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Protocol:
Miniplex STR Amplification

I. Purpose
To amplify human DNA using the Miniplex STR primer sets for degraded DNA.

II. Safety
All laboratory safety procedures will be complied with during this procedure.

III. Reagents, Supplies, and Equipment
Reagents
PCR ddH2O
10X ABI Buffer
dNTP mix (2.5mM each)
Amplitaq Gold Taq Polymerase
Nonacetylated BSA
Miniplex primers: Mini 2 (D5S818, D8S1179, D16S539)
Mini 4 (vWA, D18S51, D13S317)
Big Mini (TH01, CSF1PO, TPOX, FGA, D21S11, D7S820)
DNA Standard 9947A, 0.1ng/µL

Supplies
0.2 mL PCR tubes
0.2 mL domed caps
0.6 mL flat cap microcentrifuge tubes
0.5-10 µL pipet tips
10-100 µL pipet tips
Gloves

Equipment
Electronic multichannel pipettor, 0.5-10 µL
10-100 µL pipettor
Applied Biosystems GeneAmp 9700 Thermal Cycler

IV. General
1. Procedure will be used for preparing and amplifying extracted human genomic DNA samples.
2. Procedure will be used as necessary for research.
3. Gloves should be worn at all times.

V. Procedure

1. *Preparation of Reaction Mix*

a. Mix 200 µL dNTP mix, 250 µL 10X Buffer, and 50 µL ddH2O for a total of 500 µL reaction mix into a 0.6 mL microcentrifuge tube Vortex briefly and spin down.

2. *Preparation of Primers for each Locus*

a. Mix 5 µL of 100 µM forward primer and 5 µL of 100 µM reverse primer for each individual locus. Dilute with 40 µL of ddH2O to reach a total volume of 50µL primer mix. [If primer concentration is 200 µM, use 2.5 µL of primer(s) and adjust the amount of ddH2O accordingly so that total volume remains at 50 µL].

******NOTE: Mix the primer set for each individual locus separately. Only one forward primer and one reverse primer should be mixed at a time. Since there are 12 loci, there should be 12 different tubes, each with its own primer set for each locus.******

3. *Preparation of Miniplex Primer Mixes*

a. Mini 2: ratio of loci is 1:1:0.5 (D5, D8, D16) µL per sample – In one 0.6 mL tube, mix together 1 µL of D5, 1 µL of D8, and 0.5 µL of D16 for each sample (add 2 to the sample number for pipetting error).

b. Mini 4: ratio of loci is 1:1:1.4 (vWA, D18, D13) µL per sample – In one 0.6 mL tube, mix together 1 µL of vWA, 1 µL of D18, and 1.4 µL of D13 for each sample (add 2 to the sample number for pipetting error).

c. Big Mini: ratio of loci is 0.4:0.4:0.5:0.6:0.6:0.8 (TH01, CSF, TPOX, FGA, D21, D7) µL per sample – In one 0.6 mL tube, mix together 0.4 µL of TH01, 0.4 µL of CSF, 0.5 µL of TPOX, 0.6 µL of FGA, 0.6 µL of D21, and 0.8 µL of D7 for each sample (add 2 to the sample number for pipetting error).

******Example:** If there are 8 samples, add 2 for pipetting error and calculate the amount of each locus used for 10 total samples. The result for 10 total samples would be to mix:*
Mini 2 – 10 µL of D5, 10 µL of D8 and 5 µL of D16 together in one tube
Mini 4 – 10 µL of vWA, 10 µL of D18 and 14 µL of D13 together in one tube
*Big Mini – 4 µL of TH01, 4 µL of CSF, 5 µL of TPOX, 6 µL of FGA, 6 µL of D21, and 8 µL of D7 together in one tube.****

4. *Preparation of the Master Mix*

a. Add 5 µL per sample of reaction mix to each miniplex primer mix.
b. Add 1 µL per sample of nonacetylated BSA (if BSA is concentrated, dilute 1 µL BSA with 19 µL ddH2O) to each miniplex primer mix.
c. Add 0.4 µL per sample of Taq polymerase to each miniplex primer mix.
d. Add the appropriate amount of ddH2O per sample for each miniplex primer mix to make the final volume 24 µL per sample.
    i. Miniplex 2: 15.1 µL ddH2O per sample
    ii. Miniplex 4: 14.2 µL ddH2O per sample
    iii. Big Mini: 14.3 µL ddH2O per sample

******Example:** If there are 8 samples, add 2 for pipetting error and calculate the amount of each reagent used for 10 total samples per Miniplex set. The result for 10 total samples would be to mix:*

Mini 2 – Add 50 µL reaction mix, 10 µL nonacetylated BSA, 4 µL Taq, and 151 µL ddH2O to the Miniplex 2 primer mix.
Mini 4 – Add 50 µL reaction mix, 10 µL nonacetylated BSA, 4 µL Taq, and 142 µL ddH2O to the Miniplex 4 primer mix.
*Big Mini – Add 50 µL reaction mix, 10 µL nonacetylated BSA, 4 µL Taq, and 143 µL ddH2O to the Big Mini primer mix.****

5. Prepare 0.2 uL tubes in strips for samples to be amplified. Remember to prepare at least one reagent blank and one positive control per Miniplex kit.

6. Pipet out 24 uL of each master mix into the prepared 0.2 uL tubes.

7. Add 1 uL of DNA standard 9947A to the positive control for each set.

8. Add 0.1 ng of sample DNA to appropriately labeled tubes.

9. Cap tubes, flick to mix solution / remove bubbles, and spin down.

10. Place tubes in flat red tray and place tray in Thermal cycler.

11. Set up the following PCR conditions:

Program is: Step 1 – 95oC for 10 minutes warm up; Step 2 – cycle 94oC for 1 minute, 55oC for 1 minute, 72oC for 1 minute (33 cycles); Step 3 – 60oC for 45 minutes, 25oC for infinity.