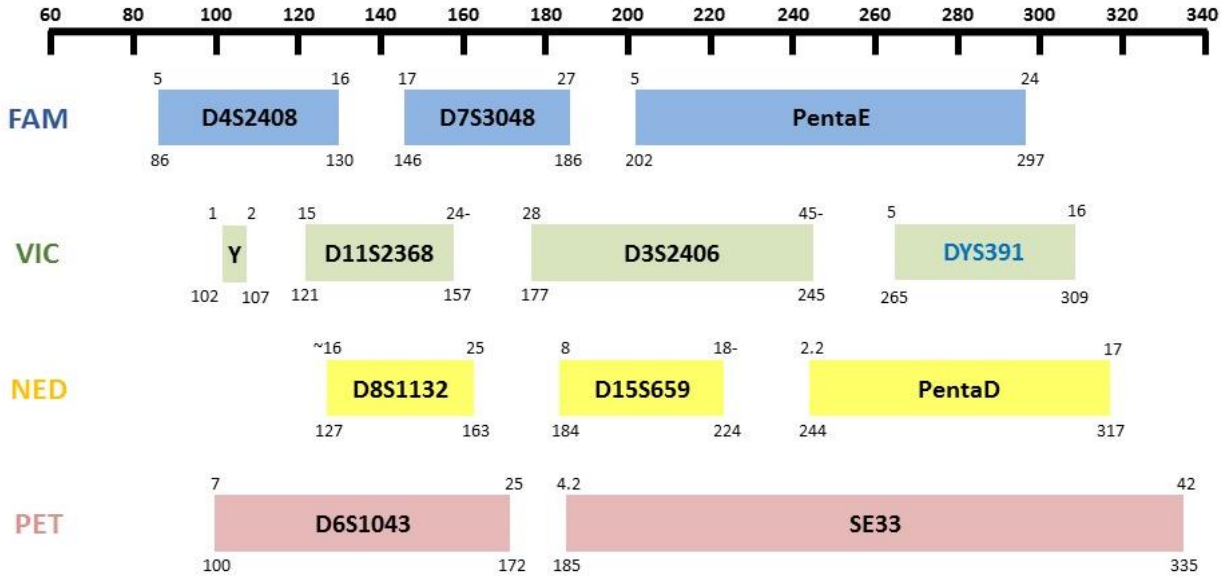
Laplex-12 PCR System Protocol

Allelic size range for Laplex-12 system



5X Primer Mix for Multiplex PCR

Locus		Primer Sequence (5'→3')	Conc. (μM)
D4S2408	F	FAM-AGG TAC ATA ACA GTT CAA TAG AAA GC	1.05
	R	CAA TTC ATC CAC TGA AAT GAC TG	1.05
D7S3048	F	TTG TAC CTT CAA GCT TCA CAA TG	1.15
	R	FAM-CCC GCA GTC AAA AAT CTG ATA	1.15
Penta E	F	GCT ACT CTG GAG GCT GAA ACA	5.00
	R	FAM-TCA TGA TTG ATA CAT GGA AAG AA	5.00
Y-M175	F	CCC AAA TCA ACT CAA CTC CA	5.00
	R	HEX-TCT ACT GAT ACC TTT GTT TCT GTT CA	5.00
D11S2368	F	TTT TAC AAT GAG GTG CAA GAA TG	1.50
	R	VIC-TGG ATG AGT TAG ATG GGT GG	1.50
D3S2406	F	VIC-CCT GGT ACT GCA GTG TTT CTT G	1.60
	R	CCA CAT GGA GAG GCT TTA GAA	1.60
DYS391	F	VIC-CAA GAC ACC CCA CCA CAG AT	4.25
	R	GTG CCA TAG AGG GAT AGG TAG G	4.25

Laplex-12 PCR System Protocol (Continued)

5X Primer Mix for Multiplex PCR (Continued)

Locus		Primer Sequence (5'→3')	Conc. (µM)
D8S1132	F	AGG AAA GGT TAG TGG CTT AAT GT	1.50
	R	NED-TCC CTC TCT CTT TCG AGC AA	1.50
D15S659	F	NED-ACC CTG AAG GCA GTA ATG GTT	1.90
	R	GCT TCC CAA CAT AAC ATA TTG CTT	1.90
Penta D	F	NED-GCA TGG TGA GGC TGA AGT AG	5.00
	R	TAG GTC ATG ATT TTG TGA TAT CTA AG	5.00
D6S1043	F	PET-CAA GGA TGG GTG GAT CAA TAG	1.65
	R	ATT GTA TGA GCC ACT TCC CAT	1.65
SE33	F	GAG TGA AAC TCC GTC RAA AGA A	5.00
	R	PET-CCC CTA CCG CTA TAG TAA CTT G	5.00

Reagents Needed

- 5X Primer Mix for Laplex-12 system
- Gold ST*R 10 X Buffer (Promega, Madison, WI)
- AmpliTaq Gold® DNA Polymerase (Applied Biosystems, Foster City, CA)

PCR Mixture

PCR Component	Vol. (µL)
dH_2O	5.5
5X Primer mix	2.0
Gold ST*R 10X Buffer	1.0
AmpliTaq Gold (5 U/µL)	0.5
Template DNA (0.5 ng/µL)	1.0
Total	10.0

Thermal Cycling

95℃ for 11 minutes, then:

94℃ for 20 seconds
59℃ for 60 seconds
72℃ for 45 seconds
for 29 cycles, then:

60℃ for 30 minutes
4℃ soak

Laplex-12 PCR System Protocol (Continued)

Capillary Electrophoresis

Materials and Reagents Needed

- Dry heating block or thermal cycler
- 3130 Capillary Array, 36 cm (Applied Biosystems, Foster City, CA)
- Performance Optimized Polymer (POP4, Applied Biosystems, Foster City, CA)
- GeneScan™ 500 LIZ™ Size Standard
- Hi-Di™ Formamide (Applied Biosystems, Foster City, CA)

Mixture for CE run

GeneScan™ 500 LIZ™ Size Standard	0.15 µL
Hi-Di™ Formamide	10.0 µL
PCR product	1.0 µL

Denaturation

95℃ for 5 minutes
4℃ soak

3130 Data Collection Software

Application Type	HID
Injection Voltage	3.0 kV
Injection Time	5 seconds
Run Time	22 minutes

Download a PDF copy of this protocol

http://forensic.yonsei.ac.kr/protocols.html

Genotyping result of 2800M control DNA