, D22 = 7.6%. 55

INTRODUCTION

Approximately 1.2 million violent crimes (on average 1 every 26 seconds) occurred in the U.S. in 2012. Of those, a firearm was used in 69.3% of the murders, 41.0% of the robberies, and 21.8% of the aggravated assaults (FBI Uniform Crime Report, 2012). Considering the prevalence of firearms used in violent crimes, it is critical that investigators have access to reliable forensic tools that can be used to identify the person(s) responsible for firing them. While recovery of the fired weapon is ideal, this often does not occur. However, fired bullets and cartridge casings ejected from a firearm *are* often abandoned by the offender and retrieved by law enforcement, which have the potential to provide a direct link between the incident, the weapon, and the perpetrator (Bentsen *et al.*, 1996).

Composition of a Cartridge and Ejection of Cartridge Casings

A cartridge consists of a casing, primer, propellant, and one or more projectiles (Figure 1). When the trigger of a loaded gun is pulled, the firing pin makes contact with the primer, which generally contains an initiating explosive, oxidizer, and fuel (Warlow, 2012). The struck primer ignites the propellant. Historically, propellants were referred to as black powder, but the modern and more efficient form is known as smokeless powder (DiMaio, 1999). Deflagration of the propellant causes buildup of gases, which force the projectile out of the casing and down the barrel of the firearm. Simultaneously, the casing is forced back against the breech face and the extractor pulls the casing to the rear until it hits the ejector, which pushes the casing out of the ejection port of the firearm (Figure 2; Doyle, 2014, Thompson, 2010, National Institute of Justice, n.d.).

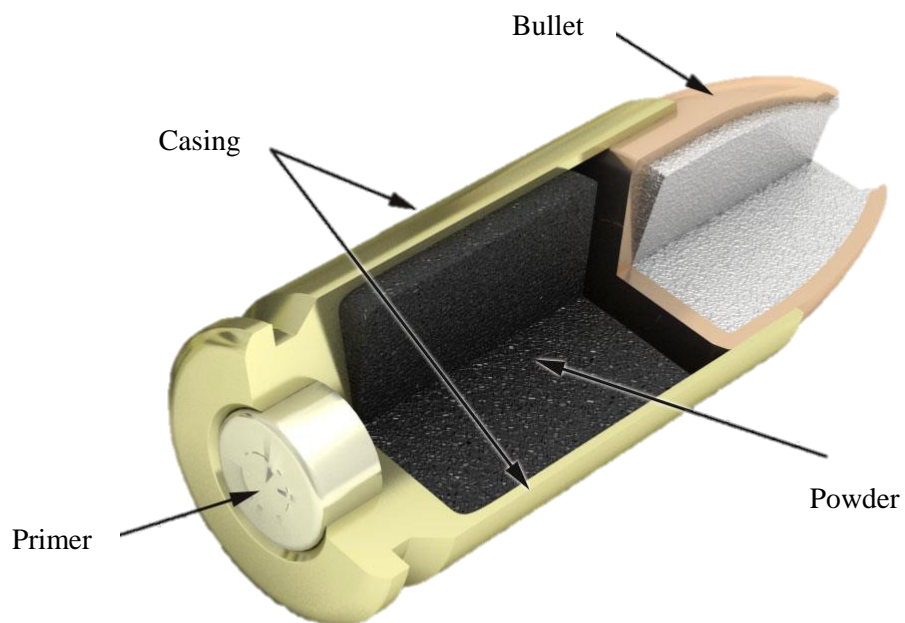


Figure 1. Anatomy of a live cartridge. Taken from Guns & Ammo Info, 2014.
<http://www.gunsandammo.info/ammo/ammo-101>.

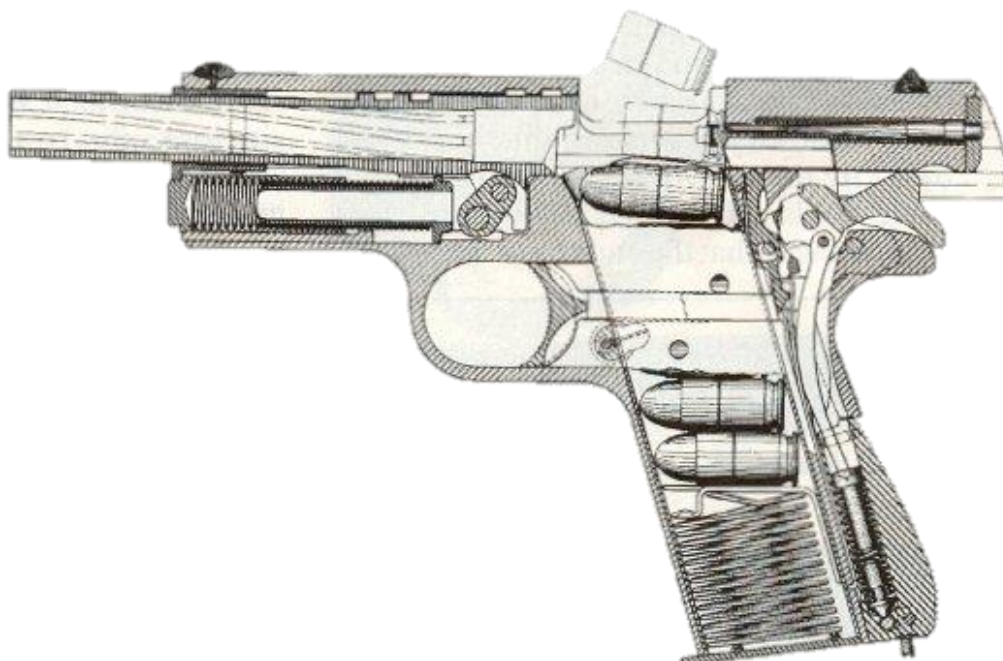


Figure 2. Extraction and ejection of a spent cartridge casing, followed by the subsequent input of a new round in the chamber of the pistol. Taken from Ruger Forum, 2014.

Class Characteristics: Identifying a Type of Firearm

A main goal of forensic firearms examiners is to determine whether a projectile, cartridge casing, or other ammunition components originated from a particular weapon. The evidence is first inspected for class characteristics (Saferstein, 2011). For example, the caliber of ammunition directly correlates with a firearm's caliber (the inner diameter of a firearm bore). If a .40-caliber casing was recovered from a shooting incident, investigators would search for a .40-caliber firearm. While this information is not individualizing, it is representative of a select group of weapons. Class characteristics of spent cartridge casings beyond caliber include the shape (*e.g.*, rimmed or rimless), the composition (*e.g.*, brass, steel, copper, or aluminum), and the headstamp containing manufacturer information (National Institute of Justice, n.d.). Class characteristics are useful for eliminating certain firearm brands, however they cannot identify a specific weapon.

Individual Characteristics: Identifying a Specific Firearm

The next step in an examination of a suspected firearm is investigation of individual characteristic. These are random imperfections and irregularities on parts of a firearm generated during the manufacturing process or as a result of natural wear and tear (*e.g.*, the amount of use, corrosion, and cleanliness) (Saferstein, 2011). Individual characteristics of a firearm will be imparted to a cartridge casing as toolmarks that can be examined via comparison microscopy. There are two general forms of toolmarks: impressed marks (impressions) and striated marks (striations). Impressed toolmarks result from the hard tool surface contacting an object at a perpendicular angle with such force that it leaves an impression. Striations are formed when the

tool surface scrapes across the softer surface of an object with substantial force (Thompson, 2010).

Thompson (2010) noted several events that occur when a firearm interacts with a cartridge to generate commonly examined toolmarks. First, marks can be generated on the side of casings by the magazine lips during loading. Additionally, when the firing pin strikes the primer, an impression of the firing pin and its microscopic imperfections are left on the casing head. As a projectile is fired, a process known as obturation occurs, in which the casing swells in the chamber and blocks the gases from traveling anywhere besides down the barrel, and, as a result of accumulated heat and pressure, chamber marks are left on the sides of the casing. Also, during discharge, toolmarks can be generated by the breech face, and if present, the extractor and ejector (Figure 3). All of these markings may include individual striations as a result of imperfections in the firearm parts. Unfortunately, although the combination of class and individual characteristics from spent cartridge casings can identify a particular weapon; they cannot directly connect an individual to a shooting incident, which is the ultimate criminal justice goal.

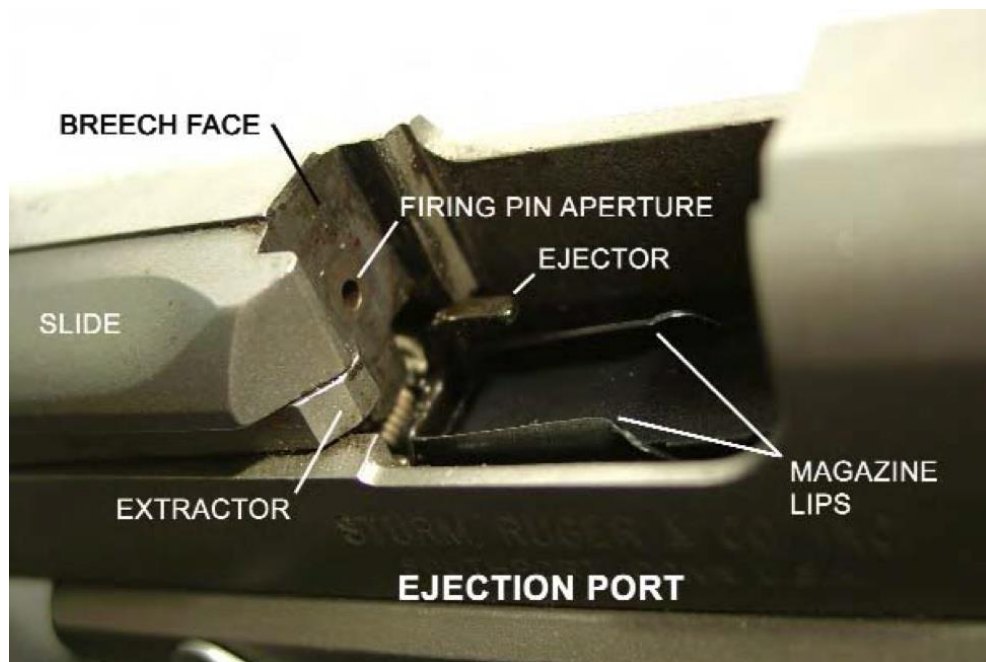


Figure 3. The anatomy of a pistol chamber, viewed through the ejection port, identifying various tools that generate marks on cartridge casings. Taken from Thompson, 2010.

Fingerprints: Identifying the Loader of a Firearm

The probative value of evidence is much greater when an examiner is able to identify the person(s) responsible for loading and/or firing a weapon in a shooting incident. For example, when a person loads a cartridge into the magazine of a firearm, fingerprints may be deposited on the ammunition. Spent casings can then be subjected to fingerprint analysis.

The most common type of fingerprint left on spent cartridge casings are latent, which can be visualized using various powders or chemicals and then “lifted” with tape or photographed. Bentsen *et al.* (1996) investigated the recovery of fingerprints deliberately rolled onto cartridges that were subsequently fired and analyzed. The authors fired the ammunition with a 0.38 Webley revolver, as they claimed it “was selected because of its lower thermodynamics of detonation and minimum handling of test rounds during loading compared to magazine or belt-fed weapons...ridge detail loss during the ejection process should be minimal in comparison to self-loading systems”. The sensitivity of multiple latent print visualization techniques was

investigated based on the amount and quality of ridge detail. The two most sensitive methods were vacuum cyanoacrylate fuming with Panacryl Brilliant Flavine staining, and selenious acid surface oxidation. Of the 21 combinations of weapons and ammunition studied post-firing using these two methods, 23.8% yielded identifiable ridge detail (16 ridge traits) and 57.1% included some ridge detail. When applied to 104 criminal incidents, two prints (one of which was associated to a CSI) were recovered using the cyanoacrylate method. The casework results clearly show fingerprints are rarely recovered from spent casings. The authors noted the loss of fingerprint ridge detail may be attributed to several variables: physical damage during cartridge loading or casing ejection, gaseous blowback during firing, or interference of propellant by-products as a result of gaseous blowback. Lastly, analysis with selenious acid treatment showed the composition of casings affected ridge detail; aluminum and nickel coated casings did not show any ridge detail while brass casings did.

Given (1976) conducted a study in which six volunteers handled nine pairs of cartridges, one of which was fired and the other not. Two variables differed among the nine pairs: the time between handling and firing cartridges (0 – 20 days), and the time between firing cartridges and lifting prints (either the same or next day). The author found more fingerprint powder adhered to the prints on casings that had been handled, fired, and lifted all in the same day. It was proposed that the decrease in powder adhesion resulted from evaporation of water in the prints. Additionally, the author found that hot, gaseous blowback normally occurred along the side of the casing that was not completely sealed against the chamber wall, and a considerable amount of fingerprint deterioration occurred in areas subjected to blowback.

Spear *et al.* (2005) examined deliberately placed fingerprints on 48 cartridges of differing caliber (.22 to .45) and casing metal (brass, nickel-plated brass, and aluminum). Three types of

fingerprints: bloody, eccrine, and oily, were impressed on the cartridges. Half of them were fired and all were stored for several months at room temperature. The bloody prints were developed with amido black, while eccrine and oily prints were processed via cyanoacrylate fuming and rhodamine 6G staining. Six fingerprints were identifiable, only one of which (bloody) was recovered from a spent cartridge casing, while none were recovered from the .22-caliber cartridges. From this it seems clear that the process of firing a weapon is destructive to fingerprints, even when they are intentionally placed on a cartridge.

Touch DNA: Identifying the Loader of a Firearm

Objects handled by an individual may contain ‘touch DNA’, or trace amounts of DNA transferred through shed skin cells and perhaps cell-free nucleic acids (Quinones and Daniel, 2012; Wickenheiser, 2002). Some efforts have been made by crime laboratories to analyze touch DNA from spent cartridge casings. Both Quinones and Daniel (2012) and Wickenheiser (2002) proposed that the amount of DNA transferred to an object during handling is dependent upon behavioral factors (*e.g.*, individuals often touch their face, eyes, nose, and hair), the texture of the substrate (*e.g.*, DNA adheres to porous substrates more readily than non-porous substrates), the individual handler (*e.g.*, some people shed cells more than others), and the amount of perspiration. Full AmpF ℓ STR[®] SGM Plus[®] profiles have been generated from touch DNA retrieved from paper (Sewell *et al.*, 2008) and bedding (Petricevic *et al.*, 2006). Additionally, Richert (2011) compared DNA yields and STR profiles from multiple regions on a firearm that were either individually swabbed and (1) DNAs were extracted from each swab separately or (2) swabs were combined then DNAs were extracted. The average DNA yield from combined swabs was more than double that of individual ones. Full Combined DNA Index System (CODIS) STR

profiles were recovered with both analysis methods, however a greater number of profiles were obtained from the combined swabs. Genetic information foreign to the handler was present in 78% of the profiles from combined swabs and 64% of the profiles from individual swabs, suggesting DNA contamination.

Techniques for DNA Extraction

Current forensic laboratory protocols used to analyze touch DNA from spent casings involve swabbing the casings and processing the swabs according to the laboratory's standard operating procedure for swabs (Forensic Scientist Sarah Rambadt, personal communication). However, touch DNA is often degraded and present in low copy number (LCN; generally less than 100 pg of DNA, Gill *et al.*, 2000) meaning analysis from spent casings has limited success. Multiple techniques exist for the isolation and purification of DNA, including organic (Comey *et al.*, 1994; Maniatis *et al.*, 1982), silica-based (Greenspoon *et al.*, 1998; Boom *et al.*, 1990), and non-binding separation (Kopka *et al.*, 2011) methods. Therefore it is possible that optimization of one or more of these may improve the amount of touch DNA recovered for subsequent analyses.

Standard phenol-chloroform DNA extractions involve digestion of the cell membrane and proteins with a lysis buffer containing a detergent (*e.g.*, SDS), proteinase K, a buffering agent (*e.g.*, Tris), and a chelating agent [*e.g.*, Ethylenedinitrilotetraacetic acid (EDTA)]. Digestion at ~56°C inactivates nucleases and breaks down cellular membranes, releasing DNA. Following the addition of phenol, the solution is vortexed and centrifuged resulting in an organic portion (containing degraded proteins and cellular debris) and an aqueous portion (containing nucleic

acids). The aqueous layer is added to chloroform to remove residual phenol. This process may be followed by additional purification and concentration methods using a centrifugal filter unit.

Silica-based extraction methods consist of silica beads or a column that selectively bind DNA under high salt conditions. Cation bridges are formed via chaotropic agents (*e.g.*, sodium iodide) between the negatively charged silica and the negatively charged DNA backbone (Melzak *et al.*, 1996). Residual proteins and impurities are washed away and a low salt solution elutes the DNAs from the silica.

Kopka *et al.* (2011) developed and validated the Fingerprint DNA Finder[®] (FDF[®]) Kit, which utilizes a non-binding DNA separation method. They stated “the DNA extraction system is based on a reversal of the silica principle”. The same set of authors (Cardozo *et al.*, 2012) described this method as using “porous matrices associated with polyanilines nano-layers, which are able to retain selectively biopolymers and potential PCR inhibiting substances, while nucleic acids are never bound and remain in solution”, based on earlier technology developed by Kapustin *et al.* (2003). The validation study of the FDF[®] Kit, performed by Kopka *et al.* (2011), included analysis of DNA samples from multiple components (trigger, magazine, slide barrel, and hammer) of four different pistols and a revolver along with cartridge casings fired from them. Only results for three partial electropherograms (samples from a trigger, magazine, and slide barrel of a single firearm) were presented, which were consistent with the handler. The authors stated “the profile was altered in the fired cartridge case (not shown). Similar results were obtained with all guns tested and with all replicate samples from the same gun”. Data presented by Kopka *et al.* (2011) are scarce, consequently it is unclear the success in DNA recovery FDF[®] Kits may have on spent cartridge casings.

Real-Time PCR: Targeting Loci for DNA Quantification

Extracted DNAs can be exponentially amplified at specific target regions (loci) using a technique developed by Mullis *et al.* (1986) known as the polymerase chain reaction (PCR). It is a doubling process that generates billions of copies of the target DNA sequence, termed the amplicon, designated by primers that flank the DNA region of interest (Figure 4). The development of this process was pivotal because it allows scientists to perform DNA analysis with very small amounts of starting material (*e.g.*, touch DNA).

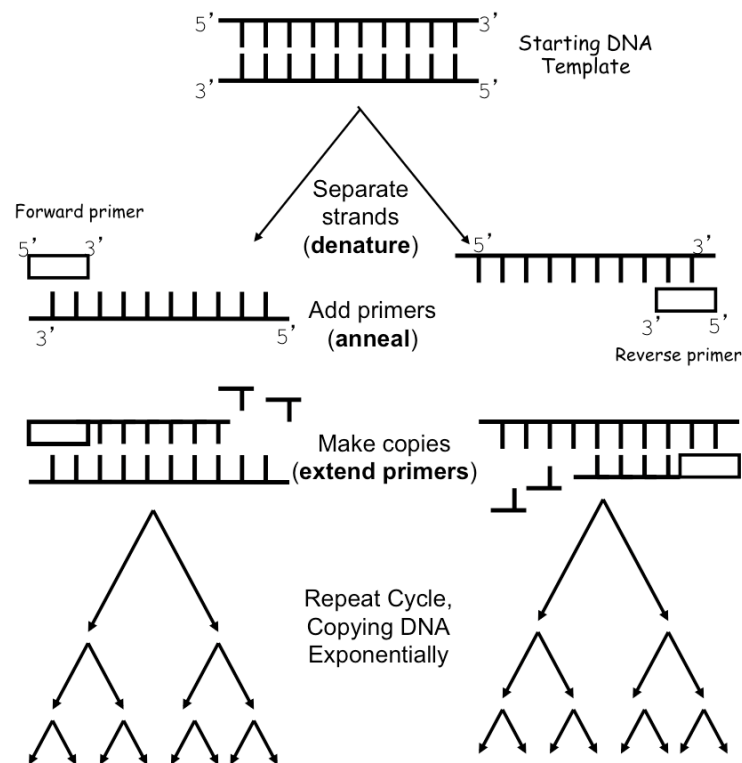


Figure 4. Diagram of the Polymerase Chain Reaction. Taken from Butler, 2005.

Real-time PCR (rtPCR) is a technique used to amplify and simultaneously quantify DNA (Higuchi *et al.*, 1993). A detection system recognizes fluorescently-labeled DNA probes annealed to the amplified target DNA sequences at each PCR cycle. When the relative fluorescence units (RFUs) reach a set fluorescence threshold, the current PCR cycle is recorded

as the cycle threshold (C_t) value for each sample. C_t values are directly proportional to the initial amount of DNA in a rtPCR reaction, so samples with more starting DNA will reach the threshold at earlier cycles than those with less starting DNA. DNA standards of known concentration are simultaneously amplified and a standard curve is generated with the DNA concentrations and C_t values associated. The concentrations of unknown samples are calculated based on their C_t values plotted on the standard curve.

A hurdle often encountered with forensic samples is PCR inhibition, which occurs when substances interact with the DNA, the polymerase, or the cofactors necessary for polymerase function, preventing DNA amplification either partially or fully. PCR inhibitors may be innate to a given sample and co-extracted with the DNA. A synthetic oligonucleotide and probe known as an internal PCR control (IPC) can be used to detect PCR inhibitors. In this process, an IPC is co-amplified with the questioned DNA sample. No amplification or poor amplification of the IPC indicates the PCR is inhibited (Figure 5). If PCR inhibitors are present, further DNA purification, DNA dilution, or the addition of certain PCR enhancers may overcome them.

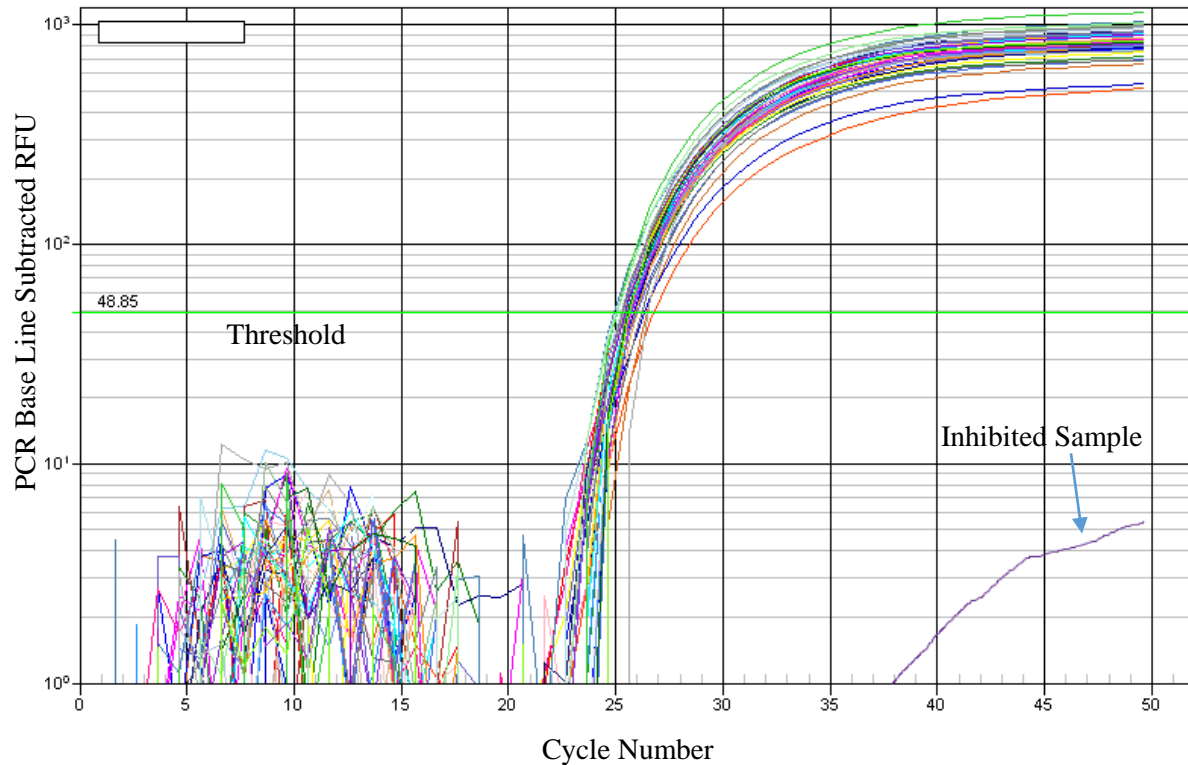


Figure 5. IPC Amplification Plot: The x-axis of the graph represents the cycle number, while the y-axis is the amount of fluorescence. The cycle threshold (C_t) value is the cycle number in which the sample passes the threshold line and this value is used to determine the starting concentration of DNA. In this example, one sample was inhibited and did not cross the threshold. The IPC for all other reactions had a C_t value of approximately 25, indicating successful amplification and no PCR inhibition.

Standard methods for quantifying DNA samples (*e.g.*, Quantifiler® Human DNA Quantification Kits) target single-copy loci. Green *et al.* (2005) found Quantifiler® could detect as little as 32 pg of DNA. However, this is disadvantageous when working with even smaller amounts of DNA, where the chance for stochastic sampling (explained below) increases and some single-copy loci may not be detected, making interpretation of STR profiles difficult. DNA quantification based on high-copy loci that are present in copious amounts throughout the human genome is one strategy for overcoming this problem. Because they are ubiquitous, some of these loci will still be available even when a DNA sample is LCN or degraded. This makes high-copy loci a sensitive target for DNA quantification.

The most abundant repetitive element in human DNA is the ~ 300 bp *Alu* sequence, which is present on every chromosome and makes up 10% of the human genome (Batzer and Deininger, 2002; Mighell *et al.*, 1997). Nicklas and Buel (2006) utilized the *Alu* subfamily Ya5 to quantify human DNA samples down to as little as 0.5 pg, or almost two orders of magnitude lower than Quantifiler[®]. However, *Alu* and other high-copy loci are sensitive to contamination by minuscule amounts of foreign DNA in the reagents and equipment used for DNA extraction and quantification. Kiley (2009) found commercially purchased *Alu* primers contained human DNA contamination, which she controlled by filtration through Microcon YM-30 columns and UV irradiation of all PCR reagents (with the exception of the polymerase and dNTPs) for 30 s – 60 s (0.25 – 0.5 J/cm², respectively).

STR Analysis: Identifying Individuals

Forensic DNA analysis utilizes PCR to amplify short tandem repeats (STRs) at multiple loci simultaneously, and capillary electrophoresis to separate amplicons by size. STRs are regions of repetitive DNA sequences that consist of core repeat units (2 – 6 bp) and the number of repeat units vary among individuals (Figure 6). A person inherits one allele, or STR variant, from each parent at a given locus. The high variability of alleles and the examination of multiple loci are what make STR analysis an effective tool for uniquely identifying individuals.

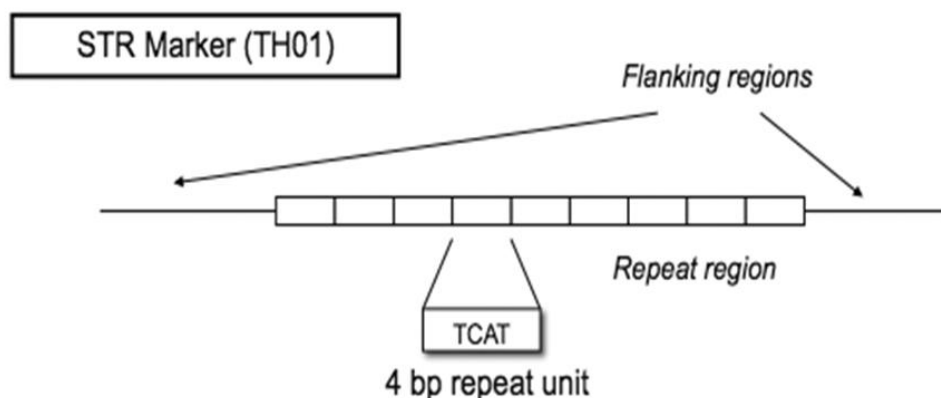


Figure 6. Diagram of a short tandem repeat (STR) allele at the TH01 locus. In this example, there are nine repeat units (TCAT) between the flanking regions, so the individual has a 9 allele. The second allele at TH01 comes from their other parent, and may contain the same or a different number of repeat units. Taken from Butler, 2005.

An STR profile is a compilation of the genetic information at multiple loci from a DNA sample. For example, if a perpetrator's blood stain is left at a scene, then the STR profile produced from it can be compared to the STR profile of a suspect. If the alleles are the same in both profiles, a random match probability (RMP) is then calculated. This value corresponds to the likelihood that a random, unrelated individual of the same ancestry (*e.g.*, Caucasian, African-American, Hispanic) as the suspect has the same STR profile as the suspect/blood stain. Even using the most common U.S. Caucasian alleles at the 13 CODIS loci generates a RMP value of 1 in 160 billion, far more than the number of people on Earth.

More often than not, the low copy, degraded state of touch DNA causes STR profiles to be partial, making analysis and interpretation challenging. DNA degradation may result from an array of mechanisms (*e.g.*, enzymatic and chemical processes) or environmental factors (*e.g.*, temperature, humidity, and pH) (Poinar, 2003; Lee and Ladd, 2001; Lindahl, 1993). Consequently, alleles that should be present may be missing from an STR profile (known as allelic drop-out). Additionally, degraded or LCN DNA is susceptible to preferential amplification and stochastic effects that lead to unequal amplification of template DNA.

Preferential amplification occurs when one allele amplifies more efficiently than the other because of differences in their length or sequence. Stochastic (random) sampling effects occur in the initial cycles of PCR amplification when due to chance, the low abundance or absence of template DNA may result in minimal to no DNA amplification (Figure 7; Butler and Hill, 2010; Gill *et al.*, 2000; Walsh *et al.*, 1992). Both of these scenarios may lead to peak height imbalance of heterozygous alleles at a given locus, which can result in drop-out—when a peak becomes indistinguishable from the background noise. Two other artifacts common to profiles generated from LCN DNA and stochastic sampling are allelic drop-in and stutter. Drop-in is the presence of STR alleles in a DNA profile that are generally not repeatable. A stutter peak is caused by the DNA strand slipping during replication, resulting in an amplicon one repeat unit smaller, or on rare occasions larger, than the true allele. In pristine DNA samples, stutter is easily identifiable, however, stutter peaks from LCN DNA can potentially have equal or greater peak heights in comparison to the true allele (Butler and Hill, 2010; Budowle *et al.*, 2009; Murray *et al.*, 1993). All four of these STR artifacts are illustrated in Figure 8.

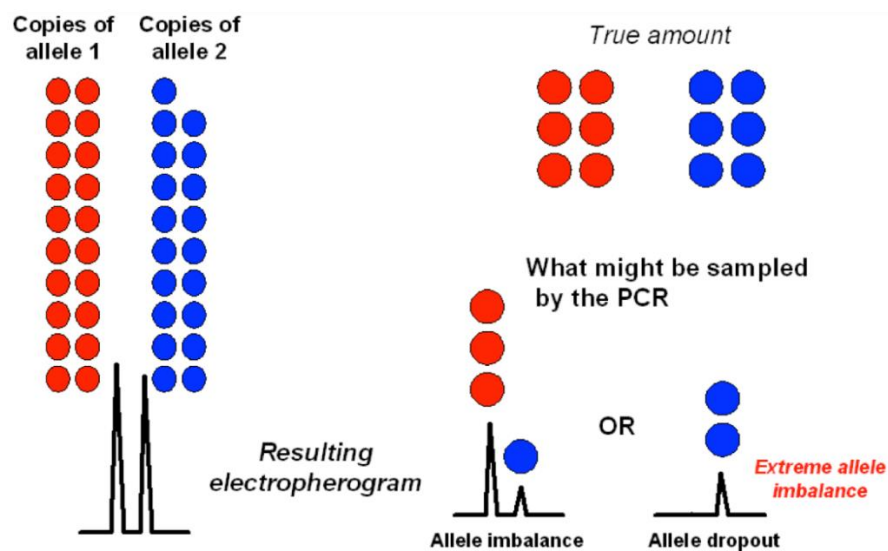


Figure 7. An example of stochastic sampling effects. When a small number of DNA templates are available from the start, there is a chance that some alleles will be amplified more than the others, resulting in imbalanced allele peak height. Taken from Krane, 2007.

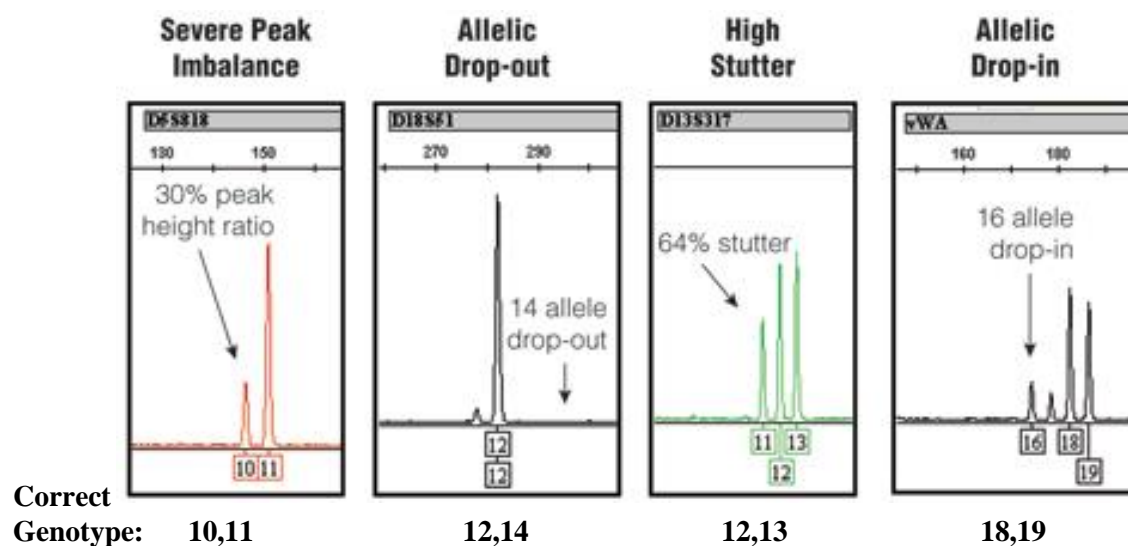


Figure 8. Common STR artifacts as a result of stochastic sampling effects and low copy, degraded DNA. Taken from Butler and Hill, 2010.

Some PCR amplification kits are designed specifically for DNA that is degraded, LCN, and/or inhibited. Table 1 shows a comparison of two such kits: the AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ PCR Amplification Kit and the PowerPlex $^{\text{®}}$ Fusion System.

Table 1. Comparison of the AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ and the PowerPlex $^{\text{®}}$ Fusion (Life Technologies, 2014; Promega, 2014).

	AmpFℓSTR$^{\text{®}}$ MiniFiler$^{\text{™}}$ PCR Amplification Kit	PowerPlex$^{\text{®}}$ Fusion System
Strengths	Optimized for genotyping degraded and/or inhibited DNA samples	Includes high inhibitor tolerance and sensitivity
Total # of Loci	8 autosomal STR loci and 1 sex-related locus (Amelogenin)	22 autosomal STR loci and 2 sex-related loci (Amelogenin and DYS391)
# of Loci < 300 bp	9	14
Minimal DNA Input for Full STR Profile	125 pg	100 pg
PCR Run Time	156 minutes	61 minutes

Previous Studies on DNA Recovered from Spent Casings

The feasibility of generating STR profiles from spent cartridge casings has been examined previously. Horsman-Hall *et al.* (2009) investigated several aspects of analyzing DNA from spent casings in order to better understand the effect of extraction methods, firing, and PCR inhibition. DNA was isolated from spent casings via four common DNA extraction methods and the yields were compared. Organic extraction followed by Microcon purification recovered a significantly lower amount of DNA than a commercial DNA extraction method (DNA IQ™ System) with varying additions (lysis buffer without proteinase K, proteinase K with 20% sarkosyl, or proteinase K with SDS) and subsequent DNA IQ™ manual purification. Pairwise t-tests showed that the DNA yields from the three DNA IQ™ extraction methods did not differ significantly. Furthermore, there was no significant difference in the total amount of DNA recovered from the casings after the weapons were fired, though full genetic profiles were only generated from the unfired casings. AmpFℓSTR® MiniFiler™, PowerPlex®16, and AmpFℓSTR® Identifiler® PCR Amplification Kits were compared for their success in allele recovery. Identifiler® did not amplify any STR alleles, and a significantly greater number of alleles were recovered with MiniFiler™ than with PowerPlex®16. At least half of the expected alleles, those consistent with the cartridge handlers, were present in over 30 percent of the MiniFiler™ profiles. PCR inhibition, most likely from the metals of the cartridge casings or residual primer components, was encountered in a portion of the samples, and MiniFiler™ was more successful in dealing with it than PowerPlex®16. Treatment with bovine serum albumin (BSA) and additional Taq polymerase did not greatly improve the PowerPlex®16 results. The results of this study demonstrated that organic extraction may not be the best method for DNA isolation from

fired cartridge casings. Furthermore, MiniFiler™ recovered the most STR alleles, which is consistent with expectations since this kit was designed for degraded and inhibited DNA.

Spear *et al.* (2005) attempted to recover DNA from planted fingerprints. After the casings were processed for fingerprints, they were swabbed and DNA was organically extracted and amplified with an AmpFℓSTR® Profiler Plus® Kit. Only three of the 48 casings generated a DNA profile, all of which came from bloody fingerprints. One of the three resulted from a fired casing and nine of the ten loci in that profile contained allelic information. It was not specified whether the alleles were consistent with the blood donor. Additionally, it is not known if fingerprint processing prior to DNA analysis had an effect on the STR results. This study accentuated the need for an optimized method for obtaining a DNA profile from spent cartridge casings.

Orlando (2012) studied different methods for DNA recovery and analysis from spent cartridge casings. Thirty-three volunteers loaded 10 cartridges directly from a box of ammunition into the magazine of a pistol. Two swabbing procedures were compared in an attempt to determine which generated higher DNA yields while minimizing contamination and PCR inhibition. An individual swabbing method used a double swab technique (first a swab wetted with 100 µL of 5% sodium dodecyl sulfate (SDS) followed by a dry swab) for each casing. A cumulative swabbing method involved multiple casings swabbed consecutively using a single wetted swab, followed by a dry swab. There was no significant difference in DNA yield between swabbing methods. An AmpFℓSTR® Identifiler® Plus Kit was used for STR analysis and consensus STR profiles were created using the five profiles from individual swabs. Among cumulative, single, and consensus profiling, 22% to 31% of the alleles recovered were consistent with the loader. The majority of the STR profiles contained only a few alleles; weak partial profiles (7 or fewer loci with alleles) were developed in 67.7% of the cumulatively swabbed

samples, 74.2% of the individually swabbed samples, and 64.5% of the consensus profiles. Only one full consensus profile was generated, which was achieved with five individually swabbed casings, three of which produced full profiles. Orlando (2012) found higher DNA yields were recovered from cumulatively swabbing casings rather than individual swabbing, although this did not necessarily mean the alleles amplified were consistent with the loader. Furthermore, while the quantity of DNA recovered from the casings was often very low and at times quantified as zero, STR alleles were still sometimes called. These results support the need and utility of high-copy loci for accurately quantifying degraded, LCN samples.

In a retrospective study at the Forensic Laboratory for DNA Research in the Netherlands, Dieltjes *et al.* (2011) developed a method to recover and extract DNA. The authors used a Qiagen QIAamp® DNA Mini Kit on 4,085 items (cartridges, bullets, and casings) collected among 616 cases and performed a modified version of the manufacturer's protocol for bloodstains. Casings were soaked in sterile 10-mL round bottom tubes with 400 µL of buffer and rotated at a non-specified angle for 30 minutes. Following soaking, casings were swabbed with a dry sterile cotton swab and the samples underwent a pre-digestion incubation for 10 minutes at 85°C. DNAs were amplified with PowerPlex® 16. The author's noted "since the success rates for cartridges and casings were rather similar, we combined their results". The success rate per criminal case was defined as "the number of criminal cases in which at least one DNA profile could be reported". The average success rate of obtaining a reproducible STR profile (defined as multiple amplifications of a locus two or more times from a single DNA extract) from cartridges/casings was 26.5%. Examining all three types of evidence, the authors obtained 283 reproducible STR profiles (98.9% contained STR data at four or more loci), 84.1% of which were consistent with a single individual (*i.e.*, 2 or fewer peaks per locus). Additionally, 51 STR

profiles were full—containing alleles from all 15 loci. However, the authors did not clarify which items yielded which results, thus it is unknown how much of the STR data was from spent casings. Furthermore, it is not clear if known STR profiles were available to make comparisons with the 4,085 cartridges, bullets, and casings analyzed.

Goals of This Study

To date, developing full STR profiles using DNA recovered from spent casings has been minimally successful. The research presented here was designed to test the hypothesis that spent cartridge casings can be a useful source of DNA if the methods for its recovery and extraction are optimized. Therefore, the first goal of this research was to optimize and compare methods for the recovery, isolation, and purification of touch DNA present on cartridge casings. Multiple variables were examined, including swabbing versus soaking casings, shaking casings during soaking, pre-digestion incubation of soaked samples at 85°C, shaking swabs during digestion, and the duration of digestion. Following optimization, DNA yields and STR results were compared among five cell recovery and DNA extraction methods: double swabbing with an organic extraction, soaking with an organic extraction, double swabbing with a silica-based extraction, soaking with a silica-based extraction, and single swabbing followed by a non-binding DNA extraction (FDF[®] Kit). The second goal was to compare the performance characteristics of two STR analysis kits—AmpF[®]STR[®] MiniFiler[™] and PowerPlex[®] Fusion—with the intent to identify the system with maximal allele recovery and the highest degree of STR allele consistency between the loader and the DNA from the casings. Overall, the research presented here was an evaluation of five optimized methods for cell recovery and DNA

extraction and two STR amplification kits, to determine an enhanced process for touch DNA analysis from spent casings.

MATERIALS AND METHODS

Cotton swabs (860-PPC, Puritan Medical Products, Guilford, ME), tubes, and pipette tips were autoclaved at 135°C for 45 min, followed by a 1 h dry cycle. All supplies (*e.g.*, tubes, racks, scissors, hemostats, cotton swabs, pipettes, tips) and reagents used in pre-amplification procedures were ultraviolet (UV) irradiated for at least 5 min (approximately 2.5 J/cm²), per side if applicable, in a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corporation, Westbury, NY) and a laboratory coat, face mask, sleeves, and two pairs of gloves were worn. Reagent blanks were created with each DNA extraction and they, along with positive and negative controls, were quantified with every rtPCR assay.

Methods for Cell Recovery

Swabbing Cartridge Casings

A double swab technique (Sweet *et al.*, 1997) was used in conjunction with organic or QIAamp® DNA Investigator Kit (Qiagen, Hilden, Germany) extractions (detailed below) on live and spent cartridge casings. The first cotton swab was wetted with 150 µL of digestion buffer (0.1% SDS, 20 mM Tris [pH 7.5], 50 mM EDTA) for organic extraction or 150 µL of Buffer ATL (tissue lysis buffer) for QIAamp® DNA Investigator Kit extraction. Casings were double-swabbed individually, and swab heads were clipped and added to 1.5 mL microcentrifuge tubes containing 400 µL of digestion/tissue lysis buffer and either 5 µL of proteinase K (20 mg/mL) for an organic extraction or 20 µL of proteinase K (Qiagen). Tubes were vortexed for 10 s and incubated overnight at 55°C. A single swab was used in combination with Fingerprint DNA Finder® Kit (FDF® Kit; NEXTTEC™ Biotechnologie GmbH, Hilgertshausen, Germany) extraction following the manufacturer's protocol. Individual swabs wetted with 30 µL of Lysis

Buffer (Buffer FP and proteinase K) were used to recover DNA from casings, swab heads were clipped and added to spin baskets in 1.5 mL microcentrifuge tubes. Fifty additional microliters of Lysis Buffer were added to the swabs, tubes were vortexed for 10 s, and incubated for 30 min at 55°C.

Soaking Cartridge Casings

A modified version of the soaking method performed by Dieltjes *et al.* (2011) was used in combination with organic extraction or QIAamp[®] extraction on spent cartridge casings. Ten milliliter beakers, 15 mL conical tubes, 15 mL culture test tubes, 5 mL stuffed pipette tips, and various sizes of the bulb portion of transfer pipettes were tested as possible vessels for soaking casings. Based on preliminary findings, the bulb portion of a Samco[™] General-Purpose Transfer Pipette (Thermo Fisher Scientific, Waltham, MA) 13 mm in diameter was selected for subsequent soakings. Casings were placed in bulbs containing enough digestion/tissue lysis buffer to fully submerge the outside of the casing (700 µL) and soaked for 30 min (Figure 9). Casings were removed and soaking solutions were transferred to 1.5 mL microcentrifuge tubes. Bulbs and the outside surface of casings were swabbed with a dry swab. Swab heads were clipped and added to soaking solutions. Either 5 µL of proteinase K (20 mg/mL) or 20 µL of proteinase K (Qiagen) were added to each tube. Tubes were vortexed for 10 s and incubated overnight at 55°C.



Figure 9. Example of a casing soaking in 700 μL of digestion/tissue lysis buffer.

Methods for DNA Extraction

Organic Extraction

Swab heads from swabbings were transferred to spin baskets (Fitzco, Spring Park, MN) using hemostats and centrifuged at 20,000 relative centrifugal force (rcf) for 4 min. Heads were discarded and flow-throughs were transferred to the original tubes. Equal volumes of phenol were added to the tubes, which were vortexed for 10 s and centrifuged at maximum speed for 5 min. Aqueous layers were transferred to new 1.5 mL microcentrifuge tubes containing equal volumes of chloroform. Tubes were vortexed for 10 s and centrifuged at maximum speed for 5 min. Amicon[®] Ultra-0.5 mL, 30 kDa filtration columns (Millipore Corporation, Billerica, MA) were pre-treated with 1 μL of 10 $\mu\text{g}/\mu\text{L}$ yeast (*Saccharomyces cerevisiae*) rRNA (Alfa Aesar, Ward Hill, MA) and 499 μL of low TE (10 mM Tris [pH 7.5], 0.1 mM EDTA), centrifuged at 14,000 rcf for 10 min, and flow-throughs were discarded. Aqueous layers were transferred to the pre-treated spin columns, centrifuged at 14,000 rcf for 10 min, and flow-throughs discarded. DNAs were washed with 300 μL of TE (10 mM Tris [pH 7.5], 1 mM EDTA), centrifuged at 14,000 rcf for 10 min, and flow-throughs discarded. Two additional washes were performed with 300 μL of low TE. Filtration columns were inverted into new Amicon[®] collection tubes and centrifuged at 1,000 rcf for 3 min to collect retentates. Organic extractions were performed on

DNAs from buccal swabs in the same manner, except two washes with TE and one with low TE were performed. DNAs were stored at -20°C.

QIAamp[®] DNA Investigator Extraction

Swab heads were transferred to spin baskets using hemostats and centrifuged at 20,000 *g* for 4 min. Heads were discarded and flow-throughs were collected in the original tubes. DNA isolations and purifications were performed per the manufacturer's protocol for surface and buccal swabs, including the addition of carrier RNA to Buffer AL, with the following modification: three elutions were collected for each DNA extraction by adding 20 µL of Buffer ATE to column membranes, incubating at room temperature for 5 min, and centrifuging at maximum speed for 3 min (Hebda *et al.*, in press).

Fingerprint DNA Finder[®] Extraction

Prior to performing FDF[®] extractions on DNA recovered from spent casings, known genomic male DNA (145 ng/µL, Promega) was extracted with solutions from an FDF[®] Kit (Buffer FP and Prep Solution) that were either UV irradiated for 10 min or not treated. Based on lower DNA yields, none of the reagents in the FDF[®] extractions were UV irradiated for subsequent experiments. DNAs were extracted and purified according to the manufacturer's protocol for isolation of genomic DNA from fingerprints and low template DNA samples.

DNA Quantification via Real-Time PCR Analysis

Volumes of the DNA extracts were measured prior to DNA quantification. PCR amplification was performed on an iCycler[™] Thermal Cycler (Bio-Rad Laboratories, Hercules,

CA) and fluorescence was detected using an iQTM5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories). Table 2 contains the sequences of primers, probes, and IPC template DNA used for quantification. *Alu* primer and probe sequences were designed by Nicklas and Buel (2005) and IPC primers, probe, and template were designed by Lindquist *et al.* (2011). Primers, probes, and IPC template were obtained from Sigma-Aldrich (St. Louis, MO) or Integrated DNA Technologies (Coralville, IA). *Alu* forward and reverse primers were filtered through Microcon YM-100 membranes (Millipore Corporation). *Alu* standards were created via serial dilutions of Standard Reference Material[®] 2372 Human DNA Quantitation Standard Component A (genomic DNA from a single male donor; 57 ng/μL; National Institute of Standards and Technology, Gaithersburg, MD) in low TE with 20 μg/mL glycogen yielding six DNA standards at concentrations of 2000, 200, 20, 2, 0.2, and 0.02 pg/μL.

Table 2. Primer, probe, and IPC template sequences for rtPCR. HEX and 6FAM fluorescent dyes were on the 5' end of the *Alu* and IPC probes, respectively. BHQ1 and Iowa Black[®] FQ (IABkFQ) are quenchers on the 3' end of the *Alu* and IPC probes, respectively.

Primer Name	Sequence	Amplicon Length
F <i>Alu</i>	5'-GAG ATC GAG ACC ATC CCG GCT AAA-3'	113 bp
R <i>Alu</i>	5'-CTC AGC CTC CCA AGT AGC TG-3'	
<i>Alu</i> probe	5'-HEX-GGG CGT AGT GGC GGG-BHQ1-3'	
F IPC	5'-AAG CGT GAT ATT GCT CTT TCG TAT AG-3'	77 bp
R IPC	5'-ACA TAG CGA CAG ATT ACA ACA TTA GTA TTG-3'	
IPC probe	5'-6FAM-TAC CAT GGC-ZEN-AAT GCT-IABkFQ-3'	
IPC template	5'-AAG CGT GAT ATT GCT CTT TCG TAT AGT TAC CAT GGC AAT GCT TAG AAC AAT ACT AAT GTT GTA ATC TGT CGC TAT GT-3'	

Real-time PCR reactions were set up in 0.2 mL optically clear flat-capped PCR strips (USA Scientific[®], Ocala, FL) with final volumes of 15 μ L. rtPCR reactions consisted of 7.5 μ L of iQ[™] Supermix (Bio-Rad Laboratories), 500 nM *Alu* forward primer, 900 nM *Alu* reverse primer, 250 nM *Alu* probe, 1 μ M IPC forward and reverse primer, 250 nM IPC probe, 1 μ L of the working concentration of IPC template DNA (1:1 billion dilution of 100 μ M stock), 0.625 units of Taq DNA polymerase (5 U/ μ L; Empirical Bioscience, Grand Rapids, MI), 0.625 μ L of deionized water (diH₂O), and 1 μ L of DNA. DNA standards were run in duplicate. rtPCR cycling parameters included: 3 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C.

Data were analyzed with iQ[™]5 Optical System Software. A standard curve was generated based on the C_t values of the DNA standards, and DNA concentrations of the samples were extrapolated. DNA yields (pg) were calculated by multiplying rtPCR concentrations (pg/ μ L) by DNA extract volumes (μ L).

STR Amplification

The highest quantifying DNAs from Collections 1 and 2 (described below) were amplified on an Applied Biosystems 2720 Thermal Cycler (Life Technologies, Carlsbad, CA) using an AmpF ℓ STR[®] MiniFiler[™] PCR Amplification Kit (Life Technologies) and a PowerPlex[®] Fusion System (Promega, Madison, WI), to determine which resulted in the most allele calls. Based on greater recovery of allelic data, all DNAs organically extracted were amplified with Fusion, as were subsets of the DNA extracts from the other optimized methods, beginning at the highest concentrations. Once samples yielded no STR results, further amplification at lower concentrations was stopped. Reagent volumes and cycling parameters are listed in Table 3. In

instances where DNA yields were low, volumes of DNAs added to STR reactions were ‘maxed out’.

Table 3. PCR Amplification of extracted DNA with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion.

AmpFℓSTR$^{\circ}$ MiniFiler$^{\text{TM}}$	10 μ L reaction: <ul style="list-style-type: none"> • 4 μL AmpFℓSTR$^{\circ}$ MiniFiler$^{\text{TM}}$ Master Mix • 1 μL AmpFℓSTR$^{\circ}$ MiniFiler$^{\text{TM}}$ Primer Set • Approx. 500 – 750 pg or 5 μL of DNA Cycling parameters: <ul style="list-style-type: none"> • 11 min at 95°C • 30 cycles of 20 s at 94°C, 2 min at 59°C, and 1 min at 72°C • 45 min at 60°C
PowerPlex$^{\circ}$ Fusion	10 μ L reaction: <ul style="list-style-type: none"> • 2 μL PowerPlex$^{\circ}$ Fusion Master Mix • 2 μL PowerPlex$^{\circ}$ Fusion Primer Pair Mix • Approx. 250 – 500 pg or 6 μL of DNA Cycling parameters: <ul style="list-style-type: none"> • 1 min at 96°C • 30 cycles of 10 s at 94°C, 1 min at 59°C, and 30 s at 72°C • 10 min at 60°C

Capillary Electrophoresis

One microliter of amplified MiniFiler $^{\text{TM}}$ PCR product was added to 8.7 μ L of Hi-Di $^{\text{TM}}$ Formamide (Life Technologies) and 0.3 μ L of GeneScan $^{\text{TM}}$ LIZ 500 Size Standard (Life Technologies). One microliter of amplified PowerPlex $^{\circ}$ Fusion PCR product was added to 10 μ L of Hi-Di $^{\text{TM}}$ Formamide and 1 μ L of CC5 Internal Lane Standard 500 (Promega). Capillary electrophoresis was performed on an Applied Biosystems 3500 Genetic Analyzer (Life Technologies) according to the parameters listed in Table 4. Data were analyzed with GeneMapper $^{\circ}$ Software v4.1 (Life Technologies) at a threshold of 100 relative fluorescence units (RFUs) for all dyes.

Table 4. Run parameters for capillary electrophoresis on an AB 3500 genetic analyzer with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion amplified products.

	AmpFℓSTR$^{\circ}$ MiniFiler$^{\text{TM}}$	PowerPlex$^{\circ}$ Fusion
Oven Temperature ($^{\circ}$C)	60	60
Pre-Run Voltage (kV)	15	15
Pre-Run Time (s)	180	180
Injection Voltage (kV)	1.6	1.2
Injection Time (s)	8	24
Run Voltage (kV)	19.5	15
Run Time (s)	1330	1500
Capillary Length (cm)	50	50

Optimization of Cell Recovery and DNA Extraction

Spent .40-caliber Smith & Wesson brass cartridge casings were used in optimization experiments. Casings were cleaned with 1% Liquinox $^{\text{TM}}$ detergent (Alconox, White Plains, NY) and water, then decontaminated with ELIMINase $^{\circ}$ (Decon Laboratories, King of Prussia, PA) as per the manufacturer's instructions. Casings were rubbed with water twice, dried with a Kimwipe (Kimberly-Clark Corporation, Irving, TX), and exposed to UV light sitting upright (casing head closest to the bulbs) for a minimum of 5 min. Volunteers handled casings for 5 s in a random order. Changes in DNA yields were calculated for each optimization experiment by dividing the difference in average yields between treatment and non-treatment by the average yield of non-treated samples.

Decontamination of Transfer Pipette Bulbs

Preliminary tests showed the presence of human mitochondrial DNA on the inner surface of Samco $^{\text{TM}}$ General-Purpose Transfer Pipettes, therefore, ten pipettes were subjected to the treatments listed in Table 5. Organic extractions were performed and DNA yields from treated and non-treated bulbs were compared.

Table 5. Examination of foreign DNA on the inner portion of transfer pipette bulbs.

Sample	Treatment of Transfer Pipettes
1	No Treatment
2	Cut off bulb + soaked in 100% Clorox [®] Ultra Bleach (6.15% NaClO; Commercial Solutions [®] , Oakland, CA) for 30 min + soaked in diH ₂ O for 5 min
3	Cut off bulb + soaked in 50% Clorox [®] Ultra Bleach (3.07% NaClO) for 30 min + soaked in diH ₂ O for 5 min
4	Cut off bulb + soaked in ELIMINase [®] for 30 min + soaked in diH ₂ O for 5 min
5	Cut off bulb + UV irradiated bulb upright in a rack for 10 min
6	Cut off bulb + soaked in 100% Clorox [®] Ultra Bleach for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min
7	Cut off bulb + soaked in 50% Clorox [®] Ultra Bleach for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min
8	Cut off bulb + soaked in ELIMINase [®] for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min
9	Drew up 100% Clorox [®] Ultra Bleach + inverted + soaked for 4 d
10	Drew up 50% Clorox [®] Ultra Bleach + inverted + soaked for 4 d
11	Drew up ELIMINase [®] + inverted + soaked for 4 d

Pre-digestion Treatments Investigated Within the Soaking Method

Three variables within the soaking recovery method were examined to either minimize DNA loss or maximize DNA recovery. (1) The inside surface of transfer pipette bulbs were pre-treated with 1 μ L of 10 μ g/ μ L yeast rRNA and 499 μ L of low TE prior to soaking handled casings. DNA yields from pre-treated and non-pre-treated bulbs were compared. (2) Casings were shaken at 900 rpm on an Orbit[™] P2 Digital Shaker (Labnet International, Edison, NJ) during the soaking period. DNA yields from shaken and non-shaken casings were compared. (3) Following the soaking period, tubes were incubated at 85°C for 10 min and vortexed every 3 min for 10 s. DNA yields from samples subjected to a pre-digestion incubation and samples not treated were compared.

Digestion Optimization

Overnight digestions at 55°C were compared to 1 h digestions at 55°C with standard organic extractions and QIAamp® extractions. FDF® extractions were also analyzed after a 30 min digestion, which is the time recommended by the manufacturer. Further, the FDF® manufacture's protocol recommends shaking the swabs during digestion at 600 rotations per minute (rpm) and this step was followed as it is a newer isolation method. Standard protocols for organic and QIAamp® extractions at the Michigan State University (MSU) Forensic Biology Laboratory do not include shaking during digestion, although the QIAamp® protocol recommends it. Following Qiagen's recommendations, organic and QIAamp® extractions were tested with shaking at 900 rpm during the digestion. DNA yields of shaken samples were compared to those that were stationary during digestion. Based on increased DNA yields, all subsequent experiments included shaking during 1 h digestions.

Comparison of Optimized Cell Recovery and DNA Extraction Methods

Obtaining Ammunition and Testing for Foreign DNA on Live Cartridges

Live rounds of .40-caliber Smith & Wesson brass cartridges (ATK Sporting Group—Anoka, MN; Remington® Arms Company, LLC—Madison, NC; Winchester—Morgan, UT) were either donated by MSU Deputy Police Chief Dave Trexler or purchased at local retail stores. Two cartridges per box of ammunition were randomly selected and tested for the presence of background DNA. Based on low DNA yields from the non-handled ammunition (avg. DNA = 3.15 pg), live cartridges were not decontaminated prior to loading into magazines.

Loading Cartridges

The use of human subjects was approved by the Michigan State University Committee on Research Involving Human Subjects (IRB 12-770). Volunteers signed a consent form and provided two buccal swabs as DNA reference samples. Each volunteer randomly selected a letter and a number, associated with the volunteer's buccal swabs and the ammunition they handled respectively. Only the letter and number were recorded for each volunteer, which deidentified all samples. Volunteers were provided live rounds of ammunition and loaded the magazine(s) (Table 6). Volunteers loaded the same magazine(s) in Collections 1 and 2, while two magazines were alternated among loaders in Collection 3.

Collection of Spent Cartridge Casings

Handled cartridges were fired and spent casings were collected as describe in Table 6. During Collection 1, six casings were collected individually (one casing per plastic bag) and the ejection order was recorded. All six plastic bags were put into a paper bag labeled with the volunteer's number. During Collections 2 and 3, casings were collected in sets of three (Set 1 = 1st, 2nd, and 3rd ejected casings; Set 2 = 4th, 5th, and 6th ejected casings; etc.) and transferred using hemostats to a brown paper bag labeled with the volunteer's number and the set number. For example, if a volunteer randomly selected H and 7, then a bag labeled 7-4 would contain the 10th, 11th, and 12th ejected casings handled by the volunteer associated with buccal H. Hemostats were wiped with 50% Clorox[®] Ultra Bleach between volunteers during Collection 2. Casing sets were assigned to a treatment on an alternating (round robin) basis (Appendix A), allowing results to be attributed to the cell recovery and DNA extraction method and not firing order. Spent cartridge casings and buccal swabs were stored at -20°C.

Table 6. Spent cartridge casing collection events. Letters (A, B, and C) indicate a different firearm/magazine.

Collection	Location of Collection	Firearm	Magazine	Ammunition	Format Rounds Were Provided to Volunteers	Rounds Loaded Per Volunteer	Collection Apparatus
1	Private property (Garden Prairie, IL)	A	A	Remington UMC® .40 S&W Full Metal Jacket	Volunteers randomly selected from a box of ammunition	6	Plastic bags held by a metal wire near the ejection port
2	MSP Forensic Science Laboratory (Lansing, MI)	B	B & C	American Eagle® Federal Premium Ammunition and Blazer® Brass .40 S&W Full Metal Jacket	Bag of pre-divided rounds of ammunition	21	Denim microscope cover with a hole cut out, the firearm shot through the hole while the cover surrounded the firearm
3	Private property (Bath, MI)	A	A & D	Winchester® .40 S&W Full Metal Jacket	Bag of pre-divided rounds of ammunition	12	New cotton pillowcase with a hole cut out, the firearm shot through the hole while the pillowcase surrounded the firearm

Optimized Method for Soaking Cartridge Casings

Samco™ General-Purpose Transfer Pipettes were UV irradiated for 10 min, bulbs were cut off, set upright in a rack, and irradiated for an additional 10 min. Casings were placed in bulbs containing 700 µL of digestion buffer (organic extraction) or Buffer ATL (QIAamp®) and soaked for 30 min. Casings were removed and buffer solutions were transferred to 1.5 mL microcentrifuge tubes. Bulbs and the outside surface of casings were swabbed with a dry swab. Swab heads were clipped and added to soaking solutions. Tubes were incubated at 85°C for 10 min, vortexing every 3 min for 10 s. Either 5 µL of proteinase K (20 mg/mL) or 20 µL of proteinase K (Qiagen) were added to tubes, which were vortexed for 10 s and incubated with shaking at 900 rpm for 1 h at 55°C. Based on minimal DNA loss and lower DNA yields, bulbs were not pre-treated and casings were not shaken during the soak period, respectively.

Comparison of DNA Yields

All statistical tests were performed using XLSTAT 2014.2.01 (Addinsoft, Paris, France) with 95% confidence. DNA yields were tested for normality using the Shapiro-Wilk test; if $p < 0.05$ then non-parametric analyses were performed. A Kruskal-Wallis test was performed on non-parametric data to examine whether samples from the five cell recovery and DNA extraction methods shared a similar distribution. Individual pairwise comparisons were performed using the Mann-Whitney test to determine whether DNA yields significantly differed between cell recovery and DNA extraction methods.

Comparison of STR Profiles

Casing STR profiles were compared to volunteers' reference profiles and alleles were designated as loader or non-loader. Descriptive statistics (average # loader alleles, # possible loader alleles, % loader profile, and # non-loader alleles) were calculated for each optimized method. The percentage of a cartridge loader's profile was determined based on the number of alleles consistent with the loader divided by the number of possible alleles that each volunteer could have provided. Homozygous alleles in the reference profiles were counted as one possible allele. Mann-Whitney pairwise comparisons were performed on the number of loader and non-loader alleles amplified with MiniFiler™ and Fusion. RMP values of MiniFiler™ and Fusion profiles generated from sample 34.4 were calculated as both contained alleles at all loci tested. Further, all Fusion profiles were evaluated for the frequency of loader alleles at each locus to assess if the DNA recovered from spent casings was degraded.

Consensus profiles were generated by combining the Fusion profiles of the organically extracted DNAs recovered via double swabbing or soaking. If an allele was present at least twice among the three individual profiles, then that allele was included in the consensus profile. Additionally, descriptive statistics were calculated for Fusion consensus profiles. The percentages of loaders' profiles present in Fusion profiles were tested for normality using the Shapiro-Wilk test; if $p < 0.05$ for any of the cell recovery and DNA extraction methods then non-parametric analyses were performed. A Kruskal-Wallis test was performed on the non-parametric data to determine if a significant difference existed among the optimized methods. Individual pairwise comparisons were performed with the Mann-Whitney test to determine if certain cell recovery and DNA extraction methods generated significantly greater percentages of

loaders' profiles. DNA quantities recovered with each optimized method were linearly correlated to the amount of loader alleles amplified in Fusion profiles.

RESULTS

Optimization of Cell Recovery and DNA Extraction Methods

Decontamination of Transfer Pipette Bulbs

Nuclear DNA yields from treated and untreated transfer pipette bulbs (avg. yield = 1.18 pg and 0.41 pg, respectively) were similar and extremely low—less than a cell's worth of DNA (Table 7). The transfer pipette bulb filled with ELIMINase® for 4 d contained the least amount of DNA (0.25 pg), however the treatment seemed impractical considering the DNA yield was only slightly different from that of the non-treated bulb. Given these results, bulbs were UV irradiated with the other pre-amplification supplies.

Table 7. DNA quantities recovered from treated and non-treated bulbs. A higher average DNA yield (1.18 pg) was recovered from transfer pipette bulbs subjected to various treatments than the untreated bulb (0.41 pg). DNA yields were calculated based on 28 μ L retention.

Sample	Treatment of Transfer Pipettes	DNA Yield (pg)
1	No Treatment	0.41
2	Cut off bulb + soaked in 100% Bleach for 30 min + soaked in diH ₂ O for 5 min	4.06
3	Cut off bulb + soaked in 50% Bleach for 30 min + soaked in diH ₂ O for 5 min	0.77
4	Cut off bulb + soaked in ELIMINase® for 30 min + soaked in diH ₂ O for 5 min	2.20
5	Cut off bulb + UV irradiated the bulb upright in a rack for 10 min	1.11
6	Cut off bulb + soaked in 100% Bleach for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min	0.54
7	Cut off bulb + soaked in 50% Bleach for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min	0.44
8	Cut off bulb + soaked in ELIMINase® for 30 min + soaked in diH ₂ O for 5 min + UV irradiated for 10 min	0.65
9	Drew up 100% Bleach + inverted + soaked for 4 d	0.94
10	Drew up 50% Bleach + inverted + soaked for 4 d	0.86
11	Drew up ELIMINase® + inverted + soaked for 4 d	0.25
12	Reagent Blank	0.50
13	Negative Control	0.01

Pre-treatment of Transfer Pipette Bulbs with Yeast rRNA

Figure 10 shows the average DNA yields from transfer pipette bulbs that were and were not pre-treated with yeast rRNA prior to soaking handled casings (n = 2 per extraction method). Organic extractions performed on pre-treated bulbs yielded an average of 108.99 pg of DNA and untreated bulbs yielded an average of 108.06 pg of DNA. A similar result was obtained when QIAamp® extractions were performed; an average of 21.54 pg of DNA was recovered from pre-treated bulbs while 17.75 pg of DNA was recovered from untreated bulbs. DNA recovery from pre-treated bulbs increased by less than 1.0% with organic extraction and 21.3% with QIAamp® extraction. Based on these results, transfer pipette bulbs were not pre-treated in subsequent experiments.

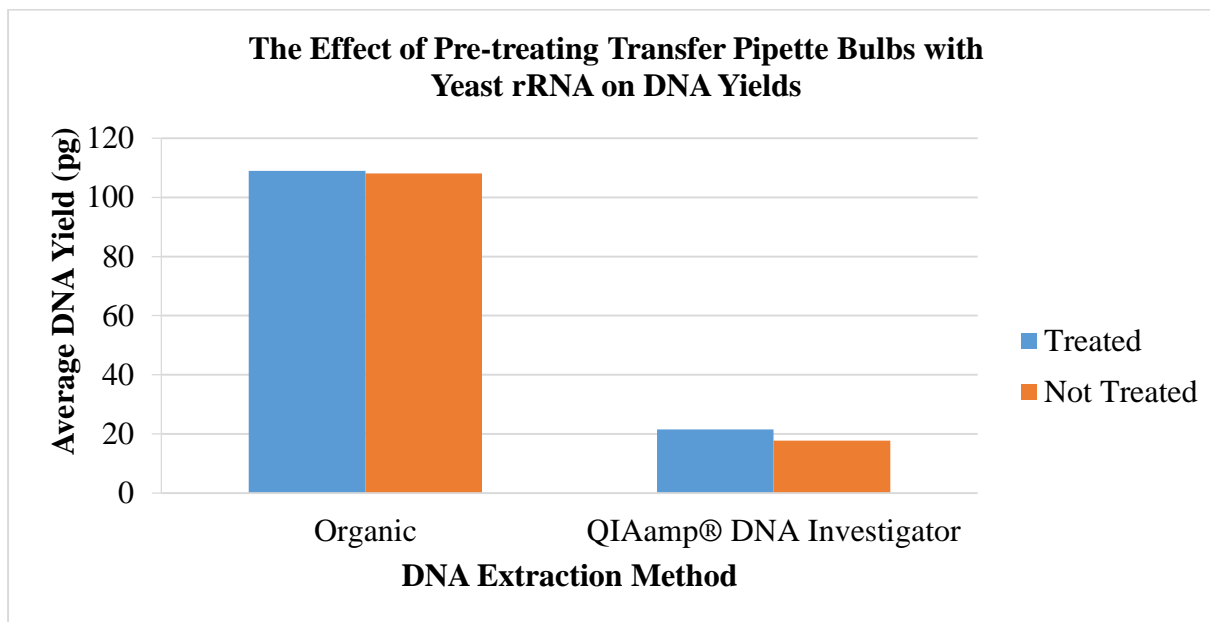


Figure 10. Average DNA yields from yeast rRNA pre-treated (organic = 108.99 pg and QIAamp® = 21.54 pg) and not pre-treated transfer pipette bulbs (organic = 108.06 pg and QIAamp® = 17.75 pg) prior to the soak period (n = 2 per extraction method). Given these results, bulbs were not pre-treated in subsequent experiments.

Shaking Casings During Soak Period

Figure 11 shows the average DNA yields from casings that were shaken at 900 rpm or kept stationary for the 30 min soak period (n = 4 per extraction method). Organic extractions on shaken casings yielded an average of 54.11 pg of DNA and stationary casings yielded an average of 377.52 pg of DNA. QIAamp[®] extractions recovered an average of 13.64 pg of DNA from shaken casings and 25.51 pg of DNA from stationary ones. The inclusion of shaking during the soak period caused an 85.7% loss in DNA using organic extraction and 46.5% DNA loss with QIAamp[®] extraction relative to non-shaking. Additionally, the digestion/tissue lysis buffer post soak period was a light blue color for those samples that were stationary and a more intense blue color for those samples that were shaken, although based on the IPC, this did not seem to affect amplification. Owing to the decreased yields, casings were not shaken in subsequent experiments.

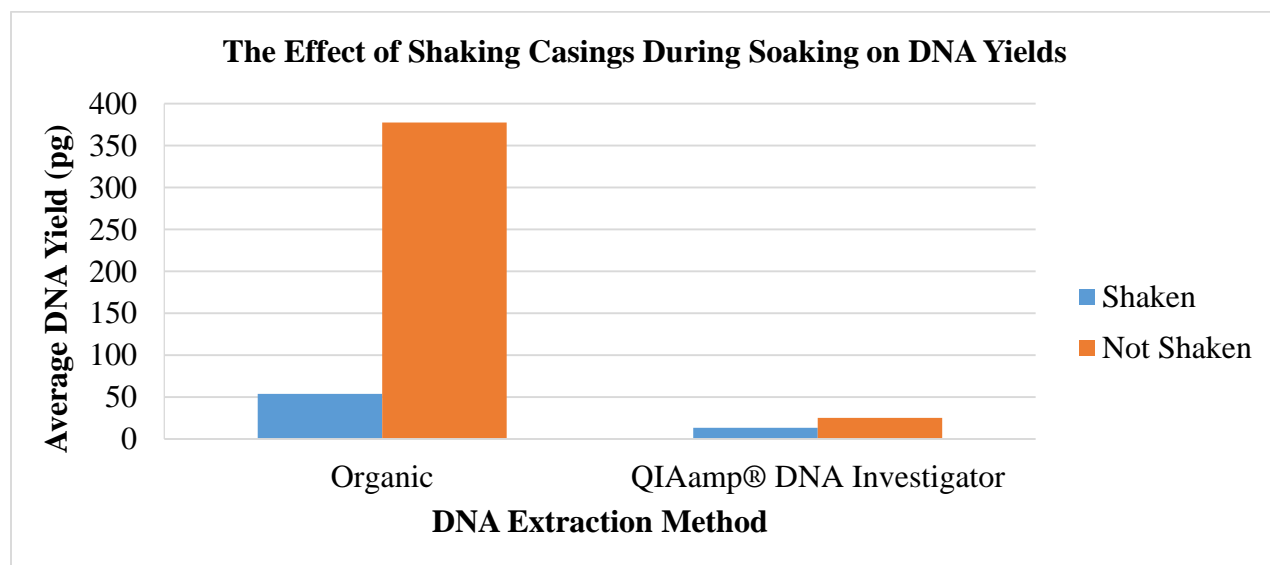


Figure 11. Average DNA yields from casings that were shaken at 900 rpm (organic = 54.11 pg and QIAamp[®] = 13.64 pg) and casings that were stationary (organic = 377.52 pg and QIAamp[®] = 25.51 pg) for the 30 min soak period (n = 4 per extraction method). Given these results, casings were not shaken in subsequent experiments.

Pre-digestion Incubation at 85°C

The average DNA yields from samples that did and did not undergo pre-digestion incubation are presented in Figure 12 (n = 2 per extraction method). Organic extractions performed on samples undergoing pre-digestion incubation yielded an average of 144.59 pg of DNA and non-incubated samples yielded an average of 59.91 pg of DNA. QIAamp® extractions recovered an average of 26.45 pg of DNA from samples undergoing incubation while 18.06 pg of DNA was recovered from samples that did not. The incubation prior to cell/DNA digestion improved DNA recovery from handled casings that were soaked by 141.3% using organic extractions and 46.4% with QIAamp® extractions, therefore future experiments included a pre-digestion incubation.

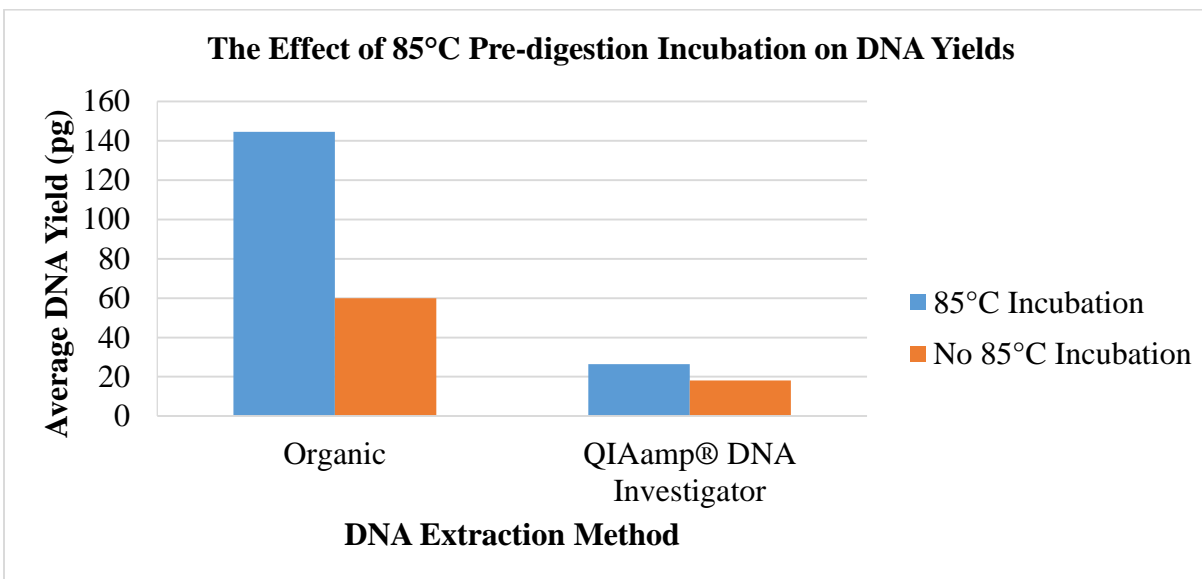


Figure 12. Average DNA yields from samples that were subjected to a pre-digestion incubation (organic = 144.59 pg and QIAamp® = 26.45 pg) and samples that were not (organic = 59.91 pg and QIAamp® = 18.06 pg) (n = 2 per extraction method). Based on these results, soaked samples were incubated for 10 min at 85°C in subsequent experiments.

Optimal Digestion Time

Figure 13 shows the average DNA yields from swabs digested for 1 h or overnight followed by organic or QIAamp[®] extraction, along with results from swabs digested for 30 min, 1 h, or overnight followed by FDF[®] extraction. Organic extraction with 1 h digestion yielded an average of 43.09 pg of DNA by double swabbing (11.7 % increase) and 85.38 pg by soaking (104.8% increase), compared to overnight digestion with double swabbing (38.59 pg) or with soaking (41.69 pg), respectively. Similar results were achieved via double swabbing and QIAamp[®] extraction: 1 h digestion yielded an average 5.5 pg (200.5% increase) while overnight yielded an average of 1.83 pg of DNA. The exception was QIAamp[®] extraction, where soaking recovered 264.5% more DNA with overnight digestion (avg. DNA = 3.39 pg) compared to 1 h digestion (avg. DNA = 0.93 pg). Finally, FDF[®] extraction recovered 144.0% more DNA with 1 h digestion (avg. DNA = 84.9 pg) than 30 min digestion (avg. DNA = 34.8 pg) and 342.2% more DNA than overnight digestion (avg. DNA = 19.2 pg). Given these results, 1 h digestion was performed in subsequent experiments.

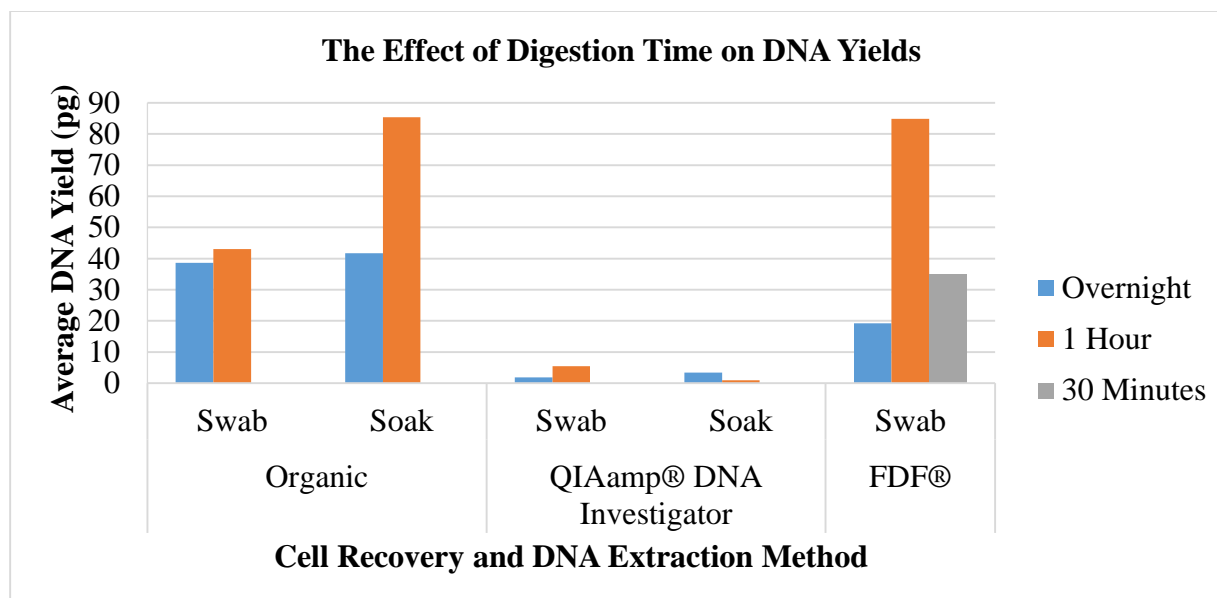


Figure 13. Average DNA yields recovered from samples digested for 1 h (double swab + organic = 43.09 pg; soak + organic = 85.38 pg; double swab + QIAamp® = 5.5 pg; soak + QIAamp® = 0.93 pg; FDF® = 84.9 pg) or digested overnight (double swab + organic = 38.59 pg; soak + organic = 41.69 pg; double swab + QIAamp® = 1.83 pg; soak + QIAamp® = 3.39 pg; FDF® = 19.2 pg) or digested for 30 min (FDF® = 34.8 pg). Owing to these results, 1 h digestion was performed in subsequent experiments.

Shaking Swabs During Digestion

Figure 14 displays the average DNA yields from samples with or without shaking at 900 rpm during the digestion (n = 2 per extraction method). An average of 95.37 pg of DNA was recovered using organic extraction and shaking during digestion, a 59.2% increase over samples not shaken (avg. DNA = 59.91 pg). QIAamp® extractions recovered 25.6% more DNA with shaking (avg. DNA 22.68 pg) than stationary samples (avg. DNA = 18.06 pg). Based on these results, subsequent experiments included shaking during digestions.

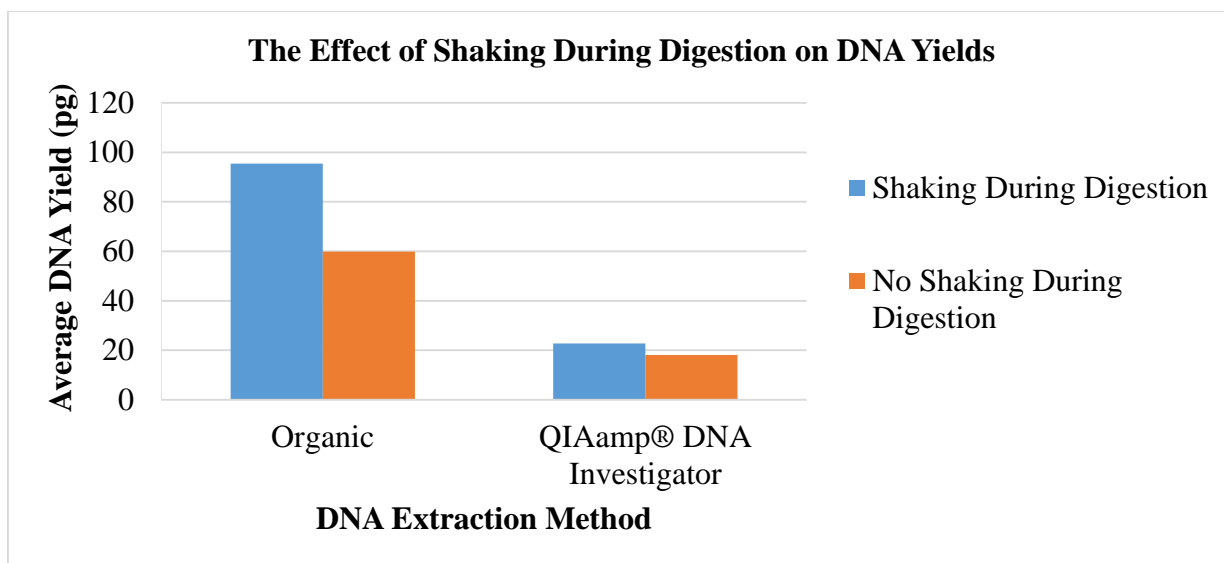


Figure 14. Average DNA quantities recovered from swabs that were either shaken (organic = 95.37 pg and QIAamp® = 22.68 pg) or stationary (organic = 59.91 pg and QIAamp® = 18.06 pg) during digestion (n = 2 per extraction method). Given these results, cell/DNA digestions included shaking in subsequent experiments.

Comparisons of Optimized Cell Recovery and DNA Extraction Methods

Comparisons of DNA Yields

A total of 420 casings were assayed using the five optimized cell recovery and DNA extraction methods: 90 casings per method with the exception of 60 casings for FDF® extraction. None of the DNA extracts contained detectable PCR inhibition. DNA concentration, extract volume, and yield from each casing are presented in Appendix B (organized according to the cell recovery and DNA extraction method). DNA yields among all five methods were not normally distributed (Shapiro Wilk, $p < 0.0001$) and there was a significant difference in DNA yields among methods (Kruskal-Wallis, $p < 0.0001$). Further, DNA yields differed significantly when pairwise relationships were analyzed between cell recovery and DNA extraction methods (Table 8). Double swabbing recovered a significantly greater amount of DNA than soaking (Mann-Whitney; organic extractions, $p = 0.0180$; QIAamp® extractions, $p < 0.0001$). Additionally,

organic extraction recovered significantly more DNA in comparison to QIAamp[®] and FDF[®] extractions (Mann-Whitney; organic vs. QIAamp[®], $p < 0.0001$ for both DNA recovery methods; organic vs. FDF[®], $p < 0.0001$). Figure 15 illustrates the median DNA yields from the optimized cell recovery and DNA extraction methods. Organic extractions had median DNA yields of 25.32 pg with double swabbing and 14.95 pg using soaking. QIAamp[®] extractions had median DNA yields of 3.81 pg with double swabbing and 1.18 pg with soaking. FDF[®] extractions recovered the least amount of DNA, with a median yield of 0.20 pg.

Table 8. Mann-Whitney pairwise comparisons (2-tailed) of DNA quantities retrieved with the optimized cell recovery and DNA extraction methods. (Bold = significantly greater DNA yields)

Pair		P-value
Double Swab + Organic	Soak + Organic	0.018
Double Swab + Organic	Double Swab + QIAamp [®]	< 0.0001
Double Swab + Organic	Soak + QIAamp [®]	< 0.0001
Double Swab + Organic	FDF [®]	< 0.0001
Soak + Organic	Double Swab + QIAamp [®]	< 0.0001
Soak + Organic	Soak + QIAamp [®]	< 0.0001
Soak + Organic	FDF [®]	< 0.0001
Double Swab + QIAamp[®]	Soak + QIAamp [®]	< 0.0001
Double Swab + QIAamp[®]	FDF [®]	< 0.0001
Soak + QIAamp[®]	FDF [®]	< 0.0001

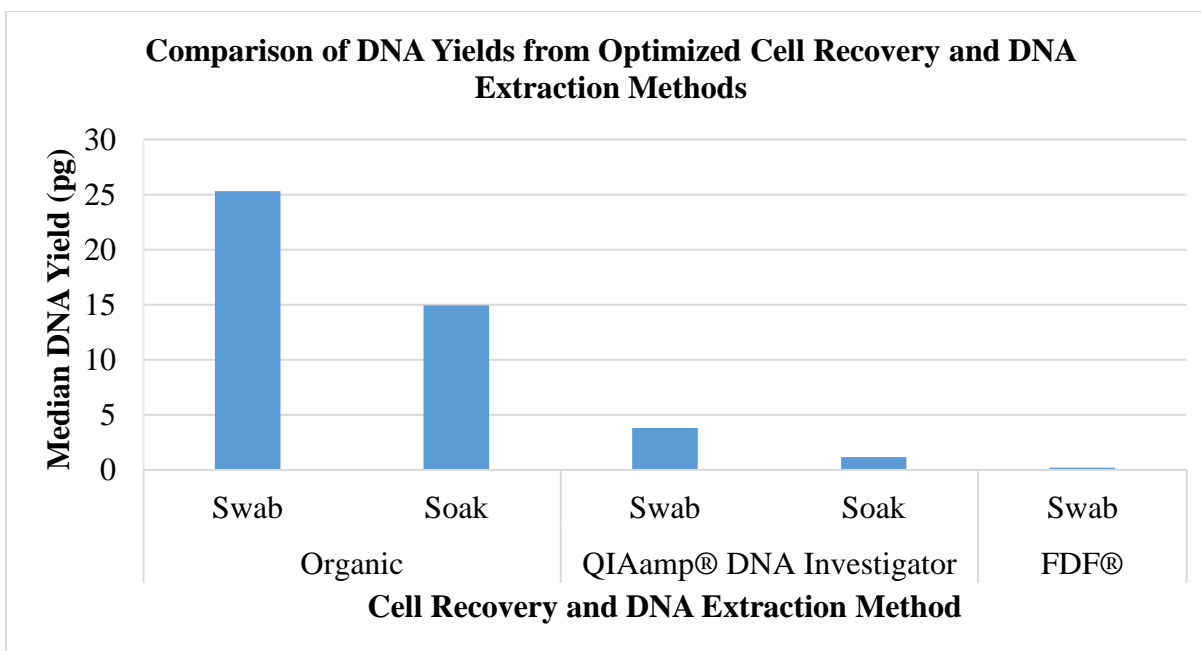


Figure 15. Median DNA quantities recovered using optimized cell recovery and DNA extraction methods. Median DNA yields from organic extractions (double swab = 25.32 pg and soak = 14.95 pg) were significantly higher than the median DNA yields from QIAamp® extractions (double swab = 3.81 pg and soak = 1.18 pg) and the median DNA yield from FDF® extractions (0.20 pg).

Comparison of MiniFiler™ and Fusion STR Profiles

Profiles from DNAs amplified using AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion are in Appendix C. Appendix D presents the number of consistent loader alleles, the number of possible loader alleles, the percentages of loaders' profile, and the number of non-loader alleles present in each profile. Table 9 summarizes the data presented in Appendix D. The average number of alleles consistent with the loader was 10.8 (MiniFiler™) and 27.33 (Fusion) with double swabbing followed by organic extractions, 10.31 (MiniFiler™) and 22.37 (Fusion) with soaking followed by organic extractions, 1.57 (MiniFiler™) and 5.71 (Fusion) with double swabbing followed by QIAamp® extractions, 2.57 (MiniFiler™) and 6.57 (Fusion) with soaking followed by QIAamp® extractions, and 0.57 (MiniFiler™) and 1.57 (Fusion) with FDF®

extractions. Overall, double swabbing followed by organic extraction generated the highest average percentage of loaders' profiles and number of non-loader alleles with both amplification kits.

Table 9. Descriptive statistics of profiles amplified with MiniFiler™ and Fusion (bold). The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp® extraction; D = soak + QIAamp® extraction; E = FDF® extraction

Method	A	A	B	B	C	C	D	D	E	E
Sample Size	15	15	16	16	14	14	14	14	7	7
Avg. # Loader Alleles	10.80	27.33	10.31	22.37	1.57	5.71	2.57	6.57	0.57	1.57
Avg. # Possible Loader Alleles	16.33	41.67	15.75	41.12	15.57	40.36	15.71	40.57	15.71	41.14
Avg. % Loader Profile	67.0	66.2	65.8	54.9	9.7	14.1	15.9	16.0	3.5	3.8
Median % Loader Profile	75.0	69.8	67.8	41.6	5.9	5.3	9.4	13.1	0.0	0.0
Avg. # Non-loader Alleles	2.47	3.47	1.94	2.94	1.29	1.36	2.07	2.93	1.43	0.71

Table 10 presents Mann-Whitney pairwise comparisons examining the number of loader and non-loader alleles present in MiniFiler™ and Fusion profiles. A significantly greater number of loader alleles was amplified from the same DNA extract with Fusion than with MiniFiler™, except for those isolated with FDF®. The number of non-loader alleles present in the profiles that

were generated using the five optimized methods did not significantly differ between MiniFiler™ and Fusion.

Table 10. Mann-Whitney pairwise comparisons (2-tailed) examining the number of loader and non-loader alleles present in MiniFiler™ and Fusion profiles generated with the optimized methods: A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp® extraction; D = soak + QIAamp® extraction; E = FDF® extraction (Bold = significantly greater number of loader alleles)

Pair		# Loader Alleles p-value	# Non-loader Alleles p-value
MiniFiler™ A	Fusion A	< 0.0001	0.4490
MiniFiler™ B	Fusion B	0.0004	0.3460
MiniFiler™ C	Fusion C	0.0120	0.9620
MiniFiler™ D	Fusion D	0.0110	0.6510
MiniFiler™ E	Fusion E	0.8100	0.3560

MiniFiler™ and Fusion were also compared by calculating RMP values of profiles from a casing handled by volunteer YY (Table 11). The RMP value of the MiniFiler™ profile 34.4 was 1 in 4.0 billion while the RMP value of the Fusion profile 34.4 was 1 in 30.5 octillion, an increase of 19 orders of magnitude.

Table 11. RMP comparison of MiniFiler™ and Fusion profiles of casing 34.4. Single alleles at a given locus were considered homozygous and frequency calculations for D13 and FGA in the MiniFiler™ profile were calculated by adding the frequencies of all allele combinations. (NT = not tested and Red = non-loader allele)

Locus	MiniFiler™ 34.4	Fusion 34.4
Amel	X,Y	X,Y
D3	NT	15
D1	NT	15,16
D2S441	NT	10,14
D10	NT	14,16
D13	9, 11 ,14	9,14
Penta E	NT	12,13
D16	12	12
D18	12,17	12,17
D2S1338	18,23	18,23
CSF	11,12	11,12
Penta D	NT	9,14
THO1	NT	6,9.3
vWA	NT	19
D21	29,30	29,30
D7	9	9
D5	NT	12,13
TPOX	NT	8,11
DYS391	NT	11
D8	NT	13
D12	NT	19
D19	NT	13
FGA	21,24, 25	21,24
D22	NT	15
RMP Value	1 in 4.0 Billion	1 in 30.5 Octillion

Comparisons of Individual and Consensus Fusion STR Profiles

All individual Fusion profiles can be found in Appendix E. The percentages of loaders' profiles from all optimized methods, except organic extractions, were not normally distributed (Shapiro Wilk, $p < 0.0001$) (Table 12). Further, there was a significant difference in the percentages of loaders' profiles among methods (Kruskal-Wallis, $p < 0.0001$). Cell recovery and

DNA extraction methods differed significantly in all but one pairwise comparison (double swabbing vs. soaking with QIAamp® extractions) when the percentages of loaders' profiles were analyzed (Table 13). In general, DNA concentrations of approximately 0.05 pg/μL or higher (~0.3 pg of input DNA) produced some allelic data (Appendix D).

Table 12. Shapiro Wilk test for normality on the percentages of loaders' profiles processed with the optimized cell recovery and DNA extraction methods and amplified using Fusion.

Cell Recovery and DNA Extraction Method	P-value
Double Swab + Organic	0.677
Soak + Organic	0.071
Double Swab + QIAamp®	0.012
Soak + QIAamp®	0.002
FDF®	< 0.0001

Table 13. Mann-Whitney pairwise comparisons (2-tailed) of the percentages of loaders' profiles processed with the optimized cell recovery and DNA extraction methods and amplified using Fusion. (Bold = significantly greater percentages of loaders' profiles)

Pair		P-value
Double Swab + Organic	Soak + Organic	0.0400
Double Swab + Organic	Double Swab + QIAamp®	< 0.0001
Double Swab + Organic	Soak + QIAamp®	< 0.0001
Double Swab + Organic	FDF®	< 0.0001
Soak + Organic	Double Swab + QIAamp®	< 0.0001
Soak + Organic	Soak + QIAamp®	< 0.0001
Soak + Organic	FDF®	< 0.0001
Double Swab + QIAamp®	Soak + QIAamp®	0.3230
Double Swab + QIAamp®	FDF®	0.0130
Soak + QIAamp®	FDF®	0.0040

Figure 16 illustrates the median percentages of loaders' profiles present in the STR results of DNAs amplified using Fusion. It should be noted that this analysis included DNA extracts that quantified much lower than those in the MiniFiler™ vs. Fusion study. The median

percentage of loaders' profiles from double swabbed and organically extracted casings was 25.8% (n = 90), while the median percentage of loaders' profiles from soaked casings was 18.2% (n = 89). The median percentage of loaders' profiles from double swabbed and QIAamp[®] extracted casings was 4.8% (n = 56), while the median percentage of loaders' profiles from soaked casings was 6.7% (n = 36). The median percentage of loaders' profiles from FDF[®] extracted casings was 0.0% (n = 14).

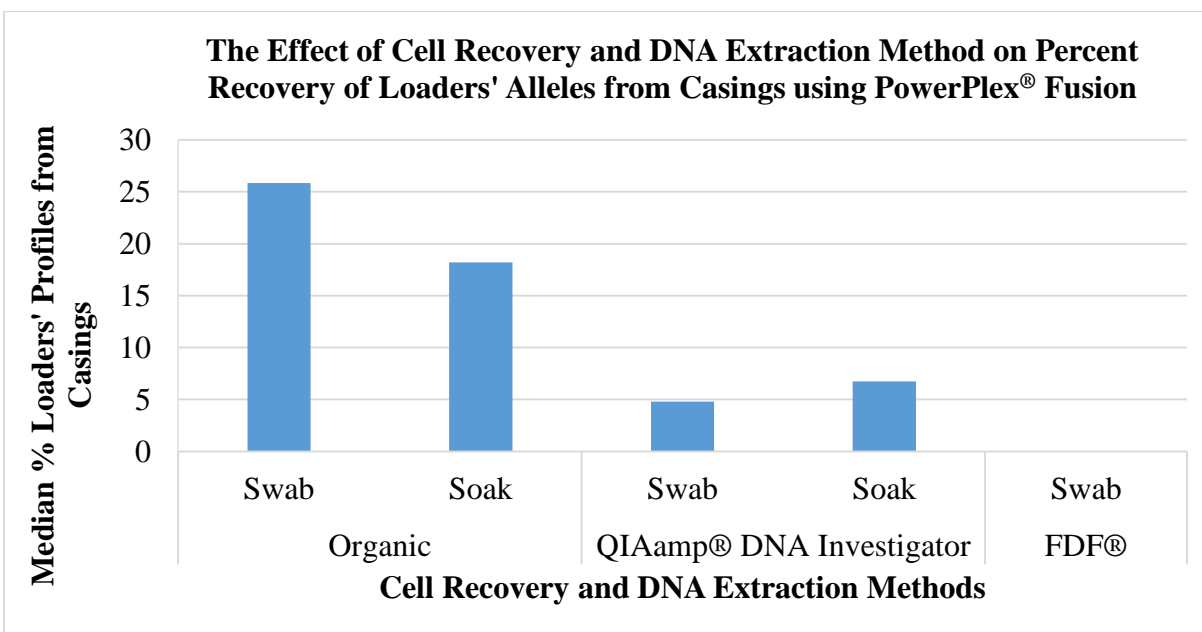


Figure 16. Median percentages of loaders' profiles recovered using optimized cell recovery and DNA extraction methods followed by amplification with Fusion. Median percentages of loaders' profiles from organic extractions (double swab = 25.8% [n = 90] and soak = 18.2% [n = 89]) were higher than loaders' profiles from QIAamp[®] extractions (double swab = 4.8% [n = 56] and soak = 6.7% [n = 36]). The median percentage of loaders' profiles from FDF[®] extractions was 0.0% (n = 14).

Table 14 shows descriptive statistics of the individual and consensus profiles of DNAs amplified with Fusion. The average number of alleles consistent with the loader was 12.4 with double swabbing and organic extractions, 9.7 with soaking and organic extractions, 3.0 with

double swabbing and QIAamp® extractions, 3.6 with soaking and QIAamp® extractions, and 1.1 with FDF® extractions. Furthermore, the average number of loader alleles in consensus profiles was 9.6 with double swabbing and 7.2 with soaking. The average number of non-loader alleles was highest in samples that were double swabbed and organically extracted (4.71 alleles). Consensus profiling of double swabbed and soaked samples decreased the number of loader alleles by 22.85% and 25.49%, and the number non-loader alleles by 76.43% and 88.17%, respectively.

Table 14. Descriptive statistics of individual and consensus profiles of DNAs amplified with Fusion. The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp® extraction; D = soak + QIAamp® extraction; E = FDF® extraction. Consensus profiles of methods A and B were generated per volunteer using the three individual profiles from casings (Collections 2 and 3), in which organic extractions were performed with either double swabbing or soaking.

PowerPlex® Fusion							
Cell Recovery and DNA Extraction Method	A	B	C	D	E	Consensus A	Consensus B
Sample Size	90	89	56	36	14	27	27
Avg. # Loader Alleles	12.43	9.69	3.05	3.64	1.07	9.59	7.22
Avg. # Possible Loader Alleles	41.76	41.75	41.59	42.11	41.36	41.81	41.81
Avg. % Loader Profile	30.0	23.3	7.4	8.7	2.7	22.9	17.3
Avg. # Non-loader Alleles	4.71	2.79	0.95	2.00	0.71	1.11	0.33

Table 15 shows the linear correlations made between DNA yields from samples recovered and extracted with the optimized methods and the number of loader alleles amplified with Fusion. There was a positive linear correlation between DNA yields and the loader alleles amplified for each method, which demonstrated more loader alleles were amplified as the amount of DNA input increased. The correlation coefficient (r) values ranged from 0.64 to 0.94.

Table 15. The degree of linear correlation between the DNA yields and the amount of loader alleles amplified in Fusion profiles. The cell recovery and DNA extraction method utilized is denoted by A = double swab + organic extraction; B = soak + organic extraction; C = double swab + QIAamp[®] extraction; D = soak + QIAamp[®] extraction; E = FDF[®] extraction.

Cell Recovery and DNA Extraction Method	A	B	C	D	E
Sample Size	90	89	56	36	14
Avg. DNA Yield (pg)	12.50	11.50	1.26	3.29	0.19
Avg. # Loader Alleles	12.43	9.69	3.05	3.64	1.07
r	0.70	0.64	0.87	0.94	0.71

Appendix F contains the consensus profiles generated from casings processed in triplicate (double swabbed or soaked followed by organic extraction). There were instances where non-loader alleles were prevalent and they were included in the consensus profile (non-loader alleles that could have originated from the preceding loader(s) are indicated with an asterisk). Further, loader alleles were sometimes not present at a high enough frequency to be included in the consensus profile. Table 16 presents an example where a consensus profile consistent with the loader was generated when a few non-loader alleles were present. Table 17 presents an example where a consensus profile inconsistent with the loader resulted from the inclusion of non-loader alleles based on their increased prevalence.

Table 16. Example of a consensus profile where non-loader alleles were rare in individual profiles and consequently excluded in the consensus, yet some alleles (*e.g.*, 16.3 and 17.3 at D1, 29 at D21, and 13 at D5) present in Profile 33-7A were consistent with the loader but not included in the consensus profile. Refer to Appendix E for explanation of table symbols.

Locus	33-7A	33-7B	33-7C	Consensus	B
Amel	X,Y	X,Y	X,Y	X,Y	X,Y
D3	16, 17 *,18	16	18	16,18	16,18
D1	16.3,17.3				16.3,17.3
D2S441	14,15		14	14	14,15
D10					13,15
D13	10		10	10	10,12
Penta E			7		7,18
D16	9,13	9, 12 *,13		9,13	9,13
D18	15	13,15	13	13,15	13,15
D2S1338	25				20,25
CSF	12	12		12	10,12
Penta D					12,13
THO1	8, 9 ,9.3	8,9.3	8	8,9.3	8,9.3
vWA	18	17,18	18	18	17,18
D21	29				29,31
D7	9		12		9,12
D5	13				11,13
TPOX					8
DYS391					11
D8	8,13	8,13	8,13	8,13	8,13
D12		22,23			22,23
D19	13, 14 *				13,15
FGA	31 ,†				21,23
D22					15,16
Method	A	A	A	A	

Table 17. Example of a consensus profile where multiple non-loader alleles were represented in the consensus profile. Refer to Appendix E for explanation of table symbols.

Locus	27-6A	27-6B	27-6C	Consensus	N
Amel	X	X,Y	X	X	X
D3	17	15*,16,17	16	16,17	16,17
D1	15	12			15.3,17.3
D2S441		11	16		11
D10					13
D13					12,14
Penta E	12*	12*		12*	13,15
D16	11*,12,13		11*	11*	12,13
D18	12,13,17*	12	12,17*	12,17*	13,14
D2S1338					20,23
CSF					11,12
Penta D			12		10,12
TH01	6,9*,9.3	6	9*,9.3	6,9*,9.3	6,9.3
vWA	14,16	17,18	17,18	17,18	17,18
D21		28			30,32.2
D7		11			11,12
D5	12	12		12	12
TPOX			8		8
DYS391					N/A
D8	10*,13	13,15	13	13	13
D12	19		20		19,20
D19			13		13,14
FGA		22,23*			21,25
D22	16*				11,15
Method	B	B	B	B	

Degradation of DNA Recovered from Spent Cartridge Casings

The frequencies of alleles consistent with the loader at each locus are presented in Figure 17. Amplification of Amelogenin, D16, TH01, and D8 generated the smallest DNA products (72 – 132 bp) and had the highest frequencies of alleles consistent with the loader, which decreased as the amplicon sizes increased. The only exceptions to this trend were in the blue channel (D13 and Penta E) and the yellow channel (D7 and D5).

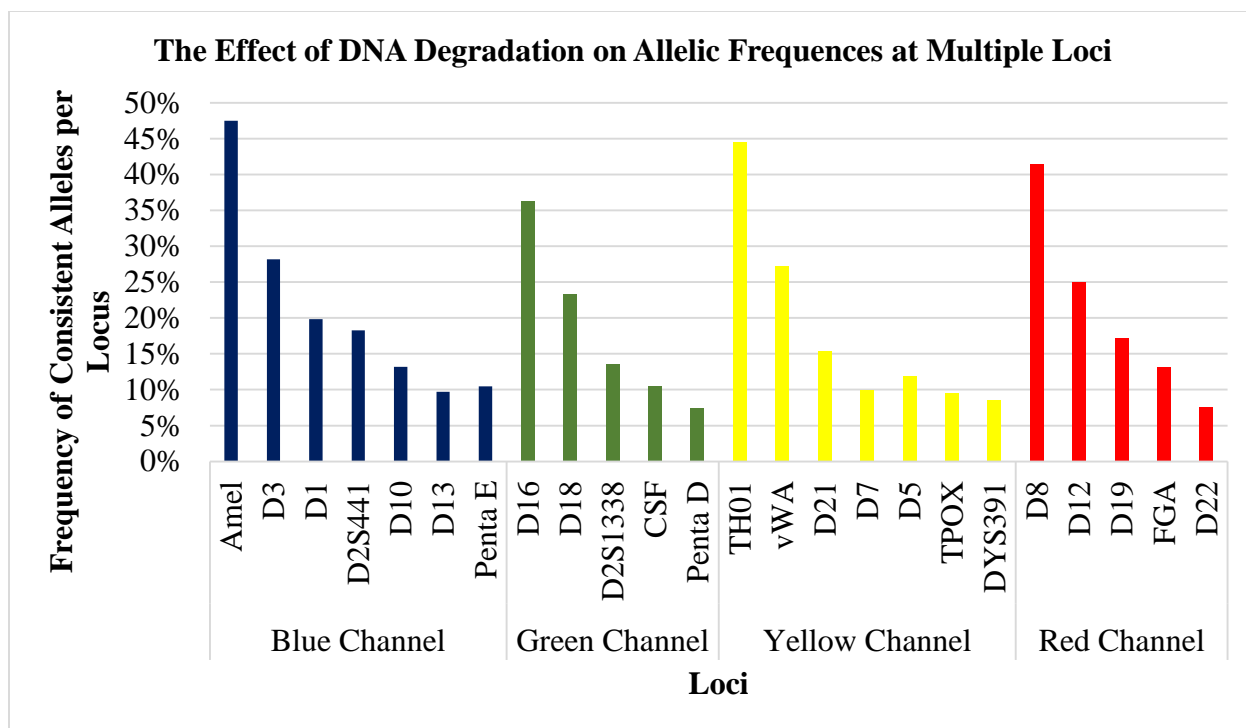


Figure 17. Frequency of consistent loader alleles amplified at each locus, illustrating preferential amplification of shorter amplicons. The loci are arranged according to their amplicon sizes (short to long) for each dye channel. With the exception of D13 (9.7%) and D7 (9.9%), the loci containing smaller amplicons had higher frequencies of amplification. Frequencies of the smallest locus in each channel: Amel = 47.5%, D16 = 36.3%, TH01 = 44.6%, D8 = 41.5%. Frequencies of the largest locus in each channel: Penta E = 10.4%, Penta D = 7.5%, DYS391 = 8.6%, D22 = 7.6%.

DISCUSSION

Spent cartridge casings, often recovered from shooting incidents, have the potential to be a valuable source of evidence used to link a perpetrator to a crime. Current forensic tools for analyzing spent casings—class characteristics, individual (toolmark) characteristics, and fingerprints—either lack specific information necessary to associate an individual to a crime, are rarely recovered, or both (Spear *et al.*, 2005; Bentsen *et al.*, 1996; Given, 1976). In contrast, DNA analysis has the potential to differentiate individuals and identify the person(s) responsible for shooting. Though DNA is a powerful tool, when recovered from spent casings it is often degraded and present in low copy numbers, causing STR analysis to be challenging. Currently, crime laboratories process spent cartridge casings for DNA as a last resort due to low DNA yields and minimal allele recovery (Forensic Scientist Sarah Rambadt, personal communication). Therefore, it is necessary to improve existing techniques for STR analysis of touch DNA from spent cartridge casings.

The overarching goal of this research was to improve the probative value of spent cartridge casings. The first step towards achieving it was to optimize and compare cell recovery and DNA extraction methods utilized on spent casings, while the second was to compare two STR analysis kits designed to target smaller amplicons (< 300 bp) in an effort to evaluate which amplified more loader alleles while simultaneously minimizing artifacts. Multiple studies have been conducted that involved different techniques to recover and isolate DNA from spent cartridge casings, including soaking evidence with rotation (Dieltjes *et al.*, 2011), pre-digestion incubation of soaked samples (Dieltjes *et al.*, 2011), organic extraction (Orlando, 2012; Horsman-Hall *et al.*, 2009), silica-based extraction (Dieltjes *et al.*, 2011; Horsman-Hall *et al.*, 2009), and a non-binding DNA extraction (Kopka *et al.*, 2011). Additionally, studies have been

performed to increase DNA yields by enhancing cell recovery and DNA extraction, including double swabbing of evidence (Sweet *et al.*, 1997), pre-treatment of purification columns (Doran and Foran, in press), and altering the duration of digestion with concurrent shaking (QIAamp® and FDF® manufactures' protocols). In the current study, these variables were tested independently to examine their influences on DNA recovery.

Multiple vessels were investigated for performing soaking as a cell/DNA recovery method. The diameters of beakers and test tubes were large, requiring a sizeable buffer volume to fully soak the outside surface of casings. Five milliliter stuffed pipette tips were smaller, which helped reduce soak solution volumes, but after a short time they started to leak. The bulb portion of transfer pipettes proved to be a useful vessel for soaking. They were close-fitting around the casings, which minimized buffer volumes and maximized the submerged surface area. Transfer pipettes are sterile, inexpensive, disposable, and offered in a variety of sizes to accommodate different ammunition calibers. The possibility of DNA loss because of binding to the plastic of the bulb led to the investigation of pre-treatment to avoid this. Recent research at the MSU Forensic Biology Laboratory found pre-treatment application of DNA purification columns with yeast rRNA substantially reduced DNA loss (Doran and Foran, in press). Yeast rRNA was applied to the bulbs to determine if it would help improve DNA recovery. Yields from pre-treated bulbs increased minimally, indicating negligible improvements in DNA recovery and little, if any, DNA adhesion onto the soaking vessel.

The inclusion of agitation during the soaking period has the potential to help loosen cells and DNA from casings, aiding in the amount of DNA recovered. Dieltjes *et al.* (2011) soaked items (cartridges, bullets, and casings) for 30 minutes with simultaneous rotation and reported the production of a blue colored lysis solution, and further reported that the soaked item itself

turned blue at longer soaking times. They attributed this to oxidation of the soaked items, and claimed to “solve the oxidation problem” by limiting the soak period to 30 minutes with subsequent swabbing. Blue soak solutions were generated with their adjusted protocol when performed in this study, and adding agitation during this step resulted in even more discoloration, indicating casings oxidized quicker. Shaken samples routinely had decreased DNA yields with both extraction methods (85.7% and 46.5% reductions for organic and QIAamp[®], respectively). It is possible that copper ions (most likely Cu⁺²) swamped out the EDTA in the soaking solution, leading to DNA degradation when other divalent cations acted as cofactors for nucleases. Furthermore, other casing metals (*e.g.*, zinc) along with primer components of the gunshot residue (GSR) could have inhibited PCR. Horsman-Hall *et al.* (2009) and Orlando (2011) noted PCR inhibition from the metals of the cartridge casings or primer components of the GSR; the former in 11% of the DNAs recovered from shotgun shells and the latter in DNA extracts from cumulative and single swabbed casings. However, PCR inhibition was not observed in the current study, thus it seems likely that the DNA loss from shaking was real.

Incubation at 85°C prior to DNA isolation is included in Qiagen’s protocol for eluting dried bloodstains off FTA[®] paper (Smith and Burgoyne, 2004). Dieltjes *et al.* (2011) followed this protocol and successfully obtained DNA, despite recovering it from ammunition and not bloodstains. In the current study, pre-digestion incubation of soaking solution and swabs increased DNA recovery by 141.3% using organic extractions and 46.4% with QIAamp[®] extractions. The increased yields may be attributable to cells being loosened from the swabs, making them more accessible for lysis. Additionally, common nucleases such as DNase I and II are inactivated at temperatures well below this (68°C and 30°C, respectively; Sigma-Aldrich

Nucleases, 2014). Thus, subjecting samples to this high temperature could have limited nuclease activity and prevented DNA degradation (further discussed below).

QIAamp® and FDF® manufacturers recommend incubating swabs in lysis buffer for at least one hour or 30 min, respectively. In this study there was no obvious correlation between digestion time and DNA yields, although only two time points (one hour and overnight) were examined. It was clear however, that yields were reduced overnight. It is conceivable that nucleases were again not inactivated by EDTA during this step due to the presence of metal ions and/or primer components. Both organic and QIAamp® extracted samples that were soaked and digested overnight recovered slightly more DNA than those double swabbed and digested overnight, which could have resulted from the former undergoing the 85°C incubation. On the other hand, the one hour incubation may not have been long enough for complete digestion of cells, and an incubation time between one hour and overnight may be advantageous. The one very odd result from digestions came from a single FDF® sample incubated for one hour, which yielded substantially more DNA (153 pg) than the others. This was most likely due to the variability of touch DNA (detailed below), and not the protocol itself. This result explains the higher average FDF® DNA yield with one hour digestion compared to the other times—as FDF® did not routinely recover more DNA than the other methods.

The standard protocols for organic and QIAamp® extractions at the MSU Forensic Biology Laboratory do not include shaking during digestion, although the QIAamp® instructions incorporate it. Since the FDF® protocol has agitation at 600 rpm during digestion, this step was incorporated into the extraction methods to determine its effect on DNA recovery. Shaking at 900 rpm was selected for organic and QIAamp® extractions, as this was the speed recommended by Qiagen. Shaking did increase DNA yields when compared to non-shaken samples, by 59.2%

and 25.6% for organic and QIAamp[®], respectively. This may have resulted from increased detachment of cells from swabs, which rendered them more accessible for lysis and DNA isolation. Further, it is possible agitation could have physically lysed the cells or aided in the process by increasing the number of cells exposed to the SDS and proteinase K.

The final optimized methods, which aimed to maximize yields associated with touch DNA on casings, include: (1) soak in transfer pipette bulbs or double swab casings, (2) pre-digest soaked samples at 85°C, (3) digest samples for one hour with concurrent shaking, (4) extract DNAs with one of the three isolation methods.

Touch DNA yields from spent casings are dependent upon two main factors: (1) the amount of DNA deposited on cartridges during loading of a magazine and (2) the effectiveness of techniques used for DNA recovery and isolation. Several authors have noted the variability between and within individuals transferring DNA on handled items (Thomasma and Foran, 2013; Phipps and Petricevic, 2007; Bright and Petricevic, 2004; Alessandrini *et al.*, 2003; Lowe *et al.*, 2002). Lowe *et al.* (2002) were the first to investigate the amount of DNA individuals deposit on handled objects. They attempted to categorize people according to ‘shedder type’ based on how much DNA they deposited or ‘shed’ 15 minutes after hand washing. The authors deemed 18 of 30 volunteers ‘good shedders’, defined as 80 – 100% of an individuals’ SGM Plus[®] profile when assessing STR results generated from handled tubes. Phipps and Petricevic (2007) attempted to replicate the study by Lowe *et al.* (2002), however, among 60 volunteers none were classified as ‘good shedders’. The authors noted differences in the protocols, where Lowe *et al.* (2002) undertook QIAamp[®] extractions and wiped tubes with a wet swab prior to handling, while they performed organic extractions and did not swab prior to handling. Phipps and Petricevic (2007) suggested discrepancies between the studies may have resulted from the

extraction performed or the damp surface created during swabbing that possibly assisted with DNA transfer. Additionally, they found the amount of DNA ‘shed’ by a single person varied day-to-day and even depended on the hand used. Beyond ‘shedder type’, it has been hypothesized that skin condition (dry or oily), substrate surfaces (porous or non-porous), and the amount of physical contact with one’s self and others impact transferred DNA quantities (Alessandrini *et al.*, 2003; Wickenheiser, 2002). Although the research presented here aimed to improve DNA yields by focusing on cell/DNA recovery and extraction techniques, the inconsistency of cells/DNA deposited on handled objects was considered during data interpretation as a possible source of variation influencing DNA yields.

The order in which casings are handled could also potentially influence the amount of DNA transferred to spent casings. The cell recovery and DNA extraction methods were assigned to casings in a round-robin manner designed to alleviate any impact that loading order had on the amount of cells deposited. However, a rigorous evaluation of the influence loading order may have on DNA transfer was not possible due to confounding variables, such as the differing number of casings loaded, the DNA recovery and extraction method used, etc. It is possible most of the cells/DNA on a loader’s fingers were transferred to the first cartridge loaded (*i.e.*, the last fired), resulting in less cells available for deposition onto subsequent cartridges. However, chamber temperature increases as more rounds of ammunition are fired, which would most drastically affect the last cartridge fired. In contrast, more force is required to load the last cartridge into a magazine, which could result in more DNA transfer (Goray *et al.*, 2010). The round robin assignments was designed to circumvent these variables, however further study will be required to determine if loading order plays a substantial role in DNA deposition.

Maximizing DNA yields is critical for touch DNA analyses, which is influenced by multiple factors. A double swab method, developed by Sweet *et al.* (1997), was designed to do just that. The method is thought to rehydrate, loosen, and collect shed cells from surfaces using a wetted swab, while a second dry swab retrieves additional cells that may not have adhered to the first one. Pang and Cheung (2007) double swabbed 20 touched items, individually extracted the swabs, and amplified the DNAs with Identifiler[®]. The authors found 80% of the first swabs and 60% of the second swabs recovered enough DNA to generate allelic data, demonstrating the importance of both swabs. Additionally, Van Oorschot *et al.* (2010) recommend swabbing objects with multiple swabs and consider it common practice to enhance DNA yields. However, double swabbing has never been compared to soaking when recovering DNA from spent cartridge casings. In this study, double swabbing recovered a significantly greater amount of DNA (69.4 % and 222.9% increase with organic and QIAamp[®] extractions, respectively) than soaking. These results were supported by Bright and Petricevic (2004) who found double swabbing yielded more DNA than soaking (avg. = 0.16 and 0.08 ng, respectively) when analyzing trace DNA from shoe insoles. In theory, similar yields from casings would have been expected using either recovery techniques, since for both the entire outer surface of a casing was exposed to digestion buffer and thoroughly swabbed. However, soaked casings were in contact with digestion buffer for a much longer period of time, which appeared to lead to oxidation, and possibly DNA degradation or loss on silica columns (explained below).

DNA recoveries were also significantly influenced by the extraction methods, with organic extractions producing the highest yields. In contrast, Horsman-Hall *et al.* (2009) reported significantly higher DNA yields from spent cartridge casings using a silica-based extraction compared to organic extraction and Microcon purification. The primary difference between these

studies was that Horsman-Hall *et al.* (2009) did not pre-treat the purification columns, as was done in the current study, which has been shown to improve DNA yields substantially (Doran and Foran, in press). It would be interesting to discover if the results of Horsman-Hall *et al.* (2009) would differ had they undertaken this step.

Lower DNA yields generated with QIAamp[®] extractions in the current study could have resulted from DNA loss on the column or problems associated with silica binding. Hebda *et al.* (in press), examined multiple elution steps with QIAamp[®] extractions and found a measurable amount of DNA was still eluting off the columns beyond three elutions. Therefore, it is feasible that yields could have increased with more elutions, but that also would have further diluted the DNA. Additionally, silica has previously been used to remove heavy metals (*e.g.*, copper, cadmium, and zinc) from aqueous solutions (Bowe, 2003). Thus, it is possible copper ions or metals from GSR (*e.g.*, lead and barium) bound to the negatively-charged DNA or silica, which could have interfered with DNA binding, causing substantial loss.

FDF[®] extractions demonstrated lower DNA yields than the other extraction methods throughout the study. Early experiments showed UV irradiation of FDF[®] reagents reduced DNA yields, with more DNA being lost using irradiated reagents. It is possible the UV irradiation modified or destroyed components within the solutions that were necessary for proper function. These experiments were performed with a known amount of input DNA, which resulted in an average loss of 50.3% and 33.6% with reagents that were and were not irradiated, respectively. The manufacturer claims that “proteins, detergents and low molecular weight compounds are retained by the nexttec[™] sorbent”. However, if the DNA was highly degraded it is possible those fragments were retained on the column, especially since the manufacturer does not provide a molecular weight cutoff for retention. It also seems likely that lower DNA yields resulted from

the single swab recovery method. The technique required 30 µL of Lysis Buffer to be applied to swabs (compared to 150 µL used with organic and QIAamp[®] extractions), and so cells may not have been rehydrated, hindering their removal. Kopka *et al.* (2011) provided limited data in their validation of the FDF[®] Kit, consequently it is difficult to make a direct comparison to the result presented here. In reference to spent casing data, they stated “these [STR profiles] are not reliable. The allele peaks are near or below the amplitude threshold of 50 rfu and should therefore be interpreted very carefully”. If the allele peaks were that weak, it is quite possible they also obtained extremely low DNA yields, similar to those obtained using FDF[®] throughout this work.

It is worth noting that all samples extracted and quantified in the optimization experiments, which were conducted on previously spent casings, yielded considerably more DNA than casings tested post loading and firing. A clear example of this was seen with organic extracts from samples shaken during digestion: an average of 95.4 pg of DNA was recovered, while an average of 50.9 pg was recovered from spent casings. The decrease in DNA yields could have been caused by DNA degradation during deflagration. Horsman-Hall *et al.* (2009) claimed average chamber temperatures can reach 1800°C for 0.5 to 5.0 ms during firing, but this is largely dependent upon the type of firearm, ammunition, and the amount of firing (*i.e.*, the chamber is cooler prior to shooting several rounds). Additionally, Bentsen *et al.* (1996) suggested that fingerprint ridge details are lost because of friction from loading and ejection, which may also affect touch DNA on the casings. Based on these findings, it is hypothesized that the heat in the chamber, coupled with friction generated during deflagration, could result in lower DNA yields from handled and fired cartridge casings compared to handled casings.

The improved cell recovery and DNA extraction method can be incorporated into research that aims to answer further questions regarding DNA yields from spent cartridge casings. Current studies in the MSU Forensic Biology Laboratory include understanding the effects of cyanoacrylate fuming on DNA recovery, the influence of ammunition caliber, and the feasibility of cumulatively swabbing multiple casings to improve DNA yields while maintaining minimal contamination. Additionally, based on the results of the optimization experiments, it would be advantageous to further investigate some variables that may clarify results of the work presented here. The first includes testing shorter soak periods, which may decrease the amount of casing oxidation and possibly increase DNA yields. If recovery is improved then quantities could be compared to those from double swabbed casings to ascertain which is the better recovery method. It would also be valuable to test soaking casings directly in an 85°C water bath, since it seems possible from the current experiments that this incubation inactivates nucleases thus reducing DNA degradation. However, soaking casings at this high temperature could also increase oxidation. Additionally, swabbed casings were never subjected to an 85°C pre-digestion incubation, so it would be worthwhile to examine if this step improves DNA yields, again presumably by inactivating nucleases. Next, since only two digestion times (one hour and overnight) were considered (plus 30 min for FDF[®]), it is difficult to say if yields would have increased or decreased if intermediate time points were tested. Therefore, it would be advantageous to test additional incubation times to determine if one hour is optimal or if longer incubation periods yield more DNA. When performing QIAamp[®] extractions it would be useful to determine if the DNAs recovered from oxidized casings are present in the final elutions or the flow-throughs of the DNA binding and wash steps. If DNAs are present in the flow-throughs then copper ions or other contaminants might be preventing binding to the column. Lastly, based

on the poor DNA recovery with FDF[®] extractions, it would be beneficial to further investigate this isolation method however given that no information is provided on the makeup or chemistry of the FDF[®] columns, optimization of this procedure may be difficult. (Preliminary experiments showed that DNA was recovered from FDF[®] columns using a second elution, however this increased volumes substantially and thus was not advantageous; data not shown.)

Low DNA yields can have a strong, negative impact on the number of alleles amplified with standard STR kits, however, recently, new kits have been developed that assay more loci and are more sensitive to low copy samples. A CODIS Core Loci Working Group recently recommended expanding the 13 CODIS loci for multiple reasons: (1) increase discrimination power, (2) decrease the chance of adventitious matches, and (3) increase international compatibility (Hares, 2012A). In 2009, the European Union added five autosomal STR loci to their European Standard Set (ESS). Inclusion of those loci in the new CODIS requirements would aid international crime investigations (Hill, 2012). The proposed expansion consists of 20 required and three recommended markers, which include 12 of the 13 previous core loci (excluding TPOX), four of the ESS loci (D12S391, D1S1656, D2S441, and D10S1248), two commonly typed loci (D2S1338 and D19S433), and two sex-related loci (Amelogenin and DYS391). Further, recommended loci (in order of preference for kit inclusion) include TPOX, D22S1045, and SE33 (Hares, 2012B). Two new STR kits—PowerPlex[®] Fusion and GlobalFiler[®] (Life Technologies)—have been manufactured to meet the expanded CODIS loci requirements. Both kits assay 24 loci and were designed with heightened sensitivity for degraded, LCN, and/or inhibited samples. Increasing the number of required CODIS loci from 13 to 20 can benefit analysis of touch DNA samples because there is a greater chance of amplifying more alleles.

The second part of this study began with a comparison of AmpFℓSTR® MiniFiler™ and PowerPlex® Fusion, both targeting loci below 300 bp, to evaluate which recovered the most alleles consistent with the loader without increasing artifactual data (*e.g.*, drop-in and drop-out). When comparing the percentage of a profile produced, MiniFiler™ outperformed Fusion for organic extractions, however this resulted from the higher number of Fusion alleles that each loader could have provided due to the increased number of loci assayed. For example, sample 23-2A amplified with MiniFiler™ generated 12 alleles consistent with the loader (85.7% of the loader's profile), while the same DNA extract amplified with Fusion yielded 22 loader alleles (57.9% of the loader's profile). When the large difference in alleles amplified is taken into account, Fusion outperformed MiniFiler™ in all respects. It amplified significantly more loader alleles from organic and QIAamp® extracts while the number of non-loader alleles did not differ significantly between the kits. These results demonstrate the improved quality and quantity of genetic information obtained with Fusion. Oostdik *et al.* (2014) validated the Fusion System and found strong amplification with minimal artifacts when analyzing less than pristine samples, confirming the findings of this study. There was no statistical difference between MiniFiler™ and Fusion when comparing the number of amplified loader alleles from FDF® extracts, however, only 2 of the 7 samples produced any allelic data and in both cases Fusion amplified more loader alleles. All in all, Fusion generated more than twice the number of alleles than did MiniFiler™.

Comparing these STR results to other studies, it is clear optimization improved genotyping success from spent casings. Orlando (2012) recovered mostly (~70%) partial Identifiler® Plus profiles (7 or less loci with alleles) and of those, most were not consistent with the loader. Horsman-Hall *et al.* (2009) utilized MiniFiler™ with a 20 s injection time (compared to 8 s in this study). The authors noted 11% of the profiles contained loaders' alleles at all 9

possible loci and 20% had loader alleles at over half of the loci. MiniFiler™ profiles (double swab + organic) in this study were more complete than those generated by Horsman-Hall *et al.* (2009), wherein 20% were full profiles and 53% contained over half of the possible loader alleles. Amplifying the same DNA extracts as were analyzed with MiniFiler™, Fusion generated 13% full profiles and 67% containing over half of the possible loader alleles. The final comparison of MiniFiler™ and Fusion in this study evaluated RMP values from profiles of DNA extract 34.4. The value generated from MiniFiler™ was 1 in 4 billion, whereas the Fusion profile had an RMP value 19 magnitudes higher (1 in 30.5 octillion). This improved discrimination developed confidence that the DNA used to generate the profile originated from the loader. It would be interesting to investigate how GlobalFiler®, which also assays 24 loci, compares to Fusion. Additionally, it may be beneficial to perform post-PCR purification techniques that can increase RFU values in samples with peaks near or slightly below the RFU threshold.

It would also be advantageous to be able to make a reasonable prediction of the number of alleles likely to amplify based on the amount of DNA in a sample. Partial Fusion profiles (containing 1 or 2 alleles) were amplified from as little as 0.3 pg of input DNA, while full Fusion profiles were produced from all reactions with 100 pg or more of DNA. A correlation between DNA yields and the number of loader alleles present was clear, with *r* values ranging from 0.64 to 0.94. These findings are valuable because they could allow for an approximate DNA cutoff at which (for instance) concentrations lower than 0.05 pg/μL would not be worth typing since the chance of obtaining allelic data is so low. Additionally, the ability to quantify DNA samples at such low concentrations is most likely due to the high-copy locus, *Alu*, targeted in this study. Although standard quantification methods were not compared to *Alu*, it is possible that only the highest quantified samples (28.8 and 50.5 pg/μL) would have been detected using Quantifiler®;

considering Green *et al.* (2005) found that it could only detect down to 32 pg of DNA. Overall, heightened sensitivity of *Alu* quantification provided more informative DNA quantities that could be correlated to amplified loader alleles.

Non-loader alleles were present in approximately 75% of Fusion profiles, ranging from 1 to 27 alleles. As with alleles consistent with loaders, smaller amplicons showed higher instances of non-loader alleles. Investigation into the average number of non-loader alleles amplified at Fusion loci demonstrated an average of one allele difference between the largest and smallest loci. Additionally, more non-loader alleles were present in the red channel than all other dye channels; an increase in the average number of non-loader alleles between the red channel and other three channels ranged from 0.5 to 0.7 (data not shown). One of the more intriguing instances of non-loader prevalence involved two volunteers (M and Q) in the first collection, where a Fusion profile from M contained 20 non-loader alleles, 16 of which were consistent with Q who loaded the magazine immediately prior. This was strong evidence that DNA can be left on parts of the pistol following firing. The seven loaders that produced Fusion profiles with the most non-loader alleles had an average of 21 that were inconsistent, approximately one-third of which (on average) could have originated either from the loader immediately prior or the loader that previously used the same magazine (two volunteers prior). While the association of non-loader alleles was not examined beyond two preceding loaders, it is possible those alleles could have originated from other volunteers or individuals that may have had physical contact with the loaders (*e.g.*, a partner) (Meakin and Jamieson, 2013). Profiles from Collection 3 were analyzed for contamination from the magazine or firearm, since every other volunteer loaded the same magazine. Of the 92 Fusion profiles generated, on average there were 2.8 non-loader alleles per profile and of those, an average of 39.3% could potentially be attributed to contamination from

the firearm, while an average of 32.1% could have originated from the magazine. Overall the difference in possible contamination from the pistol compared to the magazine was small. However, given these results, it is possible more DNA transfer resulted from the interaction between the cartridge and parts of the chamber. DNA transfer may have also occurred via the collection apparatuses (microscope cover and pillow case) or on the hemostats used in Collection 3 because they were not wiped between loaders, as was done in Collection 2.

Consensus profiling is a technique that can be used to filter out non-loader alleles and subsequently reduce the possibility of misidentification, as an increase in allele presence builds confidence that the alleles originated from the loader. It has previously been utilized with LCN DNA analysis (Taberlet *et al.*, 1996) where replicate PCR amplifications were used to develop a profile following set guidelines (Butler and Hill, 2010). In this study, alleles that were present in at least two of three individual profiles from the same loader were included in consensus profiles. Development of consensus profiles decreased the average number of non-loader alleles by 76.4% (double swab) and 88.2% (soak). However, it also decreased the average number of loader alleles from ~12 to ~10 (double swab) and ~10 to ~7 (soak). This tradeoff may generate less discriminating RMP values, however, it is more conservative and likely helps minimize the chance of incorrect identification.

Overall, the results of this study demonstrate that significantly higher DNA yields are recovered from spent cartridge casings using a double swab method and organic extraction than using a soak method or extracting DNAs with QIAamp® or FDF® Kits. Additionally, significantly more loader alleles were amplified using PowerPlex® Fusion than MiniFiler™, without substantially increasing the number of non-loader alleles. Although the DNAs recovered from spent casings were degraded and present in low copy number (over 95% had yields under

100 pg), these cell recovery and DNA extraction methods, along with Fusion amplification and consensus profiling, generated reliable and accurate data. This research has the potential to provide a strong investigative lead by associating an individual to a shooting incident; however, it should be noted that it does not necessarily identify the shooter of the weapon. Regardless, it provides a foundation for crime laboratories who wish to utilize DNA analysis as a reliable tool for investigating spent cartridge casings, increasing their probative value by aiding in identification of the loader of a firearm.

APPENDICES

APPENDIX A. ASSIGNMENT OF CELL RECOVERY AND DNA EXTRACTION METHODS TO SPENT CARTRIDGE CASINGS

Table A1. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 1.

Bag	Buccal Letter	Casing Identifier	Order Loaded in Magazine	Analysis Method	Cell Recovery Method	DNA Extraction Method
28	Q	28.1	2 nd	i	Soak	Organic Extraction
28	Q	28.2	2 nd	ii	Double Swab	Organic Extraction
28	Q	28.3	2 nd	iii	Soak	QIAamp [®] Extraction
28	Q	28.4	2 nd	iv	Double Swab	QIAamp [®] Extraction
28	Q	28.5	2 nd	v	Single Swab	FDF [®] Extraction
28	Q	28.6	2 nd	vi	Never Tested	Never Tested
43	M	43.1	3 rd	i	Soak	Organic Extraction
43	M	43.2	3 rd	vi	Never Tested	Never Tested
43	M	43.3	3 rd	ii	Double Swab	Organic Extraction
43	M	43.4	3 rd	iii	Soak	QIAamp [®] Extraction
43	M	43.5	3 rd	iv	Double Swab	QIAamp [®] Extraction
43	M	43.6	3 rd	v	Single Swab	FDF [®] Extraction
34	YY	34.1	5 th	v	Single Swab	FDF [®] Extraction
34	YY	34.2	5 th	vi	Never Tested	Never Tested
34	YY	34.3	5 th	i	Soak	Organic Extraction
34	YY	34.4	5 th	ii	Double Swab	Organic Extraction
34	YY	34.5	5 th	iii	Soak	QIAamp [®] Extraction
34	YY	34.6	5 th	iv	Double Swab	QIAamp [®] Extraction

Table A1 (cont'd).

Bag	Buccal Letter	Casing Identifier	Order Loaded in Magazine	Analysis Method	Cell Recovery Method	DNA Extraction Method
48	GG	48.1	4 th	iv	Double Swab	QIAamp [®] Extraction
48	GG	48.2	4 th	v	Single Swab	FDF [®] Extraction
48	GG	48.3	4 th	vi	Never Tested	Never Tested
48	GG	48.4	4 th	i	Soak	Soak
48	GG	48.5	4 th	ii	Double Swab	Organic Extraction
48	GG	48.6	4 th	iii	Soak	QIAamp [®] Extraction
30	LL	30.1	1 st	iii	Soak	QIAamp [®] Extraction
30	LL	30.2	1 st	iv	Double Swab	QIAamp [®] Extraction
30	LL	30.3	1 st	v	Single Swab	FDF [®] Extraction
30	LL	30.4	1 st	vi	Never Tested	Never Tested
30	LL	30.5	1 st	i	Soak	Organic Extraction
30	LL	30.6	1 st	ii	Double Swab	Organic Extraction
37	RR	37.1	9 th	ii	Double Swab	Organic Extraction
37	RR	37.2	9 th	iii	Soak	QIAamp [®] Extraction
37	RR	37.3	9 th	iv	Double Swab	QIAamp [®] Extraction
37	RR	37.4	9 th	v	Single Swab	FDF [®] Extraction
37	RR	37.5	9 th	vi	Never Tested	Never Tested
37	RR	37.6	9 th	i	Soak	Organic Extraction
19	CC	19.1	8 th	i	Soak	Organic Extraction
19	CC	19.2	8 th	ii	Double Swab	Organic Extraction
19	CC	19.3	8 th	iii	Soak	QIAamp [®] Extraction
19	CC	19.4	8 th	iv	Double Swab	QIAamp [®] Extraction
19	CC	19.5	8 th	v	Single Swab	FDF [®] Extraction
19	CC	19.6	8 th	vi	Never Tested	Never Tested

Table A1 (cont'd).

Bag	Buccal Letter	Casing Identifier	Order Loaded in Magazine	Analysis Method	Cell Recovery Method	DNA Extraction Method
37	RR	37-1A	7 th	ii	Double Swab	Organic Extraction
37	RR	37-1B	7 th	iv	Double Swab	QIAamp [®] Extraction
37	RR	37-1C	7 th	vi	Never Tested	Never Tested
37	RR	37-2A	7 th	i	Soak	Organic Extraction
37	RR	37-2B	7 th	iii	Soak	QIAamp [®] Extraction
37	RR	37-2C	7 th	v	Single Swab	FDF [®] Extraction
19	CC	19-1A	6 th	i	Soak	Organic Extraction
19	CC	19-1B	6 th	v	Soak	QIAamp [®] Extraction
19	CC	19-1C	6 th	iii	Single Swab	FDF [®] Extraction
19	CC	19-2A	6 th	ii	Double Swab	Organic Extraction
19	CC	19-2B	6 th	iv	Double Swab	QIAamp [®] Extraction
19	CC	19-2C	6 th	vi	Never Tested	Never Tested

Table A2. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 2.

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
2-1	U	1 – 3	17 th	Used for a different study	Double Swab	Organic Extraction
2-2	U	4 – 6	17 th	Used for a different study	Double Swab	Organic Extraction
2-3	U	7 – 9	17 th	i	Double Swab	Organic Extraction
2-4	U	10 – 12	17 th	ii	Soak	Organic Extraction
2-5	U	13 – 15	17 th	iii	Double Swab	QIAamp [®] Extraction
2-6	U	16 – 18	17 th	iv	Soak	QIAamp [®] Extraction
2-7	U	19 – 21	17 th	v	Single Swab	FDF [®] Extraction
3-1	MM	1 – 3	4 th	v	Single Swab	FDF [®] Extraction
3-2	MM	4 – 6	4 th	Used for a different study	Double Swab	Organic Extraction
3-3	MM	7 – 9	4 th	Used for a different study	Double Swab	Organic Extraction
3-4	MM	10 – 12	4 th	i	Double Swab	Organic Extraction
3-5	MM	13 – 15	4 th	ii	Soak	Organic Extraction
3-6	MM	16 – 18	4 th	iii	Double Swab	QIAamp [®] Extraction
3-7	MM	19 – 21	4 th	iv	Soak	QIAamp [®] Extraction
8-1	S	1 – 3	5 th	iv	Soak	QIAamp [®] Extraction
8-2	S	4 – 6	5 th	v	Single Swab	FDF [®] Extraction
8-3	S	7 – 9	5 th	Used for a different study	Double Swab	Organic Extraction
8-4	S	10 – 12	5 th	Used for a different study	Double Swab	Organic Extraction
8-5	S	13 – 15	5 th	i	Double Swab	Organic Extraction
8-6	S	16 – 18	5 th	ii	Soak	Organic Extraction
8-7	S	19 – 21	5 th	iii	Double Swab	QIAamp [®] Extraction

Table A2 (cont'd).

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
10-1	VV	1 – 3	2 nd	iii	Double Swab	QIAamp [®] Extraction
10-2	VV	4 – 6	2 nd	iv	Soak	QIAamp [®] Extraction
10-3	VV	7 – 9	2 nd	v	Single Swab	FDF [®] Extraction
10-4	VV	10 – 12	2 nd	Used for a different study	Double Swab	Organic Extraction
10-5	VV	13 – 15	2 nd	Used for a different study	Double Swab	Organic Extraction
10-6	VV	16 – 18	2 nd	i	Double Swab	Organic Extraction
10-7	VV	19 – 21	2 nd	ii	Soak	Organic Extraction
13-1	V	1 – 3	6 th	ii	Soak	Organic Extraction
13-2	V	4 – 6	6 th	iii	Double Swab	QIAamp [®] Extraction
13-3	V	7 – 9	6 th	iv	Soak	QIAamp [®] Extraction
13-4	V	10 – 12	6 th	v	Single Swab	FDF [®] Extraction
13-5	V	13 – 15	6 th	Used for a different study	Double Swab	Organic Extraction
13-6	V	16 – 18	6 th	Used for a different study	Double Swab	Organic Extraction
13-7	V	19 – 21	6 th	i	Double Swab	Organic Extraction
15-1	HH	1 – 3	11 th	i	Double Swab	Organic Extraction
15-2	HH	4 – 6	11 th	ii	Soak	Organic Extraction
15-3	HH	7 – 9	11 th	iii	Double Swab	QIAamp [®] Extraction
15-4	HH	10 – 12	11 th	iv	Soak	QIAamp [®] Extraction
15-5	HH	13 – 15	11 th	v	Single Swab	FDF [®] Extraction
15-6	HH	16 – 18	11 th	Used for a different study	Double Swab	Organic Extraction
15-7	HH	19 – 21	11 th	Used for a different study	Double Swab	Organic Extraction

Table A2 (cont'd).

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
23-1	L	1 – 3	9 th	Used for a different study	Double Swab	Organic Extraction
23-2	L	4 – 6	9 th	i	Double Swab	Organic Extraction
23-3	L	7 – 9	9 th	ii	Soak	Organic Extraction
23-4	L	10 – 12	9 th	iii	Double Swab	QIAamp [®] Extraction
23-5	L	13 – 15	9 th	iv	Soak	QIAamp [®] Extraction
23-6	L	16 – 18	9 th	v	Single Swab	FDF [®] Extraction
23-7	L	19 – 21	9 th	Used for a different study	Double Swab	Organic Extraction
25-1	T	1 – 3	16 th	Used for a different study	Double Swab	Organic Extraction
25-2	T	4 – 6	16 th	Used for a different study	Double Swab	Organic Extraction
25-3	T	7 – 9	16 th	i	Double Swab	Organic Extraction
25-4	T	10 – 12	16 th	ii	Soak	Organic Extraction
25-5	T	13 – 15	16 th	iii	Double Swab	QIAamp [®] Extraction
25-6	T	16 – 18	16 th	iv	Soak	QIAamp [®] Extraction
25-7	T	19 – 21	16 th	v	Single Swab	FDF [®] Extraction
26-1	XX	1 – 3	12 th	v	Single Swab	FDF [®] Extraction
26-2	XX	4 – 6	12 th	Used for a different study	Double Swab	Organic Extraction
26-3	XX	7 – 9	12 th	Used for a different study	Double Swab	Organic Extraction
26-4	XX	10 – 12	12 th	i	Double Swab	Organic Extraction
26-5	XX	13 – 15	12 th	ii	Soak	Organic Extraction
26-6	XX	16 – 18	12 th	iii	Double Swab	QIAamp [®] Extraction
26-7	XX	19 – 21	12 th	iv	Soak	QIAamp [®] Extraction

Table A2 (cont'd).

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
27-1	N	1 – 3	13 th	iv	Soak	QIAamp [®] Extraction
27-2	N	4 – 6	13 th	v	Single Swab	FDF [®] Extraction
27-3	N	7 – 9	13 th	Used for a different study	Double Swab	Organic Extraction
27-4	N	10 – 12	13 th	Used for a different study	Double Swab	Organic Extraction
27-5	N	13 – 15	13 th	i	Double Swab	Organic Extraction
27-6	N	16 – 18	13 th	ii	Soak	Organic Extraction
27-7	N	19 – 21	13 th	iii	Double Swab	QIAamp [®] Extraction
24-1	OO	1 – 3	15 th	iii	Double Swab	QIAamp [®] Extraction
24-2	OO	4 – 6	15 th	iv	Soak	QIAamp [®] Extraction
24-3	OO	7 – 9	15 th	v	Single Swab	FDF [®] Extraction
24-4	OO	10 – 12	15 th	Used for a different study	Double Swab	Organic Extraction
24-5	OO	13 – 15	15 th	Used for a different study	Double Swab	Organic Extraction
24-6	OO	16 – 18	15 th	i	Double Swab	Organic Extraction
24-7	OO	19 – 21	15 th	ii	Soak	Organic Extraction
33-1	B	1 – 3	3 rd	ii	Soak	Organic Extraction
33-2	B	4 – 6	3 rd	iii	Double Swab	QIAamp [®] Extraction
33-3	B	7 – 9	3 rd	iv	Soak	QIAamp [®] Extraction
33-4	B	10 – 12	3 rd	v	Single Swab	FDF [®] Extraction
33-5	B	13 – 15	3 rd	Used for a different study	Double Swab	Organic Extraction
33-6	B	16 – 18	3 rd	Used for a different study	Double Swab	Organic Extraction
33-7	B	19 – 21	3 rd	i	Double Swab	Organic Extraction

Table A2 (cont'd).

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
36-1	D	1 – 3	7 th	i	Double Swab	Organic Extraction
36-2	D	4 – 6	7 th	ii	Soak	Organic Extraction
36-3	D	7 – 9	7 th	iii	Double Swab	QIAamp [®] Extraction
36-4	D	10 – 12	7 th	iv	Soak	QIAamp [®] Extraction
36-5	D	13 – 15	7 th	v	Single Swab	FDF [®] Extraction
36-6	D	16 – 18	7 th	Used for a different study	Double Swab	Organic Extraction
36-7	D	19 – 21	7 th	Used for a different study	Double Swab	Organic Extraction
38-1	WW	1 – 3	14 th	Used for a different study	Double Swab	Organic Extraction
38-2	WW	4 – 6	14 th	i	Double Swab	Organic Extraction
38-3	WW	7 – 9	14 th	ii	Soak	Organic Extraction
38-4	WW	10 – 12	14 th	iii	Double Swab	QIAamp [®] Extraction
38-5	WW	13 – 15	14 th	iv	Soak	QIAamp [®] Extraction
38-6	WW	16 – 18	14 th	v	Single Swab	FDF [®] Extraction
38-7	WW	19 – 21	14 th	Used for a different study	Double Swab	Organic Extraction
40-1	SS	1 – 3	8 th	Used for a different study	Double Swab	Organic Extraction
40-2	SS	4 – 6	8 th	Used for a different study	Double Swab	Organic Extraction
40-3	SS	7 – 9	8 th	i	Double Swab	Organic Extraction
40-4	SS	10 – 12	8 th	ii	Soak	Organic Extraction
40-5	SS	13 – 15	8 th	iii	Double Swab	QIAamp [®] Extraction
40-6	SS	16 – 18	8 th	iv	Soak	QIAamp [®] Extraction
40-7	SS	19 – 21	8 th	v	Single Swab	FDF [®] Extraction

Table A2 (cont'd).

Set	Buccal Letter	Casings Ejected	Order Loaded in Magazine(s)	Analysis Method	Cell Recovery Method	DNA Extraction Method
41-1	Y	1 – 3	10 th	v	Single Swab	FDF [®] Extraction
41-2	Y	4 – 6	10 th	Used for a different study	Double Swab	Organic Extraction
41-3	Y	7 – 9	10 th	Used for a different study	Double Swab	Organic Extraction
41-4	Y	10 – 12	10 th	i	Double Swab	Organic Extraction
41-5	Y	13 – 15	10 th	ii	Soak	Organic Extraction
41-6	Y	16 – 18	10 th	iii	Double Swab	QIAamp [®] Extraction
41-7	Y	19 – 21	10 th	iv	Soak	QIAamp [®] Extraction
50-1	II	1 – 3	1 st	iv	Soak	QIAamp [®] Extraction
50-2	II	4 – 6	1 st	v	Single Swab	FDF [®] Extraction
50-3	II	7 – 9	1 st	Used for a different study	Double Swab	Organic Extraction
50-4	II	10 – 12	1 st	Used for a different study	Double Swab	Organic Extraction
50-5	II	13 – 15	1 st	i	Double Swab	Organic Extraction
50-6	II	16 – 18	1 st	ii	Soak	Organic Extraction
50-7	II	19 – 21	1 st	iii	Double Swab	QIAamp [®] Extraction

Table A3. Round robin assignment of cell recovery and DNA extraction methods to spent casings from Collection 3.

Set	Buccal Letter	Casing Ejected	Order Loaded in Magazine	Analysis Method	Cell Recovery Method	DNA Extraction Method
1-1	W	1 – 3	10 th	i	Double Swab	Organic Extraction
1-2	W	4 – 6	10 th	ii	Soak	Organic Extraction
1-3	W	7 – 9	10 th	iii	Double Swab	QIAamp [®] Extraction
1-4	W	10 – 12	10 th	iv	Soak	QIAamp [®] Extraction
6-1	QQ	1 – 3	1 st	iv	Soak	QIAamp [®] Extraction
6-2	QQ	4 – 6	1 st	i	Double Swab	Organic Extraction
6-3	QQ	7 – 9	1 st	ii	Soak	Organic Extraction
6-4	QQ	10 – 12	1 st	iii	Double Swab	QIAamp [®] Extraction
7-1	P	1 – 3	4 th	iii	Double Swab	QIAamp [®] Extraction
7-2	P	4 – 6	4 th	iv	Soak	QIAamp [®] Extraction
7-3	P	7 – 9	4 th	i	Double Swab	Organic Extraction
7-4	P	10 – 12	4 th	ii	Soak	Organic Extraction
11-1	DD	1 – 3	8 th	ii	Soak	Organic Extraction
11-2	DD	4 – 6	8 th	iii	Double Swab	QIAamp [®] Extraction
11-3	DD	7 – 9	8 th	iv	Soak	QIAamp [®] Extraction
11-4	DD	10 – 12	8 th	i	Double Swab	Organic Extraction
12-1	FF	1 – 3	6 th	i	Double Swab	Organic Extraction
12-2	FF	4 – 6	6 th	ii	Soak	Organic Extraction
12-3	FF	7 – 9	6 th	iii	Double Swab	QIAamp [®] Extraction
12-4	FF	10 – 12	6 th	iv	Soak	QIAamp [®] Extraction
17-1	KK	1 – 3	2 nd	iv	Soak	QIAamp [®] Extraction
17-2	KK	4 – 6	2 nd	i	Double Swab	Organic Extraction
17-3	KK	7 – 9	2 nd	ii	Soak	Organic Extraction
17-4	KK	10 – 12	2 nd	iii	Double Swab	QIAamp [®] Extraction

Table A3 (cont'd).

Set	Buccal Letter	Casing Ejected	Order Loaded in Magazine	Analysis Method	Cell Recovery Method	DNA Extraction Method
18-1	Z	1 – 3	7 th	iii	Double Swab	QIAamp [®] Extraction
18-2	Z	4 – 6	7 th	iv	Soak	QIAamp [®] Extraction
18-3	Z	7 – 9	7 th	i	Double Swab	Organic Extraction
18-4	Z	10 – 12	7 th	ii	Soak	Organic Extraction
20-1	PP	1 – 3	5 th	ii	Soak	Organic Extraction
20-2	PP	4 – 6	5 th	iii	Double Swab	QIAamp [®] Extraction
20-3	PP	7 – 9	5 th	iv	Soak	QIAamp [®] Extraction
20-4	PP	10 – 12	5 th	i	Double Swab	Organic Extraction
21-1	X	1 – 3	9 th	i	Double Swab	Organic Extraction
21-2	X	4 – 6	9 th	ii	Soak	Organic Extraction
21-3	X	7 – 9	9 th	iii	Double Swab	QIAamp [®] Extraction
21-4	X	10 – 12	9 th	iv	Soak	QIAamp [®] Extraction
35-1	UU	1 – 3	3 rd	iv	Soak	QIAamp [®] Extraction
35-2	UU	4 – 6	3 rd	i	Double Swab	Organic Extraction
35-3	UU	7 – 9	3 rd	ii	Soak	Organic Extraction
35-4	UU	10 – 12	3 rd	iii	Double Swab	QIAamp [®] Extraction

APPENDIX B. DNA QUANTITIES FROM SPENT CASINGS ASSAYED WITH OPTIMIZED CELL RECOVERY AND DNA EXTRACTION METHODS^{1, 2, 3, 4}

Table B1. DNA quantities recovered from spent cartridge casings using a double swab technique (Sweet *et al.*, 1997) and organic extraction.

Casing Identifier	DNA Concentration (pg/ μ L)	DNA Extract Volume (μ L)	DNA Yield (pg)
30.6	2.88E+01	25.40	731.52
34.4	1.77E+01	24.00	424.80
13-7B	1.61E+01	24.00	386.40
28.2	1.39E+01	27.80	386.42
19-2A	5.39E+00	24.20	130.44
23-2A	5.14E+00	25.60	131.58
1-1C	4.75E+00	26.00	123.50
21-1B	4.69E+00	27.00	126.63
23-2B	4.15E+00	24.00	99.60
41-4B	4.04E+00	29.30	118.37
13-7A	3.69E+00	25.20	92.99
50-5B	3.68E+00	25.50	93.84
43.3	3.46E+00	26.20	90.65
13-7C	2.93E+00	27.50	80.58
33-7B	2.65E+00	24.40	64.66
18-3A	2.51E+00	26.40	66.26
3-4A	2.42E+00	29.00	70.18
2-3A	2.21E+00	28.80	63.65
33-7A	2.16E+00	24.50	52.92
8-5C	1.97E+00	29.00	57.13
50-5A	1.94E+00	25.70	49.86

¹ The casings are organized based on DNA concentration arranged in descending order.

² Casings identifiers: decimal = collected individually. number hyphenated with another number & a letter = collected in triplicate (first number = loader; second number = casing set; letter = individual casing from set)

³ In Collection 1, casings loaded by RR & CC were collected in triplicate and individually due to a confusion in available supplies. As a result, Table B1 contains casings identified in both forms. The only difference between Collections 2 & 3 and triplicate casings from RR & CC is that in Collection 1 each volunteer loaded enough casings for each method to recover DNA from only one casing, rather than three.

⁴ In Collection 3, casing sets were incorrectly labeled resulting in 8 sets of “7-#”. There should have been 4 sets of “7-#” (loader P) and 4 sets of “18-#” (loader Z). Consequently, the sets were temporarily assigned to either P or Z. The volunteer’s profile that was most consistent with each casing was determined following STR analysis. In situations where minimal allelic information was available and a ‘correct’ association could not be made, then individual casings were given an identifier that could associate to either volunteer (*e.g.*, 7/18-1A.1).

Table B1 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
40-3B	1.83E+00	28.80	52.70
26-4B	1.78E+00	26.80	47.70
20-4B	1.75E+00	28.40	49.70
26-4A	1.70E+00	24.50	41.65
20-4A	1.70E+00	28.00	47.60
17-2A	1.68E+00	22.60	37.97
8-5B	1.66E+00	25.00	41.50
38-2B	1.55E+00	27.20	42.16
48.5	1.49E+00	22.40	33.38
20-4C	1.48E+00	27.40	40.55
27-5B	1.39E+00	18.80	26.13
23-2C	1.38E+00	25.20	34.78
26-4C	1.38E+00	27.00	37.26
27-5A	1.38E+00	21.20	29.26
27-5C	1.31E+00	24.50	32.10
17-2B	1.28E+00	26.00	33.28
1-1A	1.26E+00	25.80	32.51
33-7C	1.19E+00	25.60	30.46
18-3B	1.11E+00	26.00	28.86
21-1A	1.01E+00	27.20	27.47
36-1A	9.91E-01	22.20	22.00
19.2	9.89E-01	25.90	25.62
38-2A	9.87E-01	26.20	25.86
50-5C	9.58E-01	27.40	26.25
8-5A	9.57E-01	27.00	25.84
36-1C	8.94E-01	28.00	25.03
6-2A	8.87E-01	27.20	24.13
17-2C	8.61E-01	24.20	20.84
2-3C	8.25E-01	26.00	21.45
1-1B	8.04E-01	30.00	24.12
38-2C	7.87E-01	26.00	20.46
41-4C	7.36E-01	25.50	18.77
21-1C	7.36E-01	27.00	19.87
11-4C	6.89E-01	27.40	18.88
40-3A	6.83E-01	27.00	18.44
36-1B	5.80E-01	25.00	14.50
12-1A	5.47E-01	23.70	12.96
41-4A	5.42E-01	24.00	13.01

Table B1 (cont'd).

Casing Identifier	DNA Concentration (pg/ μL)	DNA Extract Volume (μL)	DNA Yield (pg)
3-4B	5.35E-01	26.00	13.91
11-4A	5.20E-01	27.30	14.20
2-3B	5.19E-01	33.00	17.13
12-1C	5.18E-01	25.00	12.95
25-3C	4.79E-01	24.00	11.50
37-1A	4.75E-01	25.20	11.97
24-6B	4.73E-01	27.00	12.77
18-3C	4.63E-01	23.30	10.79
11-4B	4.48E-01	26.00	11.65
15-1C	3.81E-01	27.60	10.52
6-2C	3.70E-01	26.50	9.81
15-1B	3.67E-01	30.80	11.30
25-3B	3.54E-01	24.80	8.78
40-3C	3.53E-01	27.20	9.60
3-4C	3.40E-01	24.00	8.16
35-2B	3.18E-01	26.60	8.46
10-6B	3.14E-01	27.80	8.73
24-6C	3.06E-01	27.40	8.38
12-1B	3.04E-01	23.60	7.17
25-3A	2.95E-01	26.80	7.91
6-2B	2.79E-01	27.00	7.53
10-6A	2.59E-01	26.20	6.79
35-2A	2.57E-01	27.30	7.02
7-3A	2.46E-01	28.00	6.89
10-6C	2.28E-01	28.40	6.48
7-3B	1.89E-01	28.40	5.37
15-1A	1.74E-01	25.20	4.38
24-6A	1.24E-01	26.80	3.32
37.1	1.06E-01	24.40	2.59
7-3C	5.61E-02	28.00	1.57
35-2C	4.07E-02	25.00	1.02

Table B2. DNA quantities recovered from spent cartridge casings using a soaking technique and organic extraction.

Casing Identifier	DNA Concentration (pg/ μL)	DNA Extract Volume (μL)	DNA Yield (pg)
34.3	5.05E+01	28.20	1424.10
30.5	1.71E+01	27.60	471.96
13-1A	1.36E+01	26.00	353.60
23-3C	5.92E+00	26.60	157.47
28.1	4.75E+00	27.80	132.05
8-6A	4.63E+00	24.00	111.12
13-1B	4.60E+00	25.00	115.00
8-6B	3.82E+00	25.00	95.50
13-1C	3.60E+00	25.80	92.88
23-3A	3.56E+00	25.60	91.14
19-1A	2.82E+00	27.50	77.55
50-6A	2.72E+00	24.60	66.91
23-3B	2.71E+00	24.00	65.04
41-5A	2.16E+00	23.00	49.68
38-3C	2.16E+00	25.60	55.30
8-6C	2.06E+00	25.60	52.74
50-6C	1.90E+00	26.00	49.40
21-2B	1.74E+00	28.40	49.42
38-3B	1.55E+00	23.20	35.96
38-3A	1.49E+00	24.80	36.95
26-5C	1.41E+00	28.00	39.48
50-6B	1.39E+00	27.00	37.53
26-5A	1.35E+00	20.20	27.27
20-1A	1.33E+00	28.00	37.24
27-6A	1.32E+00	27.60	36.43
33-1C	1.30E+00	24.40	31.72
41-5C	1.30E+00	24.80	32.24
17-3C	1.26E+00	28.30	35.66
21-2A	1.22E+00	28.50	34.77
37-2A	1.20E+00	26.40	31.68
27-6B	1.20E+00	28.00	33.60
17-3B	1.20E+00	30.00	36.00
27-6C	1.16E+00	23.00	26.68
43.1	1.14E+00	20.60	23.48
26-5B	1.11E+00	24.30	26.97
25-4C	1.02E+00	25.20	25.70
2-4C	9.15E-01	25.20	23.06
33-1B	8.86E-01	25.20	22.33

Table B2 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
33-1A	8.79E-01	24.20	21.27
41-5B	8.61E-01	22.80	19.63
3-5B	7.88E-01	25.00	19.70
20-1B	7.79E-01	28.20	21.97
11-1C	6.91E-01	30.20	20.87
48.4	6.71E-01	31.00	20.80
2-4B	6.13E-01	24.50	15.02
19.1	5.95E-01	25.00	14.88
2-4A	5.52E-01	24.60	13.58
10-7A	4.96E-01	24.00	11.90
21-2C	4.76E-01	27.80	13.23
37.6	4.67E-01	29.20	13.64
36-2A	4.65E-01	24.00	11.16
36-2B	4.22E-01	26.00	10.97
20-1C	4.06E-01	28.00	11.37
25-4A	3.95E-01	26.20	10.35
18-4C	3.88E-01	28.00	10.86
12-2C	3.69E-01	27.50	10.15
3-5A	3.67E-01	26.80	9.84
40-4C	3.27E-01	23.20	7.59
1-2C	2.94E-01	31.70	9.32
12-2B	2.88E-01	28.20	8.12
40-4A	2.76E-01	25.20	6.96
10-7B	2.75E-01	25.70	7.07
25-4B	2.72E-01	25.00	6.80
36-2C	2.70E-01	24.70	6.67
15-2C	2.59E-01	22.00	5.70
24-7C	2.54E-01	24.00	6.10
6-3C	2.46E-01	26.60	6.54
18-4A	2.40E-01	31.30	7.51
3-5C	2.38E-01	25.00	5.95
1-2B	2.35E-01	27.40	6.44
24-7A	2.22E-01	26.40	5.86
1-2A	2.09E-01	29.80	6.23
7-4A	2.00E-01	31.20	6.24
10-7C	1.93E-01	30.00	5.79
12-2A	1.91E-01	27.90	5.33
17-3A	1.75E-01	29.00	5.08

Table B2 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
35-3A	1.62E-01	28.20	4.57
15-2B	1.36E-01	25.50	3.47
40-4B	1.23E-01	26.00	3.20
18-4B	1.15E-01	29.20	3.36
11-1A	1.07E-01	28.00	3.00
6-3B	9.43E-02	29.80	2.81
11-1B	9.01E-02	31.20	2.81
15-2A	8.22E-02	22.60	1.86
24-7B	6.91E-02	28.60	1.98
7-4C	5.42E-02	28.50	1.54
35-3B	3.95E-02	29.00	1.15
6-3A	2.94E-02	28.20	0.83
7-4B	2.03E-02	27.50	0.56
35-3C	1.99E-02	28.30	0.56

Table B3. DNA quantities recovered from spent cartridge casings using a double swab technique (Sweet *et al.*, 1997) and QIAamp® DNA Investigator extraction.

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
13-2B	1.25E+00	57.00	71.25
34.6	1.17E+00	58.90	68.91
21-3B	9.04E-01	58.40	52.79
21-3A	4.79E-01	59.00	28.26
28.4	4.71E-01	58.80	27.69
12-3A	3.92E-01	58.50	22.93
20-2B	3.89E-01	56.80	22.10
13-2A	3.58E-01	57.70	20.66
23-4C	3.16E-01	59.80	18.90
17-4A	3.00E-01	58.40	17.52
38-4B	2.84E-01	57.50	16.33
2-5B	2.80E-01	57.60	16.13
17-4C	2.57E-01	57.20	14.70
21-3C	2.50E-01	56.70	14.18
8-7A	2.29E-01	57.40	13.14
38-4A	2.15E-01	56.80	12.21
23-4A	2.14E-01	58.80	12.58
26-6A	2.00E-01	59.00	11.80
23-4B	1.90E-01	57.20	10.87
8-7B	1.83E-01	58.20	10.65
41-6B	1.82E-01	58.40	10.63
13-2C	1.77E-01	57.70	10.21
48.1	1.65E-01	57.40	9.47
33-2C	1.49E-01	56.60	8.43
11-2A	1.41E-01	56.30	7.94
37-1B	1.38E-01	59.00	8.14
20-2A	1.36E-01	57.20	7.78
30.2	1.27E-01	58.20	7.39
6-4C	1.27E-01	59.20	7.52
20-2C	1.23E-01	58.40	7.18
2-5C	1.08E-01	57.20	6.18
2-5A	1.05E-01	57.60	6.05
26-6C	1.04E-01	57.80	6.01
7/18-1B.1	1.01E-01	57.40	5.80
41-6A	9.60E-02	57.70	5.54
1-3A	9.47E-02	60.00	5.68
7/18-1C.1	9.43E-02	58.00	5.47

Table B3 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
38-4C	9.31E-02	57.30	5.33
7/18-1A.2	9.18E-02	57.80	5.31
8-7C	8.74E-02	58.60	5.12
1-3C	8.69E-02	57.80	5.02
27-7C	7.50E-02	56.20	4.22
1-3B	7.28E-02	60.00	4.37
17-4B	6.70E-02	58.20	3.90
33-2B	6.69E-02	55.70	3.73
26-6B	6.55E-02	59.70	3.91
25-5C	6.23E-02	59.00	3.68
11-2C	5.97E-02	57.00	3.40
12-3B	5.79E-02	56.80	3.29
27-7B	5.66E-02	58.80	3.33
12-3C	5.54E-02	59.20	3.28
7/18-1A.1	5.51E-02	59.00	3.25
50-7B	5.45E-02	58.00	3.16
3-6C	5.33E-02	58.20	3.10
50-7C	5.04E-02	58.50	2.95
11-2B	4.91E-02	57.30	2.81
41-6C	4.74E-02	59.20	2.81
50-7A	4.42E-02	56.90	2.51
36-3B	4.26E-02	58.60	2.50
25-5B	3.99E-02	57.30	2.29
6-4A	3.86E-02	59.50	2.30
19-2B	3.79E-02	57.80	2.19
15-3B	3.32E-02	56.20	1.87
6-4B	3.22E-02	57.00	1.84
27-7A	3.20E-02	57.20	1.83
25-5A	3.14E-02	58.20	1.83
37.3	2.67E-02	58.40	1.56
7/18-1B.2	2.64E-02	57.00	1.50
33-2A	2.59E-02	57.00	1.48
10-1B	2.56E-02	58.20	1.49
3-6B	1.90E-02	58.00	1.10
19.4	1.84E-02	55.00	1.01
40-5B	1.77E-02	57.60	1.02
7/18-1C.2	1.70E-02	57.20	0.97
36-3A	1.68E-02	57.70	0.97

Table B3 (cont'd).

Casing Identifier	DNA Concentration (pg/ μL)	DNA Extract Volume (μL)	DNA Yield (pg)
3-6A	1.63E-02	58.00	0.95
40-5A	1.50E-02	57.60	0.86
15-3A	1.36E-02	56.20	0.76
40-5C	1.36E-02	59.00	0.80
36-3C	1.03E-02	56.80	0.59
24-1B	9.96E-03	56.00	0.56
10-1C	9.24E-03	57.30	0.53
15-3C	9.23E-03	56.20	0.52
35-4A	8.60E-03	57.50	0.49
24-1C	5.73E-03	57.40	0.33
10-1A	4.36E-03	58.00	0.25
35-4C	3.70E-03	58.40	0.22
35-4B	2.86E-03	56.40	0.16
43.5	1.52E-03	57.80	0.09
24-1A	0.00E+00	57.20	0.00

Table B4. DNA quantities recovered from spent cartridge casings using a soaking technique and QIAamp® DNA Investigator extraction.

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
3-7C	8.85E+00	57.00	504.45
13-3A	3.46E+00	58.00	200.68
23-5C	1.11E+00	59.30	65.82
27-1B	7.00E-01	58.00	40.60
23-5B	6.87E-01	60.60	41.63
23-5A	6.49E-01	57.20	37.12
26-7B	5.45E-01	57.40	31.28
26-7A	4.71E-01	58.20	27.41
25-6A	4.00E-01	58.30	23.32
21-4A	3.74E-01	56.60	21.17
27-1C	2.92E-01	57.00	16.64
30.1	2.28E-01	59.60	13.59
36-4B	1.70E-01	59.00	10.03
13-3C	1.63E-01	58.00	9.45
34.5	1.59E-01	59.20	9.41
12-4B	1.49E-01	58.00	8.64
36-4C	1.28E-01	57.20	7.32
8-1B	1.22E-01	60.00	7.32
7/18-2C.1	1.06E-01	56.90	6.03
33-3C	1.04E-01	59.00	6.14
11-3C	9.93E-02	58.40	5.80
38-5A	6.43E-02	59.00	3.79
11-3A	6.07E-02	56.00	3.40
26-7C	5.38E-02	57.40	3.09
10-2A	5.31E-02	59.40	3.15
17-1C	5.23E-02	57.40	3.00
8-1C	5.18E-02	57.80	2.99
33-3B	5.11E-02	57.60	2.94
25-6C	5.09E-02	57.50	2.93
25-6B	5.08E-02	59.20	3.01
37-2B	4.81E-02	59.00	2.84
41-7C	4.76E-02	58.40	2.78
50-1C	4.40E-02	59.80	2.63
17-1A	4.05E-02	56.40	2.28
6-1A	4.01E-02	57.20	2.29
7/18-2B.1	3.95E-02	59.60	2.35
40-6B	3.38E-02	60.20	2.03
50-1A	3.00E-02	58.70	1.76

Table B4 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
33-3A	2.97E-02	58.80	1.75
2-6B	2.64E-02	57.70	1.52
17-1B	2.56E-02	57.00	1.46
27-1A	2.54E-02	57.80	1.47
10-2C	2.28E-02	57.00	1.30
15-4C	2.22E-02	57.50	1.28
13-3B	2.03E-02	59.00	1.20
10-2B	1.95E-02	59.90	1.17
38-5B	1.85E-02	58.50	1.08
28.3	1.58E-02	59.20	0.94
19.3	1.47E-02	59.50	0.87
3-7A	1.46E-02	59.00	0.86
2-6C	1.37E-02	60.20	0.82
11-3B	1.35E-02	55.20	0.75
2-6A	1.32E-02	57.40	0.76
40-6A	1.22E-02	59.60	0.73
3-7B	1.13E-02	57.80	0.65
8-1A	1.11E-02	58.60	0.65
41-7B	1.11E-02	59.40	0.66
36-4A	1.07E-02	60.00	0.64
43.4	1.05E-02	58.00	0.61
1-4B	9.85E-03	57.00	0.56
6-1B	8.69E-03	58.00	0.50
20-3C	8.36E-03	56.50	0.47
12-4A	8.16E-03	56.50	0.46
50-1B	7.89E-03	59.60	0.47
19-1B	7.17E-03	60.40	0.43
41-7A	6.64E-03	58.40	0.39
6-1C	6.46E-03	57.80	0.37
24-2C	6.41E-03	57.00	0.37
15-4B	6.30E-03	59.90	0.38
38-5C	6.15E-03	58.20	0.36
40-6C	6.03E-03	59.20	0.36
24-2A	5.81E-03	59.50	0.35
48.6	5.76E-03	58.50	0.34
7/18-2A.1	5.43E-03	58.00	0.31
37.2	5.04E-03	59.00	0.30
20-3B	4.92E-03	56.60	0.28

Table B4 (cont'd).

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
20-3B	4.92E-03	56.60	0.28
35-1A	4.19E-03	57.60	0.24
15-4A	3.78E-03	56.80	0.21
1-4C	3.59E-03	57.20	0.21
24-2B	3.28E-03	58.50	0.19
21-4C	2.77E-03	56.20	0.16
1-4A	2.31E-03	56.20	0.13
35-1C	1.71E-03	56.40	0.10
7/18-2C.2	1.66E-03	56.40	0.09
12-4C	1.36E-03	58.80	0.08
7/18-2A.2	1.12E-03	59.40	0.07
35-1B	7.62E-04	57.20	0.04
20-3A	6.58E-04	56.80	0.04
21-4B	1.90E-04	56.00	0.01
7/18-2B.2	1.89E-04	56.20	0.01

Table B5. DNA quantities recovered from spent cartridge casings using a single swab technique and FDF[®] extraction.

Casing Identifier	DNA Concentration (pg/ µL)	DNA Extract Volume (µL)	DNA Yield (pg)
34.1	2.82E-01	75.00	21.15
30.3	5.14E-02	74.80	3.84
8-2A	1.65E-02	69.50	1.15
48.2	1.18E-02	67.80	0.80
37.4	1.10E-02	73.60	0.81
33-4B	1.07E-02	71.00	0.76
19-1C	9.48E-03	71.40	0.68
37-2C	9.29E-03	75.00	0.70
28.5	8.93E-03	68.60	0.61
27-2B	7.81E-03	68.40	0.53
19.5	7.30E-03	72.20	0.53
25-7C	7.19E-03	66.80	0.48
24-3B	7.19E-03	66.80	0.48
43.6	7.18E-03	68.60	0.49
50-2C	5.82E-03	70.90	0.41
26-1B	5.77E-03	70.50	0.41
27-2C	4.92E-03	69.00	0.34
50-2A	4.89E-03	69.80	0.34
3-1B	3.97E-03	71.00	0.28
50-2B	3.93E-03	72.20	0.28
27-2A	3.71E-03	65.40	0.24
8-2B	3.67E-03	70.30	0.26
2-7A	3.61E-03	63.60	0.23
13-4A	3.50E-03	74.20	0.26
41-1B	3.24E-03	68.40	0.22
25-7A	3.14E-03	70.80	0.22
26-1C	3.13E-03	69.90	0.22
41-1C	3.10E-03	69.50	0.22
26-1A	3.04E-03	70.40	0.21
23-6B	3.00E-03	67.50	0.20
3-1C	2.88E-03	69.40	0.20
8-2C	2.78E-03	68.20	0.19
38-6B	2.17E-03	66.60	0.14
23-6A	2.08E-03	68.20	0.14
33-4C	2.03E-03	68.00	0.14
15-5B	1.99E-03	67.20	0.13
10-3C	1.98E-03	72.00	0.14
15-5C	1.95E-03	75.50	0.15

Table B5 (cont'd).

Casing Identifier	DNA Concentration (pg/ μL)	DNA Extract Volume (μL)	DNA Yield (pg)
38-6C	1.84E-03	70.20	0.13
2-7C	1.71E-03	67.70	0.12
23-6C	1.69E-03	73.20	0.12
24-3A	1.68E-03	70.20	0.12
3-1A	1.54E-03	65.80	0.10
25-7B	1.53E-03	74.20	0.11
2-7B	1.51E-03	68.00	0.10
40-7A	1.29E-03	63.70	0.08
10-3A	1.28E-03	69.80	0.09
40-7C	1.10E-03	71.00	0.08
36-5C	9.65E-04	62.80	0.06
24-3C	8.56E-04	71.40	0.06
36-5B	7.38E-04	71.50	0.05
40-7B	7.08E-04	70.50	0.05
13-4B	6.15E-04	71.80	0.04
15-5A	5.98E-04	69.80	0.04
33-4A	5.84E-04	72.00	0.04
13-4C	5.16E-04	73.00	0.04
38-6A	4.95E-04	71.00	0.04
41-1A	4.09E-04	65.80	0.03
36-5A	1.29E-04	64.00	0.01
10-3B	2.47E-06	71.40	0.00

APPENDIX C. COMPARISON OF AMF_{STR}[®] MINIFILER[™] STR PROFILES AND POWERPLEX[®] FUSION STR PROFILES

Red font = non-loader allele

Italicized font = allele is consistent with the loader but could have originated from the previous loader

** = non-loader allele could have originated from the previous loader*

† = off-ladder allele (the number of † symbols represents the number of off-ladder alleles)

N/A = not applicable

NT = locus was not tested (several loci examined with PowerPlex[®] Fusion are not included in MiniFiler[™])

Blank = no alleles recovered at that locus

Table C1. Alleles amplified with AmpF_{STR}[®] MiniFiler[™] and PowerPlex[®] Fusion from spent cartridge casings loaded by volunteer CC during Collection 1.

Locus	Mini 19-1A	Fusion 19-1A	Mini 19-1C	Fusion 19-1C	Mini 19-2A	Fusion 19-2A	CC
Amel	X	X,Y*			X,Y*	X	X
D3	NT		NT		NT	15	14,15
D1	NT	12	NT		NT	11,17.3	11,17.3
D2S441	NT	14	NT		NT	10,14	10,14
D10	NT	13,14,15	NT		NT	16	14,16
D13	13	13			11,12,13	13	13
Penta E	NT		NT		NT	12,13	12,13
D16	9,10,11	11,12			11,12	11,12	11,12
D18	12,14	12,13			12,13,17*	12,16	12
D2S1338	17,21	17			17	17	17
CSF	12	11			†,12	11,12	11,12
Penta D	NT	13	NT		NT		9,12
THO1	NT	6*,7,8,9.3	NT		NT	7,9.3	7,9.3
vWA	NT	18	NT		NT	16,17	17
D21	28,32.2	32.2			32.2	27,28,32.2	28,32.2
D7	8,12	12			9,12	9,12	9,12
D5	NT	13*	NT		NT	9,12	9,12
TPOX	NT		NT		NT	8	8,11
DYS391	NT		NT		NT		N/A
D8	NT	12,13	NT		NT	12,13,15	12,13
D12	NT		NT		NT	17,24	17,24
D19	NT	14.2,15	NT		NT	14.2,15.2	14.2,15.2
FGA	24*	22.2			23,25		23,25
D22	NT		NT		NT	16	16

Table C2. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer Q during Collection 1.

Locus	Mini 28.1	Fusion 28.1	Mini 28.2	Fusion 28.2	Mini 28.4	Fusion 28.4	Mini 28.5	Fusion 28.5	Q
Amel	X,Y	Y	X,Y	X,Y					X,Y
D3	NT	17	NT	15,16,17	NT	17	NT		15,17
D1	NT		NT	12,16.3	NT		NT		12,16.3
D2S441	NT		NT	11	NT		NT		11
D10	NT		NT	13,15	NT		NT		13,15
D13	11,13		11,13	11					11,13
Penta E	NT	13	NT	7,11	NT		NT		7,11
D16	9,11,13*	11	11	11		11			11
D18	13,15*	13,14	13,14	13,14					13,14
D2S1338	19,23,24		23,24	18,23,24	24				23,24
CSF	10,11		10,11,†	10,11	10				10,11
Penta D	NT		NT	10	NT		NT		2.2,10
THO1	NT	7*,8,9	NT	8,9	NT		NT		8,9
vWA	NT	16	NT	16,18	NT	16	NT		16,18
D21	29*,30		30,32.2	30		32.2			30,32.2
D7	8,11		8,11	8,11					8,11
D5	NT		NT	13	NT		NT		13
TPOX	NT		NT	8	NT		NT		8
DYS391	NT		NT	10	NT		NT		10
D8	NT	13,17	NT	13,17	NT	17	NT		13,17
D12	NT	18	NT	18	NT		NT		18
D19	NT		NT	13,15	NT	15	NT		13,15
FGA	22,24		22,24	24		16,16.1,18			22,24
D22	NT		NT		NT		NT		11,12

Table C3. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer LL during Collection 1.

Locus	Mini 30.1	Fusion 30.1	Mini 30.2	Fusion 30.2	Mini 30.5	Fusion 30.5	Mini 30.6	Fusion 30.6	LL
Amel					X	X	X	X	X
D3	NT	15	NT	15	NT	15	NT	15	15
D1	NT		NT		NT	17,18.3	NT	17,18.3	17,18.3
D2S441	NT	14	NT		NT	11.3,14	NT	11.3,14	11.3,14
D10	NT		NT		NT	13,15	NT	13,15	13,15
D13	12				12	12	12	12	12
Penta E	NT		NT		NT	14,17	NT	14,17	14,17
D16	11	†			11,13	11,13	11,13	11,13	11,13
D18					14,15	14,15	14,15,16,17*	14,15	14,15
D2S1338	20				17,18,19*,20,26	17,20	17,18,20,23*,26	17,20	17,20
CSF	12				11,12	11,12	11,12	11,12	11,12
Penta D	NT		NT		NT	9	NT	9	9
THO1	NT		NT	7	NT	7	NT	7	7
vWA	NT	16	NT		NT	16,17	NT	16,17	16,17
D21					29,31.2	29,31.2	29,30*,31.2	29,31.2	29,31.2
D7					8	8	8	8	8
D5	NT		NT		NT	10,12	NT	10,12	10,12
TPOX	NT		NT		NT	8	NT	8	8
DYS391	NT		NT		NT		NT	10	N/A
D8	NT	13	NT		NT	13,14,20	NT	13,14	13,14
D12	NT		NT		NT	18,25	NT	18,25	18,25
D19	NT		NT		NT	13,14	NT	13,14	13,14
FGA	20,30.2,†	23			19,23,25	19,23	19,23	19,23,†	19,23
D22	NT		NT	†	NT	†,15	NT	15	15

Table C4. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer YY during Collection 1.

Locus	Mini 34.1	Fusion 34.1	Mini 34.3	Fusion 34.3	Mini 34.4	Fusion 34.4	Mini 34.5	Fusion 34.5	Mini 34.6	Fusion 34.6	YY
Amel			X,Y	X,Y	X,Y	X,Y				X,Y	X,Y
D3	NT	15	NT	15	NT	15	NT		NT	15	15
D1	NT	15	NT	15,16	NT	15,16	NT		NT	15,16	15,16
D2S441	NT		NT	10,14	NT	10,14	NT	13,14	NT		10,14
D10	NT		NT	14,16	NT	14,16	NT		NT	14	14,16
D13	14		9,14	9,14	9,11,14	9,14			9,14		9,14
Penta E	NT		NT	12,13	NT	12,13	NT		NT	12,13	12,13
D16	12	12	12	12	12	12			12	11,12	12
D18			12,17	12,17	12,17	12,17			12,17	12,17	12,17
D2S1338			18,23	18,23	18,23	18,23	23		23	18	18,23
CSF	11		11,12	11,12	11,12	11,12	11		11,12		11,12
Penta D	NT		NT	9,14	NT	9,14	NT		NT		9
THO1	NT	9.3	NT	6,9.3	NT	6,9.3	NT	6	NT	6,9.3	6,9.3
vWA	NT		NT	19	NT	19	NT		NT	19	19
D21			29,30	29,30	29,30	29,30	29		28,29,30	29	29,30
D7			9	9	9	9	9		9	9	9
D5	NT		NT	12,13	NT	12,13	NT		NT		12,13
TPOX	NT		NT	8,11	NT	8,11	NT		NT	11	8,11
DYS391	NT		NT	11	NT	11	NT		NT	11	11
D8	NT	13	NT	13	NT	13	NT	13	NT	13	13
D12	NT		NT	19	NT	19	NT	19	NT	19	19
D19	NT		NT	13	NT	13	NT		NT	13	13
FGA		†,21	21,24	21,24	21,24,25	21,24	†		24	21,23,2,24	21,24
D22	NT	†	NT	15	NT	15	NT		NT	15	15

Table C5. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer RR during Collection 1.

Locus	Mini 37-2A	Fusion 37-2A	Mini 37-2B	Fusion 37-2B	RR
Amel		<i>X</i>			X,Y
D3	NT	16	NT		16,17
D1	NT	16.3	NT		14,16.3
D2S441	NT	11	NT		11,16
D10	NT		NT		13,15
D13	14				8,14
Penta E	NT		NT		7,18
D16	9,11,12	9,12			11,12
D18	12*,16,17	15,16,17			16,17
D2S1338	20	18			20,25
CSF		10			10,13
Penta D	NT		NT		9,12
THO1	NT	7,9.3*	NT		6,7
vWA	NT	15,19	NT		15,18
D21	30				30
D7	12		10		10,12
D5	NT	12	NT		12,13
TPOX	NT		NT		8
DYS391	NT		NT		11
D8	NT	13*	NT	13*	11,15
D12	NT	22	NT		18,22
D19	NT	14.2*	NT		13,15
FGA	20				20,24
D22	NT		NT		15

Table C6. Alleles amplified with AmpF ℓ STR $^{\text{®}}$ MiniFiler $^{\text{™}}$ and PowerPlex $^{\text{®}}$ Fusion from spent cartridge casings loaded by volunteer U during Collection 2.

Locus	Mini 2-3A	Fusion 2-3A	Mini 2-5B	Fusion 2-5B	U
Amel	<i>X</i>	<i>X</i>			<i>X</i>
D3	NT	15	NT		15
D1	NT	11,17.3	NT		11,17.3
D2S441	NT	10,15	NT		10,15
D10	NT	12	NT		12,14
D13	9				9,13
Penta E	NT		NT		12,15
D16	<i>11</i>	<i>11</i> ,13			11,13
D18	14,15	13*,14,15	15		14,15
D2S1338	17,25	25			17,25
CSF	12	12	13		10,12
Penta D	NT	<i>10</i>	NT		10,11
THO1	NT	6,7	NT		6,7
vWA	NT	14	NT		14,20
D21	28,30	28,30,31			28,30
D7	11				11
D5	NT	11	NT		11
TPOX	NT	8,11	NT		8,11
DYS391	NT		NT		N/A
D8	NT	12	NT		12
D12	NT	23	NT	20	17,23
D19	NT	<i>13</i>	NT		13
FGA	24,†,†,†	17.2,24,25	†	46.2	24,25
D22	NT	16	NT		16

Table C7. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer MM during Collection 2.

Locus	Mini 3-4A	Fusion 3-4A	Mini 3-7C	Fusion 3-7C	MM
Amel		Y*	X	X	X
D3	NT	18*	NT	15	14,16
D1	NT		NT	12,15.3	12,16
D2S441	NT		NT	14*	10,11
D10	NT		NT	13*	14,15
D13			11	11	8,12
Penta E	NT		NT	11,12	7,21
D16		12	11,13*	11,13*	12
D18			16,18	16,18	14,14.2
D2S1338			17,19	17,19	17,23
CSF	†		11,12	11,12	12,13
Penta D	NT		NT	10,12*	13
THO1	NT		NT	9.3	9,9.3
vWA	NT		NT	16,17	17
D21			29,32.2	29,32.2	29,31.2
D7			8,12*	8,12*	9,11
D5	NT		NT	10,12	9,10
TPOX	NT		NT	8	8
DYS391	NT		NT		N/A
D8	NT		NT	11,13	13,15
D12	NT	22	NT	13,18,22	18,22
D19	NT		NT	14,15*	14,15.2
FGA	47.2,†,†,†	17.2	22,22.2,24	22.2,24	22,26
D22	NT		NT	16*,17	11,12

Table C8. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer S during Collection 2.

Locus	Mini 8-2A	Fusion 8-2A	Mini 8-6A	Fusion 8-6A	Mini 8-6B	Fusion 8-6B	S
Amel			X	X	X	X	X
D3	NT		NT	18	NT		18
D1	NT		NT	12,15	NT	12,15	12,15
D2S441	NT		NT	11,11.3	NT	11.3	11,11.3
D10	NT		NT	15	NT	15	13,15
D13			12,13		13	12	12,13
Penta E	NT		NT	13	NT	12	12,13
D16			11	11	11	11	11
D18			12,16	12,16	12,16	12	12,16
D2S1338	20		17,19,25	17	17,23*,25	17,25	17,25
CSF	14,†,†		10,†,10.2,11	10	10,11,12*	10,11	10,11
Penta D	NT		NT	10,13	NT		10,13
THO1	NT		NT	6,9	NT	6,9	6,9
vWA	NT		NT	17,18	NT	16,17,18	17,18
D21			28	28	28	28,31	28
D7	9*		10	10		10	10
D5	NT		NT	10,12	NT	12	10,12
TPOX	NT		NT		NT		8,11
DYS391	NT		NT		NT		N/A
D8	NT		NT	13,16	NT	13,16	13,16
D12	NT		NT	18,18.3	NT	18,18.3	18,18.3
D19	NT		NT	15	NT	15	13.2,15
FGA	29.2,†	†	22,23,†	†	22,†,†,†	22,23	22,23
D22	NT		NT	15	NT	15	15

Table C8 (cont'd).

Locus	Mini 8-7A	Fusion 8-7A	Mini 8-7B	Fusion 8-7B	S
Amel					X
D3	NT	18	NT		18
D1	NT		NT		12,15
D2S441	NT		NT		11,11.3
D10	NT		NT		13,15
D13				12	12,13
Penta E	NT		NT		12,13
D16					11
D18		12			12,16
D2S1338		17			17,25
CSF	†		†,†,†		10,11
Penta D	NT		NT		10,13
THO1	NT	6,9	NT		6,9
vWA	NT	17	NT		17,18
D21					28
D7					10
D5	NT		NT		10,12
TPOX	NT		NT		8,11
DYS391	NT		NT		N/A
D8	NT	†	NT		13,16
D12	NT		NT		18,18.3
D19	NT	†	NT	†	13.2,15
FGA	23,32.2,†,†	†	20,28,48.2,†,†,†,†,†,†	†	22,23
D22	NT		NT	20	15

Table C9. Alleles amplified with AmpF ℓ STR $^{\circledR}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circledR}$ Fusion from spent cartridge casings loaded by volunteer V during Collection 2.

Locus	Mini 13-1A	Fusion 13-1A	Mini 13-1B	Fusion 13-1B	Mini 13-1C	Fusion 13-1C	V
Amel	X,Y	X,Y	X,Y	X,Y		X,Y	X,Y
D3	NT	14	NT	14	NT	14	14
D1	NT	17.3	NT	16.3,17.3	NT	16.3	16.3,17.3
D2S441	NT	11,11.3	NT		NT	11	11,11.3
D10	NT	15,16	NT	15	NT	15,16	15,16
D13	10,12	10,12	10,12		10,12	10	10,12
Penta E	NT	5,14	NT		NT	5,14	5,14
D16	11,12	11,12	11,12	11,12		12	11,12
D18	16,17	16,17	16	17	13,16,17	13,16	16,17
D2S1338	20,22	20,22	20,22		20,22	20,22	20,22
CSF	10,11	11	10,11		10,†	10	10,11
Penta D	NT	11,12	NT		NT	11	11,12
THO1	NT	9,9.3	NT	9,9.3	NT	6*,9,9.3	9,9.3
vWA	NT	16,18	NT	16	NT	16,18	16,18
D21	28,32.2	28,32.2	28		28,29	28,29	28,32.2
D7	11,12	12			11,12	11	11,12
D5	NT	12	NT	12	NT	12	12
TPOX	NT	8	NT	8	NT	8	8
DYS391	NT	11	NT		NT		11
D8	NT	9,12	NT	9	NT	9,12,13*,†	9,12
D12	NT	21,23	NT	21,23	NT	21,23	21,23
D19	NT	12,14	NT	11,12,14	NT	14	12,14
FGA	21.2,22,†,†	21.2,22	21.2	†	22,†,†,†,†	22	21.2,22
D22	NT	11,16	NT		NT		11,16

Table C9 (cont'd).

Locus	Mini 13-2A	Fusion 13-2A	Mini 13-2B	Fusion 13-2B	Mini 13-3A	Fusion 13-3A	V
Amel	X			X,Y	X,Y	X,Y	X,Y
D3	NT		NT		NT	14	14
D1	NT		NT		NT	16.3,17.3	16.3,17.3
D2S441	NT	11	NT	11,11.3	NT		11,11.3
D10	NT		NT	15,16	NT	16	15,16
D13					10,12		10,12
Penta E	NT		NT		NT		5,14
D16			11	11,12	11,12	11,12	11,12
D18			17	17	16,17	16,17	16,17
D2S1338		22	20		20,22	20,22	20,22
CSF	9,†		16,†,†		10,11	11	10,11
Penta D	NT	12	NT	12	NT	12	11,12
THO1	NT	3,9	NT	9.3	NT	9,9.3	9,9.3
vWA	NT		NT	16,17*	NT	16,18	16,18
D21				28			28,32.2
D7						11	11,12
D5	NT		NT		NT		12
TPOX	NT		NT		NT		8
DYS391	NT		NT		NT		11
D8	NT	9,12	NT	9,15	NT	9,12	9,12
D12	NT		NT		NT	21,23	21,23
D19	NT		NT		NT	11,12,14	12,14
FGA	21,†,†,†,†,†,†,†	21.2,41.2	†,†	21.2,22	21.2,22	†	21.2,22
D22	NT	11	NT		NT	11	11,16

Table C9 (cont'd).

Locus	Mini 13-3C	Fusion 13-3C	Mini 13-7A	Fusion 13-7A	Mini 13-7B	Fusion 13-7B	Mini 13-7C	Fusion 13-7C	V
Amel				X,Y	X,Y	X,Y		X,Y	X,Y
D3	NT		NT	14,18*	NT	14	NT	14	14
D1	NT		NT	16.3,17.3	NT	16.3,17.3	NT		16.3,17.3
D2S441	NT		NT	11,11.3	NT	11,11.3	NT	11,11.3	11,11.3
D10	NT		NT		NT	15,16	NT	15,16	15,16
D13			12	10	10,12	10,12	12		10,12
Penta E	NT		NT	5,14	NT	5,14	NT	5,14	5,14
D16		12	11,12	11,12	11,12	11,12	11,12	12	11,12
D18			16	17	16,17	16,17	16	16,17	16,17
D2S1338			17*,20,22,25*	20,22	20,22	20,22	20,22	20	20,22
CSF	15		†	11	10,11	10,11	10,11	10	10,11
Penta D	NT		NT	12	NT	11,12	NT	12	11,12
THO1	NT		NT	9,9.3	NT	9,9.3	NT	9,9.3	9,9.3
vWA	NT	16,18	NT	16,18	NT	16,18	NT	14,17*,18	16,18
D21		36.2	32.2	28,32.2	28,32.2	28,32.2	28	28	28,32.2
D7				12	11	11,12	11	11,12	11,12
D5	NT		NT	12	NT	12	NT		12
TPOX	NT		NT		NT	8	NT		8
DYS391	NT		NT	11	NT	11	NT	11	11
D8	NT		NT	9,12	NT	9,12	NT	9,12	9,12
D12	NT		NT	20,21,23	NT	21,23	NT	23	21,23
D19	NT		NT		NT	12	NT	12	12,14
FGA	†,†,†,†,†,†	†,†	21.2,31.2,†,†	22	21.2,22,†	21.2,22	21.2,†,†	22,23*,32.2	21.2,22
D22	NT	†	NT		NT	11,16	NT	11,16	11,16

Table C10. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer L during Collection 2.

Locus	Mini 23-2A	Fusion 23-2A	Mini 23-2B	Fusion 23-2B	Mini 23-3A	Fusion 23-3A	L
Amel	X	X	X	X		X	X
D3	NT	16	NT	16, 17	NT	15 ,16	16
D1	NT	16,17.3	NT	16,17.3	NT	16,17.3	16,17.3
D2S441	NT	11	NT	11	NT	11	11
D10	NT		NT	15	NT		13,15
D13	13	13	13		13		13
Penta E	NT	7	NT	7	NT	7	7
D16	11	11	11	11	11	11	11
D18	15,16	15,16	15,16	15	15,16		15,16
D2S1338	17	17	17 , 19	17	17		17
CSF	12,13,†,†	13	12,13,†,†		†		12,13
Penta D	NT		NT	8.2	NT		9,11
THO1	NT	8,9.3	NT	8,9.3	NT	7 ,8,9.3	8,9.3
vWA	NT	14	NT	14,18	NT	16 ,18	14,18
D21	27,30	30		30	27	30	27,30
D7	8,10	8			8		8,10
D5	NT		NT		NT		11,12
TPOX	NT		NT		NT		8
DYS391	NT		NT		NT		N/A
D8	NT	13,14	NT	13,14	NT	13	13,14
D12	NT	20	NT	18,20	NT	18	18,20
D19	NT		NT	14,15	NT	14,15	14,15
FGA	23.2 , 30 ,†,†,†	21,23	21,23	21,†,†	23 , 28	†	21,23
D22	NT		NT	†,16	NT		15,16

Table C10 (cont'd).

Locus	Mini 23-3B	Fusion 23-3B	Mini 23-3C	Fusion 23-3C	Mini 23-4A	Fusion 23-4A	L
Amel	<i>X</i>	<i>X,Y*</i>	<i>X</i>	<i>X</i>			<i>X</i>
D3	NT	16	NT	16	NT		16
D1	NT	16	NT	<i>17.3</i>	NT		16,17.3
D2S441	NT	<i>11</i>	NT	<i>11</i>	NT		11
D10	NT		NT		NT		13,15
D13	13		13	<i>12*</i>			13
Penta E	NT		NT		NT		7
D16	<i>11</i>	<i>11</i>	<i>11</i>	<i>11</i>			11
D18	15,16	16	<i>12*,15,16</i>	16			15,16
D2S1338	<i>17</i>	<i>17</i>	<i>17,18,22</i>	<i>17</i>			17
CSF	<i>5</i>		<i>12,†</i>	<i>12</i>	†		12,13
Penta D	NT		NT	<i>6</i>	NT		9,11
THO1	NT	8,9.3	NT	8,9.3	NT	9.3	8,9.3
vWA	NT	<i>14,16</i>	NT	<i>14,17*,18</i>	NT		14,18
D21	27		27	27,30			27,30
D7	10	10		<i>9,10</i>			8,10
D5	NT	11	NT	12	NT		11,12
TPOX	NT		NT	8	NT		8
DYS391	NT		NT		NT		N/A
D8	NT		NT	<i>13,14,15,15.1</i>	NT		13,14
D12	NT	18	NT	18,20,†	NT		18,20
D19	NT	<i>15</i>	NT	<i>14,15</i>	NT	<i>15</i>	14,15
FGA	<i>23,25,32,†,†</i>	21	<i>21,23,†,†</i>	<i>21,†</i>		†	21,23
D22	NT		NT		NT		15,16

Table C10 (cont'd).

Locus	Mini 23-4B	Fusion 23-4B	Mini 23-4C	Fusion 23-4C	L
Amel					X
D3	NT		NT		16
D1	NT	16	NT	15.3,17.3	16,17.3
D2S441	NT		NT		11
D10	NT		NT		13,15
D13					13
Penta E	NT		NT	21	7
D16		11			11
D18					15,16
D2S1338					17
CSF	10		12		12,13
Penta D	NT		NT		9,11
THO1	NT		NT	8	8,9.3
vWA	NT		NT		14,18
D21					27,30
D7					8,10
D5	NT		NT		11,12
TPOX	NT		NT		8
DYS391	NT		NT		N/A
D8	NT		NT		13,14
D12	NT		NT		18,20
D19	NT		NT	†,13	14,15
FGA	22.2,24.2,†,†,†		17,19.2,†		21,23
D22	NT		NT		15,16

Table C10 (cont'd).

Locus	Mini 23-5A	Fusion 23-5A	Mini 23-5B	Fusion 23-5B	Mini 23-5C	Fusion 23-5C	L
Amel		<i>X</i>		<i>X</i>	<i>X</i>	<i>X,Y*</i>	<i>X</i>
D3	NT		NT	16	NT		16
D1	NT		NT		NT		16,17.3
D2S441	NT		NT		NT		11
D10	NT		NT		NT		13,15
D13							13
Penta E	NT		NT		NT		7
D16		<i>†,11</i>				<i>11</i>	11
D18			<i>12*</i>	15	16		15,16
D2S1338							17
CSF	<i>†</i>		<i>†,†,†</i>				12,13
Penta D	NT		NT		NT		9,11
THO1	NT	9.3	NT		NT	9.3	8,9.3
vWA	NT		NT		NT		14,18
D21							27,30
D7	8						8,10
D5	NT		NT		NT		11,12
TPOX	NT		NT		NT	8	8
DYS391	NT		NT		NT		N/A
D8	NT	<i>14,19</i>	NT	<i>13,14</i>	NT		13,14
D12	NT		NT	<i>†</i>	NT	18	18,20
D19	NT		NT	<i>14.2</i>	NT	<i>†</i>	14,15
FGA	<i>†,†,†</i>	<i>30</i>	<i>18.2,†,†,†</i>	<i>†</i>	<i>18.2,†,†</i>	21	21,23
D22	NT		NT		NT		15,16

Table C11. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer T during Collection 2.

Locus	Mini 25-6A	Fusion 25-6A	Mini 25-7C	Fusion 25-7C	T
Amel					X
D3	NT	16	NT		16,17
D1	NT		NT		16,17.3
D2S441	NT		NT		11,14
D10	NT	17	NT		14,17
D13					11
Penta E	NT		NT		11,12
D16					11,12
D18		13			13,17
D2S1338					20,24
CSF	15				10,11
Penta D	NT		NT		8,10
THO1	NT		NT		6,7
vWA	NT		NT		19,20
D21	31.2*				29
D7					8,10
D5	NT		NT		12
TPOX	NT		NT		8,11
DYS391	NT		NT		N/A
D8	NT	13	NT		13,14
D12	NT		NT		19,23
D19	NT		NT		13,16.2
FGA	27.2,†	†,†	19.2,†,†		24
D22	NT	11	NT		11,18

Table C12. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.

Locus	Mini 26-6A	Fusion 26-6A	Mini 26-7A	Fusion 26-7A	Mini 26-7B	Fusion 26-7B	XX
Amel							X
D3	NT		NT		NT	15	14,15
D1	NT		NT	14	NT		14,17.3
D2S441	NT	11.3	NT	14	NT		12,14
D10	NT		NT		NT		14,16
D13			12				12,13
Penta E	NT		NT		NT		12
D16		6				13	11,13
D18							17,18
D2S1338			17	17		16	17
CSF	†,†		8,12		6,11*,†,†		10,12
Penta D	NT		NT		NT		12
THO1	NT		NT		NT	9	9,9.3
vWA	NT		NT		NT		17,19
D21							29,32
D7				12			9,12
D5	NT		NT		NT		10,13
TPOX	NT		NT		NT		8,12
DYS391	NT		NT		NT		N/A
D8	NT		NT		NT	10	10,13
D12	NT	18.3	NT		NT	22	18,22
D19	NT		NT	13,14	NT		13,14
FGA	27.2,†,†,†,†,†	22.2	28.2,†,†,†		32.2,49.2,†,†,†	21	21,23
D22	NT		NT		NT	14	16,17

Table C13. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer N during Collection 2.

Locus	Mini 27-1B	Fusion 27-1B	Mini 27-1C	Fusion 27-1C	Mini 27-2B	Fusion 27-2B	N
Amel		X					X
D3	NT	16	NT		NT		16,17
D1	NT		NT		NT		15.3,17.3
D2S441	NT		NT		NT		11
D10	NT		NT		NT		13
D13		14					12,14
Penta E	NT		NT		NT		13,15
D16							12,13
D18	14						13,14
D2S1338							20,23
CSF	5,†,15				14,†		11,12
Penta D	NT	12	NT		NT		10,12
THO1	NT	9.3	NT		NT		6,9.3
vWA	NT	18	NT		NT		17,18
D21							30,32.2
D7							11,12
D5	NT		NT		NT		12
TPOX	NT		NT		NT		8
DYS391	NT		NT		NT		N/A
D8	NT	13	NT		NT		13
D12	NT	19,21.3	NT		NT		19,20
D19	NT	14	NT	†	NT		13,14
FGA	†,19,21,48.2	25.2,29.2	17.2,29.2,†,†	20.3,†	20,†,†	50.2	21,25
D22	NT		NT		NT	†	11,15

Table C14. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer B during Collection 2.

Locus	Mini 33-4B	Fusion 33-4B	Mini 33-7A	Fusion 33-7A	Mini 33-7B	Fusion 33-7B	B
Amel				X,Y		X,Y	X,Y
D3	NT	18	NT	16,17*,18	NT	16	16,18
D1	NT	17.3	NT	16.3,17.3	NT		16.3,17.3
D2S441	NT		NT	14,15	NT		14,15
D10	NT	13	NT		NT		13,15
D13			12	10	10,12		10,12
Penta E	NT	17.4,18	NT		NT		7,18
D16				9,13	9,13	9,12*,13	9,13
D18			15	15	13,15	13,15	13,15
D2S1338				25	20		20,25
CSF	12		10,†	12	10,12,†,†	12	10,12
Penta D	NT		NT		NT		12,13
THO1	NT	†	NT	8,9,9.3	NT	8,9.3	8,9.3
vWA	NT		NT	18	NT	17,18	17,18
D21			29	29	29		29,31
D7				9			9,12
D5	NT		NT	13	NT		11,13
TPOX	NT		NT		NT		8
DYS391	NT		NT		NT		11
D8	NT		NT	8,13	NT	8,13	8,13
D12	NT	13	NT		NT	22,23	22,23
D19	NT		NT	13,14*	NT		13,15
FGA	24,24.2,33.2	16.1,19.3,23,†,†	20.2,23,†,†	31,†	21,23,48.2		21,23
D22	NT		NT		NT		15,16

Table C15. Alleles amplified with AmpF ℓ STR $^{\circledR}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circledR}$ Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.

Locus	Mini 38-3C	Fusion 38-3C	Mini 38-4A	Fusion 38-4A	Mini 38-4B	Fusion 38-4B	WW
Amel		<i>X</i>		<i>X</i>			<i>X</i>
D3	NT	<i>16</i>	NT		NT		16,18
D1	NT		NT		NT		11,12
D2S441	NT	<i>11</i>	NT		NT	<i>11,14</i>	11,14
D10	NT		NT		NT	16	15,16
D13							8,9
Penta E	NT		NT		NT		11,12
D16	<i>12</i>	<i>12</i>		<i>12</i>	<i>12</i>	<i>11</i>	12
D18	12,15	12,15				12	12,15
D2S1338		17				<i>25</i>	17,21
CSF					†,†,†		11,12
Penta D	NT		NT		NT		10,12
THO1	NT	<i>8,9.3</i>	NT		NT		9.3
vWA	NT	<i>15,17</i>	NT		NT	15	15,17
D21							28,30
D7							10,11
D5	NT		NT		NT		13
TPOX	NT		NT		NT		8,12
DYS391	NT		NT		NT		N/A
D8	NT	10,12	NT		NT	10	10,12
D12	NT	<i>17.3,19.3</i>	NT		NT	<i>18,21.3</i>	18,19.3
D19	NT		NT		NT		13,14
FGA	16,†,†		<i>21*,†,†</i>	†,†	<i>21*</i>		20,24
D22	NT		NT		NT		16

Table C16. Alleles amplified with AmpF ℓ STR $^{\circledR}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circledR}$ Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.

Locus	Mini 41-4B	Fusion 41-4B	Mini 41-5A	Fusion 41-5A	Y
Amel		X,Y		X,Y	X,Y
D3	NT	14	NT	17,18	16,17
D1	NT	16.3,17.3*	NT		12,14
D2S441	NT	11.3	NT		14,15
D10	NT	15,16	NT		14,15
D13		12		13	13,14
Penta E	NT	5,14	NT		5,14
D16	11,12	11,12		11,12	11,12
D18	16*,17	16*,17		17	17
D2S1338	20,22	20	24		17,24
CSF	7,10,11	10	†		12,14
Penta D	NT	12	NT		8,13
THO1	NT	9,9.3	NT	9.3	9,9.3
vWA	NT	16,18*	NT	14	14,16
D21	28,32.2	28,32.2			29,30.2
D7	11,12	11,12			8,10
D5	NT	12	NT		12
TPOX	NT	8	NT		8
DYS391	NT	11	NT		11
D8	NT	9,12	NT	10,14	10,14
D12	NT	21,23	NT	20*,†	17,21
D19	NT	12,14*	NT	9,16.2	13,16.2
FGA	25.2,†,†	21.2,22	20.2,†		22,27
D22	NT	11,16	NT		11,16

Table C17. Alleles amplified with AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$ and PowerPlex $^{\circ}$ Fusion from spent cartridge casings loaded by volunteer II during Collection 2.

Locus	Mini 50-5B	Fusion 50-5B	Mini 50-6A	Fusion 50-6A	II
Amel	Y	Y		X,Y	X,Y
D3	NT	17	NT	17	17
D1	NT	15,18.3	NT	15	15,18.3
D2S441	NT	10,11	NT		10,11
D10	NT	13	NT		13,15
D13	12	11,12			11,12
Penta E	NT	13,14	NT		13,14
D16	12	11,12		10,12	12
D18	16,17	16		17	16,17
D2S1338	19,21		21	21	19,21
CSF	12	12	12,†		12
Penta D	NT		NT		9,13
THO1	NT	6,8,9,9.3	NT	8,9.3	8,9.3
vWA	NT	15,17	NT	15,17	15,17
D21	29	31	29	31	29,31
D7	10	12			10,12
D5	NT	12	NT		11,12
TPOX	NT	8	NT		8
DYS391	NT		NT		11
D8	NT	11,13	NT	11,13	11,13
D12	NT	18,20,†	NT	18	18,20
D19	NT	14	NT	13.2,15.2	14,15.2
FGA	20,21,23,25.2,†,†,†,†,†	23,†	†,†	21,†	21,23
D22	NT	15,16	NT		15,16

**APPENDIX D. ANALYSIS OF LOADER AND NON-LOADER ALLELES IN STR PROFILES AMPLIFIED WITH
AMPF_{STR}[®] MINIFILER[™] AND POWERPLEX[®] FUSION⁵**

Table D1. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a double swab technique (Sweet *et al.*, 1997) and organic extraction.

Casing Identifier	DNA Conc. (pg/μL)	AmpF _{STR} [®] MiniFiler [™]				PowerPlex [®] Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
30.6	2.88E+01	15	15	100.0	6	38	38	100.0	1
34.4	1.77E+01	16	16	100.0	2	38	38	100.0	1
13-7B	1.61E+01	17	18	94.4	0	43	44	97.7	0
28.2	1.39E+01	17	17	100	0	36	42	85.7	2
19-2A	5.39E+00	12	14	85.7	5	33	40	82.5	4
23-2A	5.14E+00	12	14	85.7	2	22	38	57.9	0
1-1C	4.75E+00	N/A	N/A	N/A	N/A	22	42	52.4	4
21-1B	4.69E+00	N/A	N/A	N/A	N/A	21	42	50.0	4
23-2B	4.15E+00	10	14	71.4	3	23	38	60.5	2
41-4B	4.04E+00	3	17	17.6	11	18	44	40.9	21
13-7A	3.69E+00	8	18	44.4	3	31	44	70.4	2
50-5B	3.68E+00	12	16	75.0	2	30	43	69.8	4
43.3	3.46E+00	N/A	N/A	N/A	N/A	8	41	19.5	20

⁵ The casings are organized based on DNA concentration arranged in descending order.

Table D1 (cont'd).

Casing Identifier	DNA Conc. (pg/μL)	AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
13-7C	2.93E+00	11	18	61.1	0	29	44	65.9	4
33-7B	2.65E+00	12	18	66.7	1	16	46	34.8	1
18-3A	2.51E+00	N/A	N/A	N/A	N/A	19	40	47.5	7
3-4A	2.42E+00	0	16	0.0	1	2	41	4.9	3
2-3A	2.21E+00	12	16	75.0	0	28	39	71.8	3
33-7A	2.16E+00	5	18	27.8	1	23	46	50.0	4
8-5C	1.97E+00	N/A	N/A	N/A	N/A	14	41	34.1	5
50-5A	1.94E+00	N/A	N/A	N/A	N/A	17	43	39.5	11
40-3B	1.83E+00	N/A	N/A	N/A	N/A	12	39	30.8	18
26-4B	1.78E+00	N/A	N/A	N/A	N/A	22	42	52.4	7
20-4B	1.75E+00	N/A	N/A	N/A	N/A	9	39	23.1	10
26-4A	1.70E+00	N/A	N/A	N/A	N/A	22	42	52.4	1
20-4A	1.70E+00	N/A	N/A	N/A	N/A	11	39	28.2	3
17-2A	1.68E+00	N/A	N/A	N/A	N/A	13	46	28.3	1
8-5B	1.66E+00	N/A	N/A	N/A	N/A	20	41	48.8	1
38-2B	1.55E+00	N/A	N/A	N/A	N/A	17	41	41.5	18
48.5	1.49E+00	N/A	N/A	N/A	N/A	9	43	20.9	2
20-4C	1.48E+00	N/A	N/A	N/A	N/A	10	39	25.6	18
27-5B	1.39E+00	N/A	N/A	N/A	N/A	12	40	30.0	6
23-2C	1.38E+00	N/A	N/A	N/A	N/A	12	38	31.6	4
26-4C	1.38E+00	N/A	N/A	N/A	N/A	24	42	57.1	3
27-5A	1.38E+00	N/A	N/A	N/A	N/A	9	40	22.5	7
27-5C	1.31E+00	N/A	N/A	N/A	N/A	15	40	37.5	9
17-2B	1.28E+00	N/A	N/A	N/A	N/A	9	46	19.6	2

Table D1 (cont'd).

Casing Identifier	DNA Conc. (pg/μL)	AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
1-1A	1.26E+00	N/A	N/A	N/A	N/A	9	41	21.9	9
33-7C	1.19E+00	N/A	N/A	N/A	N/A	12	46	26.1	0
18-3B	1.11E+00	N/A	N/A	N/A	N/A	14	40	35.0	6
21-1A	1.01E+00	N/A	N/A	N/A	N/A	12	42	28.6	2
36-1A	9.91E-01	N/A	N/A	N/A	N/A	12	44	27.3	1
19.2	9.89E-01	N/A	N/A	N/A	N/A	23	40	57.5	5
38-2A	9.87E-01	N/A	N/A	N/A	N/A	17	41	41.5	0
50-5C	9.58E-01	N/A	N/A	N/A	N/A	20	43	46.5	4
8-5A	9.57E-01	N/A	N/A	N/A	N/A	13	41	31.7	7
36-1C	8.94E-01	N/A	N/A	N/A	N/A	5	44	11.4	5
6-2A	8.87E-01	N/A	N/A	N/A	N/A	10	46	21.7	13
17-2C	8.61E-01	N/A	N/A	N/A	N/A	9	46	19.6	6
2-3C	8.25E-01	N/A	N/A	N/A	N/A	11	39	28.2	3
1-1B	8.04E-01	N/A	N/A	N/A	N/A	11	41	26.8	9
38-2C	7.87E-01	N/A	N/A	N/A	N/A	9	41	21.9	4
41-4C	7.36E-01	N/A	N/A	N/A	N/A	18	44	40.9	8
21-1C	7.36E-01	N/A	N/A	N/A	N/A	7	42	16.7	2
11-4C	6.89E-01	N/A	N/A	N/A	N/A	6	41	14.6	0
40-3A	6.83E-01	N/A	N/A	N/A	N/A	13	39	33.3	10
36-1B	5.80E-01	N/A	N/A	N/A	N/A	3	44	6.8	1
12-1A	5.47E-01	N/A	N/A	N/A	N/A	6	41	14.6	1
41-4A	5.42E-01	N/A	N/A	N/A	N/A	4	44	9.1	3
3-4B	5.35E-01	N/A	N/A	N/A	N/A	1	41	2.4	3

Table D1 (cont'd).

Casing Identifier	DNA Conc. (pg/ μ L)	AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$				PowerPlex $^{\circ}$ Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
11-4A	5.20E-01	N/A	N/A	N/A	N/A	0	41	0.0	0
2-3B	5.19E-01	N/A	N/A	N/A	N/A	16	39	41.0	1
12-1C	5.18E-01	N/A	N/A	N/A	N/A	6	41	14.6	6
25-3C	4.79E-01	N/A	N/A	N/A	N/A	3	41	7.3	12
37-1A	4.75E-01	N/A	N/A	N/A	N/A	12	44	27.3	0
24-6B	4.73E-01	N/A	N/A	N/A	N/A	5	41	12.2	14
18-3C	4.63E-01	N/A	N/A	N/A	N/A	9	40	22.5	1
11-4B	4.48E-01	N/A	N/A	N/A	N/A	3	41	7.3	2
15-1C	3.81E-01	N/A	N/A	N/A	N/A	1	41	2.4	1
6-2C	3.70E-01	N/A	N/A	N/A	N/A	3	46	6.5	2
15-1B	3.67E-01	N/A	N/A	N/A	N/A	2	41	4.9	2
25-3B	3.54E-01	N/A	N/A	N/A	N/A	7	41	17.1	1
40-3C	3.53E-01	N/A	N/A	N/A	N/A	0	39	0.0	1
3-4C	3.40E-01	N/A	N/A	N/A	N/A	9	41	21.9	4
35-2B	3.18E-01	N/A	N/A	N/A	N/A	18	42	42.8	25
10-6B	3.14E-01	N/A	N/A	N/A	N/A	4	41	9.7	2
24-6C	3.06E-01	N/A	N/A	N/A	N/A	5	41	12.2	2
12-1B	3.04E-01	N/A	N/A	N/A	N/A	2	41	4.9	3
25-3A	2.95E-01	N/A	N/A	N/A	N/A	5	41	12.2	3
6-2B	2.79E-01	N/A	N/A	N/A	N/A	6	46	13.0	6
10-6A	2.59E-01	N/A	N/A	N/A	N/A	0	41	0.0	1
35-2A	2.57E-01	N/A	N/A	N/A	N/A	3	42	7.1	6
7-3A	2.46E-01	N/A	N/A	N/A	N/A	2	45	4.4	1
10-6C	2.28E-01	N/A	N/A	N/A	N/A	0	41	0.0	0

Table D1 (cont'd).

Casing Identifier	DNA Conc. (pg/μL)	AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
7-3B	1.89E-01	N/A	N/A	N/A	N/A	4	45	8.9	1
15-1A	1.74E-01	N/A	N/A	N/A	N/A	4	41	9.7	3
24-6A	1.24E-01	N/A	N/A	N/A	N/A	1	41	2.4	2
37.1	1.06E-01	N/A	N/A	N/A	N/A	1	44	2.3	2
7-3C	5.61E-02	N/A	N/A	N/A	N/A	5	45	11.1	3
35-2C	4.07E-02	N/A	N/A	N/A	N/A	1	42	2.4	2

Table D2. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a soaking technique and organic extraction. DNA extract 3-5A is the only sample extracted with an organic extraction and amplified with PowerPlex® Fusion that does not have allelic data due to high levels of contamination.

Casing Identifier	DNA Conc. (pg/μL)	AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
34.3	5.05E+01	16	16	100.0	0	38	38	100.0	1
30.5	1.71E+01	15	15	100.0	4	38	38	100.0	1
13-1A	1.36E+01	18	18	100.0	0	41	44	93.2	0
23-3C	5.92E+00	10	14	71.4	3	24	38	63.1	6
28.1	4.75E+00	15	17	88.2	5	11	42	26.6	2
8-6A	4.63E+00	14	14	100.0	2	29	41	70.7	0
13-1B	4.60E+00	13	18	72.2	0	19	44	43.2	1
8-6B	3.82E+00	11	14	78.6	2	28	41	68.3	2
13-1C	3.60E+00	11	18	61.1	2	30	44	68.2	4
23-3A	3.56E+00	8	14	57.1	1	15	38	39.5	3
19-1A	2.82E+00	9	14	64.3	6	16	40	40.0	11
50-6A	2.72E+00	3	16	18.7	0	17	43	39.5	2
23-3B	2.71E+00	9	14	64.3	3	15	38	39.5	2
41-5A	2.16E+00	1	17	5.9	1	12	44	27.3	3
38-3C	2.16E+00	3	16	18.7	0	13	41	31.7	2
8-6C	2.06E+00	N/A	N/A	N/A	N/A	23	41	56.1	5
50-6C	1.90E+00	N/A	N/A	N/A	N/A	8	43	18.6	1
21-2B	1.74E+00	N/A	N/A	N/A	N/A	20	42	47.6	5
38-3B	1.55E+00	N/A	N/A	N/A	N/A	10	41	24.4	2
38-3A	1.49E+00	N/A	N/A	N/A	N/A	17	41	41.5	13
26-5C	1.41E+00	N/A	N/A	N/A	N/A	17	42	40.5	0
50-6B	1.39E+00	N/A	N/A	N/A	N/A	9	43	20.9	2

Table D2 (cont'd).

		AmpF ℓ STR® MiniFiler™				PowerPlex® Fusion			
Casing Identifier	DNA Conc. (pg/ μ L)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
26-5A	1.35E+00	N/A	N/A	N/A	N/A	17	42	40.5	1
20-1A	1.33E+00	N/A	N/A	N/A	N/A	9	39	23.1	1
27-6A	1.32E+00	N/A	N/A	N/A	N/A	10	40	25.0	10
33-1C	1.30E+00	N/A	N/A	N/A	N/A	11	46	23.9	4
41-5C	1.30E+00	N/A	N/A	N/A	N/A	9	44	20.4	3
17-3C	1.26E+00	N/A	N/A	N/A	N/A	10	46	21.7	0
21-2A	1.22E+00	N/A	N/A	N/A	N/A	12	42	28.6	2
37-2A	1.20E+00	9	17	52.9	2	12	44	27.3	7
27-6B	1.20E+00	N/A	N/A	N/A	N/A	10	40	25.0	9
17-3B	1.20E+00	N/A	N/A	N/A	N/A	12	46	26.1	0
27-6C	1.16E+00	N/A	N/A	N/A	N/A	10	40	25.0	5
43.1	1.14E+00	N/A	N/A	N/A	N/A	6	41	14.6	2
26-5B	1.11E+00	N/A	N/A	N/A	N/A	17	42	40.5	2
25-4C	1.02E+00	N/A	N/A	N/A	N/A	6	41	14.6	3
2-4C	9.15E-01	N/A	N/A	N/A	N/A	18	39	46.1	8
33-1B	8.86E-01	N/A	N/A	N/A	N/A	12	46	26.1	1
33-1A	8.79E-01	N/A	N/A	N/A	N/A	12	46	26.1	1
41-5B	8.61E-01	N/A	N/A	N/A	N/A	14	44	31.8	1
3-5B	7.88E-01	N/A	N/A	N/A	N/A	4	41	9.7	10
20-1B	7.79E-01	N/A	N/A	N/A	N/A	4	39	10.2	4
11-1C	6.91E-01	N/A	N/A	N/A	N/A	13	41	31.7	6
48.4	6.71E-01	N/A	N/A	N/A	N/A	5	43	11.6	5
2-4B	6.13E-01	N/A	N/A	N/A	N/A	7	39	17.9	4
19.1	5.95E-01	N/A	N/A	N/A	N/A	5	40	12.5	2

Table D2 (cont'd).

		AmpF ℓ STR $^{\circ}$ MiniFiler $^{\text{TM}}$				PowerPlex $^{\circ}$ Fusion			
Casing Identifier	DNA Conc. (pg/ μ L)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
2-4A	5.52E-01	N/A	N/A	N/A	N/A	8	39	20.5	3
10-7A	4.96E-01	N/A	N/A	N/A	N/A	5	41	12.2	5
21-2C	4.76E-01	N/A	N/A	N/A	N/A	5	42	11.9	2
37.6	4.67E-01	N/A	N/A	N/A	N/A	8	44	18.2	3
36-2A	4.65E-01	N/A	N/A	N/A	N/A	8	44	18.2	4
36-2B	4.22E-01	N/A	N/A	N/A	N/A	3	44	6.8	2
20-1C	4.06E-01	N/A	N/A	N/A	N/A	9	39	23.1	1
25-4A	3.95E-01	N/A	N/A	N/A	N/A	4	41	9.7	3
18-4C	3.88E-01	N/A	N/A	N/A	N/A	5	40	12.5	0
12-2C	3.69E-01	N/A	N/A	N/A	N/A	3	41	7.3	4
3-5A	3.67E-01	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-4C	3.27E-01	N/A	N/A	N/A	N/A	8	39	20.5	3
1-2C	2.94E-01	N/A	N/A	N/A	N/A	3	41	7.3	2
12-2B	2.88E-01	N/A	N/A	N/A	N/A	4	41	9.7	1
40-4A	2.76E-01	N/A	N/A	N/A	N/A	3	39	5.1	4
10-7B	2.75E-01	N/A	N/A	N/A	N/A	3	41	7.3	2
25-4B	2.72E-01	N/A	N/A	N/A	N/A	4	41	9.7	5
36-2C	2.70E-01	N/A	N/A	N/A	N/A	3	44	6.8	0
15-2C	2.59E-01	N/A	N/A	N/A	N/A	3	41	7.3	7
24-7C	2.54E-01	N/A	N/A	N/A	N/A	5	41	12.2	3
6-3C	2.46E-01	N/A	N/A	N/A	N/A	12	46	26.1	5
18-4A	2.40E-01	N/A	N/A	N/A	N/A	4	40	10.0	1
3-5C	2.38E-01	N/A	N/A	N/A	N/A	4	41	9.7	1

Table D2 (cont'd).

Casing Identifier	DNA Conc. (pg/μL)	AmpFlSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
1-2B	2.35E-01	N/A	N/A	N/A	N/A	5	41	12.2	0
24-7A	2.22E-01	N/A	N/A	N/A	N/A	3	41	7.3	3
1-2A	2.09E-01	N/A	N/A	N/A	N/A	1	41	2.4	1
7-4A	2.00E-01	N/A	N/A	N/A	N/A	2	45	4.4	8
10-7C	1.93E-01	N/A	N/A	N/A	N/A	2	41	4.9	2
12-2A	1.91E-01	N/A	N/A	N/A	N/A	7	41	17.7	0
17-3A	1.75E-01	N/A	N/A	N/A	N/A	10	46	21.7	1
35-3A	1.62E-01	N/A	N/A	N/A	N/A	0	42	0.0	0
15-2B	1.36E-01	N/A	N/A	N/A	N/A	1	41	2.4	3
40-4B	1.23E-01	N/A	N/A	N/A	N/A	2	39	5.1	1
18-4B	1.15E-01	N/A	N/A	N/A	N/A	5	40	12.5	1
11-1A	1.07E-01	N/A	N/A	N/A	N/A	2	41	4.9	3
6-3B	9.43E-02	N/A	N/A	N/A	N/A	1	46	2.2	1
11-1B	9.01E-02	N/A	N/A	N/A	N/A	0	41	0.0	0
15-2A	8.22E-02	N/A	N/A	N/A	N/A	0	41	0.0	0
24-7B	6.91E-02	N/A	N/A	N/A	N/A	1	41	2.4	1
7-4C	5.42E-02	N/A	N/A	N/A	N/A	4	45	8.9	1
35-3B	3.95E-02	N/A	N/A	N/A	N/A	3	42	7.1	0
6-3A	2.94E-02	N/A	N/A	N/A	N/A	1	46	2.2	2
7-4B	2.03E-02	N/A	N/A	N/A	N/A	1	45	2.2	0
35-3C	1.99E-02	N/A	N/A	N/A	N/A	2	42	4.8	0

Table D3. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a double swab technique (Sweet *et al.*, 1997) and QIAamp® DNA Investigator extraction.

Casing Identifier	DNA Conc. (pg/μL)	AmpFSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
13-2B	1.25E+00	3	18	16.7	1	17	44	38.6	2
34.6	1.17E+00	12	16	75.0	1	25	38	65.8	2
21-3B	9.04E-01	N/A	N/A	N/A	N/A	11	42	26.2	1
21-3A	4.79E-01	N/A	N/A	N/A	N/A	6	42	14.3	3
28.4	4.71E-01	2	17	11.8	0	6	42	14.3	3
12-3A	3.92E-01	N/A	N/A	N/A	N/A	5	41	12.2	0
20-2B	3.89E-01	N/A	N/A	N/A	N/A	5	39	12.8	1
13-2A	3.58E-01	1	18	5.6	2	8	44	18.2	2
23-4C	3.16E-01	1	14	7.1	3	2	38	5.3	0
17-4A	3.00E-01	N/A	N/A	N/A	N/A	2	46	4.3	0
38-4B	2.84E-01	1	16	6.2	1	7	41	17.1	3
2-5B	2.80E-01	1	16	6.2	1	0	39	0.0	2
17-4C	2.57E-01	N/A	N/A	N/A	N/A	0	46	0.0	3
21-3C	2.50E-01	N/A	N/A	N/A	N/A	9	42	21.4	1
8-7A	2.29E-01	1	14	7.1	1	6	41	14.6	0
38-4A	2.15E-01	0	16	0.0	1	2	41	4.9	0
23-4A	2.14E-01	0	14	0.0	0	2	38	5.3	0
26-6A	2.00E-01	0	16	0.0	1	0	42	0.0	4
23-4B	1.90E-01	0	14	0.0	3	2	38	5.3	0
8-7B	1.83E-01	0	14	0.0	3	1	41	2.4	1
41-6B	1.82E-01	N/A	N/A	N/A	N/A	7	44	15.9	2
13-2C	1.77E-01	N/A	N/A	N/A	N/A	0	44	0.0	3
48.1	1.65E-01	N/A	N/A	N/A	N/A	2	43	4.6	1

Table D3 (cont'd).

		AmpF [®] STR [®] MiniFiler [™]				PowerPlex [®] Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
33-2C	1.49E-01	N/A	N/A	N/A	N/A	5	46	10.9	0
11-2A	1.41E-01	N/A	N/A	N/A	N/A	2	41	4.9	0
37-1B	1.38E-01	N/A	N/A	N/A	N/A	3	44	6.8	1
20-2A	1.36E-01	N/A	N/A	N/A	N/A	2	39	5.1	0
30.2	1.27E-01	0	15	0.0	0	2	38	5.3	0
6-4C	1.27E-01	N/A	N/A	N/A	N/A	3	46	6.5	2
20-2C	1.23E-01	N/A	N/A	N/A	N/A	3	39	7.7	1
2-5C	1.08E-01	N/A	N/A	N/A	N/A	3	39	7.7	0
2-5A	1.05E-01	N/A	N/A	N/A	N/A	1	39	2.6	2
26-6C	1.04E-01	N/A	N/A	N/A	N/A	0	42	0.0	0
7/18-1B.1	1.01E-01	N/A	N/A	N/A	N/A	4	40	10.0	3
41-6A	9.60E-02	N/A	N/A	N/A	N/A	1	44	2.3	0
1-3A	9.47E-02	N/A	N/A	N/A	N/A	0	41	0.0	2
7/18-1C.1	9.43E-02	N/A	N/A	N/A	N/A	0	40	0.0	0
38-4C	9.31E-02	N/A	N/A	N/A	N/A	1	41	2.4	1
7/18-1A.2	9.18E-02	N/A	N/A	N/A	N/A	1	45	2.2	0
8-7C	8.74E-02	N/A	N/A	N/A	N/A	6	41	14.6	1
1-3C	8.69E-02	N/A	N/A	N/A	N/A	0	41	0.0	1
27-7C	7.50E-02	N/A	N/A	N/A	N/A	0	40	0.0	0
1-3B	7.28E-02	N/A	N/A	N/A	N/A	0	41	0.0	1
17-4B	6.70E-02	N/A	N/A	N/A	N/A	0	46	0.0	0
33-2B	6.69E-02	N/A	N/A	N/A	N/A	0	46	0.0	1
26-6B	6.55E-02	N/A	N/A	N/A	N/A	2	42	4.7	0
25-5C	6.23E-02	N/A	N/A	N/A	N/A	0	41	0.0	0

Table D3 (cont'd).

		AmpF [®] STR [®] MiniFiler [™]				PowerPlex [®] Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
11-2C	5.97E-02	N/A	N/A	N/A	N/A	1	41	2.4	0
12-3B	5.79E-02	N/A	N/A	N/A	N/A	0	41	0.0	1
27-7B	5.66E-02	N/A	N/A	N/A	N/A	1	40	2.5	0
12-3C	5.54E-02	N/A	N/A	N/A	N/A	1	41	2.4	0
7/18-1A.1	5.51E-02	N/A	N/A	N/A	N/A	2	40	5.0	0
50-7B	5.45E-02	N/A	N/A	N/A	N/A	0	43	0.0	1
3-6C	5.33E-02	N/A	N/A	N/A	N/A	2	41	4.9	1
50-7C	5.04E-02	N/A	N/A	N/A	N/A	0	43	0.0	0
11-2B	4.91E-02	N/A	N/A	N/A	N/A	0	41	0.0	0
41-6C	4.74E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50-7A	4.42E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-3B	4.26E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25-5B	3.99E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6-4A	3.86E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19-2B	3.79E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-3B	3.32E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6-4B	3.22E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
27-7A	3.20E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25-5A	3.14E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
37.3	2.67E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-1B.2	2.64E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33-2A	2.59E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-1B	2.56E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-6B	1.90E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D3 (cont'd).

		AmpF [®] STR [®] MiniFiler [™]				PowerPlex [®] Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
19.4	1.84E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-5B	1.77E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-1C.2	1.70E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-3A	1.68E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-6A	1.63E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-5A	1.50E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-3A	1.36E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-5C	1.36E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-3C	1.03E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-1B	9.96E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-1C	9.24E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-3C	9.23E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-4A	8.60E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-1C	5.73E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-1A	4.36E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-4C	3.70E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-4B	2.86E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
43.5	1.52E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-1A	0.00E+00	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D4. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a soaking technique and QIAamp® DNA Investigator extraction.

Casing Identifier	DNA Conc. (pg/μL)	AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
3-7C	8.85E+00	5	14	35.7	11	13	41	31.7	27
13-3A	3.46E+00	14	18	77.8	0	26	44	59.0	1
23-5C	1.11E+00	2	14	14.3	1	6	38	15.8	1
27-1B	7.00E-01	2	17	11.8	4	9	40	22.5	3
23-5B	6.87E-01	0	14	0.0	2	5	38	13.2	1
23-5A	6.49E-01	1	14	7.1	0	4	38	10.5	2
26-7B	5.45E-01	0	16	0.0	2	6	42	14.3	2
26-7A	4.71E-01	3	16	18.7	1	6	42	14.3	0
25-6A	4.00E-01	0	14	0.0	3	5	41	12.2	0
21-4A	3.74E-01	N/A	N/A	N/A	N/A	3	42	7.1	6
27-1C	2.92E-01	0	17	0.0	2	0	40	0.0	1
30.1	2.28E-01	4	15	26.7	2	5	38	13.1	0
36-4B	1.70E-01	N/A	N/A	N/A	N/A	3	44	6.8	5
13-3C	1.63E-01	0	18	0.0	1	3	44	6.8	1
34.5	1.59E-01	4	16	25.0	0	4	38	10.5	1
12-4B	1.49E-01	N/A	N/A	N/A	N/A	6	41	14.6	3
36-4C	1.28E-01	N/A	N/A	N/A	N/A	3	44	6.8	0
8-1B	1.22E-01	N/A	N/A	N/A	N/A	3	41	7.3	3
7/18-2C.1	1.06E-01	N/A	N/A	N/A	N/A	4	45	8.9	3
33-3C	1.04E-01	N/A	N/A	N/A	N/A	3	46	6.5	2
11-3C	9.93E-02	N/A	N/A	N/A	N/A	2	41	4.9	3
38-5A	6.43E-02	N/A	N/A	N/A	N/A	0	41	0.0	1
11-3A	6.07E-02	N/A	N/A	N/A	N/A	1	41	2.4	2

Table D4 (cont'd).

		AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
26-7C	5.38E-02	N/A	N/A	N/A	N/A	1	42	2.4	0
10-2A	5.31E-02	N/A	N/A	N/A	N/A	0	41	0.0	1
17-1C	5.23E-02	N/A	N/A	N/A	N/A	1	46	2.2	0
8-1C	5.18E-02	N/A	N/A	N/A	N/A	0	41	0.0	0
33-3B	5.11E-02	N/A	N/A	N/A	N/A	1	46	2.2	0
25-6C	5.09E-02	N/A	N/A	N/A	N/A	2	41	4.9	0
25-6B	5.08E-02	N/A	N/A	N/A	N/A	1	41	2.4	0
37-2B	4.81E-02	1	17	5.8	0	0	44	0.0	1
41-7C	4.76E-02	N/A	N/A	N/A	N/A	1	44	2.3	0
50-1C	4.40E-02	N/A	N/A	N/A	N/A	0	43	0.0	1
17-1A	4.05E-02	N/A	N/A	N/A	N/A	1	46	2.2	0
6-1A	4.01E-02	N/A	N/A	N/A	N/A	0	46	0.0	1
7/18-2B.1	3.95E-02	N/A	N/A	N/A	N/A	3	45	6.7	0
40-6B	3.38E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50-1A	3.00E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33-3A	2.97E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-6B	2.64E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
17-1B	2.56E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
27-1A	2.54E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-2C	2.28E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-4C	2.22E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13-3B	2.03E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-2B	1.95E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38-5B	1.85E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D4 (cont'd).

		AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
28.3	1.58E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19.3	1.47E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-7A	1.46E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-6C	1.37E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11-3B	1.35E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-6A	1.32E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-6A	1.22E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-7B	1.13E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8-1A	1.11E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
41-7B	1.11E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-4A	1.07E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
43.4	1.05E-02	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1-4B	9.85E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6-1B	8.69E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
20-3C	8.36E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
12-4A	8.16E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50-1B	7.89E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
19-1B	7.17E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
41-7A	6.64E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6-1C	6.46E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-2C	6.41E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-4B	6.30E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38-5C	6.15E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-6C	6.03E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D4 (cont'd).

		AmpFtSTR® MiniFiler™				PowerPlex® Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
24-2A	5.81E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
48.6	5.76E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-2A.1	5.43E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
37.2	5.04E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
20-3B	4.92E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-1A	4.19E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-4A	3.78E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1-4C	3.59E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-2B	3.28E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
21-4C	2.77E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
1-4A	2.31E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-1C	1.71E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-2C.2	1.66E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
12-4C	1.36E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-2A.2	1.12E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
35-1B	7.62E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
20-3A	6.58E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
21-4B	1.90E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
7/18-2B.2	1.89E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D5. Summary of alleles recovered in STR profiles generated from spent cartridge casings using a single swab and FDF® extraction.

Casing Identifier	DNA Conc. (pg/μL)	AmpFSTR® MiniFiler™				PowerPlex® Fusion			
		# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
34.1	2.82E-01	3	16	18.7	0	6	38	15.8	0
30.3	5.14E-02	N/A	N/A	N/A	N/A	4	38	10.5	1
8-2A	1.65E-02	0	14	0.0	4	0	41	0.0	0
48.2	1.18E-02	N/A	N/A	N/A	N/A	0	43	0.0	1
37.4	1.10E-02	N/A	N/A	N/A	N/A	0	44	0.0	1
33-4B	1.07E-02	1	18	5.6	3	5	46	10.9	4
19-1C	9.48E-03	0	14	0.0	0	0	40	0.0	0
37-2C	9.29E-03	N/A	N/A	N/A	N/A	0	44	0.0	0
28.5	8.93E-03	0	17	0.0	0	0	42	0.0	0
27-2B	7.81E-03	0	17	0.0	2	0	40	0.0	1
19.5	7.30E-03	N/A	N/A	N/A	N/A	0	40	0.0	1
25-7C	7.19E-03	0	14	0.0	1	0	41	0.0	0
24-3B	7.19E-03	N/A	N/A	N/A	N/A	0	41	0.0	1
43.6	7.18E-03	N/A	N/A	N/A	N/A	0	41	0.0	0
50-2C	5.82E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
26-1B	5.77E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
27-2C	4.92E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50-2A	4.89E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-1B	3.97E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
50-2B	3.93E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
27-2A	3.71E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8-2B	3.67E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-7A	3.61E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D5 (cont'd).

		AmpF STR ® MiniFiler™				PowerPlex® Fusion			
Casing Identifier	DNA Conc. (pg/μL)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
13-4A	3.50E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
41-1B	3.24E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25-7A	3.14E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
26-1C	3.13E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
41-1C	3.10E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
26-1A	3.04E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
23-6B	3.00E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-1C	2.88E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
8-2C	2.78E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38-6B	2.17E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
23-6A	2.08E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33-4C	2.03E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-5B	1.99E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-3C	1.98E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-5C	1.95E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38-6C	1.84E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-7C	1.71E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
23-6C	1.69E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-3A	1.68E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3-1A	1.54E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
25-7B	1.53E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
2-7B	1.51E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-7A	1.29E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D5 (cont'd).

		AmpF ℓ STR [®] MiniFiler [™]				PowerPlex [®] Fusion			
Casing Identifier	DNA Conc. (pg/ μ L)	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
10-3A	1.28E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-7C	1.10E-03	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-5C	9.65E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
24-3C	8.56E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-5B	7.38E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
40-7B	7.08E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13-4B	6.15E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15-5A	5.98E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
33-4A	5.84E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
13-4C	5.16E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38-6A	4.95E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
41-1A	4.09E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
36-5A	1.29E-04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
10-3B	2.47E-06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table D6. Summary of alleles recovered in consensus and individual STR profiles generated from DNA extracts retrieved via a double swab technique (Sweet *et al.*, 1997) and organic extraction. Consensus profiles are presented first (Con. = Consensus) and the next three casing identifiers are the individual profiles.

	PowerPlex® Fusion			
Casing Identifier	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 2-3	18	39	46.15	0
2-3A	28	39	71.8	3
2-3B	16	39	41	1
2-3C	11	39	28.2	3
Con. 3-4	1	41	2.44	0
3-4A	2	41	4.9	3
3-4B	1	41	2.4	3
3-4C	9	41	21.9	4
Con. 8-5	14	41	34.15	0
8-5A	13	41	31.7	7
8-5B	20	41	48.8	1
8-5C	14	41	34.1	5
Con. 10-6	0	41	0	0
10-6A	0	41	0	1
10-6B	4	41	9.7	2
10-6C	0	41	0	0
Con. 13-7	40	44	90.91	0
13-7A	31	44	70.4	2
13-7B	43	44	97.7	0
13-7C	29	44	65.9	4
Con. 15-1	1	41	2.44	0
15-1A	4	41	9.7	3
15-1B	2	41	4.9	2
15-1C	1	41	2.4	1
Con. 23-2	19	38	50	0
23-2A	22	38	57.9	0
23-2B	23	38	60.5	2
23-2C	12	38	31.6	4

Table D6 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 24-6	2	41	4.88	2
24-6A	1	41	2.4	2
24-6B	5	41	12.2	14
24-6C	5	41	12.2	2
Con. 25-3	2	41	4.88	1
25-3A	5	41	12.2	3
25-3B	7	41	17.1	1
25-3C	3	41	7.3	12
Con. 26-4	22	42	52.38	0
26-4A	22	42	52.4	1
26-4B	22	42	52.4	7
26-4C	24	42	57.1	3
Con. 27-5	9	40	22.5	3
27-5A	9	40	22.5	7
27-5B	12	40	30	6
27-5C	15	40	37.5	9
Con. 33-7	16	46	34.78	0
33-7A	23	46	50	4
33-7B	16	46	34.8	1
33-7C	12	46	26.1	0
Con. 36-1	3	44	6.82	0
36-1A	12	44	27.3	1
36-1B	3	44	6.8	1
36-1C	5	44	11.4	5
Con. 38-2	13	41	31.71	1
38-2A	17	41	41.5	0
38-2B	17	41	41.5	18
38-2C	9	41	21.9	4
Con. 40-3	7	39	17.95	4
40-3A	13	39	33.3	10
40-3B	12	39	30.8	18
40-3C	0	39	0	1

Table D6 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 41-4	7	44	15.91	2
41-4A	4	44	9.1	3
41-4B	18	44	40.9	21
41-4C	18	44	40.9	8
Con. 50-5	20	43	46.51	2
50-5A	17	43	39.5	11
50-5B	30	43	69.8	4
50-5C	20	43	46.5	4
Con. 1-1	13	41	31.71	4
1-1A	9	41	21.9	9
1-1B	11	41	26.8	9
1-1C	22	42	52.4	4
Con. 6-2	5	46	10.87	3
6-2A	10	46	21.7	13
6-2B	6	46	13	6
6-2C	3	46	6.5	2
Con. 7-3	2	45	4.44	0
7-3A	2	45	4.4	1
7-3B	4	45	8.9	1
7-3C	5	45	11.1	3
Con. 11-4	0	41	0	0
11-4A	0	41	0	0
11-4B	3	41	7.3	2
11-4C	6	41	14.6	0
Con. 12-1	3	41	7.32	0
12-1A	6	41	14.6	1
12-1B	2	41	4.9	3
12-1C	6	41	14.6	6
Con. 17-2	9	46	19.57	0
17-2A	13	46	28.3	1
17-2B	9	46	19.6	2
17-2C	9	46	19.6	6

Table D6 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 18-3	10	40	25	0
18-3A	19	40	47.5	7
18-3B	14	40	35	6
18-3C	9	40	22.5	1
Con. 20-4	7	39	17.95	5
20-4A	11	39	28.2	3
20-4B	9	39	23.1	10
20-4C	10	39	25.6	18
Con. 21-1	13	42	30.95	0
21-1A	12	42	28.6	2
21-1B	21	42	50	4
21-1C	7	42	16.7	2
Con. 35-2	3	42	7.14	3
35-2A	3	42	7.1	6
35-2B	18	42	42.8	25
35-2C	1	42	2.4	2

Table D7. Summary of alleles recovered in consensus and individual STR profiles generated from DNA extracts retrieved via a soaking technique and organic extraction. Consensus profiles are presented first (Con. = Consensus) and the next three casing identifiers are the individual profiles.

	PowerPlex® Fusion			
Casing Identifier	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 2-4	7	39	17.95	0
2-4A	8	39	20.5	3
2-4B	7	39	17.9	4
2-4C	18	39	46.1	8
Con. 3-5	2	41	4.88	0
3-5A	N/A	N/A	N/A	N/A
3-5B	4	41	9.7	10
3-5C	4	41	9.7	1
Con. 8-6	28	41	68.29	0
8-6A	29	41	70.7	0
8-6B	28	41	68.3	2
8-6C	23	41	56.1	5
Con. 10-7	1	41	2.44	1
10-7A	5	41	12.2	5
10-7B	3	41	7.3	2
10-7C	2	41	4.9	2
Con. 13-1	32	44	72.73	0
13-1A	41	44	93.2	0
13-1B	19	44	43.2	1
13-1C	30	44	68.2	4
Con. 15-2	0	41	0	1
15-2A	0	41	0	0
15-2B	1	41	2.4	3
15-2C	3	41	7.3	7
Con. 23-3	19	38	50	1
23-3A	15	38	39.5	3
23-3B	15	38	39.5	2
23-3C	24	38	63.1	6

Table D7 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 24-7	2	41	4.88	0
24-7A	3	41	7.3	3
24-7B	1	41	2.4	1
24-7C	5	41	12.2	3
Con. 25-4	4	41	9.76	0
25-4A	4	41	9.7	3
25-4B	4	41	9.7	5
25-4C	6	41	14.6	3
Con. 26-5	18	42	42.86	0
26-5A	17	42	40.5	1
26-5B	17	42	40.5	2
26-5C	17	42	40.5	0
Con. 27-6	9	40	22.5	5
27-6A	10	40	25	10
27-6B	10	40	25	9
27-6C	10	40	25	5
Con. 33-1	10	46	21.74	0
33-1A	12	46	26.1	1
33-1B	12	46	26.1	1
33-1C	11	46	23.9	4
Con. 36-2	2	44	4.55	1
36-2A	8	44	18.2	4
36-2B	3	44	6.8	2
36-2C	3	44	6.8	0
Con. 38-3	12	41	29.27	0
38-3A	17	41	41.5	13
38-3B	10	41	24.4	2
38-3C	13	41	31.7	2
Con. 40-4	1	39	2.56	0
40-4A	3	39	5.1	4
40-4B	2	39	5.1	1
40-4C	8	39	20.5	3

Table D7 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 41-5	8	44	18.18	0
41-5A	12	44	27.3	3
41-5B	14	44	31.8	1
41-5C	9	44	20.4	3
Con. 50-6	10	43	23.26	0
50-6A	17	43	39.5	2
50-6B	9	43	20.9	2
50-6C	8	43	18.6	1
Con. 1-2	1	41	2.44	0
1-2A	1	41	2.4	1
1-2B	5	41	12.2	0
1-2C	3	41	7.3	2
Con. 6-3	1	46	2.17	0
6-3A	1	46	2.2	2
6-3B	1	46	2.2	1
6-3C	12	46	26.1	5
Con. 7-4	0	45	0	0
7-4A	2	45	4.4	8
7-4B	1	45	2.2	0
7-4C	4	45	8.9	1
Con. 11-1	0	41	0	0
11-1A	2	41	4.9	3
11-1B	0	41	0	0
11-1C	13	41	31.7	6
Con. 12-2	3	41	7.32	0
12-2A	7	41	17.7	0
12-2B	4	41	9.7	1
12-2C	3	41	7.3	4
Con. 17-3	8	46	17.39	0
17-3A	10	46	21.7	1
17-3B	12	46	26.1	0
17-3C	10	46	21.7	0

Table D7 (cont'd).

Casing Identifier	PowerPlex® Fusion			
	# Loader Alleles	# Possible Loader Alleles	% Loader Profile	# Non-loader Alleles
Con. 18-4	3	40	7.5	0
18-4A	4	40	10	1
18-4B	5	40	12.5	1
18-4C	5	40	12.5	0
Con. 20-1	7	39	17.95	0
20-1A	9	39	23.1	1
20-1B	4	39	10.2	4
20-1C	9	39	23.1	1
Con. 21-2	7	42	16.67	0
21-2A	12	42	28.6	2
21-2B	20	42	47.6	5
21-2C	5	42	11.9	2
Con. 35-3	0	42	0	0
35-3A	0	42	0	0
35-3B	3	42	7.1	0
35-3C	2	42	4.8	0

APPENDIX E. POWERPLEX® FUSION STR PROFILES^{6,7}

Red = non-loader allele

Italicized = allele is consistent with the loader but could have originated from the previous loader

* = non-loader allele could have originated from the previous loader

† = off-ladder allele (each † symbol represents a different off-ladder allele)

N/A = not applicable

Blank = no alleles recovered at that locus

The cell recovery and DNA extraction method utilized to recover and extract DNAs from spent cartridge casings is denoted with one of the following letters:

A = double swab + organic extraction

B = soak + organic extraction

C = double swab + QIAamp® extraction

D = soak + QIAamp® extraction

E = single swab + FDF® extractions

⁶ Two magazines were alternated among loaders in Collection 3, therefore two sets of STR profiles are presented for each volunteer from that collection. first set (blue) = alleles italicized/asterisk based on the loader immediately prior (contamination from firearm); second set (green) = alleles italicized/asterisk based on the preceding magazine loader (contamination from magazine)

⁷ Volunteer P = 1st to load & fire the pistol; Thus, profiles from P were compared to vol. DD (owner of the firearm). Volunteer KK = 1st to load the 2nd magazine; Thus, profiles from KK were only compared to the immediately prior loader.

Table E1. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer CC and collected individually during Collection 1.

Locus	19.1	19.2	19.5	CC
Amel	X	X,Y*		X
D3		15		14,15
D1				11,17.3
D2S441		14		10,14
D10		14,16		14,16
D13				13
Penta E				12,13
D16	11	11,12		11,12
D18				12
D2S1338		16,17		17
CSF		11,12		11,12
Penta D				9,12
THO1	7,9.3	6*,7,8,9.3		7,9.3
vWA		17		17
D21	29	28		28,32.2
D7				9,12
D5		9		9,12
TPOX				8,11
DYS391				N/A
D8	12,14	12,13		12,13
D12		17,24		17,24
D19		14.2,15*,15.2	16.2	14.2,15.2
FGA		25		23,25
D22				16
Method	B	A	E	

Table E2. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer CC and collected in triplicate during Collection 1.

Locus	19-1A	19-1C	19-2A	CC
Amel	X,Y*		X	X
D3			15	14,15
D1	12		11,17.3	11,17.3
D2S441	14		10,14	10,14
D10	13,14,15		16	14,16
D13	13		13	13
Penta E			12,13	12,13
D16	11,12		11,12	11,12
D18	12,13		12,16	12
D2S1338	17		17	17
CSF	11		11,12	11,12
Penta D	13			9,12
THO1	6*,7,8,9.3		7,9.3	7,9.3
vWA	18		16,17	17
D21	32.2		27,28,32.2	28,32.2
D7	12		9,12	9,12
D5	13*		9,12	9,12
TPOX			8	8,11
DYS391				N/A
D8	12,13		12,13,15	12,13
D12			17,24	17,24
D19	14.2,15		14.2,15.2	14.2,15.2
FGA	22.2			23,25
D22			16	16
Method	B	E	A	

Table E3. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Q and collected individually during Collection 1.

Locus	28.1	28.2	28.4	28.5	Q
Amel	Y	X,Y			X,Y
D3	17	15,16,17	17		15,17
D1		12,16.3			12,16.3
D2S441		11			11
D10		13,15			13,15
D13		11			11,13
Penta E	13	7,11			7,11
D16	11	11	11		11
D18	13,14	13,14			13,14
D2S1338		18,23,24			23,24
CSF		10,11			10,11
Penta D		10			2.2,10
THO1	7*,8,9	8,9			8,9
vWA	16	16,18	16		16,18
D21		30	32.2		30,32.2
D7		8,11			8,11
D5		13			13
TPOX		8			8
DYS391		10			10
D8	13,17	13,17	17		13,17
D12	18	18			18
D19		13,15	15		13,15
FGA		24	16,16.1,18		22,24
D22					11,12
Method	B	A	C	E	

Table E4. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer LL and collected individually during Collection 1.

Locus	30.1	30.2	30.3	30.5	30.6	LL
Amel				<i>X</i>	<i>X</i>	<i>X</i>
D3	<i>15</i>	<i>15</i>	<i>15</i>	<i>15</i>	<i>15</i>	<i>15</i>
D1				17,18.3	17,18.3	17,18.3
D2S441	<i>14</i>		11.3	11.3, <i>14</i>	11.3, <i>14</i>	11.3,14
D10				<i>13</i> ,15	<i>13</i> ,15	13,15
D13				<i>12</i>	<i>12</i>	12
Penta E				14,17	14,17	14,17
D16	†			11,13	11,13	11,13
D18				14,15	14,15	14,15
D2S1338				17,20	17,20	17,20
CSF				11, <i>12</i>	11, <i>12</i>	11,12
Penta D				9	9	9
THO1		7	7	7	7	7
vWA	<i>16</i>			<i>16</i> ,17	<i>16</i> ,17	16,17
D21				29,31.2	29,31.2	29,31.2
D7				8	8	8
D5				10, <i>12</i>	10, <i>12</i>	10,12
TPOX				8	8	8
DYS391					10	N/A
D8	13		†, 13.3	<i>13</i> , <i>14</i> , 20	<i>13</i> , <i>14</i>	13,14
D12			25	18,25	18,25	18,25
D19				<i>13</i> , <i>14</i>	<i>13</i> , <i>14</i>	13,14
FGA	23		†	19,23	19,23,†	19,23
D22		†		†, <i>15</i>	<i>15</i>	15
Method	D	C	E	B	A	

Table E5. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer YY and collected individually during Collection 1.

Locus	34.1	34.3	34.4	34.5	34.6	YY
Amel		X,Y	X,Y		X,Y	X,Y
D3	15	15	15		15	15
D1	15	15,16	15,16		15,16	15,16
D2S441		10,14	10,14	13,14		10,14
D10		14,16	14,16		14	14,16
D13		9,14	9,14			9,14
Penta E		12,13	12,13		12,13	12,13
D16	12	12	12		11,12	12
D18		12,17	12,17		12,17	12,17
D2S1338		18,23	18,23		18	18,23
CSF		11,12	11,12			11,12
Penta D		9,14	9,14			9
THO1	9.3	6,9.3	6,9.3	6	6,9.3	6,9.3
vWA		19	19		19	19
D21		29,30	29,30		29	29,30
D7		9	9		9	9
D5		12,13	12,13			12,13
TPOX		8,11	8,11		11	8,11
DYS391		11	11		11	11
D8	13	13	13	13	13	13
D12		19	19	19	19	19
D19		13	13		13	13
FGA	†,21	21,24	21,24		21,23.2,24	21,24
D22	†	15	15		15	15
Method	E	B	A	D	C	

Table E6. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer RR and collected individually during Collection 1.

Locus	37.1	37.4	37.6	RR
Amel			Y	X,Y
D3			14*,17	16,17
D1	14.3		14,16.3	14,16.3
D2S441				11,16
D10				13,15
D13				8,14
Penta E				7,18
D16			11	11,12
D18				16,17
D2S1338			25	20,25
CSF	11*	12*		10,13
Penta D				9,12
THO1			3,7	6,7
vWA			15	15,18
D21				30
D7				10,12
D5				12,13
TPOX				8
DYS391				11
D8			13*	11,15
D12	18			18,22
D19			†	13,15
FGA	†			20,24
D22				15
Method	A	E	B	

Table E7. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer RR and collected in triplicate during Collection 1.

Locus	37-1A	37-1B	37-2A	37-2B	37-2C	RR
Amel	X	Y	X			X,Y
D3			16			16,17
D1		16	16.3			14,16.3
D2S441			11			11,16
D10	13					13,15
D13	14					8,14
Penta E						7,18
D16	11,12		9,12			11,12
D18			15,16,17			16,17
D2S1338	20		18			20,25
CSF			10			10,13
Penta D						9,12
THO1	6,7		7,9.3*			6,7
vWA	15,18		15,19			15,18
D21						30
D7						10,12
D5	13		12			12,13
TPOX						8
DYS391						11
D8	11	11	13*	13*		11,15
D12			22			18,22
D19			14.2*			13,15
FGA		20				20,24
D22						15
Method	A	C	B	D	E	

Table E8. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer M and collected individually during Collection 1.

Locus	43.1	43.3	43.6	M
Amel	<i>Y</i>	<i>X,Y</i>		X,Y
D3	<i>15</i>	<i>15,17*</i>		15,16
D1	16	12*		16
D2S441		11*		10,14
D10				13,14
D13		11*,13*		12,14
Penta E		7		7,12
D16	11	11*		9,12
D18		13*,14*		12,17
D2S1338				19,23
CSF				12
Penta D		2.2*,10*		9
THO1	6,9.3	8*,9*		9.3
vWA		18*		16,19
D21		24.2,30		26.2,30
D7				8,9
D5	13*			12
TPOX		8		8,11
DYS391		10*		11
D8		13		13,14
D12		18*,27		19,21
D19	13	13,15*		13,14
FGA	22	18.2,27.3		21,22
D22				15
Method	B	A	E	

Table E9. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer GG and collected individually during Collection 1.

Locus	48.1	48.2	48.4	48.5	GG
Amel	<i>Y</i>		<i>X,Y</i>	<i>X,Y</i>	<i>X,Y</i>
D3	<i>15</i>				14,15
D1			16.3		15.3,17.3
D2S441					11,12
D10					13,15
D13				12	12
Penta E					13,19
D16			†	11,12	12,13
D18					13,16
D2S1338					19,25
CSF				11	11,13
Penta D				10	10,13
TH01			7,9.3	8	8,9.3
vWA					15,17
D21			30*,31.2		29,31.2
D7					9,10
D5					11
TPOX					8
DYS391				10	10
D8			14*	12	12,13
D12			25		20,21
D19			16	19.2	13,16
FGA	25	18			21
D22		†			11,17
Method	C	E	B	A	

Table E10. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer U during Collection 2.

Locus	2-3A	2-3B	2-3C	2-4A	2-4B	2-4C	2-5A	2-5B	2-5C	U
Amel	X	X,Y*	X	X	X	X				X
D3	15	15	15	17*		15,18			15	15
D1	11,17.3	11	17.3			11				11,17.3
D2S441	10,15	15				10,15				10,15
D10	12	12	14			12,14				12,14
D13										9,13
Penta E		15		12		13,15				12,15
D16	11,13		11,13	11,13	11,13	11,13				11,13
D18	13*,14,15		14,15		16	14				14,15
D2S1338	25	17,25			25					17,25
CSF	12	10				10				10,12
Penta D	10									10,11
THO1	6,7	6,7	7	9.3	7	†,6,9.3				6,7
vWA	14	14	15			18	18			14,20
D21	28,30,31			28	28	32.2				28,30
D7						9				11
D5	11	11		11		11				11
TPOX	8,11	11		11	11	8,11				8,11
DYS391										N/A
D8	12	12	12,13*,15		13*,13.2,16	13*			12	12
D12	23		17	17	†	17	14	20		17,23
D19	13	13		14			13			13
FGA	17.2,24,25					19.2		46.2	25,†	24,25
D22	16					16				16
Method	A	A	A	B	B	B	C	C	C	

Table E11. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer MM during Collection 2.

Locus	3-4A	3-4B	3-4C	3-5B	3-5C	3-6C	3-7C	MM
Amel	Y*		X				X	X
D3	18*			15			15	14,16
D1				18.3		12	12,15.3	12,16
D2S441			11				14*	10,11
D10							13*	14,15
D13			8				11	8,12
Penta E							11,12	7,21
D16	12	11		†,12,13*	11		11,13*	12
D18			15*,17	13*	14		16,18	14,14.2
D2S1338				25*			17,19	17,23
CSF				11			11,12	12,13
Penta D			13			13	10,12*	13
THO1		9.3	6,9,9.3	7			9.3	9,9.3
vWA							16,17	17
D21				32.2			29,32.2	29,31.2
D7		8					8,12*	9,11
D5			11*				10,12	9,10
TPOX							8	8
DYS391								N/A
D8		12	†,13,15	13,15	15,†	9	11,13	13,15
D12	22		18	18,23*	18,22		13,18,22	18,22
D19							14,15*	14,15.2
FGA	17.2			24			22.2,24	22,26
D22							16*,17	11,12
Method	A	A	A	B	B	C	D	

Table E12. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer S during Collection 2.

Locus	8-1B	8-1C	8-2A	8-5A	8-5B	8-5C	S
Amel				X	X	X	X
D3				18	18	18	18
D1				11,15	12,15		12,15
D2S441						11	11,11.3
D10				15		15	13,15
D13				13	13		12,13
Penta E					13		12,13
D16				11	11	11	11
D18				12	12	12	12,16
D2S1338						17	17,25
CSF				11	13*		10,11
Penta D							10,13
TH01	6			7,9	6,9		6,9
vWA					18		17,18
D21				29*,34			28
D7				12	10	10	10
D5					12	10	10,12
TPOX				8	11		8,11
DYS391							N/A
D8	13,16			10,13,16	13,16	6,13,14,16	13,16
D12	17.3			18	18,18.3	18.3	18,18.3
D19	†				13.2,15	7,15,16,19.2	13.2,15
FGA	16.1,22.1		†	21		23,†	22,23
D22							15
Method	D	D	E	A	A	A	

Table E12 (cont'd).

Locus	8-6A	8-6B	8-6C	8-7A	8-7B	8-7C	S
Amel	<i>X</i>	<i>X</i>	<i>X</i>				<i>X</i>
D3	18		18	18			18
D1	<i>12,15</i>	<i>12,15</i>	<i>12,15</i>			15	12,15
D2S441	<i>11,11.3</i>	11.3	11.3, 14				11,11.3
D10	<i>15</i>	<i>15</i>	<i>15</i>				13,15
D13		<i>12</i>	13		<i>12</i>	<i>12</i>	12,13
Penta E	13	12	12,13				12,13
D16	11	11	11				11
D18	12,16	12	12,16	12		16	12,16
D2S1338	<i>17</i>	<i>17,25</i>		<i>17</i>			17,25
CSF	10	10,11					10,11
Penta D	<i>10,13</i>		10				10,13
THO1	6,9	6,9	6	6,9			6,9
vWA	<i>17,18</i>	16 , <i>17,18</i>	<i>17,18</i>	<i>17</i>			17,18
D21	28	28, 31	28, 29 *				28
D7	10	10				10	10
D5	<i>10,12</i>	12					10,12
TPOX			8				8,11
DYS391							N/A
D8	<i>13,16</i>	<i>13,16</i>	†, <i>13,16,19</i>	†			13,16
D12	<i>18,18.3</i>	<i>18,18.3</i>	18.3			†,18.3	18,18.3
D19	15	15	13.2, 14	†	†	13.2, 18.2	13.2,15
FGA	†	22,23	23,†	†	†	†	22,23
D22	15	15	16		20		15
Method	B	B	B	C	C	C	

Table E13. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer VV during Collection 2.

Locus	10-2A	10-6A	10-6B	10-6C	10-7A	10-7B	10-7C	VV
Amel			X		X	X	Y	X,Y
D3			16			17		14,17
D1					14		14	15,17.3
D2S441			14					11,14
D10					13			12,13
D13								11
Penta E								7,8
D16								12
D18							12	12,16
D2S1338								17,18
CSF	12*					12*		11
Penta D								9,12
THO1					7			9.3
vWA					17	15*		17
D21					32.2			28,32.2
D7						10		10,11
D5			13					11,13
TPOX								11
DYS391								11
D8			8,13*		13*,14			8,12
D12					15			15,25
D19								14,15.2
FGA		32.2,†					27.3	22,23
D22					16*			11,15
Method	D	A	A	A	B	B	B	

Table E14. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer V during Collection 2.

Locus	13-1A	13-1B	13-1C	13-2A	13-2B	13-2C	13-3A	13-3C	V
Amel	X,Y	X,Y	X,Y		X,Y		X,Y		X,Y
D3	14	14	14			16	14		14
D1	17.3	16.3,17.3	16.3				16.3,17.3		16.3,17.3
D2S441	11,11.3		11	11	11,11.3				11,11.3
D10	15,16	15	15,16		15,16		16		15,16
D13	10,12		10						10,12
Penta E	5,14		5,14						5,14
D16	11,12	11,12	12		11,12		11,12	12	11,12
D18	16,17	17	13,16		17		16,17		16,17
D2S1338	20,22		20,22	22			20,22		20,22
CSF	11		10				11		10,11
Penta D	11,12		11	12	12		12		11,12
THO1	9,9.3	9,9.3	6*,9,9.3	3,9	9.3		9,9.3		9,9.3
vWA	16,18	16	16,18		16,17*		16,18	16,18	16,18
D21	28,32.2		28,29		28			36.2	28,32.2
D7	12		11				11		11,12
D5	12	12	12			11			12
TPOX	8	8	8						8
DYS391	11								11
D8	9,12	9	9,12,13*,†	9,12	9,15	†,10	9,12		9,12
D12	21,23	21,23	21,23				21,23		21,23
D19	12,14	11,12,14	14				11,12,14		12,14
FGA	21.2,22	†	22	21.2,41.2	21.2,22		†	†,†	21.2,22
D22	11,16			11			11	†	11,16
Method	B	B	B	C	C	C	D	D	

Table E14 (cont'd).

Locus	13-7A	13-7B	13-7C	V
Amel	X,Y	X,Y	X,Y	X,Y
D3	14,18*	14	14	14
D1	16.3,17.3	16.3,17.3		16.3,17.3
D2S441	11,11.3	11,11.3	11,11.3	11,11.3
D10		15,16	15,16	15,16
D13	10	10,12		10,12
Penta E	5,14	5,14	5,14	5,14
D16	11,12	11,12	12	11,12
D18	17	16,17	16,17	16,17
D2S1338	20,22	20,22	20	20,22
CSF	11	10,11	10	10,11
Penta D	12	11,12	12	11,12
THO1	9,9.3	9,9.3	9,9.3	9,9.3
vWA	16,18	16,18	14,17*,18	16,18
D21	28,32.2	28,32.2	28	28,32.2
D7	12	11,12	11,12	11,12
D5	12	12		12
TPOX		8		8
DYS391	11	11	11	11
D8	9,12	9,12	9,12	9,12
D12	20,21,23	21,23	23	21,23
D19		12	12	12,14
FGA	22	21.2,22	22,23*,32.2	21.2,22
D22		11,16	11,16	11,16
Method	A	A	A	

Table E15. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer HH during Collection 2.

Locus	15-1A	15-1B	15-1C	15-2A	15-2B	15-2C	HH
Amel	X	X			X		X
D3		15					14,18
D1							16,17.3
D2S441							11,14
D10	13						13,15
D13						8	10,11
Penta E	12						10,14
D16	†				11*	11*	9,12
D18	12					15	16
D2S1338						16	17,19
CSF							11,13
Penta D							10
THO1	9.3*		9		9.3*	6	9
vWA							14,16
D21							30,31
D7							11,12
D5	12						9,12
TPOX						11	9,11
DYS391							N/A
D8		10,11	14.1			16	10,13
D12	20					20	20,21
D19						14.2	13,14
FGA					32.2	22	22,25
D22							16
Method	A	A	A	B	B	B	

Table E16. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer L during Collection 2.

Locus	23-2A	23-2B	23-2C	23-3A	23-3B	23-3C	L
Amel	X	X	X	X	X,Y*	X	X
D3	16	16,17		15,16	16	16	16
D1	16,17.3	16,17.3		16,17.3	16	17.3	16,17.3
D2S441	11	11		11	11	11	11
D10		15	14*				13,15
D13	13					12*	13
Penta E	7	7		7			7
D16	11	11	11,12*	11	11	11	11
D18	15,16	15	15		16	16	15,16
D2S1338	17	17			17	17	17
CSF	13		12			12	12,13
Penta D		8.2	9			6	9,11
THO1	8,9.3	8,9.3	3,8,9.3	7,8,9.3	8,9.3	8,9.3	8,9.3
vWA	14	14,18	14	16,18	14,16	14,17*,18	14,18
D21	30	30		30		27,30	27,30
D7	8				10	9,10	8,10
D5					11	12	11,12
TPOX						8	8
DYS391							N/A
D8	13,14	13,14	11,13,14	13		13,14,15,15.1	13,14
D12	20	18,20	18	18	18	18,20,†	18,20
D19		14,15	15	14,15	15	14,15	14,15
FGA	21,23	21,†,†		†	21	21,†	21,23
D22		†,16					15,16
Method	A	A	A	B	B	B	

Table E16 (cont'd).

Locus	23-4A	23-4B	23-4C	23-5A	23-5B	23-5C	L
Amel				<i>X</i>	<i>X</i>	<i>X,Y*</i>	<i>X</i>
D3					16		16
D1		16	15.3,17.3				16,17.3
D2S441							11
D10							13,15
D13							13
Penta E			21				7
D16		11		†,11		11	11
D18					15		15,16
D2S1338							17
CSF							12,13
Penta D							9,11
THO1	9.3		8	9.3		9.3	8,9.3
vWA							14,18
D21							27,30
D7							8,10
D5							11,12
TPOX						8	8
DYS391							N/A
D8				14,19	13,14		13,14
D12					†	18	18,20
D19	15		†,13		14.2	†	14,15
FGA	†			30	†	21	21,23
D22							15,16
Method	C	C	C	D	D	D	

Table E17. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer OO during Collection 2.

Locus	24-3B	24-6A	24-6B	24-6C	24-7A	24-7B	24-7C	OO
Amel			X	Y	X	X	X	X
D3			15					15,18
D1								14,18.3
D2S441								11,14
D10								14,15
D13								9,12
Penta E			12*					10,13
D16			11		8,12		12	12
D18			12*	11,14				11,14
D2S1338		20	20					17,25
CSF								10,11
Penta D			10*					9,12
THO1		6,9	6,7,9,9.3*	6,9.3*		9.3*		6,9
vWA			16				14,17	17,18
D21					29			28,31.2
D7		8	10		10			10
D5								11
TPOX			12*				8	8
DYS391			8					N/A
D8			13,16				16,17	12,17
D12			18*	19,20	19		18*	18.3,20
D19								14,16
FGA	28.2		22					18,24
D22	†							11,15
Method	E	A	A	A	B	B	B	

Table E18. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer T during Collection 2.

Locus	25-3A	25-3B	25-3C	25-4A	25-4B	25-4C	25-5C	25-6A	25-6B	25-6C	25-7C	T
Amel	X				X	X						X
D3	15*		16,18*	16				16				16,17
D1			11		15	17.3						16,17.3
D2S441			14									11,14
D10		14	15*					17				14,17
D13	9*				12*							11
Penta E												11,12
D16	12					11						11,12
D18		13	15	14*	17	15,16		13				13,17
D2S1338												20,24
CSF												10,11
Penta D			12*									8,10
THO1	6,7,9.3	6,8	9.3	6	9*	6						6,7
vWA			15,17*	17*	16,18*							19,20
D21		29		29,34.2								29
D7												8,10
D5			13									12
TPOX			12									8,11
DYS391												N/A
D8			10	14	13,14	11,13,14		13		13		13,14
D12		19,23	22									19,23
D19										11		13,16.2
FGA		24	24					†,†	24			24
D22	11							11				11,18
Method	A	A	A	B	B	B	C	D	D	D	E	

Table E19. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.

Locus	26-4A	26-4B	26-4C	26-5A	26-5B	26-5C	XX
Amel	X	X	X	X	X	X	X
D3	14,15	14,15	14,15	14,15,16	15	14	14,15
D1		14,17.3	14,17.3	17.3	14,17.3		14,17.3
D2S441	14	14	12			12	12,14
D10	14,16	13*,14	14				14,16
D13		11*,12			13	12	12,13
Penta E					12	12	12
D16	11,13	13	11,12*,13	11,13	12*,13	11,13	11,13
D18	17	17,17.2	15,17,18	17,18		17,18	17,18
D2S1338		17	17				17
CSF		10					10,12
Penta D							12
THO1	9,9.3	7,9,9.3	9.3	9,9.3	9,9.3	9	9,9.3
vWA	17,19	17,19	17,19	17,19	17,19	17	17,19
D21			32	29	29	29	29,32
D7			9		9		9,12
D5	10		10			10	10,13
TPOX	12						8,12
DYS391							N/A
D8	10,13	10,13	10,13,17	10,13	10,13	10,13	10,13
D12	18,22	19,22	18,22		22,23	22	18,22
D19	13	14	14	13		13	13,14
FGA	21,23	21.2,23	23	21,†			21,23
D22	6	16,18			16		16,17
Method	A	A	A	B	B	B	

Table E19 (cont'd).

Locus	26-6A	26-6B	26-6C	26-7A	26-7B	26-7C	XX
Amel							X
D3		15			15		14,15
D1		<i>17.3</i>		14			14,17.3
D2S441	11.3			<i>14</i>			12,14
D10							14,16
D13							12,13
Penta E							12
D16	6				13		11,13
D18							17,18
D2S1338				<i>17</i>	16		17
CSF							10,12
Penta D							12
THO1					9		9,9.3
vWA						17	17,19
D21							29,32
D7				<i>12</i>			9,12
D5							10,13
TPOX							8,12
DYS391							N/A
D8			†		<i>10</i>		10,13
D12	18.3				22		18,22
D19				<i>13,14</i>			13,14
FGA	22.2				21		21,23
D22					14		16,17
Method	C	C	C	D	D	D	

Table E20. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer N during Collection 2.

Locus	27-1B	27-1C	27-2B	27-5A	27-5B	27-5C	N
Amel	X			X	X,Y	X,Y	X
D3	16			16	17	14*,17,18	16,17
D1				15.3	14*	15.3,17.3	15.3,17.3
D2S441					11		11
D10						13	13
D13	14				14		12,14
Penta E							13,15
D16				11*,12	12	11*,12	12,13
D18				18*	13,18*	14,16	13,14
D2S1338						20	20,23
CSF							11,12
Penta D	12					12	10,12
TH01	9.3			6,9.3	6,9.3	9.3	6,9.3
vWA	18			16,17,18	17	18	17,18
D21						32.2	30,32.2
D7							11,12
D5						11,13*	12
TPOX							8
DYS391							N/A
D8	13			11,13	13,14	13,15	13
D12	19,21.3			25	17,19	20	19,20
D19	14	†				15.2	13,14
FGA	25.2,29.2	20.3,†	50.2	24	21,46.2		21,25
D22			†	17*		11	11,15
Method	D	D	E	A	A	A	

Table E20 (cont'd).

Locus	27-6A	27-6B	27-6C	27-7B	27-7C	N
Amel	<i>X</i>	<i>X,Y</i>	<i>X</i>	<i>X</i>		<i>X</i>
D3	17	15*,16,17	16			16,17
D1	15	12				15.3,17.3
D2S441		11	16			11
D10						13
D13						12,14
Penta E	12*	12*				13,15
D16	11*,12,13		11*			12,13
D18	12,13,17*	12	12,17*			13,14
D2S1338						20,23
CSF						11,12
Penta D			12			10,12
THO1	6,9*,9.3	6	9*,9.3			6,9.3
vWA	14,16	17,18	17,18			17,18
D21		28				30,32.2
D7		11				11,12
D5	12	12				12
TPOX			8			8
DYS391						N/A
D8	10*,13	13,15	13			13
D12	19		20			19,20
D19			13			13,14
FGA		22,23*				21,25
D22	16*					11,15
Method	B	B	B	C	C	

Table E21. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer B during Collection 2.

Locus	33-1A	33-1B	33-1C	33-2B	33-2C	33-3B	33-3C	B
Amel	X,Y	X,Y	X,Y				Y	X,Y
D3		15	18					16,18
D1	16.3							16.3,17.3
D2S441	10		14					14,15
D10								13,15
D13								10,12
Penta E								7,18
D16		9,13					9	9,13
D18	15	13,15	15					13,15
D2S1338								20,25
CSF	10	10						10,12
Penta D								12,13
THO1	8,9.3	9.3	6,9.3	3				8,9.3
vWA	17,18	17,18	15,17					17,18
D21					31			29,31
D7		9						9,12
D5								11,13
TPOX								8
DYS391								11
D8	8,13		8,11,13,14		8,13	13	8	8,13
D12			23					22,23
D19			15		13,†		14.2	13,15
FGA							22.1	21,23
D22	16	16			†,15			15,16
Method	B	B	B	C	C	D	D	

Table E21 (cont'd).

Locus	33-4B	33-7A	33-7B	33-7C	B
Amel		<i>X,Y</i>	<i>X,Y</i>	<i>X,Y</i>	<i>X,Y</i>
D3	18	16, 17* ,18	16	18	16,18
D1	<i>17.3</i>	<i>16.3,17.3</i>			<i>16.3,17.3</i>
D2S441		<i>14,15</i>		14	14,15
D10	<i>13</i>				13,15
D13		10		10	10,12
Penta E	17.4 ,18			7	7,18
D16		9,13	9, 12* ,13		9,13
D18		15	13,15	13	13,15
D2S1338		25			20,25
CSF		12	12		10,12
Penta D					12,13
THO1	†	8, 9 ,9.3	8,9.3	8	8,9.3
vWA		18	<i>17,18</i>	18	17,18
D21		29			29,31
D7		9		12	9,12
D5		<i>13</i>			11,13
TPOX					8
DYS391					11
D8		8,13	8,13	8,13	8,13
D12	13		22,23		22,23
D19		13, 14*			13,15
FGA	16.1,19.3 ,23,†,†	31 ,†			21,23
D22					15,16
Method	E	A	A	A	

Table E22. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer D during Collection 2.

Locus	36-1A	36-1B	36-1C	36-2A	36-2B	36-2C	36-4B	36-4C	D
Amel	X,Y	Y	Y	Y	X	Y		Y	X,Y
D3	17		15				18,18.3,19		17,18
D1				15			14		15
D2S441				11.3*				†,†	11,14
D10									13,14
D13	11								11
Penta E									7,13
D16			12*,13		13		13		13
D18	14				14		†,†		12,14
D2S1338			20	20					17,20
CSF						12		12	11,12
Penta D									9,11
THO1	8,9.3	9*,9.3	9.3	6,9.3		9.3			8,9.3
vWA	15			15				17	15,17
D21							28		28,30
D7									9,12
D5			9						11,12
TPOX									9,11
DYS391									10
D8	8,13	13	9*,13	8,9*,13	9*,12*				8,13
D12	19		18	19,20					15,19
D19	15								14,15
FGA	22.1,†		†				13		21,26
D22	†						18		12,17
Method	A	A	A	B	B	B	D	D	

Table E23. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.

Locus	38-2A	38-2B	38-2C	38-3A	38-3B	38-3C	WW
Amel	X	X,Y	X	X,Y	X	X	X
D3	16,18	16,17*	15,17*	16,17*		16	16,18
D1	11	15		11,15			11,12
D2S441		11,14		11		11	11,14
D10		13*,16	15				15,16
D13	8	11	9	13,14*			8,9
Penta E		7,8		11			11,12
D16	12	12	12	10,11,12	12	12	12
D18	12	12,16,17	14*,15	17	12,15	12,15	12,15
D2S1338		18	18	21		17	17,21
CSF				11			11,12
Penta D		9,12	12				10,12
THO1	9.3	9.3	8,9.3	7,9.3	9.3	8,9.3	9.3
vWA	15,17	15,17		17,18*	17	15,17	15,17
D21	28	28,32.2*		30			28,30
D7		10		10			10,11
D5	13	11					13
TPOX	8	11					8,12
DYS391		11					N/A
D8	10	10,12		10,12	10,12,13*	10,12	10,12
D12	18,19.3	19.3,25		19*	26	17.3,19.3	18,19.3
D19	14	14,15.2	13	13,14	13,14		13,14
FGA		23		20,21*,25*			20,24
D22							16
Method	A	A	A	B	B	B	

Table E23 (cont'd).

Locus	38-4A	38-4B	38-4C	38-5A	WW
Amel	<i>X</i>				X
D3			17*		16,18
D1					11,12
D2S441		11,14	11		11,14
D10		16			15,16
D13					8,9
Penta E					11,12
D16	12	11			12
D18		12			12,15
D2S1338		25		†	17,21
CSF					11,12
Penta D					10,12
THO1					9,3
vWA		15			15,17
D21					28,30
D7					10,11
D5					13
TPOX					8,12
DYS391					N/A
D8		10			10,12
D12		18,21.3			18,19.3
D19					13,14
FGA	†,†			49.2	20,24
D22					16
Method	C	C	C	D	

Table E24. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer SS during Collection 2.

Locus	40-3A	40-3B	40-3C	40-4A	40-4B	40-4C	SS
Amel		X,Y		X			X,Y
D3	14,17*	14,17*		15			14,18
D1		15*,18.3					14,17.3
D2S441	10	11		14*		11	11
D10	13*,14	13*,15					14
D13	11*						10,12
Penta E	7	14					7,19
D16	12	10,12		11	10	12	11,12
D18	10	16				10,12	10,12
D2S1338	22	19				18	17,20
CSF	11,12	12				12	11,12
Penta D	9	9					9,12
THO1	8	7,8		6,8	8		8
vWA	18	15*		15*		16,17	17
D21	29	29,31					29,32.2
D7							12
D5		11*					13
TPOX	8	8					9
DYS391	11						11
D8	†,10,11,13	11,13				12,13,14	12,13
D12	15,20	18	†				15,24
D19		13,14			15		14,15
FGA			23.1				22,24
D22		13					16
Method	A	A	A	B	B	B	

Table E25. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.

Locus	41-4A	41-4B	41-4C	41-5A	41-5B	41-5C	41-6A	41-6B	41-7C	Y
Amel	Y	X,Y	X,Y	X,Y	X,Y	X,Y				X,Y
D3		14	17	17,18	16	17		16		16,17
D1		16.3,17.3*			14	16.3				12,14
D2S441		11.3	14			14		14		14,15
D10	14	15,16			14					14,15
D13		12	9,13	13						13,14
Penta E		5,14								5,14
D16	11	11,12	13	11,12	11	11				11,12
D18	16*	16*,17	17	17	15*,17			17		17
D2S1338		20	17,24		24					17,24
CSF		10								12,14
Penta D		12	8		8					8,13
THO1	6	9,9.3	6,9.3	9.3	9.3	8*,9,9.3		9.3		9,9.3
vWA		16,18*	14	14	16		14			14,16
D21		28,32.2	30.2							29,30.2
D7		11,12	10,12							8,10
D5		12								12
TPOX		8	11							8
DYS391		11								11
D8	10,15	9,12	10,14	10,14	10,14	13*,14		14		10,14
D12		21,23	17,20*	20*,†	17	21		17,24.3		17,21
D19		12,14*	13,15.2	9,16.2				12,13		13,16.2
FGA		21.2,22	20,27						†, 22	22,27
D22		11,16								11,16
Method	A	A	A	B	B	B	C	C	D	

Table E26. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer II during Collection 2.

Locus	50-1C	50-5A	50-5B	50-5C	50-6A	50-6B	50-6C	50-7B	50-7C	II
Amel		X,Y	Y	X,Y	X,Y	X	Y			X,Y
D3		17	17	17	17	17				17
D1		12,15	15,18.3		15	15				15,18.3
D2S441		11,11.3	10,11	10,11			10,11			10,11
D10		15	13	15						13,15
D13		12	11,12	11		11				11,12
Penta E			13,14							13,14
D16		11,12	11,12	11,12	10,12	11,12				12
D18			16	16	17					16,17
D2S1338				19	21			10		19,21
CSF		10	12							12
Penta D						12				9,13
THO1		7,8,9.3	6,8,9,9.3	8	8,9.3	8,9.3	8,9.3			8,9.3
vWA		17,18	15,17	15,17	15,17					15,17
D21		29	31		31					29,31
D7			12							10,12
D5		11	12	12						11,12
TPOX		11	8				8			8
DYS391				11						11
D8		11,13,14,16	11,13	11,13,15,16	11,13	11,13	†,13			11,13
D12	†	18	18,20,†	18,19	18		22			18,20
D19		16.2	14	15.2	13.2,15.2		†,15.2			14,15.2
FGA	22.2	22,23	23,†	21,†	21,†		†			21,23
D22			15,16							15,16
Method	D	A	A	A	B	B	B	C	C	

Table E27. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.

Locus	1-1A	1-1B	1-1C	1-2A	1-2B	1-2C	1-3A	1-3B	1-3C	W
Amel	X	X,Y*	X		X	X,Y*				X
D3	14,15*,16		14,16			14				14,16
D1	14									14,15.3
D2S441		11.3	14		14					11.3,14
D10			15							13,15
D13		10,11*					14			10,12
27-7cPenta E			12							12
D16	9*,13	9*,13	11,13							11,13
D18										12
D2S1338		17*								18,22
CSF		10	12							10,12
Penta D										9,11
THO1		6,7,8*,9.3	6,9.3	9.3	6					6,9.3
vWA	15*,16*	16*	15*,17		17			16*		17
D21	30*,32,33.2		28,33.2							28,33.2
D7			9							9,10
D5		13	12,13							12,13
TPOX		12	12							8,12
DYS391										N/A
D8	15	8,10	10,15			15				10,15
D12	18,19*		18,22	17	21		†			18,21
D19	13*,14	13*	7,15							14,15
FGA	†	21	†,18*			22*	†			21,23
D22	10*						6		10*	16
Method	A	A	A	B	B	B	C	C	C	

Table E28. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.

Locus	1-1A	1-1B	1-1C	1-2A	1-2B	1-2C	1-3A	1-3B	1-3C	W
Amel	X	X,Y*	X		X	X,Y*				X
D3	14,15*,16		14,16			14				14,16
D1	14									14,15.3
D2S441		11.3	14		14					11.3,14
D10			15							13,15
D13		10,11					14*			10,12
Penta E			12							12
D16	9*,13	9*,13	11,13							11,13
D18										12
D2S1338		17								18,22
CSF		10	12							10,12
Penta D										9,11
THO1		6,7,8,9.3	6,9.3	9.3	6					6,9.3
vWA	15,16*	16*	15,17		17			16*		17
D21	30*,32,33.2		28,33.2							28,33.2
D7			9							9,10
D5		13	12,13							12,13
TPOX		12	12							8,12
DYS391										N/A
D8	15	8,10	10,15			15				10,15
D12	18,19*		18,22	17	21		†			18,21
D19	13*,14	13*	7,15							14,15
FGA	†	21	†,18			22*	†			21,23
D22	10*						6		10*	16
Method	A	A	A	B	B	B	C	C	C	

Table E29. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer QQ during Collection 3.

Locus	6-1A	6-2B	6-2A	6-2C	6-3A	6-3B	6-3C	6-4C	QQ
Amel		Y	X,Y				X		X,Y
D3			16		16	15*	16		16,18
D1		17.3		14	14				16.3,17.3
D2S441			10*				14		14,15
D10			12				14*		13,15
D13			14*						10,12
Penta E							7	7	7,18
D16		9,11	9,12*				11		9,13
D18		12*,15	12*				15		13,15
D2S1338			23*		17			25	20,25
CSF									10,12
Penta D			13						12,13
THO1		8	9,3	8,9,3			8,9,3		8,9,3
vWA	15	16*	15,16*,19*				16*,18	15	17,18
D21			26.2*	27					29,31
D7			8*						9,12
D5			12*				12*		11,13
TPOX						8			8
DYS391									11
D8		13,15	13				13,15	13	8,13
D12			21*				22	21*	22,23
D19		11.1	13				13		13,15
FGA		24	†				23		21,23
D22				16					15,16
Method	D	A	A	A	B	B	B	C	

Table E30. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.

Locus	7-3A	7-3B	7-3C	7-4A	7-4B	7-4C	P
Amel		X	X			X,Y	X,Y
D3				16*		14	15,17
D1	11		11	11			11,17.3
D2S441			11.3				11,12
D10		14	13				13,14
D13				12*			8,10
Penta E							11,21
D16			†,11	11			11
D18			16				14,18
D2S1338						19	25,26
CSF							10
Penta D							9,12
THO1		6*,7		9*		9.3	7,9.3
vWA		17			16		16,17
D21							29,32.2
D7							8,11
D5						12	12,13
TPOX							8,11
DYS391							10
D8	12		11,13			†	13,15
D12	17			15,19	20		17,21
D19							13,14
FGA				22*,25			19,21
D22				14			15,17
Method	A	A	A	B	B	B	

Table E31. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.

Locus	7/18-1A.2	7/18-2B.1	7/18-2C.1	P (coincides w/7)	Z (coincides w/ 18)
Amel	X			X,Y	X
D3		17	15	15,17	15,16
D1				11,17.3	15,18.3
D2S441				11,12	12,14
D10			12,13	13,14	13
D13				8,10	9,11
Penta E				11,21	7,14
D16				11	9,12
D18				14,18	12,15
D2S1338			17*	25,26	19,25
CSF				10	11,13
Penta D			12	9,12	10,14
THO1		7	3	7,9.3	6,9.3
vWA				16,17	18,19
D21		29		29,32.2	30
D7				8,11	10,11
D5				12,13	12,13
TPOX				8,11	8,11
DYS391				10	N/A
D8			15	13,15	11,13
D12				17,21	20
D19				13,14	14
FGA				19,21	21,24
D22				15,17	15
Method	C	D	D		

Table E32. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.

Locus	7-3A	7-3B	7-3C	7-4A	7-4B	7-4C	P
Amel		X	X			X,Y	X,Y
D3				16*		14	15,17
D1	11		11	11			11,17.3
D2S441			11.3				11,12
D10		14	13				13,14
D13				12*			8,10
Penta E							11,21
D16			†,11	11			11
D18			16*				14,18
D2S1338						19	25,26
CSF							10
Penta D							9,12
THO1		6*,7		9		9.3	7,9.3
vWA		17			16		16,17
D21							29,32.2
D7							8,11
D5						12	12,13
TPOX							8,11
DYS391							10
D8	12		11,13			†	13,15
D12	17			15,19	20		17,21
D19							13,14
FGA				22,25*			19,21
D22				14			15,17
Method	A	A	A	B	B	B	

Table E33. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.

Locus	7/18-1A.2	7/18-2B.1	7/18-2C.1	P (coincides w/7)	Z (coincides w/ 18)
Amel	X			X,Y	X
D3		17	15	15,17	15,16
D1				11,17.3	15,18.3
D2S441				11,12	12,14
D10			12,13	13,14	13
D13				8,10	9,11
Penta E				11,21	7,14
D16				11	9,12
D18				14,18	12,15
D2S1338			17	25,26	19,25
CSF				10	11,13
Penta D			12	9,12	10,14
TH01		7	3	7,9.3	6,9.3
vWA				16,17	18,19
D21		29		29,32.2	30
D7				8,11	10,11
D5				12,13	12,13
TPOX				8,11	8,11
DYS391				10	N/A
D8			15	13,15	11,13
D12				17,21	20
D19				13,14	14
FGA				19,21	21,24
D22				15,17	15
Method	C	D	D		

Table E34. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.

Locus	11-1A	11-1B	11-1C	11-2A	11-2B	11-2C	11-3A	11-3C	11-4A	11-4B	11-4C	DD
Amel	Y							X			Y	X,Y
D3			15	16								15,16
D1			11,15*,16							15*		16
D2S441			10,14									10,14
D10			14									13,14
D13			12									12,14
Penta E			14*									7,12
D16			9,12				9			12	9	9,12
D18	18										17	12,17
D2S1338			18									19,23
CSF												12
Penta D												9
THO1			6*,9.3							9	9.3	9.3
vWA			19					15		16		16,19
D21			30					30				26.2,30
D7						9					8	8,9
D5												12
TPOX												8,11
DYS391												11
D8	12,13		11*,14					15				13,14
D12	†			19			17				21	19,21
D19			13							14		13,14
FGA	18						31.2	17				21,22
D22												15
Method	B	B	B	C	C	C	D	D	A	A	A	

Table E35. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.

Locus	11-1A	11-1B	11-1C	11-2A	11-2B	11-2C	11-3A	11-3C	11-4A	11-4B	11-4C	DD
Amel	Y							X			Y	X,Y
D3			15	16								15,16
D1			11,15,16							15		16
D2S441			10,14									10,14
D10			14									13,14
D13			12									12,14
Penta E			14									7,12
D16			9,12				9			12	9	9,12
D18	18										17	12,17
D2S1338			18*									19,23
CSF												12
Penta D												9
THO1			6*,9.3							9	9.3	9.3
vWA			19					15		16		16,19
D21			30					30				26.2,30
D7						9					8	8,9
D5												12
TPOX												8,11
DYS391												11
D8	12,13		11,14					15*				13,14
D12	†			19			17				21	19,21
D19			13							14		13,14
FGA	18						31.2	17				21,22
D22												15
Method	B	B	B	C	C	C	D	D	A	A	A	

Table E36. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.

Locus	12-1A	12-1B	12-1C	12-2A	12-2B	12-2C	12-3A	12-3B	12-3C	12-4B	FF
Amel			X,Y	X	X,Y		X			X	X
D3	18					15	15				15,18
D1	16.3,17.3	17.3									14,17.3
D2S441											10,11
D10											14,17
D13				9							9,11
Penta E											12
D16	10,12		†,11*								10,12
D18			18*				17			17	12,17
D2S1338											17,18
CSF					9						9,10
Penta D											8,16
THO1	6		6,9.3	6			9.3				6,9.3
vWA				16	16					16	16
D21					30					30	30,33.2
D7											10,12
D5			11,12	10							10,12
TPOX											8,11
DYS391											N/A
D8	14	11*	14	15		13*,14,15		6		12,15	14,15
D12		20				23	20		20	18*	20
D19		12	13,13.1			7				14*,15	13,15
FGA			23	20		28.3,†		†			20
D22		12							†		11,16
Method	A	A	A	B	B	B	C	C	C	D	

Table E37. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.

Locus	12-1A	12-1B	12-1C	12-2A	12-2B	12-2C	12-3A	12-3B	12-3C	12-4B	FF
Amel			X,Y*	X	X,Y*		X			X	X
D3	18					15	15				15,18
D1	16.3,17.3	17.3									14,17.3
D2S441											10,11
D10											14,17
D13				9							9,11
Penta E											12
D16	10,12		†,11*								10,12
D18			18*				17			17	12,17
D2S1338											17,18
CSF					9						9,10
Penta D											8,16
THO1	6		6,9.3	6			9.3				6,9.3
vWA				16	16					16	16
D21					30					30	30,33.2
D7											10,12
D5			11,12	10							10,12
TPOX											8,11
DYS391											N/A
D8	14	11	14	15		13*,14,15		6		12,15	14,15
D12		20				23	20		20	18	20
D19		12	13,13.1			7				14*,15	13,15
FGA			23	20		28.3,†		†			20
D22		12							†		11,16
Method	A	A	A	B	B	B	C	C	C	D	

Table E38. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer KK during Collection 3.

Locus	17-1A	17-1C	17-2A	17-2B	17-2C	KK
Amel		<i>Y</i>	<i>X,Y</i>		<i>X</i>	<i>X,Y</i>
D3			15			15,16
D1						17,17.3
D2S441			<i>14</i>	10		10,14
D10			16			14,16
D13						12
Penta E						7,18
D16			11,13*	9	9,11	9,11
D18				12	16	16,18
D2S1338					23	23,25
CSF						11,12
Penta D			<i>13</i>			12,13
THO1	6		6,7	6,7	6,8*,9.3*	6,7
vWA			15	15	15,17*,18*	15,16
D21						31,32.2
D7			10			10,11
D5						11,12
TPOX						8,10
DYS391						10
D8			<i>13</i>	<i>13,14</i>	14,15	13,14
D12			17	17,19	†, 17	17,23
D19		†			13*	14,15
FGA				25		23,25
D22						11,16
Method	D	D	A	A	A	

Table E38 (cont'd).

Locus	17-3A	17-3B	17-3C	17-4A	17-4B	17-4C	KK
Amel	X,Y	X,Y	X,Y				X,Y
D3	15,16	16	15				15,16
D1	17,17.3						17,17.3
D2S441		14					10,14
D10							14,16
D13	12						12
Penta E			7				7,18
D16	9	9	11				9,11
D18	13*	16,18				15*	16,18
D2S1338				23			23,25
CSF							11,12
Penta D							12,13
THO1		6,7	7				6,7
vWA		15					15,16
D21				32.2			31,32.2
D7			10				10,11
D5							11,12
TPOX							8,10
DYS391							10
D8	14	14	14	†			13,14
D12	17		17,23	†			17,23
D19						12.2	14,15
FGA		25	†	†			23,25
D22						20	11,16
Method	B	B	B	C	C	C	

Table E39. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.

Locus	18-3A	18-3B	18-3C	18-4A	18-4B	18-4C	Z
Amel	X	X,Y	X	X	X		X
D3	15,16	15	15			16	15,16
D1							15,18.3
D2S441	12,14						12,14
D10			13				13
D13							9,11
Penta E		7					7,14
D16	9,11,14	12	10*		†		9,12
D18	18	12,15	12	15			12,15
D2S1338	19	25					19,25
CSF		11					11,13
Penta D		10					10,14
THO1	6,7,9.3	6,9.3	6,9.3	6	9.3	6	6,9.3
vWA	18,19	15				18	18,19
D21	30	29	30	29		30	30
D7	10	10					10,11
D5	10*,12			13			12,13
TPOX	8						8,11
DYS391							N/A
D8	†,11,13	13,15*	13	†	11,13	11	11,13
D12	19.1,20		20		20,25		20
D19	14	14					14
FGA	19.3	21,24			†		21,24
D22							15
Method	A	A	A	B	B	B	

Table E40. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.

Locus	7/18-1A.1	7/18-1B.1	7/18-1C.1	P (coincides w/7)	Z (coincides w/ 18)
Amel				X,Y	X
D3				15,17	15,16
D1		15		11,17.3	15,18.3
D2S441		11*		11,12	12,14
D10				13,14	13
D13				8,10	9,11
Penta E				11,21	7,14
D16				11	9,12
D18				14,18	12,15
D2S1338		24		25,26	19,25
CSF				10	11,13
Penta D				9,12	10,14
THO1	6			7,9.3	6,9.3
vWA		18		16,17	18,19
D21				29,32.2	30
D7		10		8,11	10,11
D5		12		12,13	12,13
TPOX				8,11	8,11
DYS391				10	N/A
D8	13			13,15	11,13
D12		23		17,21	20
D19				13,14	14
FGA				19,21	21,24
D22				15,17	15
Method	C	C	C		

Table E41. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.

Locus	18-3A	18-3B	18-3C	18-4A	18-4B	18-4C	Z
Amel	X	X,Y	X	X	X		X
D3	15,16	15	15			16	15,16
D1							15,18.3
D2S441	12,14						12,14
D10			13				13
D13							9,11
Penta E		7					7,14
D16	9,11*,14	12	10		†		9,12
D18	18*	12,15	12	15			12,15
D2S1338	19	25					19,25
CSF		11					11,13
Penta D		10					10,14
THO1	6,7,9.3	6,9.3	6,9.3	6	9.3	6	6,9.3
vWA	18,19	15				18	18,19
D21	30	29*	30	29*		30	30
D7	10	10					10,11
D5	10*,12			13			12,13
TPOX	8						8,11
DYS391							N/A
D8	†,11,13	13,15*	13	†	11,13	11	11,13
D12	19.1,20		20		20,25		20
D19	14	14					14
FGA	19.3	21,24*			†		21,24
D22							15
Method	A	A	A	B	B	B	

Table E42. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by either volunteer P or volunteer Z during Collection 3. Due to miss labeling of bags and minimal STR data, these results could not confidently be associated with a particular volunteer.

Locus	7/18-1A.1	7/18-1B.1	7/18-1C.1	P (coincides w/7)	Z (coincides w/ 18)
Amel				X,Y	X
D3				15,17	15,16
D1		15		11,17.3	15,18.3
D2S441		11		11,12	12,14
D10				13,14	13
D13				8,10	9,11
Penta E				11,21	7,14
D16				11	9,12
D18				14,18	12,15
D2S1338		24		25,26	19,25
CSF				10	11,13
Penta D				9,12	10,14
THO1	6			7,9.3	6,9.3
vWA		18		16,17	18,19
D21				29,32.2	30
D7		10		8,11	10,11
D5		12		12,13	12,13
TPOX				8,11	8,11
DYS391				10	N/A
D8	13			13,15	11,13
D12		23		17,21	20
D19				13,14	14
FGA				19,21	21,24
D22				15,17	15
Method	C	C	C		

Table E43. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.

Locus	20-1A	20-1B	20-1C	20-2A	20-2B	20-2C	20-4A	20-4B	20-4C	PP
Amel	X		X	X	X		X	Y*	X,Y*	X
D3	15	14,15	15	15			15			15
D1	12		15.3				12,18.3	17	17.3*	12,15.3
D2S441							13	14	10,14	14
D10										13
D13									12	11
Penta E								7		11,12
D16	11		13		11	11	†,11,13	9,13	9,12	11,13
D18	18		18				18		18,19	16,18
D2S1338	19	19							19,23	17,19
CSF										11,12
Penta D					12				12	10,12
THO1	9,9.3		9.3				9.3	9.3	6,7*,8,9.3	9.3
vWA						16	16	17	16	16,17
D21		28	29					31,32.2	33.2	29,32.2
D7					8			10	10,11*	8,12
D5		11							12	10,12
TPOX									10	8
DYS391										N/A
D8	13	11,13	11,†,14		11,15*	13	11,13	11,13,14,17	13,14	11,13
D12	18	21*					22	17*,†	27	18,22
D19						13.1		14,15		14,15
FGA			24				15	25	22.2,25	22.2,24
D22										16,17
Method	B	B	B	C	C	C	A	A	A	

Table E44. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.

Locus	20-1A	20-1B	20-1C	20-2A	20-2B	20-2C	20-4A	20-4B	20-4C	PP
Amel	X		X	X	X		X	Y*	X,Y*	X
D3	15	14,15	15	15			15			15
D1	12		15.3				12,18.3	17	17.3	12,15.3
D2S441							13	14	10*,14	14
D10										13
D13									12*	11
Penta E								7		11,12
D16	11		13		11	11	†,11,13	9,13	9,12	11,13
D18	18		18				18		18,19	16,18
D2S1338	19	19							19,23	17,19
CSF										11,12
Penta D					12				12	10,12
THO1	9*,9.3		9.3				9.3	9.3	6*,7,8,9.3	9.3
vWA						16	16	17	16	16,17
D21		28	29					31,32.2	33.2	29,32.2
D7					8			10*	10*,11	8,12
D5		11							12	10,12
TPOX									10	8
DYS391										N/A
D8	13	11,13	11,†,14		11,15	13	11,13	11,13,14,17	13,14	11,13
D12	18	21*					22	17,†	27	18,22
D19						13.1		14,15		14,15
FGA			24				15	25	22.2,25	22.2,24
D22										16,17
Method	B	B	B	C	C	C	A	A	A	

Table E45. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.

Locus	21-1A	21-1B	21-1C	21-2A	21-2B	21-2C	X
Amel		X,Y	Y	X,Y	X		X,Y
D3	16	15,16	15	15,16	15	15	15,16
D1	11,14	11,14		11	14		11,14
D2S441		14		14	14		14
D10		12,14	14				12,14
D13						11	11,12
Penta E					12,14		12,14
D16	9,11,13	9,13	9	9	9,13	9	9,13
D18	15			15	15		15
D2S1338					17,21		17,21
CSF					11		11
Penta D							12,14
THO1	8,9.3*	8	8	8	8	8	8
vWA	15,16	15,16		15			15,16
D21		27,30		27		26.2*	27,30
D7					9*		10,11
D5					11		11,12
TPOX		8					8,9
DYS391							10
D8	15	13*,15	15	15	15	11,15	15
D12	18	18,19,20.3,23,†	18,21*	20,21*	18,19,23		18,19
D19		13.2	18		12,13		12,13
FGA	18				14,22,30.2		18,22
D22		14			15*		10,14
Method	A	A	A	B	B	B	

Table E45 (cont'd).

Locus	21-3A	21-3B	21-3C	21-4A	X
Amel		<i>Y</i>			X,Y
D3		<i>15,16</i>		<i>15</i>	15,16
D1		11	11,14	17.3	11,14
D2S441			<i>14</i>	11	14
D10		12			12,14
D13					11,12
Penta E					12,14
D16	9,13	13	9,13	11	9,13
D18		15	15		15
D2S1338			17		17,21
CSF					11
Penta D					12,14
THO1	8	8		9	8
vWA		<i>16</i>			15,16
D21			27	30	27,30
D7	11				10,11
D5					11,12
TPOX					8,9
DYS391					10
D8	10,13.2,15	9,15	15		15
D12	†,19	19		18,18.3,†	18,19
D19	16.2				12,13
FGA		†		19	18,22
D22			12	†	10,14
Method	C	C	C	D	

Table E46. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.

Locus	21-1A	21-1B	21-1C	21-2A	21-2B	21-2C	X
Amel		X,Y	Y	X,Y	X		X,Y
D3	16	15,16	15	15,16	15	15	15,16
D1	11,14	11,14		11	14		11,14
D2S441		14		14	14		14
D10		12,14	14				12,14
D13						11	11,12
Penta E					12,14		12,14
D16	9,11,13	9,13	9	9	9,13	9	9,13
D18	15			15	15		15
D2S1338					17,21		17,21
CSF					11		11
Penta D							12,14
THO1	8,9.3*	8	8	8	8	8	8
vWA	15,16	15,16		15			15,16
D21		27,30		27		26.2	27,30
D7					9		10,11
D5					11		11,12
TPOX		8					8,9
DYS391							10
D8	15	13*,15	15	15	15	11*,15	15
D12	18	18,19,20.3,23,†	18,21	20*,21	18,19,23		18,19
D19		13.2	18		12,13		12,13
FGA	18				14,22,30.2		18,22
D22		14			15*		10,14
Method	A	A	A	B	B	B	

Table E46 (cont'd).

Locus	21-3A	21-3B	21-3C	21-4A	X
Amel		Y			X,Y
D3		15,16		15	15,16
D1		11	11,14	17.3	11,14
D2S441			14	11	14
D10		12			12,14
D13					11,12
Penta E					12,14
D16	9,13	13	9,13	11	9,13
D18		15	15		15
D2S1338			17		17,21
CSF					11
Penta D					12,14
THO1	8	8		9	8
vWA		16			15,16
D21			27	30	27,30
D7	11				10,11
D5					11,12
TPOX					8,9
DYS391					10
D8	10,13.2,15	9,15	15		15
D12	†,19	19		18,18.3,†	18,19
D19	16.2				12,13
FGA		†		19	18,22
D22			12	†	10,14
Method	C	C	C	D	

Table E47. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.

Locus	35-2A	35-2B	35-2C	35-3A	35-3B	35-3C	UU
Amel	X	X,Y			X		X,Y
D3	14	15*,16,17					16,17
D1		11,12,14,17.3*					12,16.3
D2S441		14*					8,10
D10							13,15
D13		13					11,12
Penta E		13					12
D16	9*,12	11,12	11			11	11
D18		16*,17					13,15
D2S1338	17	17,21					17
CSF							10,12
Penta D							12,13
THO1	9.3	7*,9,9.3			6,9		6,9
vWA	15*,17	14,16*,17,18					14,20
D21		29,30.2					29,30
D7		8,10					10,13
D5						10	10,12
TPOX	11	8					8,11
DYS391							11
D8		10,12,13,14*					13
D12		17*,18			†		18,21
D19		13,14,15*					13.2,14
FGA		14,22	18,30				22
D22		15,16					15,16
Method	A	A	A	B	B	B	

Table E48. Alleles obtained with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.

Locus	35-2A	35-2B	35-2C	35-3A	35-3B	35-3C	UU
Amel	X	X,Y			X		X,Y
D3	14	15,16,17					16,17
D1		11,12,14,17.3*					12,16.3
D2S441		14*					8,10
D10							13,15
D13		13					11,12
Penta E		13					12
D16	9*,12	11,12	11			11	11
D18		16,17					13,15
D2S1338	17	17,21					17
CSF							10,12
Penta D							12,13
THO1	9.3*	7,9,9.3*			6,9		6,9
vWA	15,17*	14,16,17*,18*					14,20
D21		29,30.2					29,30
D7		8,10					10,13
D5						10	10,12
TPOX	11	8					8,11
DYS391							11
D8		10,12,13,14					13
D12		17,18			†		18,21
D19		13*,14,15*					13.2,14
FGA		14,22	18,30				22
D22		15,16					15,16
Method	A	A	A	B	B	B	

APPENDIX F. CONSENSUS POWERPLEX® FUSION STR PROFILES

Red = non-loader allele

Italicized = allele is consistent with the loader but could have originated from the previous loader

* = non-loader allele could have originated from the previous loader

† = off-ladder allele (each † symbol represents a different off-ladder allele)

N/A = not applicable

Blank = no alleles recovered at that locus

Con. = consensus profile

The cell recovery and DNA extraction method utilized to recover and extract DNAs from spent cartridge casings is denoted with one of the following letters:

A = double swab + organic extraction

B = soak + organic extraction

Table F1. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer U during Collection 2.

Locus	2-3A	2-3B	2-3C	Con.	2-4A	2-4B	2-4C	Con.	U
Amel	X	X,Y*	X	X	X	X	X	X	X
D3	15	15	15	15	17*		15,18		15
D1	11,17.3	11	17.3	11,17.3			11		11,17.3
D2S441	10,15	15		15			10,15		10,15
D10	12	12	14	12			12,14		12,14
D13									9,13
Penta E		15			12		13,15		12,15
D16	11,13		11,13	11,13	11,13	11,13	11,13	11,13	11,13
D18	13*,14,15		14,15	14,15		16	14		14,15
D2S1338	25	17,25		25		25			17,25
CSF	12	10					10		10,12
Penta D	10								10,11
THO1	6,7	6,7	7	6,7	9.3	7	†,6,9.3		6,7
vWA	14	14	15	14			18		14,20
D21	28,30,31				28	28	32.2	28	28,30
D7							9		11
D5	11	11		11	11		11	11	11
TPOX	8,11	11		11	11	11	8,11	11	8,11
DYS391									N/A
D8	12	12	12,13*,15	12		13*,13.2,16	13*		12
D12	23		17		17	†	17	17	17,23
D19	13	13		13	14				13
FGA	17.2,24,25						19.2		24,25
D22	16						16		16
Method	A	A	A	A	B	B	B	B	

Table F2. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer MM during Collection 2. Casings 3-5A was excluded from analysis due to contamination, consequently it was not used in construction of the consensus STR profile.

Locus	3-4A	3-4B	3-4C	Con.	3-5B	3-5C	Con.	MM
Amel	Y*		X					X
D3	18*				15			14,16
D1					18.3			12,16
D2S441			11					10,11
D10								14,15
D13			8					8,12
Penta E								7,21
D16	12	11			†,12,13*	11		12
D18			15*,17		13*	14		14,14.2
D2S1338					25*			17,23
CSF					11			12,13
Penta D			13					13
TH01		9.3	6,9,9.3	9.3	7			9,9.3
vWA								17
D21					32.2			29,31.2
D7		8						9,11
D5			11*					9,10
TPOX								8
DYS391								N/A
D8		12	†,13,15		13,15	15,†	15	13,15
D12	22		18		18,23*	18,22	18	18,22
D19								14,15.2
FGA	17.2				24			22,26
D22								11,12
Method	A	A	A	A	B	B	B	

Table F3. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer S during Collection 2.

Locus	8-5A	8-5B	8-5C	Con.	8-6A	8-6B	8-6C	Con.	S
Amel	X	X	X	X	X	X	X	X	X
D3	18	18	18	18	18		18	18	18
D1	11,15	12,15		15	12,15	12,15	12,15	12,15	12,15
D2S441			11		11,11.3	11.3	11.3,14	11.3	11,11.3
D10	15		15	15	15	15	15	15	13,15
D13	13	13		13		12	13		12,13
Penta E		13			13	12	12,13	12,13	12,13
D16	11	11	11	11	11	11	11	11	11
D18	12	12	12	12	12,16	12	12,16	12,16	12,16
D2S1338			17		17	17,25		17	17,25
CSF	11	13*			10	10,11		10	10,11
Penta D					10,13		10	10	10,13
THO1	7,9	6,9		9	6,9	6,9	6	6,9	6,9
vWA		18			17,18	16,17,18	17,18	17,18	17,18
D21	29*,34				28	28,31	28,29*	28	28
D7	12	10	10	10	10	10		10	10
D5		12	10		10,12	12		12	10,12
TPOX	8	11					8		8,11
DYS391									N/A
D8	10,13,16	13,16	6,13,14,16	13,16	13,16	13,16	†,13,16,19	13,16	13,16
D12	18	18,18.3	18.3	18,18.3	18,18.3	18,18.3	18.3	18,18.3	18,18.3
D19		13.2,15	7,15,16,19.2	15	15	15	13.2,14	15	13.2,15
FGA	21		23,†		†	22,23	23,†	23	22,23
D22					15	15	16	15	15
Method	A	A	A	A	B	B	B	B	

Table F4. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer VV during Collection 2.

Locus	10-6A	10-6B	10-6C	Con.	10-7A	10-7B	10-7C	Con.	VV
Amel		X			X	X	Y	X	X,Y
D3		16				17			14,17
D1					14		14	14	15,17.3
D2S441		14							11,14
D10					13				12,13
D13									11
Penta E									7,8
D16									12
D18							12		12,16
D2S1338									17,18
CSF						12*			11
Penta D									9,12
THO1					7				9.3
vWA					17	15*			17
D21					32.2				28,32.2
D7						10			10,11
D5		13							11,13
TPOX									11
DYS391									11
D8		8,13*			13*,14				8,12
D12					15				15,25
D19									14,15.2
FGA	32.2,†						27.3		22,23
D22					16*				11,15
Method	A	A	A	A	B	B	B	B	

Table F5. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer V during Collection 2.

Locus	13-7A	13-7B	13-7C	Con.	13-1A	13-1B	13-1C	Con.	V
Amel	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D3	14,18*	14	14	14	14	14	14	14	14
D1	16.3,17.3	16.3,17.3		16.3,17.3	17.3	16.3,17.3	16.3	16.3,17.3	16.3,17.3
D2S441	11,11.3	11,11.3	11,11.3	11,11.3	11,11.3		11	11	11,11.3
D10		15,16	15,16	15,16	15,16	15	15,16	15,16	15,16
D13	10	10,12		10	10,12		10	10	10,12
Penta E	5,14	5,14	5,14	5,14	5,14		5,14	5,14	5,14
D16	11,12	11,12	12	11,12	11,12	11,12	12	11,12	11,12
D18	17	16,17	16,17	16,17	16,17	17	13,16	16,17	16,17
D2S1338	20,22	20,22	20	20,22	20,22		20,22	20,22	20,22
CSF	11	10,11	10	10,11	11		10		10,11
Penta D	12	11,12	12	11,12	11,12		11	11	11,12
THO1	9,9.3	9,9.3	9,9.3	9,9.3	9,9.3	9,9.3	6*,9,9.3	9,9.3	9,9.3
vWA	16,18	16,18	14,17*,18	16,18	16,18	16	16,18	16,18	16,18
D21	28,32.2	28,32.2	28	28,32.2	28,32.2		28,29	28	28,32.2
D7	12	11,12	11,12	11,12	12		11		11,12
D5	12	12		12	12	12	12	12	12
TPOX		8			8	8	8	8	8
DYS391	11	11	11	11	11				11
D8	9,12	9,12	9,12	9,12	9,12	9	9,12,13*,†	9,12	9,12
D12	20,21,23	21,23	23	21,23	21,23	21,23	21,23	21,23	21,23
D19		12	12	12	12,14	11,12,14	14	12,14	12,14
FGA	22	21.2,22	22,23*,32.2	22	21.2,22	†	22	22	21.2,22
D22		11,16	11,16	11,16	11,16				11,16
Method	A	A	A	A	B	B	B	B	

Table F6. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer HH during Collection 2.

Locus	15-1A	15-1B	15-1C	Con.	15-2A	15-2B	15-2C	Con.	HH
Amel	X	X		X		X			X
D3		15							14,18
D1									16,17.3
D2S441									11,14
D10	13								13,15
D13							8		10,11
Penta E	12								10,14
D16	†					11*	11*	11*	9,12
D18	12						15		16
D2S1338							16		17,19
CSF									11,13
Penta D									10
THO1	9.3*		9			9.3*	6		9
vWA									14,16
D21									30,31
D7									11,12
D5	12								9,12
TPOX							11		9,11
DYS391									N/A
D8		10,11	14.1				16		10,13
D12	20						20		20,21
D19							14.2		13,14
FGA						32.2	22		22,25
D22									16
Method	A	A	A	A	B	B	B	B	

Table F7. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer L during Collection 2.

Locus	23-2A	23-2B	23-2C	Con.	23-3A	23-3B	23-3C	Con.	L
Amel	X	X	X	X	X	X,Y*	X	X	X
D3	16	16,17		16	15,16	16	16	16	16
D1	16,17.3	16,17.3		16,17.3	16,17.3	16	17.3	16,17.3	16,17.3
D2S441	11	11		11	11	11	11	11	11
D10		15	14*						13,15
D13	13						12*		13
Penta E	7	7		7	7				7
D16	11	11	11,12*	11	11	11	11	11	11
D18	15,16	15	15	15		16	16	16	15,16
D2S1338	17	17		17		17	17	17	17
CSF	13		12				12		12,13
Penta D		8.2	9				6		9,11
THO1	8,9.3	8,9.3	3,8,9.3	8,9.3	7,8,9.3	8,9.3	8,9.3	8,9.3	8,9.3
vWA	14	14,18	14	14	16,18	14,16	14,17*,18	14,16,18	14,18
D21	30	30		30	30		27,30	30	27,30
D7	8					10	9,10	10	8,10
D5						11	12		11,12
TPOX							8		8
DYS391									N/A
D8	13,14	13,14	11,13,14	13,14	13		13,14,15,15.1	13	13,14
D12	20	18,20	18	18,20	18	18	18,20,†	18	18,20
D19		14,15	15	15	14,15	15	14,15	14,15	14,15
FGA	21,23	21,†,†		21	†	21	21,†	21	21,23
D22		†,16							15,16
Method	A	A	A	A	B	B	B	B	

Table F8. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer OO during Collection 2.

Locus	24-6A	24-6B	24-6C	Con.	24-7A	24-7B	24-7C	Con.	OO
Amel		X	Y		X	X	X	X	X
D3		15							15,18
D1									14,18.3
D2S441									11,14
D10									14,15
D13									9,12
Penta E		12*							10,13
D16		11			8,12		12	12	12
D18		12*	11,14						11,14
D2S1338	20	20		20					17,25
CSF									10,11
Penta D		10*							9,12
THO1	6,9	6,7,9,9.3*	6,9.3*	6,9,9.3*		9.3*			6,9
vWA		16					14,17		17,18
D21					29				28,31.2
D7	8	10			10				10
D5									11
TPOX		12*					8		8
DYS391		8							N/A
D8		13,16					16,17		12,17
D12		18*	19,20		19		18*		18.3,20
D19									14,16
FGA		22							18,24
D22									11,15
Method	A	A	A	A	B	B	B	B	

Table F9. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer T during Collection 2.

Locus	25-3A	25-3B	25-3C	Con.	25-4A	25-4B	25-4C	Con.	T
Amel	X					X	X	X	X
D3	15*		16,18*		16				16,17
D1			11			15	17.3		16,17.3
D2S441			14						11,14
D10		14	15*						14,17
D13	9*					12*			11
Penta E									11,12
D16	12						11		11,12
D18		13	15		14*	17	15,16		13,17
D2S1338									20,24
CSF									10,11
Penta D			12*						8,10
THO1	6,7,9.3	6,8	9.3	6,9.3	6	9*	6	6	6,7
vWA			15,17*		17*	16,18*			19,20
D21		29			29,34.2				29
D7									8,10
D5			13						12
TPOX			12						8,11
DYS391									N/A
D8			10		14	13,14	11,13,14	13,14	13,14
D12		19,23	22						19,23
D19									13,16.2
FGA		24	24	24					24
D22	11								11,18
Method	A	A	A	A	B	B	B	B	

Table F10. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer XX during Collection 2.

Locus	26-4A	26-4B	26-4C	Con.	26-5A	26-5B	26-5C	Con.	XX
Amel	X	X	X	X	X	X	X	X	X
D3	14,15	14,15	14,15	14,15	14,15,16	15	14	14,15	14,15
D1		14,17.3	14,17.3	14,17.3	17.3	14,17.3		17.3	14,17.3
D2S441	14	14	12	14			12		12,14
D10	14,16	13*,14	14	14					14,16
D13		11*,12				13	12		12,13
Penta E						12	12	12	12
D16	11,13	13	11,12*,13	11,13	11,13	12*,13	11,13	11,13	11,13
D18	17	17,17.2	15,17,18	17	17,18		17,18	17,18	17,18
D2S1338		17	17	17					17
CSF		10							10,12
Penta D									12
THO1	9,9.3	7,9,9.3	9.3	9,9.3	9,9.3	9,9.3	9	9,9.3	9,9.3
vWA	17,19	17,19	17,19	17,19	17,19	17,19	17	17,19	17,19
D21			32		29	29	29	29	29,32
D7			9			9			9,12
D5	10		10	10			10		10,13
TPOX	12								8,12
DYS391									N/A
D8	10,13	10,13	10,13,17	10,13	10,13	10,13	10,13	10,13	10,13
D12	18,22	19,22	18,22	18,22		22,23	22	22	18,22
D19	13	14	14	14	13		13	13	13,14
FGA	21,23	21.2,23	23	23	21,†				21,23
D22	6	16,18				16			16,17
Method	A	A	A	A	B	B	B	B	

Table F11. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer N during Collection 2.

Locus	27-5A	27-5B	27-5C	Con.	27-6A	27-6B	27-6C	Con.	N
Amel	X	X,Y	X,Y	X,Y	X	X,Y	X	X	X
D3	16	17	14*,17,18	17	17	15*,16,17	16	16,17	16,17
D1	15.3	14*	15.3,17.3	15.3	15	12			15.3,17.3
D2S441		11				11	16		11
D10			13						13
D13		14							12,14
Penta E					12*	12*		12*	13,15
D16	11*,12	12	11*,12	11*,12	11*,12,13		11*	11*	12,13
D18	18*	13,18*	14,16	18*	12,13,17*	12	12,17*	12,17*	13,14
D2S1338			20						20,23
CSF									11,12
Penta D			12				12		10,12
THO1	6,9.3	6,9.3	9.3	6,9.3	6,9*,9.3	6	9*,9.3	6,9*,9.3	6,9.3
vWA	16,17,18	17	18	17,18	14,16	17,18	17,18	17,18	17,18
D21			32.2			28			30,32.2
D7						11			11,12
D5			11,13*		12	12		12	12
TPOX							8		8
DYS391									N/A
D8	11,13	13,14	13,15	13	10*,13	13,15	13	13	13
D12	25	17,19	20		19		20		19,20
D19			15.2				13		13,14
FGA	24	21,46.2				22,23*			21,25
D22	17*		11		16*				11,15
Method	A	A	A	A	B	B	B	B	

Table F12. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer B during Collection 2.

Locus	33-7A	33-7B	33-7C	Con.	33-1A	33-1B	33-1C	Con.	B
Amel	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D3	16,17*,18	16	18	16,18		15	18		16,18
D1	16.3,17.3				16.3				16.3,17.3
D2S441	14,15		14	14	10		14		14,15
D10									13,15
D13	10		10	10					10,12
Penta E			7						7,18
D16	9,13	9,12*,13		9,13		9,13			9,13
D18	15	13,15	13	13,15	15	13,15	15	15	13,15
D2S1338	25								20,25
CSF	12	12		12	10	10		10	10,12
Penta D									12,13
THO1	8,9,9.3	8,9.3	8	8,9.3	8,9.3	9.3	6,9.3	9.3	8,9.3
vWA	18	17,18	18	18	17,18	17,18	15,17	17,18	17,18
D21	29								29,31
D7	9		12			9			9,12
D5	13								11,13
TPOX									8
DYS391									11
D8	8,13	8,13	8,13	8,13	8,13		8,11,13,14	8,13	8,13
D12		22,23					23		22,23
D19	13,14*						15		13,15
FGA	31,†								21,23
D22					16	16		16	15,16
Method	A	A	A	A	B	B	B	B	

Table F13. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer D during Collection 2.

Locus	36-1A	36-1B	36-1C	Con.	36-2A	36-2B	36-2C	Con.	D
Amel	X,Y	Y	Y	Y	Y	X	Y	Y	X,Y
D3	17		15						17,18
D1					15				15
D2S441					11.3*				11,14
D10									13,14
D13	11								11
Penta E									7,13
D16			12*,13			13			13
D18	14					14			12,14
D2S1338			20		20				17,20
CSF							12		11,12
Penta D									9,11
THO1	8,9.3	9*,9.3	9.3	9.3	6,9.3		9.3	9.3	8,9.3
vWA	15				15				15,17
D21									28,30
D7									9,12
D5			9						11,12
TPOX									9,11
DYS391									10
D8	8,13	13	9*,13	13	8,9*,13	9*,12*		9*	8,13
D12	19		18		19,20				15,19
D19	15								14,15
FGA	22.1,†		†						21,26
D22	†								12,17
Method	A	A	A	A	B	B	B	B	

Table F14. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer WW during Collection 2.

Locus	38-2A	38-2B	38-2C	Con.	38-3A	38-3B	38-3C	Con.	WW
Amel	X	X,Y	X	X	X,Y	X	X	X	X
D3	16,18	16,17*	15,17*	16,17*	16,17*		16	16	16,18
D1	11	15			11,15				11,12
D2S441		11,14			11		11	11	11,14
D10		13*,16	15						15,16
D13	8	11	9		13,14*				8,9
Penta E		7,8			11				11,12
D16	12	12	12	12	10,11,12	12	12	12	12
D18	12	12,16,17	14*,15	12	17	12,15	12,15	12,15	12,15
D2S1338		18	18	18	21		17		17,21
CSF					11				11,12
Penta D		9,12	12	12					10,12
THO1	9.3	9.3	8,9.3	9.3	7,9.3	9.3	8,9.3	9.3	9.3
vWA	15,17	15,17		15,17	17,18*	17	15,17	17	15,17
D21	28	28,32.2*		28	30				28,30
D7		10			10				10,11
D5	13	11							13
TPOX	8	11							8,12
DYS391		11							N/A
D8	10	10,12		10	10,12	10,12,13*	10,12	10,12	10,12
D12	18,19.3	19.3,25		19.3	19*	26	17.3,19.3		18,19.3
D19	14	14,15.2	13	14	13,14	13,14		13,14	13,14
FGA		23			20,21*,25*				20,24
D22									16
Method	A	A	A	A	B	B	B	B	

Table F15. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer SS during Collection 2.

Locus	40-3A	40-3B	40-3C	Con.	40-4A	40-4B	40-4C	Con.	SS
Amel		X,Y			X				X,Y
D3	14,17*	14,17*		14,17*	15				14,18
D1		15*,18.3							14,17.3
D2S441	10	11			14*		11		11
D10	13*,14	13*,15		13*					14
D13	11*								10,12
Penta E	7	14							7,19
D16	12	10,12		12	11	10	12		11,12
D18	10	16					10,12		10,12
D2S1338	22	19					18		17,20
CSF	11,12	12		12			12		11,12
Penta D	9	9		9					9,12
THO1	8	7,8		8	6,8	8		8	8
vWA	18	15*			15*		16,17		17
D21	29	29,31		29					29,32.2
D7									12
D5		11*							13
TPOX	8	8		8					9
DYS391	11								11
D8	†,10,11,13	11,13		11,13			12,13,14		12,13
D12	15,20	18	†						15,24
D19		13,14				15			14,15
FGA			23.1						22,24
D22		13							16
Method	A	A	A	A	B	B	B	B	

Table F16. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Y during Collection 2.

Locus	41-4A	41-4B	41-4C	Con.	41-5A	41-5B	41-5C	Con.	Y
Amel	Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y	X,Y
D3		14	17		17,18	16	17	17	16,17
D1		16.3,17.3*				14	16.3		12,14
D2S441		11.3	14				14		14,15
D10	14	15,16				14			14,15
D13		12	9,13		13				13,14
Penta E		5,14							5,14
D16	11	11,12	13	11	11,12	11	11	11	11,12
D18	16*	16*,17	17	16*,17	17	15*,17		17	17
D2S1338		20	17,24			24			17,24
CSF		10							12,14
Penta D		12	8			8			8,13
THO1	6	9,9.3	6,9.3	6,9.3	9.3	9.3	8*,9,9.3	9.3	9,9.3
vWA		16,18*	14		14	16			14,16
D21		28,32.2	30.2						29,30.2
D7		11,12	10,12	12					8,10
D5		12							12
TPOX		8	11						8
DYS391		11							11
D8	10,15	9,12	10,14	10	10,14	10,14	13*,14	10,14	10,14
D12		21,23	17,20*		20*,†	17	21		17,21
D19		12,14*	13,15.2		9,16.2				13,16.2
FGA		21.2,22	20,27						22,27
D22		11,16							11,16
Method	A	A	A	A	B	B	B	B	

Table F17. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer II during Collection 2.

Locus	50-5A	50-5B	50-5C	Con.	50-6A	50-6B	50-6C	Con.	II
Amel	X,Y	Y	X,Y	X,Y	X,Y	X	Y	X,Y	X,Y
D3	17	17	17	17	17	17		17	17
D1	12,15	15,18.3		15	15	15		15	15,18.3
D2S441	11,11.3	10,11	10,11	10,11			10,11		10,11
D10	15	13	15	15					13,15
D13	12	11,12	11	11,12		11			11,12
Penta E		13,14							13,14
D16	11,12	11,12	11,12	11,12	10,12	11,12		12	12
D18		16	16	16	17				16,17
D2S1338			19		21				19,21
CSF	10	12							12
Penta D						12			9,13
THO1	7,8,9.3	6,8,9,9.3	8	8,9.3	8,9.3	8,9.3	8,9.3	8,9.3	8,9.3
vWA	17,18	15,17	15,17	15,17	15,17				15,17
D21	29	31			31				29,31
D7		12							10,12
D5	11	12	12	12					11,12
TPOX	11	8					8		8
DYS391			11						11
D8	11,13,14,16	11,13	11,13,15,16	11,13,16	11,13	11,13	†,13	11,13	11,13
D12	18	18,20,†	18,19	18	18		22		18,20
D19	16.2	14	15.2		13.2,15.2		†,15.2	15.2	14,15.2
FGA	22,23	23,†	21,†	23	21,†		†		21,23
D22		15,16							15,16
Method	A	A	A	A	B	B	B	B	

Table F18. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer W during Collection 3.

Locus	1-1A	1-1B	1-1C	Con.	1-2A	1-2B	1-2C	Con.	W
Amel	X	X,Y*	X	X		X	X,Y*	X	X
D3	14,15*,16		14,16	14,16			14		14,16
D1	14								14,15.3
D2S441		11.3	14			14			11.3,14
D10			15						13,15
D13		10,11*							10,12
Penta E			12						12
D16	9*,13	9*,13	11,13	9*,13					11,13
D18									12
D2S1338		17*							18,22
CSF		10	12						10,12
Penta D									9,11
THO1		6,7,8*,9.3	6,9.3	6,9.3	9.3	6			6,9.3
vWA	15*,16*	16*	15*,17	15*,16*,17		17			17
D21	30*,32,33.2		28,33.2	33.2					28,33.2
D7			9						9,10
D5		13	12,13	13					12,13
TPOX		12	12	12					8,12
DYS391									N/A
D8	15	8,10	10,15	10,15			15		10,15
D12	18,19*		18,22	18	17	21			18,21
D19	13*,14	13*	7,15	13*					14,15
FGA	†	21	†,18*				22*		21,23
D22	10*								16
Method	A	A	A	A	B	B	B	B	

Table F19. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer QQ during Collection 3.

Locus	6-2B	6-2A	6-2C	Con.	6-3A	6-3B	6-3C	Con.	QQ
Amel	Y	X,Y		Y			X		X,Y
D3		16			16	15*	16	16	16,18
D1	17.3		14		14				16.3,17.3
D2S441		10*					14		14,15
D10		12					14*		13,15
D13		14*							10,12
Penta E							7		7,18
D16	9,11	9,12*		9,12*			11		9,13
D18	12*,15	12*		12*			15		13,15
D2S1338		23*			17				20,25
CSF									10,12
Penta D		13							12,13
THO1	8	9.3	8,9.3	8,9.3			8,9.3		8,9.3
vWA	16*	15,16*,19*		16*			16*,18		17,18
D21		26.2*	27						29,31
D7		8*							9,12
D5		12*					12*		11,13
TPOX						8			8
DYS391									11
D8	13,15	13		13			13,15		8,13
D12		21*					22		22,23
D19	11.1	13					13		13,15
FGA	24	†					23		21,23
D22			16						15,16
Method	A	A	A	A	B	B	B	B	

Table F20. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer P during Collection 3.

Locus	7-3A	7-3B	7-3C	Con.	7-4A	7-4B	7-4C	Con.	P
Amel		X	X	X			X,Y		X,Y
D3					16*		14		15,17
D1	11		11	11	11				11,17.3
D2S441			11.3						11,12
D10		14	13						13,14
D13					12*				8,10
Penta E									11,21
D16			†,11		11				11
D18			16						14,18
D2S1338							19		25,26
CSF									10
Penta D									9,12
THO1		6*,7			9*		9.3		7,9.3
vWA		17				16			16,17
D21									29,32.2
D7									8,11
D5							12		12,13
TPOX									8,11
DYS391									10
D8	12		11,13				†		13,15
D12	17				15,19	20			17,21
D19									13,14
FGA					22*,25				19,21
D22					14				15,17
Method	A	A	A	A	B	B	B	B	

Table F21. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer DD during Collection 3.

Locus	11-4A	11-4B	11-4C	Con.	11-1A	11-1B	11-1C	Con.	DD
Amel			Y		Y				X,Y
D3							15		15,16
D1		15*					11,15*,16		16
D2S441							10,14		10,14
D10							14		13,14
D13							12		12,14
Penta E							14*		7,12
D16		12	9				9,12		9,12
D18			17		18				12,17
D2S1338							18		19,23
CSF									12
Penta D									9
THO1		9	9.3				6*,9.3		9.3
vWA		16					19		16,19
D21							30		26.2,30
D7			8						8,9
D5									12
TPOX									8,11
DYS391									11
D8					12,13		11*,14		13,14
D12			21		†				19,21
D19		14					13		13,14
FGA					18				21,22
D22									15
Method	A	A	A	A	B	B	B	B	

Table F22. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer FF during Collection 3.

Locus	12-1A	12-1B	12-1C	Con.	12-2A	12-2B	12-2C	Con.	FF
Amel			X,Y		X	X,Y		X	X
D3	18						15		15,18
D1	16.3,17.3	17.3		17.3					14,17.3
D2S441									10,11
D10									14,17
D13					9				9,11
Penta E									12
D16	10,12		†,11*						10,12
D18			18*						12,17
D2S1338									17,18
CSF						9			9,10
Penta D									8,16
THO1	6		6,9.3	6	6				6,9.3
vWA					16	16		16	16
D21						30			30,33.2
D7									10,12
D5			11,12		10				10,12
TPOX									8,11
DYS391									N/A
D8	14	11*	14	14	15		13*,14,15	15	14,15
D12		20					23		20
D19		12	13,13.1				7		13,15
FGA			23		20		28.3,†		20
D22		12							11,16
Method	A	A	A	A	B	B	B	B	

Table F23. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer KK during Collection 3.

Locus	17-2A	17-2B	17-2C	Con.	17-3A	17-3B	17-3C	Con.	KK
Amel	X,Y		X	X	X,Y	X,Y	X,Y	X,Y	X,Y
D3	15				15,16	16	15	15,16	15,16
D1					17,17.3				17,17.3
D2S441	14	10				14			10,14
D10	16								14,16
D13					12				12
Penta E							7		7,18
D16	11,13*	9	9,11	9,11	9	9	11	9	9,11
D18		12	16		13*	16,18			16,18
D2S1338			23						23,25
CSF									11,12
Penta D	13								12,13
THO1	6,7	6,7	6,8*,9.3*	6,7		6,7	7	7	6,7
vWA	15	15	15,17*,18*	15		15			15,16
D21									31,32.2
D7	10						10		10,11
D5									11,12
TPOX									8,10
DYS391									10
D8	13	13,14	14,15	13,14	14	14	14	14	13,14
D12	17	17,19	†, 17	17	17		17,23	17	17,23
D19			13*						14,15
FGA		25				25	†		23,25
D22									11,16
Method	A	A	A	A	B	B	B	B	

Table F24. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer Z during Collection 3.

Locus	18-3A	18-3B	18-3C	Con.	18-4A	18-4B	18-4C	Con.	Z
Amel	X	X,Y	X	X	X	X		X	X
D3	15,16	15	15	15			16		15,16
D1									15,18.3
D2S441	12,14								12,14
D10			13						13
D13									9,11
Penta E		7							7,14
D16	9,11,14	12	10*			†			9,12
D18	18	12,15	12	12	15				12,15
D2S1338	19	25							19,25
CSF		11							11,13
Penta D		10							10,14
THO1	6,7,9.3	6,9.3	6,9.3	6,9.3	6	9.3	6	6	6,9.3
vWA	18,19	15					18		18,19
D21	30	29	30	30	29		30		30
D7	10	10		10					10,11
D5	10*,12				13				12,13
TPOX	8								8,11
DYS391									N/A
D8	†,11,13	13,15*	13	13	†	11,13	11	11	11,13
D12	19.1,20		20	20		20,25			20
D19	14	14		14					14
FGA	19.3	21,24				†			21,24
D22									15
Method	A	A	A	A	B	B	B	B	

Table F25. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer PP during Collection 3.

Locus	20-4A	20-4B	20-4C	Con.	20-1A	20-1B	20-1C	Con.	PP
Amel	X	Y*	X,Y*	X,Y*	X		X	X	X
D3	15				15	14,15	15	15	15
D1	12,18.3	17	17.3*		12		15.3		12,15.3
D2S441	13	14	10,14	14					14
D10									13
D13			12						11
Penta E		7							11,12
D16	†,11,13	9,13	9,12	9	11		13		11,13
D18	18		18,19	18	18		18	18	16,18
D2S1338			19,23		19	19		19	17,19
CSF									11,12
Penta D			12						10,12
THO1	9.3	9.3	6,7*,8,9.3	9.3	9,9.3		9.3	9.3	9.3
vWA	16	17	16	16					16,17
D21		31,32.2	33.2			28	29		29,32.2
D7		10	10,11*	10					8,12
D5			12			11			10,12
TPOX			10						8
DYS391									N/A
D8	11,13	11,13,14,17	13,14	11,13,14	13	11,13	11,†,14	11,13	11,13
D12	22	17*,†	27		18	21*			18,22
D19		14,15							14,15
FGA	15	25	22.2,25	25			24		22.2,24
D22									16,17
Method	A	A	A	A	B	B	B	B	

Table F26. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer X during Collection 3.

Locus	21-1A	21-1B	21-1C	Con.	21-2A	21-2B	21-2C	Con.	X
Amel		X,Y	Y	Y	X,Y	X		X	X,Y
D3	16	15,16	15	15,16	15,16	15	15	15	15,16
D1	11,14	11,14		11,14	11	14			11,14
D2S441		14			14	14		14	14
D10		12,14	14	14					12,14
D13							11		11,12
Penta E						12,14			12,14
D16	9,11,13	9,13	9	9,13	9	9,13	9	9	9,13
D18	15				15	15		15	15
D2S1338						17,21			17,21
CSF						11			11
Penta D									12,14
THO1	8,9.3*	8	8	8	8	8	8	8	8
vWA	15,16	15,16		15,16	15				15,16
D21		27,30			27		26.2*		27,30
D7						9*			10,11
D5						11			11,12
TPOX		8							8,9
DYS391									10
D8	15	13*,15	15	15	15	15	11,15	15	15
D12	18	18,19,20.3,23,†	18,21*	18	20,21*	18,19,23			18,19
D19		13.2	18			12,13			12,13
FGA	18					14,22,30.2			18,22
D22		14				15*			10,14
Method	A	A	A	A	B	B	B	B	

Table F27. Consensus profiles created from alleles amplified with PowerPlex® Fusion from spent cartridge casings loaded by volunteer UU during Collection 3.

Locus	35-2A	35-2B	35-2C	Con.	35-3A	35-3B	35-3C	Con.	UU
Amel	X	X,Y		X		X			X,Y
D3	14	15*,16, 17							16,17
D1		11,12, 14,17.3*							12,16.3
D2S441		14*							8,10
D10									13,15
D13		13							11,12
Penta E		13							12
D16	9*,12	11,12	11	11,12			11		11
D18		16*,17							13,15
D2S1338	17	17,21		17					17
CSF									10,12
Penta D									12,13
THO1	9.3	7*,9,9.3		9.3		6,9			6,9
vWA	15*,17	14,16*, 17,18		17					14,20
D21		29,30.2							29,30
D7		8,10							10,13
D5							10		10,12
TPOX	11	8							8,11
DYS391									11
D8		10,12,13 ,14*							13
D12		17*,18				†			18,21
D19		13,14, 15*							13.2,14
FGA		14,22	18,30						22
D22		15,16							15,16
Method	A	A	A	A	B	B	B	B	

REFERENCES

REFERENCES

- Alessandrini F, Cecati M, Pesaresi M, Turchi C, Carle F, Tagliabracci A. Fingerprints as evidence for a genetic profile: morphological study on fingerprints and analysis of exogenous and individual factors affecting DNA typing. *J Forensic Sci* 2003;48(3):1–7.
- Batzer MA and Deininger PL. Alu repeats and human genomic diversity. *Nat Rev Genet* 2002;3(5):370–9.
- Bentsen RK, Brown JK, Dinsmore A, Harvey KK, Kee TG. Post firing visualization fingerprints on spent cartridge cases. *Sci Justice* 1996;36(1):3–8.
- Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;28(3):495–503.
- Bowe CAI. Use of silica-supported adsorbents, modified silica gel, modified montmorillonite KSF of magnetite in the remediation of selected heavy metals from aqueous media [dissertation]. Tampa (FL):Univ. of South Florida., 2003.
- Bright J and Petricevic SF. Recovery of trace DNA and its application to DNA profiling of shoe insoles. *Forensic Sci Int* 2004;145:7–12.
- Budowle B, Eisenberg AJ, van Daal A. Validity of low copy number typing and applications to forensic science. *Croat Med J* 2009;50(3):207–17.
- Butler JM. *Forensic DNA Typing*. 2nd ed. Burlington: Elsevier Academic Press, 2005.
- Butler JM and Hill CR. Scientific issues with analysis of low amounts of DNA. Promega Corporation web site. 2010. <http://www.promega.com/resources/profiles-in-dna/2010/scientific-issues-with-analysis-of-low-amounts-of-dna/>
- Cardozo BR, Jaureguiberry SM, Boselli GO. Touch DNA-the forensic use of solid and porous matrices coated with polyanilines in the selective separation of biopolymers from nucleic acids. *Proceedings of the 23rd International Symposium on Human Identification*; 2012 Oct 15–18; Nashville (TN) Madison (WI): Promega, 2012.
- Comey CT, Koons BW, Presley KW, Smerick JB, Sobieralski CA, Stanley DM *et al*. DNA extraction strategies for amplified fragment length polymorphism analysis. *J Forensic Sci* 1994;39(5):1254–69.
- Dieltjes P, Mieremet R, Zuniga S, Kraaijenbrink T, Pijpe J, De Knijff P. A sensitive method to extract DNA from biological traces present on ammunition for the purpose of genetic profiling. *Int J Legal Med* 2011;125:597–602.

- DiMaio VJM. Gunshot wounds: practical aspects of firearms, ballistics, and forensic techniques. 2nd ed. Boca Raton: CRC Press, 1999.
- Doran AE and Foran DR. Assessment and Mitigation of DNA Loss Utilizing Centrifugal Filtration Devices. Forensic Sci Int: Genet In press.
- Doyle JS. FirearmsID. An Introduction to Forensic Firearm Identification. 2014. www.firearmsid.com
- FBI. Uniform Crime Reports. Violent Crime. 2012. <http://www.fbi.gov/about-us/cjis/ucr/crime-in-the-u.s/2012/crime-in-the-u.s.-2012>
- Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. Forensic Sci Int 2000;112:17–40.
- Given BW. Latent fingerprints on cartridges and expended cartridge casings. J Forensic Sci 1976;21(3):587–94.
- Goray M, Eken E, Mitchell RJ, van Oorschot RAH. Secondary DNA transfer of biological substances under varying test conditions. Forensic Sci Int: Genet 2010;(4):62–7.
- Green RL, Roinestad IC, Boland C, Hennessy LK. Developmental Validation of the Quantifiler™ real-time PCR kits for the quantification of human nuclear DNA samples. J Forensic Sci 2005;50(4):1–17.
- Greenspoon SA, Scarpetta MA, Drayton ML, Turek SA. QIAamp spin columns as a method of DNA isolation for forensic casework. J Forensic Sci 1998;43(5):1024–30.
- Guns & Ammo Info. The Bullet Cartridge. 2014. <http://www.gunsandammo.info/ammo/ammo-101>
- Hares DR. Expanding the CODIS core loci in the United States. Forensic Sci Int: Genet 2012A;(6):e52–4.
- Hares DR. Addendum to expanding the CODIS core loci in the United States. Forensic Sci Int: Genet 2012B;(6):e135.
- Hebda LM, Doran AE, Foran DR. Collecting and analyzing DNA evidence from fingernails: a comparative study. J Forensic Sci. In press.
- Higuchi R, Fockler C, Dollinger G, Watson R. Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Nat Biotechnol 1993;11(9):1026–30.

- Hill CR, Kline MC, Duewer DL, Coble MD, Butler JM. Population statistics on the proposed expanded U.S. core loci. Proceedings of the 23rd International Symposium on Human Identification; 2012 Oct 15–18; Nashville (TN) Madison (WI): Promega, 2012.
- Horsman-Hall KM, Orihuela Y, Karczynski SL, Davis AL, Ban JD, and Greenspoon SA. Development of STR profiles from firearms and fired cartridge cases. *Forensic Sci Int: Genet* 2009;3:242–50.
- Kapustin DV, Yagudaeva EY, Zavada LL, Zhigis LS, Zubov VP, Yaroshevskaya EM *et al.* A composite polyaniline-containing silica sorbent for DNA isolation. *Russian Journal of Bioorganic Chemistry* 2003;29(3):281–5.
- Kiley BM. A highly sensitive sex determination assay for low quality DNA [thesis]. East Lansing (MI): Michigan State Univ., 2009.
- Kopka J, Leder M, Jaureguiberry SM, Brem G, Boselli GO. New optimized DNA extraction protocol for fingerprints deposited on a special self-adhesive security seal and other latent samples used for human identification. *J Forensic Sci* 2011;56(5):1235–40.
- Krane DE. Some of the problems associated with LCN (low copy number) testing. The Forensic Institute 2007 Forensic e-Symposium on Human Identification: Profiling of degraded and low amounts of DNA. <http://www.bioforensics.com/downloads/index.html>
- Lee HC and Ladd C. Preservation and collection of biological evidence. *Croat Med J* 2001;42(3):225–8.
- Life Technologies. AmpF ℓ STR[®] MiniFiler[™] PCR Amplification Kit. 2014. <http://www.lifetechnologies.com/us/en/home/industrial/human-identification/ampflstr-minifiler-pcr-amplification-kit.html>
- Lindahl T. Instability and decay of the primary structure of DNA. *Nature* 1993;362:709–15.
- Lindquist CD, Evans JJ, Wictum EJ. Developmental validation of feline, bovine, equine, and cervid quantitative PCR assays. *J For Sci* 2011;56(S1):S29–S35.
- Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. *For Sci Int* 2002;129:25–34.
- Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory, 1982;458–9.
- Meakin G and Jamieson A. DNA transfer: review and implications for casework. *For Sci Int: Genet* 2013;7:434–43.

- Melzak KA, Sherwood CS, Turner RFB, Haynes CA. Driving forces for DNA adsorption to silica in perchlorate solutions. *J Colloid Interface Sci* 1996;181:635–44.
- Mighell AJ, Markham AF, Robinson PA. *Alu* sequences. *Federation of European Biochemical Societies* 1997;417:1–5.
- Mullis KF, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harb Symp Quant Biol* 1986;51:263–73.
- Murray V, Monchawin C, England PR. The determination of the sequences present in the shadow bands of a dinucleotide repeat PCR. *Nucleic Acids Res* 1993;21(10):2395–98.
- National Institute of Justice. Firearms Examiner Training. <https://firearms-examiner.dna.gov>
- NEXTTEC™ Biotechnologie GmbH. FDF® Kit User Manual Version 2.1. Hilgertshausen, Germany.
- Nicklas JA and Buel E. An Alu-based, MGB Eclipse real-time PCR method for quantitation of human DNA in forensic samples. *J Forensic Sci* 2005 Sep;50(5):1081–90.
- Nicklas JA and Buel E. Simultaneous determination of total human and male DNA using a duplex real-time PCR assay. *J Forensic Sci* 2006;51(5):1005–15.
- Oostdik K, Lenz K, Nye J, Schelling K, Yet D, Bruski S *et al.* Developmental validation of the PowerPlex® Fusion System for analysis of casework and reference samples: a 24-locus multiplex for new database standards. *Forensic Sci Int: Genet* 2014;(12):69–76.
- Orlando A. The recovery and analysis of DNA from fired cartridge casings [thesis]. East Lansing (MI): Michigan State Univ., 2012.
- Pang BCM and Cheung BKK. Double swab technique for collecting touched evidence. *Leg Med* 2007;9:181–4.
- Petricevic SF, Bright J, Crockerton SL. DNA profiling of trace DNA recovered from bedding. *Forensic Sci Int* 2006;159:21–6.
- Phipps M and Petricevic S. The tendency of individuals to transfer DNA to handled items. *Forensic Sci Int* 2007;168:162–8.
- Poinar HN. The top 10 list: criteria of authenticity for DNA from ancient and forensic samples. *Int Congress Series* 2003;1239:575–9.
- Promega. PowerPlex® Fusion System. 2014. <http://www.promega.com/products/genetic-identity/str-analysis-for-forensic-and-paternity-testing/powerplex-fusion-system/>

- Qiagen. 2012. QIAamp® DNA Investigator Handbook. User's manual. Hilden, Germany.
- Quinones I and Daniel B. Cell free DNA as a component of forensic evidence recovered from touched surfaces. *Forensic Sci Int: Genet* 2012;6:26–30.
- Richert NJ. Swabbing firearms for handler's DNA. *J Forensic Sci* 2011;56(4):972–5.
- Ruger Forum. SR1911 Commander barrel lockup. 2014. <http://rugerforum.net/ruger-pistols/98275-sr1911-commander-barrel-lockup.html>
- Saferstein R. *Criminalistics: an introduction to forensic science*. 10th ed. Upper Saddle River: Prentice Hall, 2011.
- Sewell J, Quinones I, Ames C, Multaney B, Curtis S, Seeboruth H *et al*. Recovery of DNA and fingerprints from touched documents. *Forensic Sci Int: Genet* 2008;2:281–5.
- Sigma-Aldrich. Nucleases. 2014. <http://www.sigmaaldrich.com/life-science/metabolomics/enzyme-explorer/learning-center/nucleases.html>
- Smith LM and Burgoyne LA. Collecting, archiving and processing DNA from wildlife samples using FTA® databasing paper. *BMC Ecology* 2004;4:1–11.
- Spear T, Clark J, Giusto M, Khoshkebari N, Murphy M, and Rush J. Fingerprints and cartridge cases: how often are fingerprints found on handled cartridge cases and can these fingerprints be successfully typed for DNA? *Proceedings of the California Association of Criminalistics*; 2005 May 9–15; Oakland, CA. Sacramento: California Criminalistics Institute, 2005.
- Sweet D, Lorente M, Lorente JA, Valenzuela A, Villanueva E. An improved method to recover saliva from human skin: the double swab technique. *J Forensic Sci* 1997;42(2):320–2.
- Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N *et al*. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Res* 1996;24(16):3189–94.
- Thomasma SM and Foran DR. The influence of swabbing solutions on DNA recovery from touch samples. *J Forensic Sci* 2013;58(2):465–9.
- Thompson RM. *Firearm Identification in the Forensic Science Laboratory. Gun Violence Prosecution Program: The National District Attorney Association*, 2010.
- van Oorschot RAH, Ballantyne KN, Mitchell RJ. Forensic trace DNA: a review. *Investig Genet* 2010;1:14–31.
- Walsh PS, Erlich HA, Higuchi R. Preferential PCR amplification of alleles: mechanisms and solutions. *Genome Research* 1992;1:241–50.

Warlow T. Firearms, the Law, and Forensic Ballistics. 3rd ed. Boca Raton: CRC Press, 2012.

Wickenheiser RA. Trace DNA: a review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact. J Forensic Sci 2002;47(3)442–50.