1. Scope

This protocol is to be used to amplify DNA extracted from known reference samples or biological material found on items of evidence at the following loci:

- 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317,

  D7S820, SE33, D10S1248, D1S1656, D12S391, and D2S1338)

- 1 Y-STR locus (DYS391)
- 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel)
- Amelogenin (sex determining marker)

The Applied Biosystems™ (AB) GlobalFiler™ PCR Amplification Kit utilizes the polymerase chain reaction (PCR) to make copies of specific regions of the extracted DNA samples. These regions allow the analyst to characterize the source of the DNA. The GlobalFiler™ PCR Amplification Kit allows for the simultaneous amplification of 20 autosomal tetranucleotide repeat regions (loci), 1 autosomal trinucleotide repeat region (D22S1045), 1 Y-STR, 1 insertion/deletion polymorphic marker (indel) on the Y chromosome, and the sex marker Amelogenin in one reaction tube. Amplification is performed in a thermal cycler. These 21 autosomal STR loci represent the expanded set of 20 Combined DNA Index System (CODIS) core loci plus SE33.

The GlobalFiler™ PCR Amplification Kit contains all reagents needed for amplification, which includes: primer sets specific for the various loci, PCR reaction buffer, enzyme, and the positive control DNA 007. 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 five 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 AB 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™ GlobalFiler™ PCR Amplification Kit User's Manual, Revision E, July 2016.

2.2. V. Bogasa, M. Carvalho, F. Corte-Real, M.J. Porto, Testing the behavior of GlobalFiler® PCR amplification kit with degraded and/or inhibited biological samples, Forensic Sc. Int. Genet. Suppl. Ser. 5 (2015) e21–e23.

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

2.4. D. Hares, Selection and implementation of expanded CODIS core loci in the United States, Forensic Sc. Int. Genet. 17 (2015) 33–34.

2.5. J.M. Butler, Commonly used short tandem repeat markers and commercial kits, biology of STRs, and forensic issues, in: Forensic DNA Typing, second ed., Elsevier Academic Press, Burlington, 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.

2.7. H.A. Hammond, L. Jin, Y. Zhong, C.T. Caskey, R. Chakraborty, Evaluation of 13 short tandem repeat loci for use in personal identification applications, Am. J. Hum. Genet. 55 (1994) 175–189.

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} (10mM Tris-HCl , 0.1mM EDTA, pH 8.0)
3.6. AB GlobalFiler™ PCR Amplification Kit
    3.6.1. GlobalFiler™ Primer Set
    3.6.2. GlobalFiler™ Master Mix
    3.6.3. DNA Control 007
3.7. GeneAmp® PCR System 9700 or Veriti® 96-well Thermal Cycler
3.8. 0.2 mL thin-walled PCR tubes
3.9. Microcentrifuge tubes
3.10. 10% bleach solution and/or 70% ethanol
3.11. Tube racks
3.12. Pipette
3.13. Disposable pipette tips
3.14. Vortexer
3.15. 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 unknown samples shall be performed separately from PCR setup for known samples.
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 and/or 70% ethanol prior to 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 amount of reagent blank amplified must equal or exceed the amount amplified for 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 (Control DNA 007) must be initiated.
4.14. A negative amplification control must be initiated. Add 15 µL 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. Once the PCR reagents have been thawed, do not refreeze the reagents.
4.18. 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 aliquoted to 0.2 mL PCR tubes.
5.4. 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.
5.5. Determine 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 0.5 ng.
NOTE: If necessary, lesser or greater quantities of DNA may be used to obtain the desired result. Factors such as degradation, inhibition, and mixture ratio may affect this decision.

5.6. Aliquot required TE^{-4} into 0.2 mL PCR tubes and/or 1.5 mL dilution tubes (if necessary). The final reaction volume in each PCR tube is 25 µL (10 µL master mix and 15 µL sample).
5.7. Vortex and briefly spin DNA extracts.
5.8. Add appropriate volume of DNA extracts to 1.5 mL dilution tubes (if necessary).
5.9. Vortex and briefly spin dilution tubes (if necessary).
5.10. Determine total number of sample and control tubes.
5.11. Vortex and briefly spin the amplification reagents (GlobalFiler™ Primer Set and GlobalFiler™ Master Mix).
5.12. Add appropriate volumes of reagents to 1.5 mL tube(s) to create the PCR master mix. The following volumes are 'per sample.' Include additional reactions to the total

number of samples to account for loss of reagent during transfers. Multiply the volumes by the number of samples to be amplified including controls.

Globalfiler™ Component	Volume (µL)
GlobalFiler™ Master Mix	7.5
GlobalFiler™ Primer Set	2.5

5.13. Vortex and briefly spin master mix.
5.14. Aliquot 10 µL master mix to each sample and control 0.2 mL tube.
5.15. Add appropriate volume of DNA extracts or dilutions, and reagent blank(s) to 0.2 mL PCR tubes. Add a total of 15 µL DNA extract/TE^{-4} to each PCR tube for a final volume of 25 µL.
5.16. Vortex and briefly spin Control DNA 007 positive control DNA.
5.17. Add appropriate volume of Control DNA 007 to positive control 0.2 mL tube.
5.18. Add 15 µL TE^{-4} to negative control 0.2 mL tube.
5.19. Clean the bench top hood with 10% bleach and/or 70% ethanol. Turn on UV light when leaving bench top hood.
5.20. Briefly spin 0.2 mL tubes in centrifuge.
5.21. Place tubes in thermal cycler.
5.22. Ensure that all 0.2 mL tube caps are tightly sealed.
5.23. Close thermal cycler cover.
5.24. Select GlobalFiler™ protocol ("GF-28") and press start. If using the GeneAmp® PCR System 9700, verify the "Max" ramping mode has been selected. If you are using the Veriti™ Thermal Cycler, select the 100% ramping rate. Verify that thermal cycling conditions are as follows:

Initial Incubation Step	Hold	95°C	1 minute
Denature	Cycle (28 cycles)	94°C	10 seconds
Anneal/Extend		59°C	90 seconds
Final Extension Step	Hold	60°C	10 minutes
Final Hold	Hold	4°C	Up to 24 hours

5.25. When the amplification is complete (approximately 80 minutes), samples can be stored in the refrigerator or freezer according to the following chart:

Two weeks or less	2-8°C
More than two weeks	-15 to -25°C

6. Supplemental Information

Dyes Used in Globalfiler™ PCR Amplification Kit

Dye	Color	Label
6-FAM™	Blue	Samples, allelic ladders, and controls
VIC™	Green	
NED™	Yellow	
TAZ™	Red	
SID™	Purple	
LIZ™	Orange	GeneScan™ 600 LIZ™ Size Standard v2.0

Table source: Applied Biosystems™ GlobalFiler™ PCR Amplification Kit User's Manual, Revision E, July 2016

Description of Loci Amplified with the Globalfiler™ PCR Amplfication Kit

Locus designation	Chromosome location	Alleles included in Allelic Ladder	Dye label	DNA Control 007
D3S1358	3p21.31	9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20	6-FAM™	15, 16
vWA	12p13.31	11,12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24		14, 16
D16S539	16q24.1	5, 8, 9, 10, 11, 12,13, 14, 15		9, 10
CSF1PO	5q33.3-34	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		11, 12
TPOX	2p23-2per	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15		8, 8
Y indel	Yq11.221	1, 2	VIC™	2
Amelogenin	X: p22.1-22.3 Y: p11.2	X, Y		X, Y
D8S1179	8q24.13	5, 6, 7, 8, 9 10, 11, 12, 13, 14, 15, 16, 17, 18, 19		12, 13
D21S11	21q11.2-q21	24, 24.2, 25, 26, 27, 28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36, 37, 38		28, 31
D18S51	18q21.33	7, 9, 10, 10.2, 11, 12, 13, 13.2, 14, 14.2, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27		12, 15
DYS391	Yq11.21	7, 8, 9, 10, 11, 12, 13		11
D2S441	2p14	8, 9, 10, 11, 11.3, 12, 13, 14, 15, 16, 17	NED™	14, 15
D19S433	19q12	6, 7, 8, 9, 10, 11, 12, 12.2, 13, 13.2, 14, 14.2, 15, 15.2, 16, 16.2, 17, 17.2, 18.2, 19.2		14, 15
TH01	11p15.5	4, 5, 6, 7, 8, 9, 9.3, 10, 11, 13.3		7, 9.3
FGA	4q28	13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 26.2, 27, 28, 29, 30, 30.2, 31.2, 32.2, 33.2, 42.2, 43.2, 44.2, 45.2, 46.2, 47.2, 48.2, 50.2, 51.2		24, 26
D22S1045	22q12.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	TAZ™	11, 16
D5S818	5q21-31	7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18		11, 11
D13S317	13q22-31	5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16		11, 11
D7S820	7q11.21-22	6, 7, 8, 9, 10, 11, 12, 13, 14, 15		7, 12
SE33	6q14	4.2, 6.3, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20.2, 21, 21.2, 22.2, 23.2, 24.2, 25.2, 26.2, 27.2, 28.2, 29.2, 30.2, 31.2, 32.2, 33.2, 34.2, 35, 35.2, 36, 37		17, 25.2
D10S1248	10q26.3	8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19	SID™	12, 15
D1S1656	1q42.2	9, 10, 11, 12, 13, 14, 14.3, 15, 15.3, 16, 16.3, 17, 17.3, 18.3, 19.3, 20.3	SID™	13, 16
D12S391	12p13.2	14, 15, 16, 17, 18, 19, 19.3, 20, 21, 22, 23, 24, 25, 26, 27		18, 19
D2S1338	2q35	11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28		20, 23

Table source: Applied Biosystems™ GlobalFiler™ PCR Amplification Kit User's Manual, Revision E, July 2016

Original CODIS Core 13 Loci with additional Expanded Core Loci

Locus
CSF1PO
D3S1358
D5S818
D7S820
D8S1179
D13S317
D16S539
D18S51
D21S11
FGA
TH01
TPOX
vWA
D1S1656
D2S441
D2S1338
D10S1248
D12S391
D19S433
D22S1045

Red is for original CODIS Core 13 Loci.
Blue is for new additional CODIS Core Loci.

Table Source: D. Hares, Selection and implementation of expanded CODIS core loci in the United States, Forensic Sci. Int. Genet. 17 (2015) 33–34.