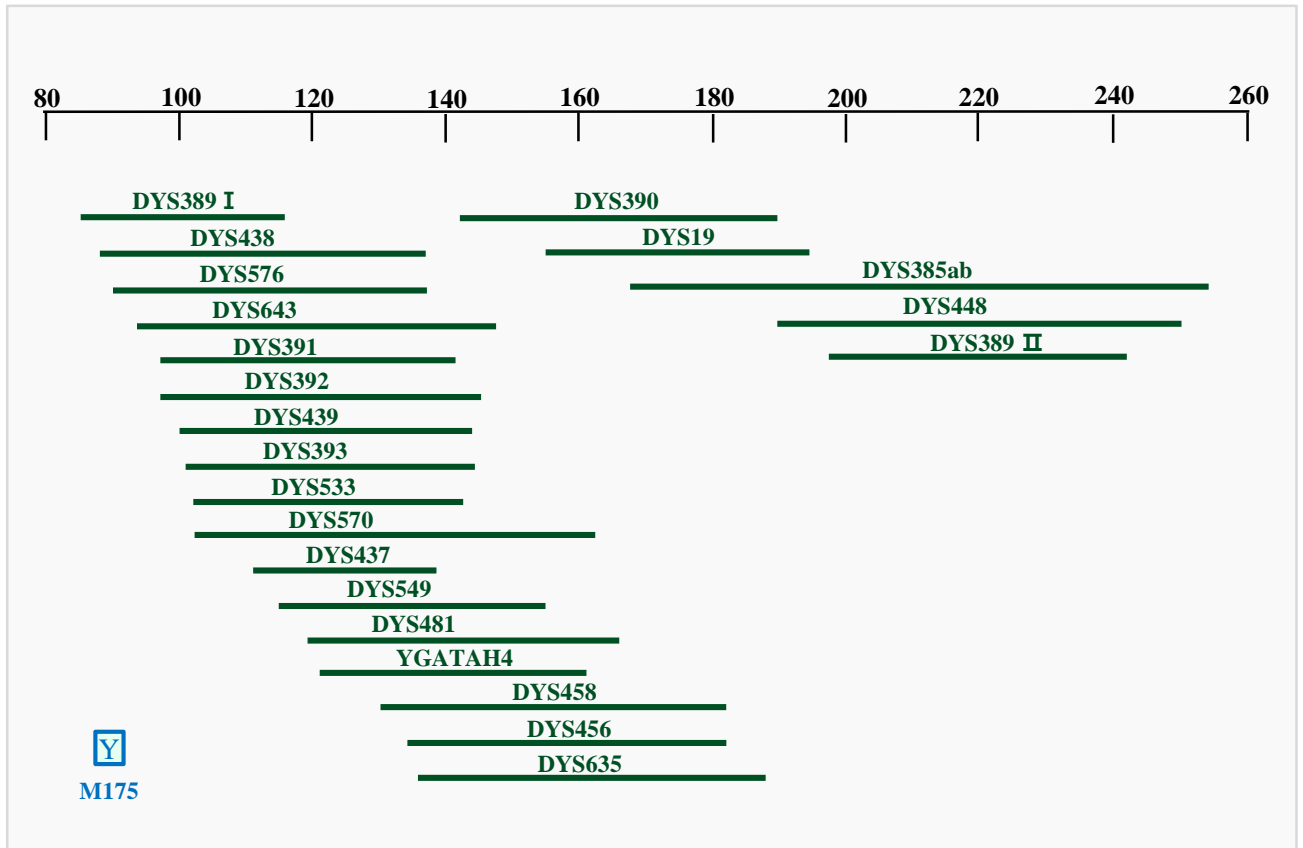
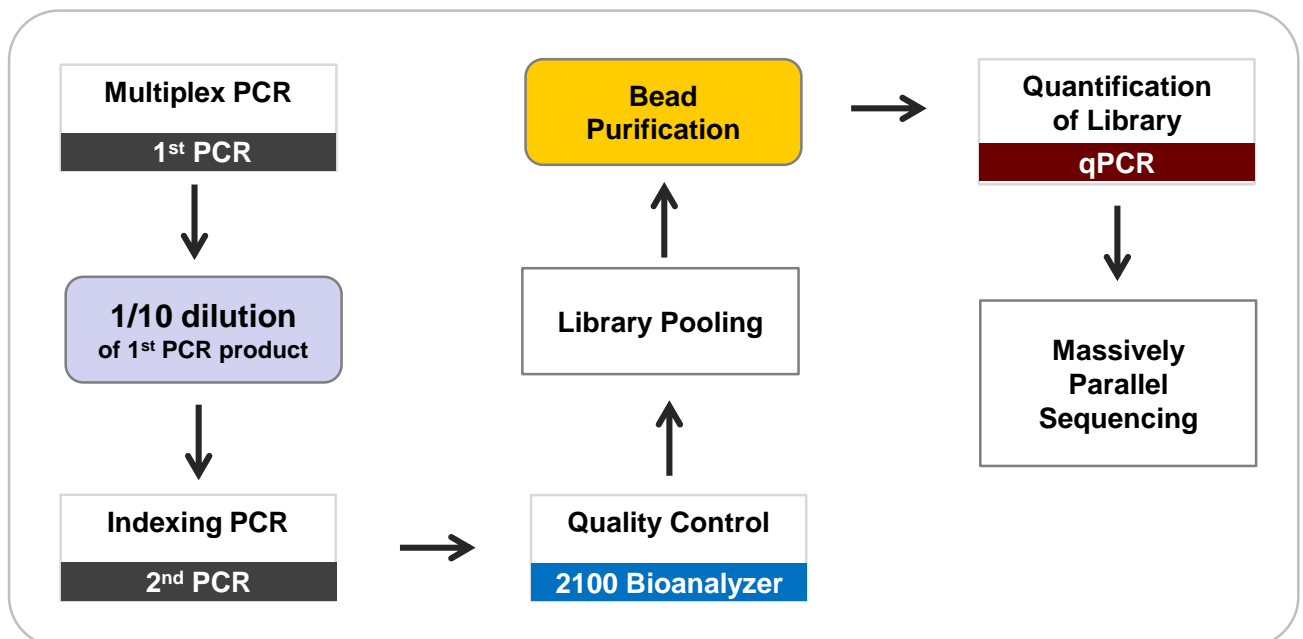


# Y-STR Analysis of Reference Sample using In-house Massively Parallel Sequencing Panel

## Target Loci and Size Range



## Workflow



# Y-STR Analysis of Reference Sample 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
DYS389II	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-gtc tgc taa aag gaa tgt ggc	1.35

\*Primer was used to amplify DYS389 I and DYS389II commonly.

# Y-STR Analysis of Reference Sample 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 <sub>2</sub> O	12.0
10 X Gold ST*R Buffer	2.0
5 X Primer Mix †	4.0
AmpliTaq Gold (5U/µl)	1.0
Template DNA (1ng/ul)	1.0
<b>Total</b>	<b>20.0</b>

## Thermal Cycling

95°C for 11 minutes, then:

94°C for 20 seconds

60°C for 60 seconds

72°C for 45 seconds

for 27 cycles, then:

72°C for 5 minutes

4°C soak

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

# Y-STR Analysis of Reference Sample using In-house MPS Panel (continued)

## 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 <sub>2</sub> O	12.5
10 X Gold ST*R Buffer	2.0
Index 1 (i7)	2.0
Index 2 (i5)	2.0
AmpliTaq Gold (5U/µl)	0.5
1/10 diluted 1 <sup>st</sup> PCR product	1.0
<b>Total</b>	<b>20.0</b>

### Thermal Cycling

95°C for 15 minutes, then:

94°C for 20 seconds  
61°C for 30 seconds  
72°C for 45 seconds  
for 16 cycles, then:

72°C for 5 minutes  
4°C 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  
Finally, normalize the MPS libraries to 10 ng/ul

# Y-STR Analysis of Reference Sample 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<sub>2</sub>O  
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 x1.2 well-mixed AMPure® XP beads to 50µl pooled library, then gently pipette the entire volume up and down 10 times to mix thoroughly
3. Incubate the sample tubes at 26°C heating block for 10 minutes by gently vortex every 2 minutes
4. Place the sample tubes on the magnetic stand at room temperature, for 2~3 minutes until the solution is clear
5. Carefully remove the supernatant from the each sample tubes, then store on the new EP tube
6. 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
7. Carefully discard the supernatant without disturbing the pellet
8. Repeat step 6-7 for a second wash
9. 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
10. Carefully discard the remain EtOH without disturbing the pellet
11. 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 Reference Sample using In-house MPS Panel (continued)

## Protocol (continued)

12. Add 52µl of the elution buffer or dH<sub>2</sub>O, then vortex
13. Incubate the sample tubes at 37°C heating block for 10 minutes by gently vortex every 2 minutes
14. 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>