Massively Parallel Sequencing of STRs has Gained Number of Typed Markers in the Analysis of Degraded DNA

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Introduction

The highly degraded and low copy number features of the DNA from old skeletal remains still makes short tandem repeat (STR) genotyping challenging. To increase the chance of successful recover of STR genotypes, massively parallel sequencing (MPS), which makes it possible to simultaneously amplify STRs with small sized amplicons, has been suggested to be promising for the analysis of degraded DNA. In this study, two in-house MPS panels which were upgraded by removing minor PCR interferences observed in STR analysis of degraded DNA with the previously developed MPS panels that can analyze 23 autosomal STRs with Amelogenin and 23 Y-STRs. And also, we analyzed 20 DNAs extracted from old skeletal remains using the developed MPS panel and commercial capillary electrophoresis (CE) kits, and will show MPS of STRs could generate more DNA profiles than conventional CE method in the analysis of highly degraded DNA.

Materials and Methods

DNA extraction and quantification A total of 20 degraded DNAs were extracted from more than 50-years-old skeletal remains and the 2800M control DNA (Promega®) was diluted to 100pg, 50pg and 33pg for the sensitivity test. Molecular characteristics of DNA samples were assessed by the Quantifiler® Trio DNA Quantification Kit (Thermo Fisher Scientific).

MPS library preparation The two in-house MPS panels were used to amplify autosomal and Y-STRs including the PowerPlex® Fusion and Y23 ® systems (Promega®). A two-round PCR was performed using Nextera XT v2 index kit (Illumina) to generate MPS libraries, and 1st PCR products and final libraries were purified using a Agencourt® AMPure® XP beads (Beckman Coulter). The quality and quantity of constructed libraries were assessed by an Agilent 2100 Bioanalyzer (Agilent Technologies) and KAPA Library Quantification Kits (KAPA Biosystems), respectively.

Massively parallel sequencing and data analysis The MPS was performed using a MiSeq Reagent Kit v3 (2 x 300 cycles) on the MiSeq System (Illumina). Generated FASTQ files for each sample were sorted as their index information and size-based STR allele was called based on read counts of each markers using the STRait Razor v3.0 program.

Capillary electrophoresis-based STR genotyping Amplification of autosomal and Y-STRs target loci were carried out using the PowerPlex® Fusion and Y23 ® systems (Promega®) and analyzed with an AB 3130 Genetic Analyzer (Thermo Fisher Scientific).

Genotype analysis All samples has been analyzed on the MPS and CE systems respectively in duplicate, and 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 and the degradation index value ranged from 1.3 to 21.5.

Table 1. The number of typed samples per autosomal markers

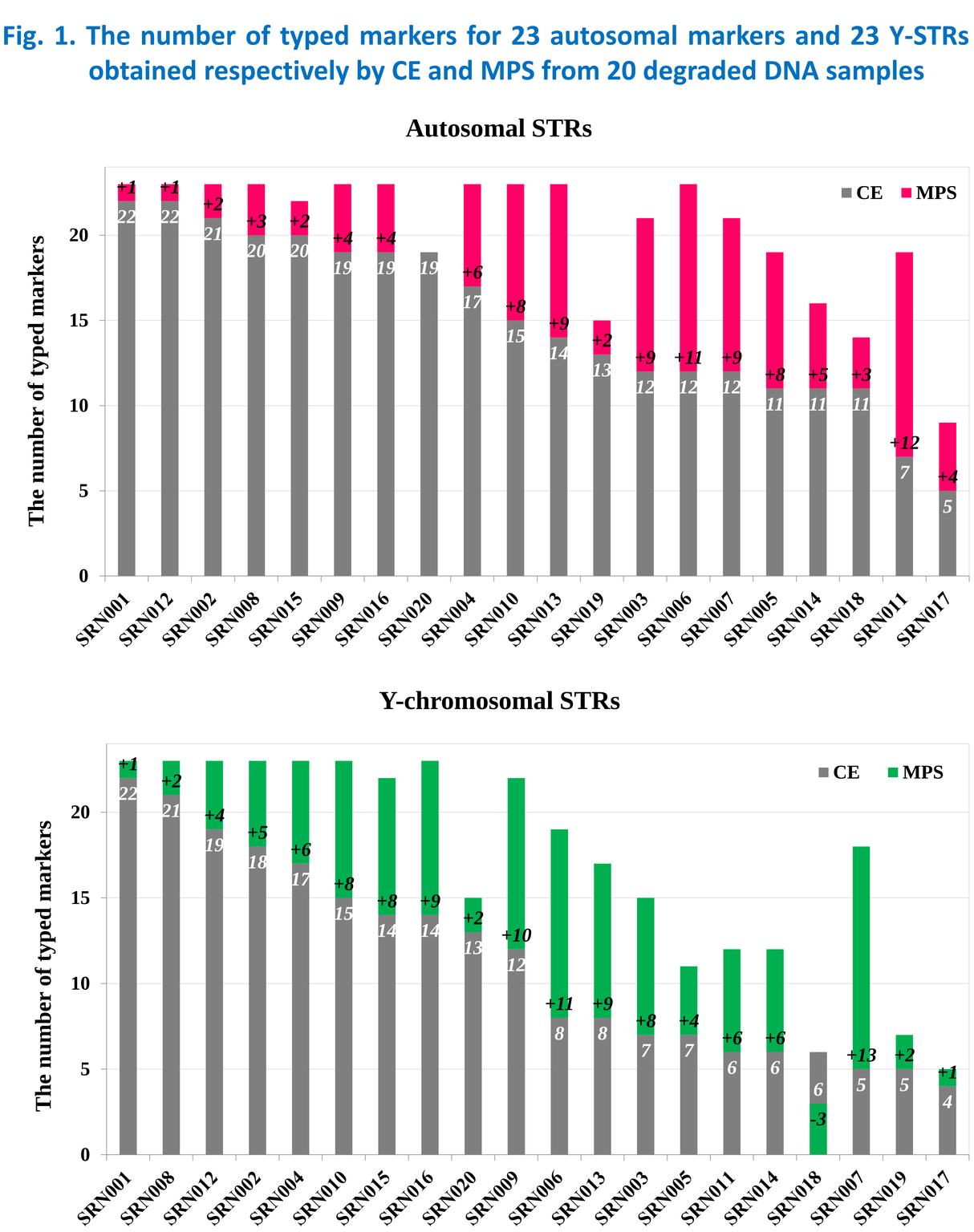
Markers	via CE	via MPS	Gain
Penta D	1	18	+17 (85%)
D22S1045	2	18	+16 (80%)
Penta E	8	20	+12 (60%)
CSF1PO	6	17	+11 (55%)
D2S1338	9	18	+9 (45%)
D10S1248	10	19	+9 (45%)
D13S317	11	19	+8 (40%)
TPOX	11	19	+8 (40%)
D5S818	10	17	+7 (35%)
D7S820	9	15	+6 (30%)
D19S433	14	15	+1 (5%)
D18S51	15	16	+1 (5%)
D12S391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

Table 2. The number of typed samples per Y-chromosomal STRs

Markers	via CE	via MPS	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	14	+5 (25%)
DYS533	10	15	+5 (25%)
DYS385	9	13	+4 (20%)
DYS481	14	18	+4 (20%)
DYS389II	8	11	+3 (15%)
DYS576	17	18	+1 (5%)
DYS635	14	14	-
DYS448	14	13	-1 (-5%)
DYS458	14	13	-1 (-5%)
DYS393	16	14	-2 (-10%)
DYS391	19	16	-3 (-15%)
DYS570	17	14	-3 (-15%)
DYS389I	18	10	-8 (-40%)

- The percentage of the gain was calculated by dividing the number of typed samples by total number of the analyzed sample (n=20).

Fig. 1. The number of typed markers for 23 autosomal markers and 23 Y-STRs obtained respectively by CE and MPS from 20 degraded DNA samples



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

Conclusion

- The two in-house MPS panels for autosomal and Y-STRs was able to generate reliable STR genotypes even if the DNA input was as low as 50 pg of the 2800M control DNA.
- MPS 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.
- The developed MPS panels could generate DNA profiles for long sized STRs that could not amplified by conventional CE kits with degraded DNA.
- MPS 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.