Protocol for Re-amplifying Allelic Ladders

Reagents/Materials Needed

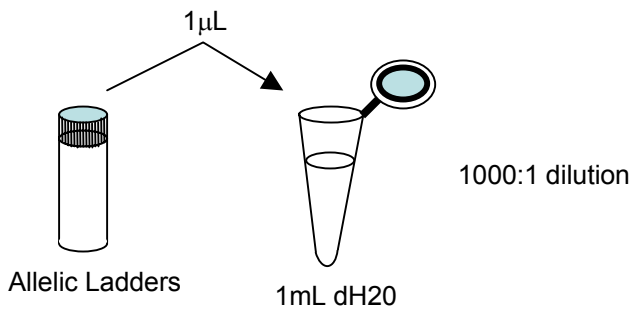
Primer Mix (e.g. NC01, SGM+, etc…)
$MgCl_2$ (25mM)
10X PCR Buffer
10mM dNTPs
BSA (3.2 mg/mL)

TaqGold DNA Polymerase (5 U/μL)
dH_2O
Allelic Ladders (PCR product)

ABI 3100 Capillary Array (36cm - ABI P/N 4315931)
Matrix Standard DS-33 (ABI P/N 4318159)
Hi-Di formamide (ABI P/N 4311320)
GS500 LIZ size standard (ABI P/N 4322682)
1X Genetic Analyzer Buffer w/EDTA (ABI P/N 402824)
POP-6 polymer (ABI P/N 4316357)
GeneScan and Genotyper Software

NOTE !!! Amplified product (do not use in pre-PCR area as it may lead to contamination)

Step 1 – Dilute Allelic Ladders by adding 1 μL of allelic ladder PCR product to 1 mL of dH_20



Step 2 – Use this dilution in the PCR reaction

	20μL PCR 1X Reaction (μL)
PCR Buffer	(n+1)[a] * 2.0
$MgCl_2$	(n+1) * 1.6
Primer Mix	(n+1) * 4.0
dNTPs	(n+1) * 0.5
BSA	(n+1) * 1.0
TaqGold	(n+1) * 0.4
dH_2O	(n+1) * 8.5
M.Mix vol. + diluted ladders (μL)	(n+1) * 18.0 2.0 per sample

[a]The (n+1) refers to the total number of reactions (plus an additional reaction for overfill).

Step 3 – PCR Amplification – Thermal Cycling Conditions

We use the GeneAmp 9700 (Applied Biosystems) in 9600-emulation mode (i.e., ramp speeds of 1 °C/s):

95 °C for 10 minutes

94 °C for 1 minute
55 °C for 1 minute
72 °C for 1 minute
} **15 cycles**

60 °C for 240 minutes **(4 hours)**

25 °C forever

It is necessary to have an extension soak time of 4 hours to promote full adenylation of the PCR products following the amplification cycles.

Step 4 – Run ladders on CE instrument (ABI 3100)

Use 1μL of PCR product to access quality of amplification.

References:
Butler, J.M., Shen, Y., McCord, B.R. (2003) The development of reduced size STR amplicons as tools for analysis of degraded DNA. *J. Forensic Sci* 48(5) 1054-1064.

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