Human DNA authentication using Combined DNA Index System (CODIS) profiling

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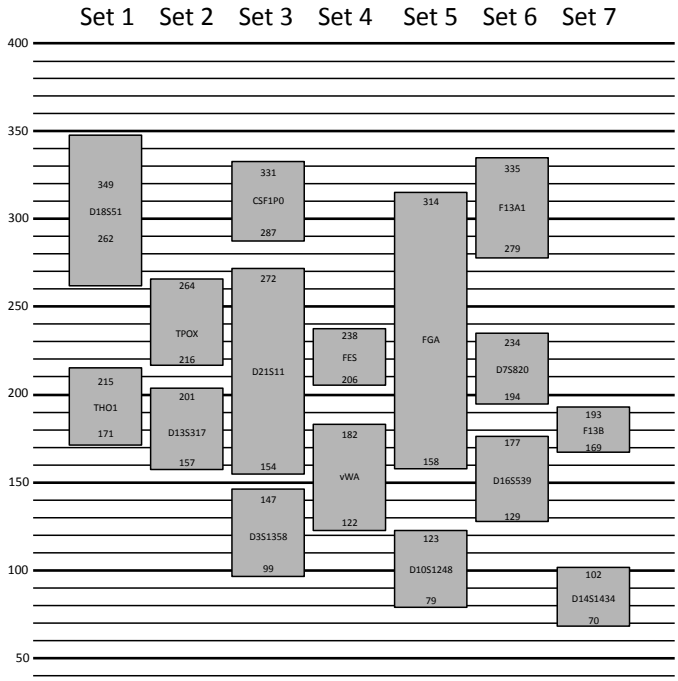
This protocol describes the procedure for the authentication of human DNA using microsatellite (STR) profiling. The protocol amplifies 16 different loci (HUMTH01, D18S51, D13S317, TPOX, D3S1358, D21S11, CSF1PO, vWA, FES, D10S1248, FGA, D16S539, D7S820, F13A1, D14S1434, F13B) in seven different PCR multiplex reactions. This technique can be used to validate the authenticity of human cell lines and to identify biologic relationship between humans (genealogy, corpse identification and paternity tests). CODIS is the generic term used to describe the Federal Bureau of Investigation (FBI's) program of support for criminal justice DNA databases. The National DNA Index System or NDIS is considered one part of CODIS, the national level, containing the DNA profiles contributed by federal, state, and local participating forensic laboratories. The DNA working group of the European Network of Forensic Science Institutes (ENFSI) decided on a European Standard Set (ESS), which includes seven loci: TH01, vWA, FGA, D21S11, D3S1358, D8S1179 and D18S51. These loci have been confirmed by a resolution of the European Council in 2001 (4) and now form the core of all national DNA databases in Europe.

Oligonucleotides

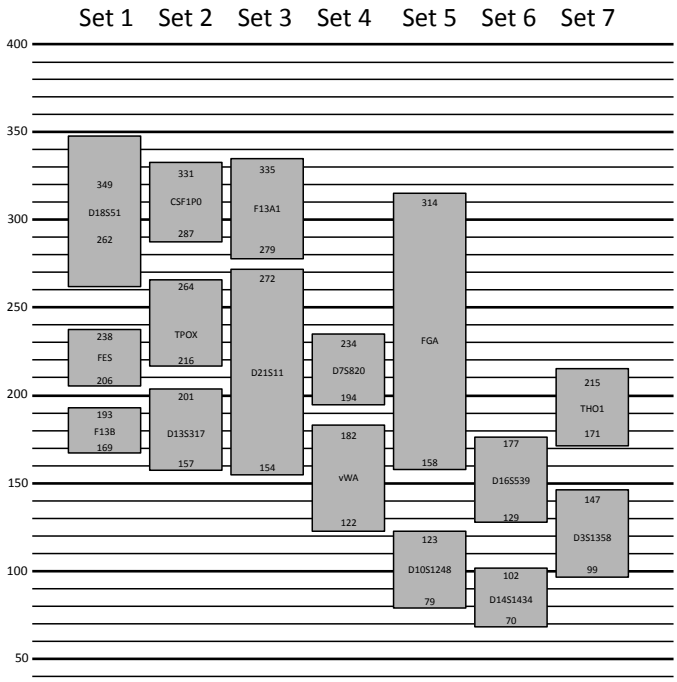
Name	Sequence	Set	Location	Ref
TH01-F	GTGGGCTGAAAAGCTCCCGATTAT	Set 1	11p15.5	1
TH01-R	ATTCAAAGGGTATCTGGGCTCTGG			
TPOX -F	ACTGGCACAGAACAGGCACTTAGG	Set 1	2p25.3	1
TPOX-R	GGAGGAACTGGGAACCACACAGGT			
CSF1P0-F	AACCTGAGTCTGCCAAGGACTAGC	Set 1	5q33.1	1
CSF1P0-R	TTCCACACACCACTGGCCATCTTC			
VWA-F	CCCTAGTGGATGATAAGAATAATCAGTATG	Set 1'	12p13.31	1
VWA-R	GGACAGATGATAAATACATAGGATGGATGG			
FES/FPS-F	GGGATTTCCCTATGGATTGG	Set 1	15q25-qter;	1
FES/FPS-R	GCGAAAGAATGAGACTACAT			
D3S1358-F	ACTGCAGTCCAATCTGGGT	Set 1	3p21.31	1
D3S1358-R	ATGAAATCAACAGAGGCTTG			
D13S317-F	ACAGAAGTATGGGATGTGGA	Set 2	13q31.1	1
D13S317-R	GCCCAAAAAGACAGACAGAA			
D10S1248-F	TTAATGAATTGAACAAATGAGTGAG	Set 2	10q26.3	2
D10S1248-R	GCAACTCTGGTTGTATTGTCTTCAT			
D16S539-F	GATCCCAAGCTCTTCCTCTT	Set 2	16q24.1	1
D16S539-R	ACGTTTGTGTGTGCATCTGT			
D7S820-F	TGTCATAGTTTAGAACGAACTAACG	Set 3	7q21.11	1
D7S820-R	CTGAGGTATCAAAAACTCAGAGG			
D18S51-F	CAAACCCGACTACCAGCAAC	Set 1	18q21.33	1
D18S51-R	AGCCATGTTCATGCCACTG			
D14S1434-F	TGTAATAACTCTACGACTGTCTGTCTG	Set 2	14q32.13	2
D14S1434-R	GAATAGGAGGTGGATGGATGG			
F13A1 -F	GAGGTTGCACTCGAGCCTTTGCAA	Set 2	6p24-p25	2
F13A1 -R	TTCCTGAATCATCCCAGAGCCACA			
D21S11-F	ATATGTGAGTCAATTCCCCAAG	Set 2	21q21.1	1

D21S11-R	TGTATTAGTCAATGTTCTCCAG			
FGA-F	GCCCCATAGGTTTTGAACTCA	Set 1	4q28	1
FGA-R	TGATTTGTCTGTAATTGCCAGC			
F13B-F	TGAGGTGGTGTACTACCATA	Set 1	1q31-q32.1	2
F13B-R	GATCATGCCATTGCACTCTA			

Electrophoretic multiplex groupings



Multiplex PCR setup



ALFexpress loading of individual PCR's (non-multiplexed)

PCR setup procedure

1. Prepare enough WBT (Water-Buffer-Taq) mix for the number of samples to process based on the next table:

	1x	2x	3x	4x
dH_20	66.3 µl	139.23 µl	205.53 µl	271.83 µl
10x Buffer	8.5 µl	17.85 µl	26.35 µl	34.85 µl
Taq	1.7 µl	3.57 µl	5.27 µl	6.97 µl

2. Add 76.5 µL of WBT mix to each 0.2 ml microtube, previously labelled with a unique identifier.

3. Add 8.5 µL of 100 ng/µL genomic DNA dilution to each tube, corresponding to each of the samples to process.

4. Label 2 PCR strips with 8 tubes each (16 in total) with the numbers 1 to 16 for each sample that will be processed. Use different colours for each pair of strips or add a unique identifier for each sample in the first tube of each strip.

5. Add 7.5 µL of the corresponding oligonucleotide mix to each one of the 16 PCR microtubes, where tube #1 will have the F13B mix, tube #8 the F13A1 mix and so on.

PCR components

	Cf	1x	2x	3x	4x
dH_2O		9.425 µl	28.275 µl	37.7 µl	47.125 µl
Buffer	1 x	1.25 µl	3.75 µl	5 µl	6.25 µl
$MgCl_2$	0.5 mM	0.125 µl	0.375 µl	0.5 µl	0.625 µl
dNTPs	0.08 mM	0.1 µl	0.3 µl	0.4 µl	0.5 µl
Primers	0.5 µM	1 µl	3 µl	4 µl	5 µl
Taq	0.04 IU/µL	0.1 µl	0.3 µl	0.4 µl	0.5 µl
DNA 100 ng/µL	25-50 ng	0.5 µl	1.5 µl	2 µl	2.5 µl
Final volumen		12.5 µl	37.5 µl	50 µl	62.5 µl

PCR conditions

STRs	Temperature (°C)	95º	95º	62º	72º	72º	5º	RT
	Time	5’	30”	50”	30”	35’	5’	∞
	Cycles	1	33			1	1	1

Agarose gel electrophoresis conditions

1. Prepare a 3% agarose gel.

2. Mix 5 µl of PCR product with 3 ml of Orange G loading buffer.

3. Add 8 µl of the PCR product-loading buffer mix to each well of the agarose gel and load molecular weight marker in the first and last well of each gel strip.

4. Run for 90 min at 120 VDC.

ALFexpress cassette preparation

1. Clean thermoplates perfectly with a sponge using diluted alkaline extran.

2. Rinse immediately with tap water followed by distilled water flushes.

3. Wipe thermoplates thoroughly with 70% ethanol.

4. Place quartz spacers in their corresponding positions.

5. Add a layer of bind silane to area of gel where wells are to be formed using a small gauze. This must cover the first 4 upper centimetres of the inner face of both thermoplates (the face that is in contact with the comb).

6. Lock the two thermoplates and spacer using corresponding clips (three on each side, one on the upper region of the plate, another in the middle and another in the lower part of the plate).

Polyacrylamide gel preparation (PAGE)

1. Place 30 g of urea in a 100 ml vessel and add 25.2 ml of Triple-distilled water.

2. Mix at 40ºC until the urea is fully dissolved (between 15 - 30 minutes).

3. Calibrate a P100 micropipette to dispense 28µL and defrost a previously prepared 10% ammonium persulfate tube microtube (which is to be kept frozen at -20ºC until use).

4. Add 6 ml of 10x TBE to 100 ml vessel containing water and urea followed by 6 ml of acrylamide, 28 µl of TEMED and 700 µl of 10% ammonium persulfate 10% (10% APS).

5. Mix quickly with a syringe while avoiding bubble formation. Fill syringe completely with the mixture and pour slowly but constantly through the lower edge of the ALFexpress cassette (between both thermoplates).

6. Gently pound thermoplates with finger to prevent bubble formation while casting the gel, especially in the site to be traversed by the laser beam.

7. If a bubble forms in the lower third of the cassette, continue pouring the gel and try to remove the bubble with a small plastic hook (bubble extractor). This device must be available before casting the gel as acrylamide polymerizes readily.

8. Keep pouring the gel until the acrylamide reaches the well region. Keep at least 1 to 5 mL of acrylamide in 100 mL vessel as a polymerization control

9. Place three or four additional clips on well region to press both thermoplates against the comb to form the wells.

10. Allow the gel to polymerize for at least 40 minutes but not longer than 1.5 hours.

11. After the polymerization, place assembled thermoplate cassette in the ALFexpress sequencer and carefully remove the comb, take care to avoid breaking the wells!

12. Prepare 2 l of 1x TBE running buffer and fill both upper and lower ALFexpress chambers to their maximum level. NOTE. ETHIDIUM BROMIDE SHOULD NOT BE ADDED TO THE TBE BUFFER.

13. Use a 60 mL syringe fitted with a P10 tip, using 1X TBE to remove the excess of gel and urea from within the wells.

Polyacrylamide electrophoresis conditions

1. **PRERUN:**

    a. Turn on the computer.

    b. Turn on the ALFexpress instrument.

    c. Open Alf win program.

    d. Choose instrument 1.

    e. File/Open casebook/STRs

    f. Start. Keep running for 30 minutes.

2. **RUN:**

    a. Set voltage to 1500 V

    b. Set power to 30 W

    c. Set laser to ~600

    d. Set run temperature to 50°C

    e. Set run time to 150 minutes.

    f. Set sample volume to 2 ml

PCR products denaturing

1. The PCR product is mixed with an equal volume of formamide denaturing buffer (7.5 ml PCR product plus 7.5 mL denaturing buffer) and the mixture is placed in a thermocycler set at 95°C for 5 min followed by immediate cooling to 5° until removed from cycler.

2. Keep denatured PCR product in ice and load 2 µL into corresponding wells of the polyacrylamide gel.

3. Continue running gel.

Bind silane preparation

1. For 1 mL add 250 mL 10% acetic acid and 3 mL of 3-aminoaminopropiltrietoxi silane to 1 ml of 96º ethanol.

2. Mix and store at 4ºC protected from light with aluminium foil.

10% Ammoniun persulfate preparation

1. Weight 1 gram of ammonium persulfate (APS) and add 10 mL of dH_2O and mix.

2. Dispense 25 aliquots of 700 µl each into 1.5 mL Eppendorf tubes.

3. Store at -20 °C until use.

Molecular weight ladder preparation

- This ladder is based in the amplification of some STR from reference cell lines (Daudi).

DAUDI (all the STRs)		
Set1	HUMTH01	183, 187
	D18S51	(322, 381) (262, 349)
Set2	D13S317	186, 190
	TPOX	230, 242
Set3	D3S1358	135, 142
	D21S11	241, 245
	CSF1PO	318
Set4	vWA	144, 151
	FES	217, 225
Set5	D10S1248	106
	FGA	189, 209
Set6	D16S539	153, 161
	D7S820	203, 211
	F13A1	203, 277
Set7	D14S1434	66
	F13B	169, 176, 220

Daudi (ladder)	
D10S1248	106
vWA	144,151
D16S539	153,161
TH01	183,187
TPOX	230,242
CSF1PO	318

References

1. Moretti TR, Moreno LI, Smerick JB, Pignone ML, Hizon R, Buckleton JS, Bright JA, Onorato AJ. Population data on the expanded CODIS core STR loci for eleven populations of significance for forensic DNA analyses in the United States. Forensic Sci Int Genet. 2016 Nov;25:175-181. doi: 10.1016/j.fsigen.2016.07.022. PMID: 27620707

2. Phillips C, Ballard D, Gill P, Court DS, Carracedo A, Lareu MV. The recombination landscape around forensic STRs: Accurate measurement of genetic distances between syntenic STR pairs using HapMap high density SNP data. Forensic Sci Int Genet. 2012 May;6(3):354-65. doi: 10.1016/j.fsigen.2011.07.012. PMID: 21871851

Revision history

1.0 Original document.

2.0 Changes to document format only.