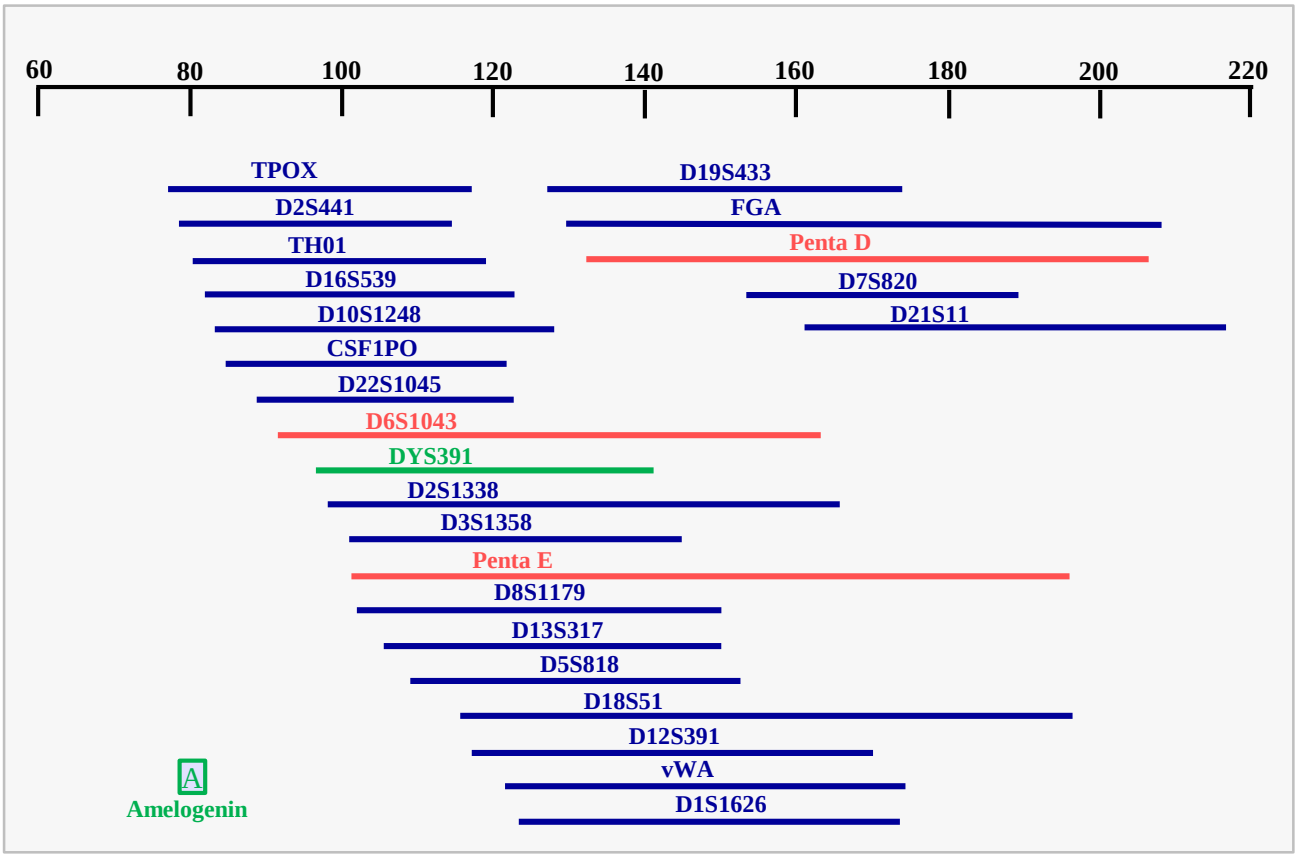
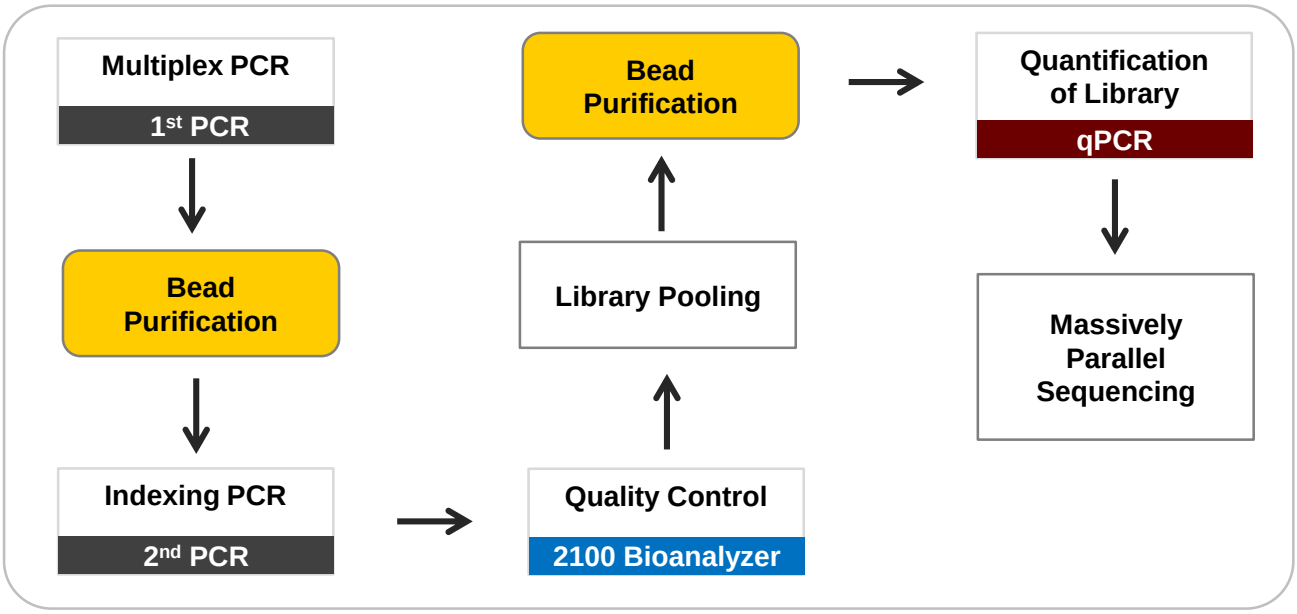
Autosomal STR Analysis of Degraded DNA
using In-house Massively Parallel Sequencing Panel

Target Loci and Size Range



Workflow



Autosomal STR Analysis of Degraded DNA using In-house MPS Panel (continued)

Multiplex PCR

5x Primer Mix for Multiplex PCR

Illumina® Nextera® Adapters	
NT1 sequence	5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG
NT2 sequence	5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G

Locus		Primer Sequence (5'→3')	Conc. (uM)
D1S1656	F	5'-NT1-ctg tgt tgc tca agg gtc aa	1.20
	R	5'-NT2-gag aaa tag aat cac tag gga acc a	1.20
TPOX	F	5'-NT1-gca cag aac agg cac tta gg	1.00
	R	5'-NT2-tcc ttg tca gcg ttt att tgc	1.00
D2S441	F	5'-NT1-gaa ctg tgg ctc atc tat gaa aa	2.25
	R	5'-NT2-aag tgg ctg tgg tgt tat gat	2.25
D2S1338	F	5'-NT1-tgg aaa cag aaa tgg ctt gg	3.25
	R	5'-NT2-agt tat tca gta agt taa agg att gc	3.25
D3S1358	F	5'-NT1-ccc act gca gtc caa tct g	1.65
	R	5'-NT2-atc aac aga ggc ttg cat gt	1.65
FGA	F	5'-NT1-ctg taa cca aaa taa aat tag gca	8.00
	R	5'-NT2-tgt ctg taa ttg cca gca aaa	8.00
D5S818	F	5'-NT1-agg gtg att ttc ctc ttt ggt	2.50
	R	5'-NT2-caa cat ttg tat ctt tat ctg tat cct	2.50
CSF1PO	F	5'-NT1-act gcc ttc ata gat aga aga t	6.00
	R	5'-NT2-ctg tgt cag acc ctg ttc taa gt	6.00
D6S1043	F	5'-NT1-caa gga tgg gtg gat caa tag	1.45
	R	5'-NT2-att gta tga gcc act tcc ca	1.45
D7S820	F	5'-NT1-aag ggt atg ata gaa cac ttg tca t	2.50
	R	5'-NT2-ctc att gac aga att gca cca	2.50
D8S1179	F	5'-NT1-ggc ctg gca act tat atg tat tt	3.25
	R	5'-NT2-cct gta gat tat ttt cac tgt ggg	3.25
D10S1248	F	5'-NT1-aat gaa ttg aac aaa tga gtg ag	2.50
	R	5'-NT2-gga aca act ctg gtt gta ttg tct	2.50
TH01	F	5'-NT1-gat tcc cat tgg cct gtt c	1.00
	R	5'-NT2-cag gtc aca ggg aac aca ga	1.00
vWA	F	5'-NT1-agc cct agt gga tga taa gaa	2.75
	R	5'-NT2-tga taa ata cat agg atg gat gg	2.75
D12S391	F	5'-NT1-cca gag aga aag aat caa cag g	4.50
	R	5'-NT2-ttc ctc taa taa atc ccc tct c	4.50
D13S317	F	5'-NT1-tct gac cca tct aac gcc ta	2.50
	R	5'-NT2-ata ggc agc cca aaa aga ca	2.50

Autosomal STR Analysis of Degraded DNA using In-house MPS Panel (continued)

5x Primer Mix for Multiplex PCR (continued)

Locus		Primer Sequence (5'→3')	Conc. (uM)
Penta E	F	5'-NT1-ggc gac tga gca aga ctc a	8.00
	R	5'-NT2-tgg aaa gaa ttc tct tat ttg gg	8.00
D16S539	F	5'-NT1-ata cag aca gac aga cag gtg	1.10
	R	5'-NT2-agc atg tat cta tca tcc atc tc	1.10
D18S51	F	5'-NT1-gtt gct act att tct ttt ctt ttt ctc	4.00
	R	5'-NT2-gcc act gca ctt cac tct ga	4.00
D19S433	F	5'-NT1-gca aaa agc tat aat tgt acc ac	2.50
	R	5'-NT2-ccc gaa taa aaa tct tct ctc ttt	2.50
D21S11	F	5'-NT1-aat tcc cca agt gaa ttg cc	4.25
	R	5'-NT2-aat agg agg tag ata gac tgg at	4.25
Penta D	F	5'-NT1-tgg aag gtc gaa gct gaa gt	10.00
	R	5'-NT2-tga ttc tct ttt ttt ccc ctt c	10.00
D22S1045	F	5'-NT1-ttt ccc cga tga tag tag tct	7.00
	R	5'-NT2-gtg agt gat cac gcg aat gt	7.00
DYS391	F	5'-NT1-ttc aat cat aca ccc ata tct gtc	3.50
	R	5'-NT2-tgc aag caa ttg cca tag ag	3.50
Amelogenin	F	5'-NT1-cct ttg aag tgg tac cag agc at	2.50
	R	5'-NT2-gca tgc cta ata ttt tca ggg aat aa	2.50

Reagents Needed

- 5 X Primer Mix
- AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)
- Gold ST*R 10 X Buffer (Promega, Madison, WI)

PCR Mixture

PCR Component	Vol. (µl)
dH_2O	10.9
10 X Gold ST*R Buffer	2.0
5 X Primer Mix ¥	4.0
AmpliTaq Gold (5U/µl)	1.1
Degraded DNA	2.0
Total	20.0

¥ 5 x primer mix can be divided into 2 ~ 3 sets

Thermal Cycling

95℃ for 11 minutes, then:

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

72℃ for 5 minutes
4℃ soak

Autosomal STR Analysis of Degraded DNA using In-house MPS Panel (continued)

Bead-based Purification of the 1st PCR Product

Materials and Reagents Needed

Agencourt® AMPure® XP beads (Beckman Coulter, Indianapolis, IN, USA)

80% Ethanol (freshly prepared)

DynaMag™-2 Magnet (ThermoFisher Scientific, Waltham, MA)

Elution buffer or dH_2O

Dry heating block for 26°C and 37°C

Pre-Preparation

- Remove the AMPure® XP beads from storage and let stand for at least 30 minutes to bring them to room temperature
- Pre-heat the heating block to 26˚C
- Freshly prepared 80% ethanol (Needed 600µl of EtOH per each samples)

Protocol

1. Add 30µl of the dH_2O to each sample tubes that include 20µl of the 1st PCR product, and mix by tapping
2. Vortex the AMPure® XP beads until they are well dispersed
3. Add x1.5 well-mixed AMPure® XP beads to each sample tubes, then gently pipette the entire volume up and down 10 times to mix thoroughly
4. Incubate the sample tubes at 26˚C heating block for 10 minutes by gently vortex every 2 minutes
5. Place the sample tubes on the magnetic stand at room temperature, for 2~3 minutes until the solution is clear
6. Carefully remove the supernatant from the each sample tubes, then store on the new EP tube
7. With the sample tubes on the magnetic stand, add 300µl of freshly prepared 80% EtOH to each tube, then move the tube side-to-side in the two positions of the magnet to wash the beads
8. Carefully discard the supernatant without disturbing the pellet
9. Repeat step 7-8 for a second wash
10. Remove the sample tube from the magnetic stand, quickly vortex and spin down, then return to the magnetic stand and incubate for 2 minutes or until the solution clear
11. Carefully discard the remain EtOH without disturbing the pellet
12. Keeping the sample tubes in the magnetic stand, air-dry the beads at room temperature for 5 minutes (Set the heating block to 37°C)

Autosomal STR Analysis of Degraded DNA using In-house MPS Panel (continued)

Protocol (continued)

13. Add 27µl of the elution buffer or dH_2O, then vortex
14. Incubate the sample tubes at 37˚C heating block for 10 minutes by gently vortex every 2 minutes
15. Transfer 25µl supernatant from the sample tubes to the new-EP tubes

Indexing PCR

Reagents Needed

Nextera® XT v2 index kit (Illumina®, Inc., San Diego, CA, USA)

AmpliTaq Gold® DNA Polymerase (ThermoFisher Scientific, Waltham, MA)

Gold ST*R 10X Buffer (Promega, Madison, WI)

PCR Mixture

PCR Component	Vol. (µl)
dH_2O	3.5
10 X Gold ST*R Buffer	2.0
Index 1 (i7)	2.0
Index 2 (i5)	2.0
AmpliTaq Gold (5U/µl)	0.5
Purified 1st PCR product	10.0
Total	20.0

Thermal Cycling

95℃ for 15 minutes, then:

94℃ for 20 seconds
61℃ for 30 seconds
72℃ for 45 seconds
for 12 cycles, then:

72℃ for 5 minutes
4℃ soak

Quality Control

Materials and Reagents Needed

Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA)

Agilent DNA 1000 kit (Agilent Technologies, Inc., Santa Clara, CA, USA)

Protocol

According to the Agilent 2100 Bioanalyzer and DNA 1000 kit User's Manuals

Autosomal STR Analysis of Degraded DNA using In-house MPS Panel (continued)

Bead-based Purification of the Pooled Library

Materials and Reagents Needed

Agencourt® AMPure® XP beads (Beckman Coulter, Indianapolis, IN, USA)

80% Ethanol (freshly prepared)

DynaMag™-2 Magnet (ThermoFisher Scientific, Waltham, MA)

Elution buffer or dH_2O

Dry heating block for 26°C and 37°C

Pre-Preparation

- Remove the AMPure® XP beads from storage and let stand for at least 30 minutes to bring them to room temperature
- Pre-heat the heating block to 26˚C
- Freshly prepared 80% ethanol (Needed 600µl of EtOH per each samples)

Protocol

1. Vortex the AMPure® XP beads until they are well dispersed
2. Add x1.1 well-mixed AMPure® XP beads to 50µl pooled library, then gently pipette the entire volume up and down 10 times to mix thoroughly
3. Same procedure described as bead-based purification of the 1st PCR product
4. Transfer 50µl supernatant from the sample tubes to the new-EP tubes

Quantify Libraries

Materials and Reagents Needed

KAPA Library Quantification Kits (KAPA Biosystems, Wilmington, MA, USA)

AB 7500 Real-Time PCR System (ThermoFisher Scientific, Waltham, MA)

Protocol

According to KAPA Library Quantification Kits User's Manual

Download a PDF copy of this protocol:

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

Updated in 2019/11