1. Scope

This protocol is to be used with the Applied Biosystems (AB) AmpFlSTR™ Identifiler™ PCR Amplification Kit to amplify DNA extracted from known reference samples or biological material found on items of evidence at the following loci: D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA, and the sex-determining locus Amelogenin. The AB AmpFlSTR™ Identifiler™ PCR Amplification Kit utilizes the polymerase chain reaction (PCR) to make copies of specific regions of extracted DNA samples. These regions are called short tandem repeats (STR) and, taken together, allow the analyst to characterize the source of the DNA. Identifiler allows for the simultaneous amplification of 15 tetranucleotide repeat regions (loci) and the gender marker Amelogenin in one reaction tube. Amplification is performed in a thermal cycler.

The Identifiler kit contains all reagents needed for amplification which includes: primer sets specific for the various loci, PCR reaction buffer, AmpliTaq® Gold DNA polymerase, and the positive control DNA (9947A). Allelic ladders are also included in the kit; however, they are not needed during amplification. The primer sets contained within each kit consist of both unlabeled primers and those that are labeled with one of four distinctive fluorescent dyes. The incorporation of fluorescent dyes during the amplification process allows for the subsequent detection, characterization, and sizing of the fragments on the Applied Biosystems 3130 Genetic Analyzer. The use of multicolor dyes permits the analysis of loci with overlapping size ranges.

2. References

The procedures described here are derived from a variety of sources. Portions of the protocol come directly from some of the references cited below.

2.1. Applied Biosystems AmpFlSTR™ Identifiler™ PCR Amplification Kit User's Manual, 2005.
2.2. Prince George's County (MD) Police Forensic Services Division, Serology/DNA Laboratory Short Tandem Repeat (STR) Analysis Protocol, 2006.
2.3. P.J. Collins, L.K. Hennessy, C.S. Leibelt, R.K. Roby, D.J. Reeder, P.A. Foxall, Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: The AmpFlSTR™ Identifiler™ PCR Amplification Kit, J. Forensic Sci. 49 (2004) 1265-1277.
2.4. C. Leibelt, B. Budowle, P. Collins, Y. Daoudi, T. Moretti, G. Nunn, D. Reeder, R. Roby, Identification of a D8S1179 primer binding site mutation and the validation of a primer designed to recover null alleles, Forensic Sci. Int. 133 (2003) 220-227.
2.5. J.M. Butler, Commonly Used Short Tandem Repeat Markers and Commercial Kits, Biology of STRs, and Forensic Issues in Forensic DNA Typing, in: Biology, Technology, and Genetics of STR Markers, second ed., Elsevier Academic Press, 2005, pp 85-180.
2.6. A. Edwards, A. Civitello, H. Hammond, C.T. Caskey, DNA typing and genetic mapping with trimeric and tetrameric tandem repeats, Am. J. Hum. Genet. 49 (1991) 746–756.

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2.8. B. Budowle, T.R. Moretti, K.M. Keys, B.W. Koons, J.B. Smerick, Validation studies of the CTT STR multiplex system, J. Forensic Sci. 42 (1997) 701–707.
2.9. C.J. Fregeau, R.M. Fourney, DNA typing with fluorescently tagged short tandem repeats: A sensitive and accurate approach to human identification, BioTechniques 15 (1993) 100–119.
2.10. P.S. Walsh, N.J. Fildes, R. Reynolds, Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA, Nucleic Acids Res. 24 (1996) 2807-2812.
2.11. R.K. Saiki, T.L. Bugawan, G.T. Horn, K.B. Mullis, H.A. Erlich, Analysis of enzymatically amplified beta-globin and HLA-DQ alpha DNA with allele-specific oligonucleotide probes, Nature 324 (1986) 163-166.
2.12. R.K. Saiki, D.H. Gelfand, S. Stoffel, S.J. Scharf, R. Higuchi, G.T. Horn, K.B. Mullis, H.A. Erlich, Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase, Science 239 (1988) 487-491.

3. Equipment

3.1. Disposable gloves
3.2. Lab coat
3.3. Eye protection
3.4. Benchtop hood
3.5. TE^{-4} (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0)
3.6. AB Identifiler PCR Amplification Kit
  3.6.1. AmpFlSTR® Identifiler® Primer Set
  3.6.2. AmpFlSTR® PCR Reaction Mix
  3.6.3. AmpliTaq Gold® DNA Polymerase
  3.6.4. AmpFlSTR® Control DNA 9947A
3.7. Thermal cycler
3.8. 0.2mL thin-walled PCR tubes
3.9. Microcentrifuge tubes
3.10. 10% bleach solution
3.11. 70% ethanol (EtOH)
3.12. Tube racks
3.13. Pipette
3.14. Disposable pipette tips
3.15. Vortexer
3.16. Centrifuge

4. Safety/Quality Assurance

4.1. Disposable gloves shall be worn when handling reagents and DNA extracts.
4.2. Change gloves frequently.
4.3. Lab coat and eye protection must be worn at all times while performing this procedure.
4.4. PCR setup for evidence samples shall be performed separately from PCR setup for known samples. PCR setup for known samples shall be performed in a dedicated area.

4.5. Samples thought to contain lower levels of DNA will be handled before those thought to contain large amounts of DNA.
4.6. Sample setup must be performed in a PCR setup hood. Clean surfaces with 10% bleach solution followed by 70% EtOH prior to and after use. After exiting hood, turn on UV light.
4.7. Exercise caution when opening tubes.
4.8. Use aerosol-resistant pipette tips when transferring liquids containing DNA.
4.9. Change pipette tips after transferring any liquids potentially containing DNA.
4.10. Only tubes associated with one sample shall be open at a time.
4.11. The reagent blank must be amplified at a sensitivity level equal to or exceeding the sensitivity of its associated DNA extracts.
4.12. Tubes containing DNA extract shall not be opened in benchtop hood prior to aliquoting master mix into 0.2 mL tubes.
4.13. A positive amplification control (9947A) must be initiated.
4.14. Add 10 uL TE^{-4} to the negative control tube last, after all DNA samples have been added to the other tubes. This tube functions as a negative control for the PCR setup.
4.15. Record the lot number of each reagent used. Do not use the reagents after the expiration date.
4.16. Store the DNA amplification reagents in a refrigerator or freezer separate from the DNA extracts and evidence.
4.17. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Procedure

5.1. Turn on the thermal cycler.
5.2. If DNA extracts have been frozen, allow them to thaw.
5.3. Dilutions can be prepared at any time prior to adding DNA extracts and/or dilutions to 0.2 mL PCR tubes. If preparing dilutions in PCR setup hood, do not prepare them until after master mix has been aliquotted to 0.2 mL PCR tubes. Determine the amount of DNA extract and TE^{-4} required for each sample and control based on quantification results. Optimal target amount of template DNA per sample and positive control is approximately 0.8 ng.
NOTE: If necessary, lesser or greater quantities of DNA may be used to obtain the desired result.

5.4. Determine total number of sample and control tubes.
5.5. In the PCR setup hood, appropriately label 0.2 mL PCR tubes and 1.5 mL master mix tube(s). Place the 0.2 mL PCR tubes in a clean tray dedicated for PCR setup.
5.6. Vortex and briefly spin the amplification reagents (Identifiler primers, PCR Reaction mix, and AmpliTaq® Gold DNA polymerase).
5.7. Add appropriate volumes of reagents to 1.5 mL tube(s) to create the PCR master mix. The following volumes are 'per sample' and include excess to allow for volume lost in pipetting. Multiply the volumes by the number of samples and controls to be amplified.

PCR Reaction Mix: 10.5 µL

Identifiler® Primer Set: 5.5 µL

AmpliTaq® Gold DNA Polymerase: 0.5 µL

5.8. Vortex and briefly spin master mix.
5.9. Aliquot 15 µL master mix to each sample and control 0.2 mL tube.
5.10. Vortex and briefly spin DNA extracts and dilutions (if necessary).
5.11. Add appropriate volume of DNA extracts or dilutions, and reagent blank(s) to 0.2 mL PCR tubes. Add TE^{-4} as necessary to bring the final total volume of the PCR reaction to 25 µL.
5.12. Vortex and briefly spin 9947A positive control DNA.
5.13. Add appropriate volume of 9947A to positive control 0.2 mL tube.
5.14. Add 10 µL TE^{-4} to negative control 0.2 mL tube.
5.15. Clean the benchtop hood with 10% bleach followed by 70% EtOH. Turn on the UV light when leaving the benchtop hood.
5.16. Briefly spin tray on plate centrifuge.
5.17. Place tubes in thermal cycler.
5.18. Ensure that all 0.2 mL tube caps are tightly sealed.
5.19. Close thermal cycler cover.
5.20. Select Identifiler protocol (*ID-28*), verify that thermal cycling conditions are as follows, and press start.

HOLD	95° C	11 min	Initial Incubation Step
CYCLE (28 cycles)	94° C	1 min	Denature
	59° C	1 min	Anneal
	72° C	1 min	Extend
HOLD	60° C	60 min	Final Extension
HOLD	4° C	∞	Final Step

5.21. When the amplification is complete, samples can be stored in the refrigerator or freezer. Amplicons can be discarded after the case has been completed and the report has undergone technical and administrative reviews.