The Genetics Society of Korea **ICGSK 2019**

STR Analysis of Degraded DNA from Old Skeletal Remains Using Two In-House Next Generation Sequencing Panels

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Introduction

Excavating old skeletal remains of Korean War and Jeju 4.3 victims

➡ **Identifying victims by STR typing**

Introduction

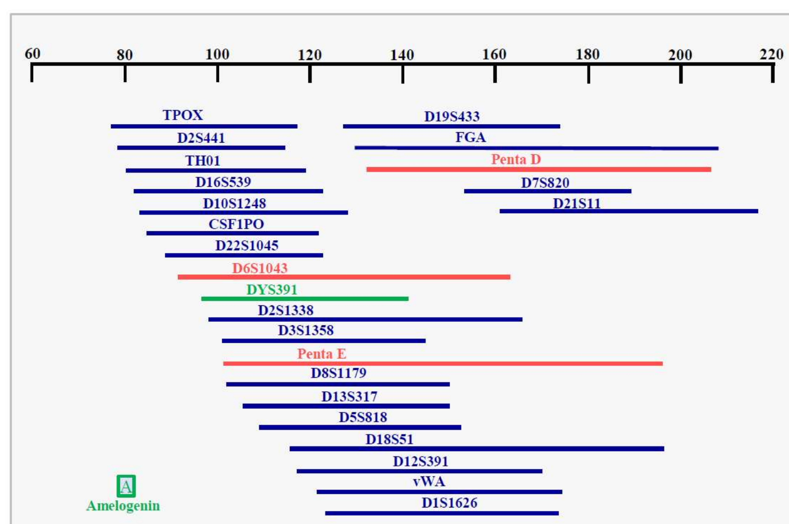
❖ The highly degraded and low copy number (LCN) features of the DNA extracted from old skeletal remains still makes short tandem repeat (STR) genotyping challenging.

❖ Next generation sequencing (NGS) of STRs, which simultaneously could amplify STRs with small sized amplicons, has been suggested to be promising for the analysis of degraded DNA.

❖ Optimized NGS panel and protocol for the STR genotyping of degraded DNA are not available.

Target Autosomal STR and Size Range

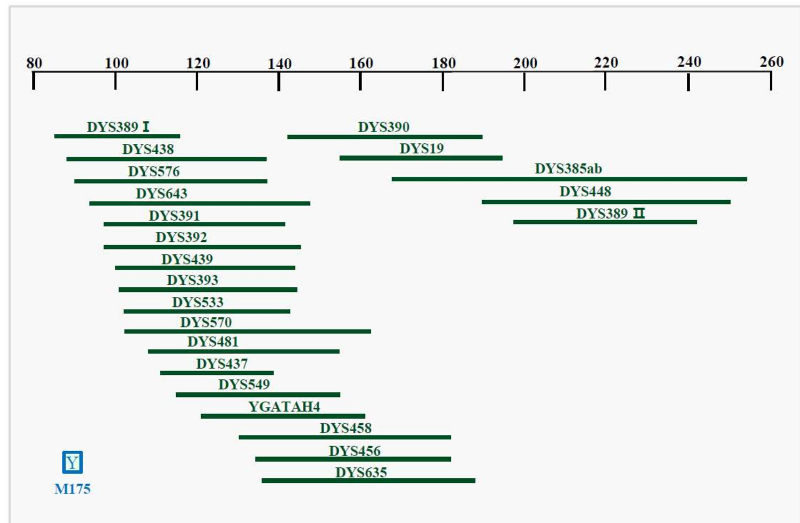
PowerPlex® Fusion Loci and D61043 *upgraded from Kim et al. FSI Genet. 30 (2017)*



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

Y-STR Target and Size Range

PowerPlex® Y23 Loci and Y-M175 *upgraded from Kwon et al. FSI Genet. 25 (2016)*

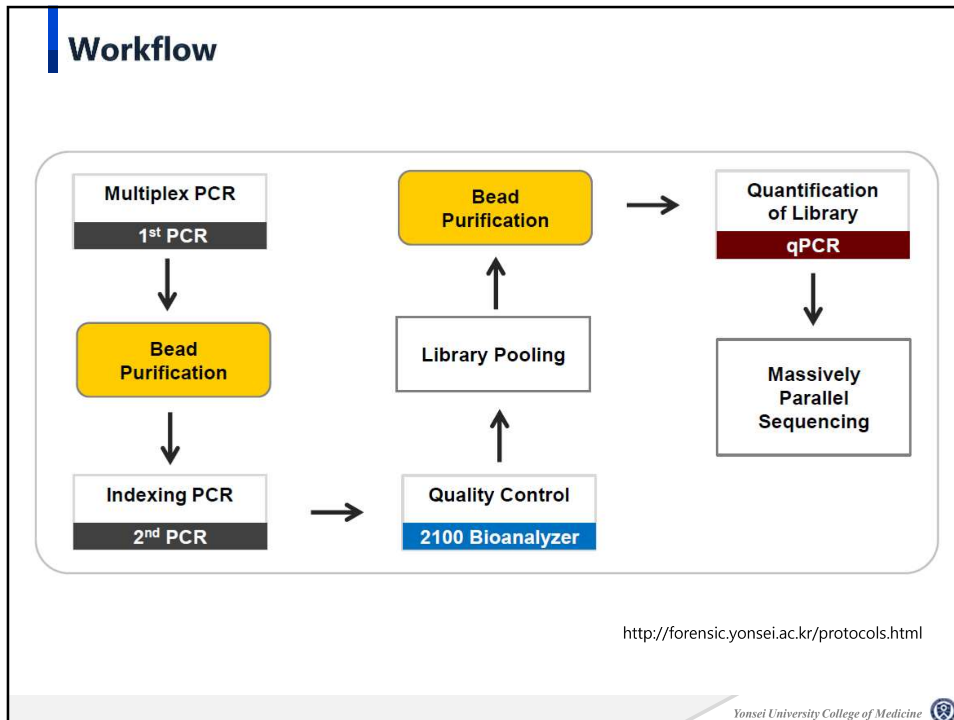


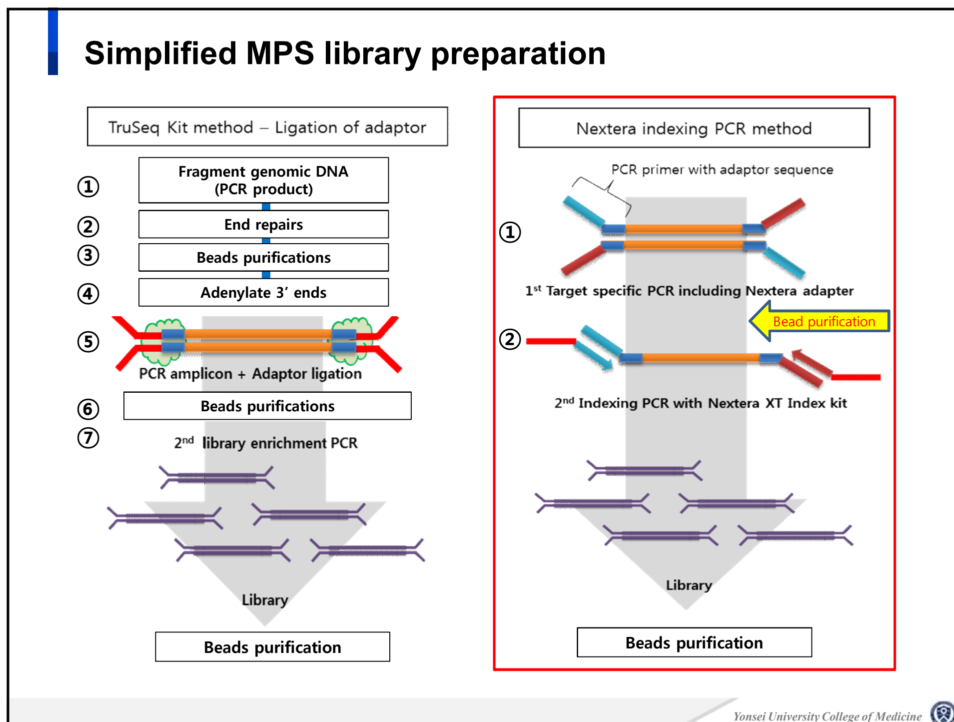
http://forensic.yonsei.ac.kr/protocols.html

Object

❖ **Evaluation of the in-house NGS panels for Autosomal and Y chromosomal STR analysis of degraded DNA**

- Establishing optimized protocol for NGS analysis of STR genotype from degraded DNA
- Comparison of STRs genotyping results between the NGS and capillary electrophoresis results


Workflow
Multiplex PCR
1st PCR
Bead Purification
Indexing PCR
2nd PCR
Quality Control
2100 Bioanalyzer
Library Pooling
Bead Purification
Quantification of Library
qPCR
Massively Parallel Sequencing
http://forensic.yonsei.ac.kr/protocols.html
Yonsei University College of Medicine


Simplified MPS library preparation
TruSeq Kit method – Ligation of adaptor
① Fragment genomic DNA (PCR product)
② End repairs
③ Beads purifications
④ Adenylate 3' ends
⑤ PCR amplicon + Adaptor ligation
⑥ Beads purifications
⑦ 2nd library enrichment PCR
Library
Beads purification
Nextera indexing PCR method
PCR primer with adaptor sequence
① 1st Target specific PCR including Nextera adapter
Bead purification
② 2nd Indexing PCR with Nextera XT Index kit
Library
Beads purification
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Materials and Methods

❖ Two-step PCR condition for Autosomal STR analysis

1st PCR Amplification

PCR mixture	Volume
dH_2O	3.0 µl
10 X Gold ST*R Buffer	2.0 µl
5 X Primer Mix¥	12.0 µl
AmpliTaq Gold (5U/µl)	1.1 µl
Template DNA*	2.0 µl
Fill up to with dH_2O	20.0 µl

Thermal Cycling		
95℃	11 min	
94℃	20 sec	X 29 cycles
59℃	60 sec	
72℃	45 sec	
72℃	5 min	
4℃	Soak	

2nd PCR Amplification

PCR mixture	Volume
dH_2O	3.5 µl
10 X Gold ST*R Buffer	2.0 µl
Index 1 (i7)	2.0 µl
Index 2 (i5)	2.0 µl
AmpliTaq Gold (5U/µl)	0.5 µl
Purified 1st PCR product	10.0 µl
Fill up to with dH_2O	20.0 µl

Thermal Cycling		
95℃	15 min	
94℃	20 sec	X 12 cycles
61℃	30 sec	
72℃	45 sec	
72℃	5 min	
4℃	Soak	

* Template; 100pg, 50pg, 33pg of control 2800M DNA,
2µl of degraded DNA from old skeletal remain DNA

Materials and Methods

❖ Two-step PCR condition for Y-STR analysis

1st PCR Amplification

PCR mixture	Volume
dH_2O	3.0 µl
10 X Gold ST*R Buffer	2.0 µl
5 X Primer Mix¥	12.0 µl
AmpliTaq Gold (5U/µl)	1.1 µl
Template DNA*	2.0 µl
Fill up to with dH_2O	20.0 µl

Thermal Cycling		
95℃	11 min	
94℃	20 sec	X 30 cycles
60℃	60 sec	
72℃	45 sec	
72℃	5 min	
4℃	Soak	

2nd PCR Amplification

PCR mixture	Volume
dH_2O	3.5 µl
10 X Gold ST*R Buffer	2.0 µl
Index 1 (i7)	2.0 µl
Index 2 (i5)	2.0 µl
AmpliTaq Gold (5U/µl)	0.5 µl
Purified 1st PCR product	10.0 µl
Fill up to with dH_2O	20.0 µl

Thermal Cycling		
95℃	15 min	
94℃	20 sec	X 13 cycles
61℃	30 sec	
72℃	45 sec	
72℃	5 min	
4℃	Soak	

* Template; 100pg, 50pg, 33pg of control 2800M DNA,
2µl of degraded DNA from old skeletal remain DNA

Materials and Methods

❖ **DNA samples in duplicate**

- A total of 20 degraded DNAs from more than 50-years-old skeletal remains
  - by using total demineralization and silica column method
- 100pg, 50pg and 33pg of diluted 2800M control DNAs

❖ **Molecular characteristics of DNA samples**

- Assessed by the Quantifiler® Trio DNA Quantification kit (Thermo Fisher Scientific)

❖ **Construction of library by 2-step PCR and quality control**

- Library QC using Agilent 2100 Bioanalyzer

Materials and Methods

❖ **Library pooling and bead purification**

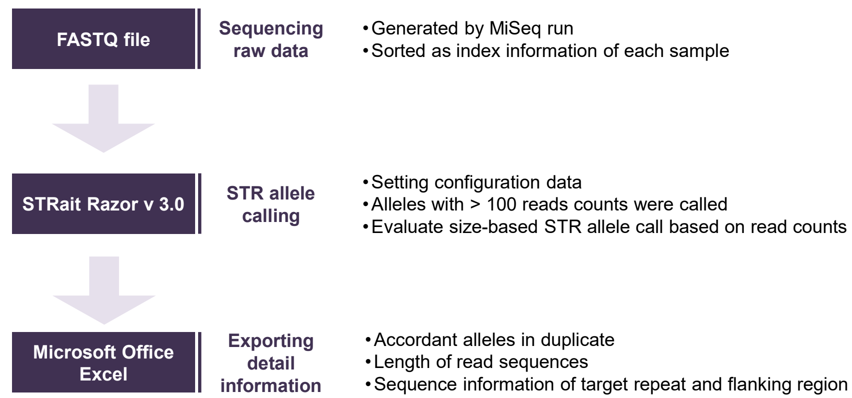
- Normalization and pooling of each library was carried out to 10ng/μl for autosomal STR and Y-STR, respectively
- Pooled libraries were purified with x1.1 Agencourt® AMPure® XP beads

❖ **Library quantification and NGS run**

- Library quantification using KAPA library quantification kit
- NGS on Illumina MiSeq system
  - Illumina MiSeq Reagent Kit v3 (2 x 300 cycles)

Materials and Methods

❖ **MPS data analysis**



Materials and Methods

❖ **Capillary electrophoresis-based STR genotyping**

- The PowerPlex® Fusion System for autosomal STR genotyping
- The PowerPlex® Y-23 System for Y-STR genotyping
- LCN 2800M control DNA and 2μl of degraded DNA from old skeletal remains
- Analyzed with an AB 3130 Genetic Analyzer and GeneMapper®ID v3.2 software

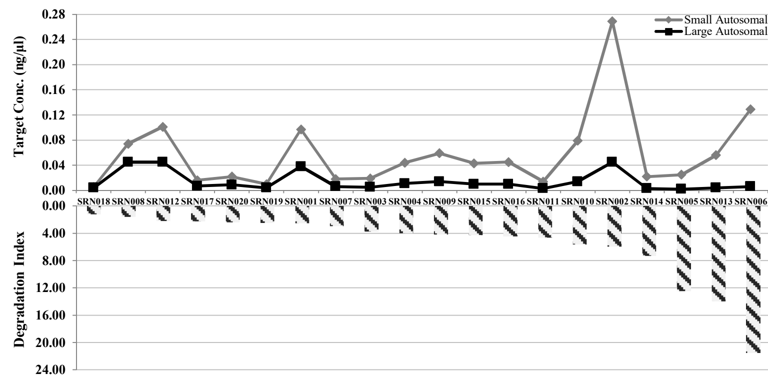
❖ **Genotype analysis**

- All samples has been analyzed on the NGS and CE systems respectively in duplicate
- Repeatedly identified genotype was determined as a final genotype for each markers

Results

❖ **Molecular characteristics for 20 degraded remain DNA**

- The mean concentration of 20 old skeletal remain DNA was 57 pg/μl
- The degradation index value ranged from 1.3 to 21.5



Results

❖ **Library QC using Agilent 2100 Bioanalyzer**

❖ **Sensitivity test using LCN 2800M control DNA**

- Genotype recover rate for 50 pg of 2800M DNA were > 95% on NGS of both autosomal and Y-STRs

Results

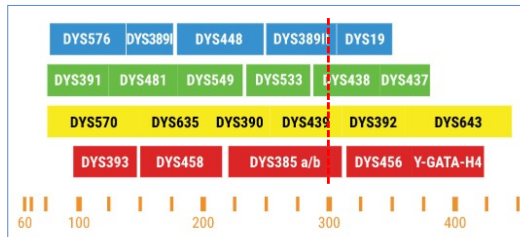
The number of typed samples per Autosomal markers

Markers	via CE	via NGS	Gain
Penta D	**1**	**18**	**+17 (85%)**
D22S1045	**2**	**18**	**+16 (80%)**
Penta E	8	20	+12 (60%)
CSF1PO	6	17	+11 (55%)
D2S1338	9	18	+9 (45%)
D10S1248	10	19	+9 (45%)
D13S317	11	19	+8 (40%)
TPOX	11	19	+8 (40%)
D5S818	10	17	+7 (35%)
D7S820	9	15	+6 (30%)
D19S433	14		
D18S51	15		
D12S391	17		
D2S441	18		
Amelogenin	19		
TH01	20		
D8S1179	19		
D1S1656	16		
FGA	16		
D21S11	14		
D16S539	20	19	-1 (-5%)
D3S1358	18	17	-1 (-5%)
vWA	19	16	-3 (-15%)

Results

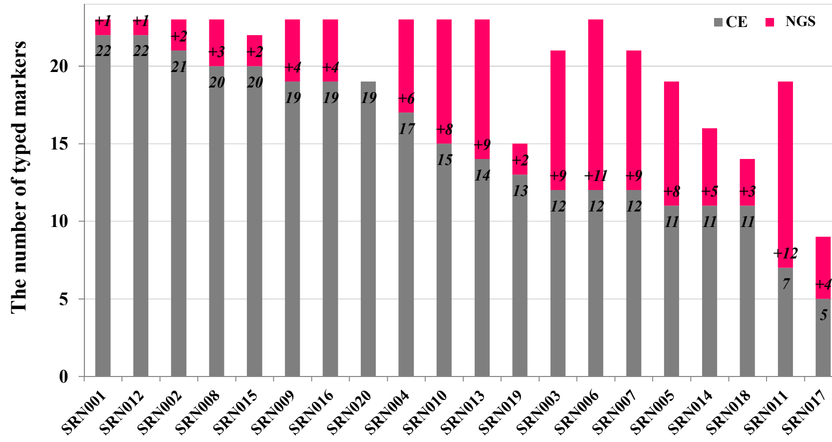
The number of typed samples per Y-STR markers

Markers	via CE	via NGS	Gain
DYS643	**2**	**17**	**+15 (75%)**
DYS19	0	13	+13 (65%)
YGATAH4	2	15	+13 (65%)
DYS438	3	16	+13 (65%)
DYS392	3	15	+12 (60%)
DYS439	5	17	+12 (60%)
DYS437	6	18	+12 (60%)
DYS456	9	17	+8 (40%)
DYS390	9	15	+6 (30%)
DYS549	9		
DYS533	10		
DYS385	9		
DYS481	14		
DYS389II	8		
DYS576	17		
DYS635	14		
DYS448	14		
DYS458	14		
DYS393	16		
DYS391	19	16	-3 (-15%)
DYS570	17	14	-3 (-15%)
DYS389I	18	10	-8 (-40%)



Results

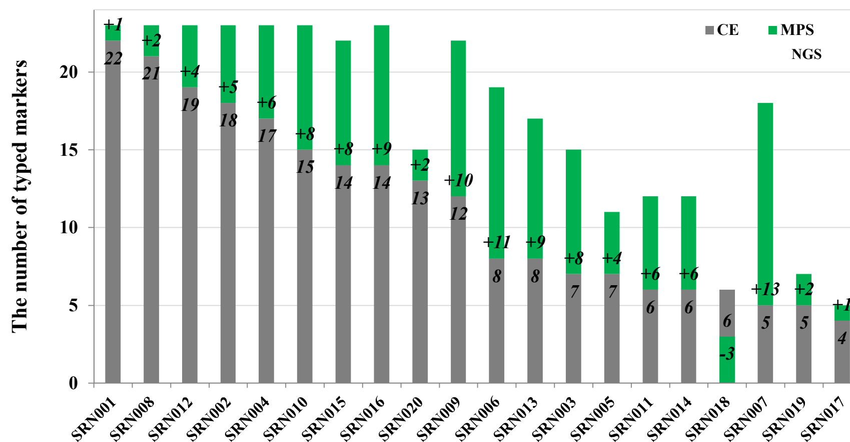
The number of typed markers for 23 autosomal STR markers obtained respectively by CE and NGS from 20 degraded DNA samples



- Samples were presented in descending order of the number of typed markers obtained in the CE analysis

Results

The number of typed markers for 23 Y-STR markers obtained respectively by CE and NGS from 20 degraded DNA samples



- Samples were presented in descending order of the number of typed markers obtained in the CE analysis

Summary

- The in-house NGS panels for autosomal and Y-STRs analysis was able to generate reliable STR genotypes even if the input DNA was as low as 50 pg of the 2800M control DNA.

- NGS of STRs gained more than 5 typed markers on average than the CE methods on both autosomal and Y-STRs analysis for the 20 degraded DNAs.

- Most of gains in the number of typed makers by NGS analysis of STRs for degraded DNA were mainly achieved in the long-length target in CE methods.

- NGS of STRs with small sized amplicons facilitates to increase discrimination power in the identification of old skeletal remains by obtaining quantitatively and qualitatively reliable STR genotypes.

Thank you for your attention!

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Yonsei DNA Profiling Group

Protocols

DNA extraction

- Blood, Buccal Swabs, or Bodyfluid using the QIAamp® DNA Mini Kit
- Hair or Casework sample using the QIAamp® DNA Investigator Kit
- Skeletal remains (bone and tooth)

DNA quantitation

- Using the NanoDrop 1000 Spectrophotometer
- Using the Quantifiler™ Duo DNA Qunatification Kit
- Using the Quantifiler® HP and Trio DNA Quantification Kits

STR genotyping

- Autosomal STRs
  - PowerPlex® Fusion System
  - GlobalFiler™ PCR Amplification Kit
  - Euplex-13 System (Autosomal STRs incorporated for expanded CODIS system)
- X-chromosomal STRs
  - Mentype® Argus X-8 PCR Amplification Kit
  - Multiplex III (DXS10146, DXS10103, DXS10148, DXS10079)
- Y-chromosomal STRs
  - PowerPlex® Y23 System
  - Kplex-Y18 System (Y-STRs compatible with Yfiler PCR Amplification Kit)
  - Euplex-Y17 System (Y-STRs incorporated for PowerPlex Y23 and RM-Y system)

MPS STR analysis

- Referenec sample
  - Autosomal STR protocol and Config file for STRait Razor v3.0
  - Y-STR protocol and Config file for STRait Razor v3.0
- Degraded DNA
  - Autosomal STR protocol and Config file for STRait Razor v3.0
  - Y-STR protocol and Config file for STRait Razor v3.0

Acknowledgement
Most of this presentation materials were prepared by Su-Jin Bae.