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

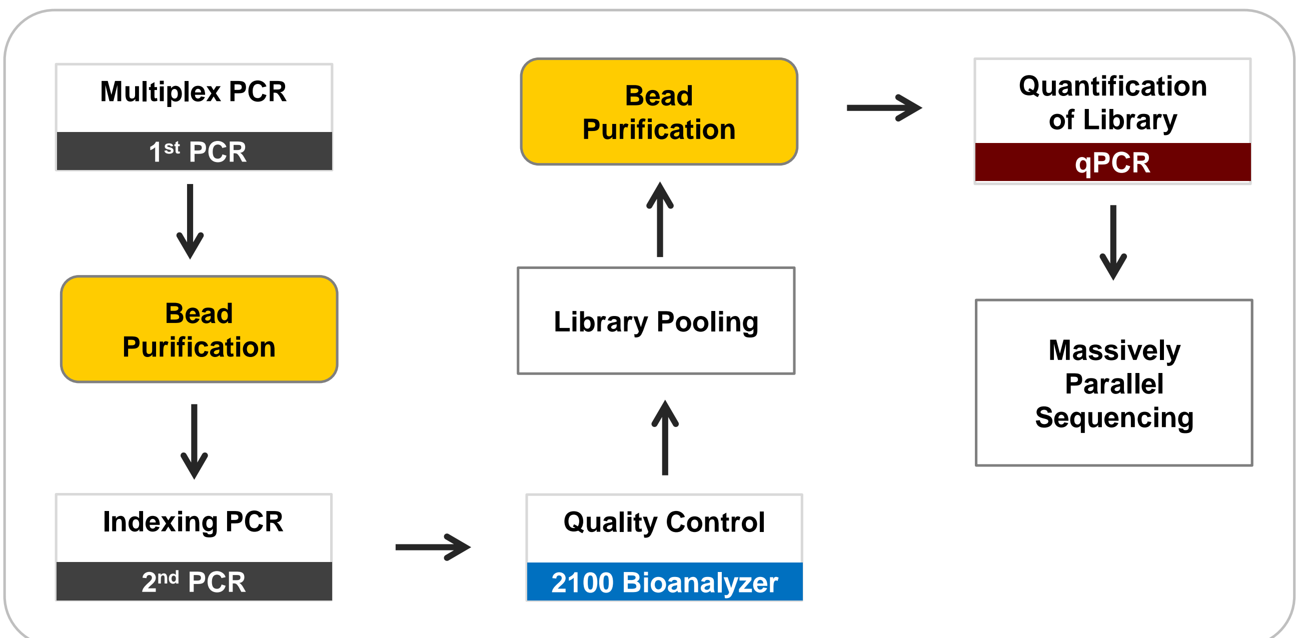
Target Loci and Size Range

80 100 120 140 160 180 200 220 240 260

DYS389 I
DYS438
DYS576
DYS643
DYS391
DYS392
DYS439
DYS393
DYS533
DYS570
DYS481
DYS437
DYS549
YGATAH4
DYS458
DYS456
DYS635
DYS390
DYS19
DYS385ab
DYS448
DYS389 II

Y
M175

Workflow



Y-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)
DYS19	F	5'-NT1-cca tct ggg tta agg aga gtg t	6.50
	R	5`-NT2-ttc act atg act act gag ttt ctg tt	6.50
DYS385ab	F	5`-NT1-gga agg aga aag aaa gta aaa aag aa	9.50
	R	5`-NT2-ttc caa tta cat agt cct cct ttc t	9.50
DYS389 I	F	5`-NT1-caa ctc tca tct gta tta tct atg tat c	2.75
DYS389 II	F	5`-NT1-caa ctc tca tct gta tta tct atg tgt g	8.50
	R	5`-NT2-gat aga ttg ata gag gga ggg a*	8.50
DYS390	F	5`-NT1-caa tgt gta tac tca gaa aca agg a	2.00
	R	5`-NT2-ctg cat ttt ggt acc cca ta	2.00
DYS391	F	5`-NT1-ttc aat cat aca ccc ata tct gtc	1.90
	R	5`-NT2-tgc aag caa ttg cca tag ag	1.90
DYS392	F	5`-NT1-taa acc tac caa tcc cat tcc t	4.00
	R	5`-NT2-ttt gtt att taa aag cca aga agg	4.00
DYS393	F	5`-NT1-tgt ggt ctt cta ctt gtg tca a	1.60
	R	5`-NT2-aaa ctc aag tcc aaa aaa tga gg	1.60
DYS437	F	5`-NT1-gac tat ggg cgt gag tgc at	1.25
	R	5`-NT2-gat aag tag ata gac atc att cac aga	1.25
DYS438	F	5`-NT1-tgg gga ata gtt gaa cgg taa	2.25
	R	5`-NT2-gca aca aga gtg aaa ctc cat t	2.25
DYS439	F	5`-NT1-aca tag gtg gag aca gat aga tga	1.40
	R	5`-NT2-ggc ttg gaa ttc ttt tac cca	1.40
DYS448	F	5`-NT1-aga aag gga gat aga gac atg ga	4.50
	R	5`-NT2-tgg ccg gtc tgg aaa ttt at	4.50
DYS456	F	5`-NT1-ctg ttg tgg gac ctt gtg ata	2.40
	R	5`-NT2-act cag ccc aaa act tct taa a	2.40
DYS458	F	5`-NT1-gca aca gga atg aaa ctc caa	2.30
	R	5`-NT2-ccc aaa gtt ctg gca tta caa	2.30
DYS481	F	5`-NT1-ctc acc aga agg ttg caa gac	1.35
	R	5`-NT2-agg aat gtg gct aac gct gt	1.35

*Primer was used to amplify DYS389 I and DYS389 II commonly.

Y-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)
DYS533	F	5`-NT1-tct tct acc tat cat ctt tct agc	2.75
	R	5`-NT2-tca gtt ctt aac tca acc aaa caa	2.75
DYS549	F	5`-NT1-gtc ccc ttt tcc att tgt ga	3.50
	R	5`-NT2-gca att agg tag gta aag agg aag a	3.50
DYS570	F	5`-NT1-ggc aac cta agc tga aat gc	1.50
	R	5`-NT2-gct gtg tcc tcc aag ttc ct	1.50
DYS576	F	5`-NT1-gcg tat ttg tct tgg ctt ttt	1.65
	R	5`-NT2-cat agc aag acc tca tct ctg aa	1.65
DYS635	F	5`-NT1-tgg ctt ctc act ttg cat aga a	3.50
	R	5`-NT2-gtg gaa cca gcc caa ata tc	3.50
DYS643	F	5`-NT1-tgc ctg gtt aaa cta ctg tgc	1.90
	R	5`-NT2-tcc ccc caa aat tct act ga	1.90
YGATAH4	F	5`-NT1-cta ttc atc cat cta atc tat cca	9.00
	R	5`-NT2-atg ctg agg aga att tcc aa	9.00
Y-M175	F	5`-NT1-tga ttt aaa ctc tct gaa tca ggc	1.30
	R	5`-NT2-tga tac ctt ttt ttc tac tga tac ctt t	1.30

Reagents Needed

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

PCR Mixture

PCR Component	Vol. (μl)
dH_2O	11.0
10 X Gold ST*R Buffer	2.0
5 X Primer Mix ¥	4.0
AmpliTaq Gold (5U/μl)	1.0
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
60℃ for 60 seconds
72℃ for 45 seconds
for 30 cycles, then:

72℃ for 5 minutes
4℃ soak

Y-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)

Y-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 10 X 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 13 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

Y-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 (Thermo Fisher 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