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**CASE REPORT**

**PHYSICAL ANTHROPOLOGY**

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# Autosomal Short Tandem Repeat Analysis of Ancient DNA by Coupled Use of Mini- and Conventional STR Kits*

**ABSTRACT:** Multiplex autosomal short tandem repeat (STR) genotyping enables researchers to obtain genetic information from ancient human samples. In this study, we tested newly developed AmpFℓSTR® MiniFiler™ kit for autosomal STR analysis of ancient DNA (aDNA), using human femurs ($n$ = 8) collected from medieval Korean tombs. After extracting aDNA from the bones, autosomal STR analyses were repeated for each sample using the AmpFℓSTR® MiniFiler™ and Identifiler™ kits. Whereas only 21.87% of larger-sized loci profiles could be obtained with the Identifiler™ kit, 75% of the same loci profiles were determined by MiniFiler™ kit analysis. This very successful amplification of large-sized STR markers from highly degraded aDNA suggests that the MiniFiler™ kit could be a useful complement to conventional STR kit analysis of ancient samples.

**KEYWORDS:** forensic science, autosomal short tandem repeat genotyping, medieval human sample, AmpFℓSTR® MiniFiler™ kit, AmpFℓSTR® Identifiler™ kit, ancient DNA, Korea

Multiplex autosomal short tandem repeat (STR) genotyping enables anthropologists to obtain invaluable genetic information from ancient human samples unearthed in archaeological fields. For instance, Di Nunno et al. (1) identified the discrepancy in STR genotype frequencies between medieval and the present-day populations living in the same geographical region of Italy. This finding supported the hypothesis that there might have been strong Germanic and Asian (Goths, Lombards, Avars) gene flows into the region during the Middle Ages. Various multiplex STR kits also have been used in reconstructing the family tree of historically important people (2–5) and in ruling out possible contamination of ancient samples by modern DNA (4,6,7).

In autosomal STR genotyping of ancient samples, experts preferentially use various commercial kits such as AmpFℓSTR® Profiler Plus™ (Applied Biosystems, Foster City, CA) (4,6–9), AmpFℓSTR® Identifiler™ (Applied Biosystems) (1,10–12), or Powerplex® ES (Promega, Madison, WI) (12) PCR amplification kits. Commercial STR kits effectively reduce the total amount of sample needed for ancient DNA (aDNA) study by simultaneously amplifying numerous STR loci in a single reaction of multiplex PCR. The development of multiplex STR kits was very important to forensic scientists or anthropologists. Because the kits enabled the repetition of experiments to be carried out with smaller amounts of DNA, which may be in the range of low template DNA amounts, they confirmed the autosomal STR profiles of ancient people much more conveniently (4,12,13).

Even so, full autosomal STR profiling of aDNA samples remains difficult to achieve with conventional STR analysis kits, because the fact that endogenous DNA extant in ancient samples often is damaged to the extent that PCR amplification is ineffective. In conventional STR kit analysis of aDNA, the success rate drops with increased PCR product size in each STR locus; accordingly, most of the failures in multiplex STR typing were observed in large-sized loci profiles (4).

In this regard, the newly developed miniSTR kit (e.g., AmpFℓSTR® MiniFiler™ PCR Amplification kit; Applied Biosystems) has PCR primers positioned as close as possible to the STR region. In the case of highly degraded DNA samples, larger-sized loci (i.e., D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, and FGA in Identifiler™ kit assay) have been more efficiently amplified by MiniFiler™ than by conventional STR kits (13–20). What is more, the MiniFiler™ kit's STR profiles have shown a high concordance with those of standard STR kits in agreement with previous data (13,20).

Nevertheless, it has remained uncertain whether the miniSTR kit could be used complementary to conventional STR kit analysis of aDNA samples, given that endogenous DNA therein is far more degraded than in forensic samples. To augment the very limited

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data on the merits in miniSTR kit assay for archaeologically obtained samples, we analyzed aDNA extracted from medieval Korean skeletons using mini- and conventional STR kits.

## Materials and Methods

A total of eight human femurs collected from 16th to 18th century Korean tombs were used in this study. The surfaces of the bones were removed using a sterilized knife, after which they were exposed to UV irradiation for 20 min, and subsequently immersed in 5.4% (w/v) sodium hypochlorite. After the samples were washed with distilled water and absolute ethanol, they were air-dried and pulverized to a fine powder using a SPEX 6750 Freezer/Mill (SPEX SamplePrep, Metuchen, NJ) (21,22). Bone fragments (0.3–0.5 g) were then removed from the femurs and incubated in 1 mL of lysis buffer (EDTA 50 mM, pH 8.0; 1 mg/mL of proteinase K; SDS 1%; 0.1 M DTT) at 56°C for 24 h. Total DNA was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) and then treated with chloroform/isoamyl alcohol (24:1). DNA isolation and purification was performed using a QIAmp PCR purification kit (Qiagen, Hilden, Germany). The purified DNA was eluted in 70 μL of EB buffer (Qiagen) (23–26).

The DNA extracted from the femur samples was amplified with the Quantifiler™ kit (Applied Biosystems), showing the total amount of *amplifiable* human DNA remaining in each sample. All of the procedures were carried out in accordance with the manufacturer's instructions. The Quantifiler™ data were analyzed by 7000 system SDS software version 1.2.3 (Applied Biosystems).

Autosomal STR analyses were repeated 10 times for each sample with AmpFℓSTR® MiniFiler™ and Identifiler™ kits (i.e., five times for each). Briefly, after 10 μL of sample DNA was amplified by MiniFiler™ or Identifiler™ kit in accordance with manufacturer's instructions, amplified products were analyzed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). When allelic profiles were reproducible in at least three of the five replicates, they were regarded authentic, consensus profiles (27). Peak height thresholds for the MiniFiler™ and Identifiler™ kits were set at 50 and 100 relative fluorescence units, respectively.

In the course of sampling and laboratory works, we always wore protection gloves, masks, gowns, and head caps. Our aDNA laboratory facilities were set up in accordance with the protocol of Hofreiter et al. (28): the rooms for aDNA extraction or PCR preparation were physically separated from our main PCR laboratory; further, the DNA extraction/PCR preparation rooms were equipped with night UV irradiation, isolated ventilation, and laminated flow hoods. Other criteria for authentic aDNA analysis, suggested by Willerslev and Cooper (29), were also followed (Data S1). To determine whether the samples had incurred any modern DNA contamination, the autosomal STR profiles of the researchers involved in this study were determined (under the permission of Institutional Review Board of Seoul National University, H-0909-049-295) to be then compared with the ancient sample STR profiles.

## Results

The overall results of MiniFiler™ and Identifiler™ kit analyses are summarized in Table 1. As can be seen, none of the STR profiles of the ancient samples was found in those of the researchers participating in the current study, confirming the authenticity of those results. In the Identifiler™ kit analysis (total locus number = 15), consensus profiles were obtained in 44 of 120 autosomal STR loci (36.7%). Of these, 53.57% (30 of 56) were smaller-sized loci (i.e., D8S1179, D3S1358, TH01, D19S433, vWA, TPOX, and D5S818), while 21.87% (14 of 64) were larger-sized loci (i.e., D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, and FGA). The range of the number of loci in which consensus profiles were obtained by Identifiler™ kit analysis was 0–12 per sample (average = 5.5 ± 5.3). The range of the number of larger-sized loci showing consensus profiles was 0–5 per sample (average = 1.8 ± 2.2).

Considering that large-sized locus profile cannot easily be obtained from degraded or inhibited DNA samples (4), the failure of the Identifiler™ kit analysis in large-sized STR locus profiling is understandable. To increase the success rate of large-sized locus profiling, we performed MiniFiler™ kit analysis (total locus number = 8) on the same eight skeletons. At this time, 75% (48 of 64) of the STR loci profiles could be determined. The range of the number of larger-sized loci where consensus profiles were obtained also increased to 2–8 per sample (average = 6 ± 2).

We found no significant improvement in the number of loci profiles when the single use analysis of MiniFiler™ kit (48/49 = 97.96%) was compared with combined Identifiler™ and MiniFiler™ kit analyses (49/49 = 100%). However, the number of 15-Identifiler locus profiles reached as high as 65.83% (79/120 loci) with the combined use of MiniFiler™ and Identifiler™ kit analyses, a remarkable improvement over the result attained with the Identifiler™ kit alone (36.7%, 44/120 loci).

Full concordance of loci profiles between the MiniFiler™ and Identifiler™ kit analyses was observed only in 12.24% (6 of 49) of STR loci. The number of locus profile differences was 43, accounting for all of the STR loci in the MiniFiler™ kit, including D7S820 ($n$ = 4), D21S11 ($n$ = 4), D18S51 ($n$ = 5), CSF1PO ($n$ = 8), D13S317 ($n$ = 7), D16S539 ($n$ = 4), D2S1338 ($n$ = 7), and FGA ($n$ = 4) (Table 2). In the Identifiler™ and MiniFiler™ kit analyses, we found some loci showed potential dropouts in the small-sized alleles (six in Identifiler™ and 10 in MiniFiler™ analysis), possibly as a result of processing a small number of starting DNA templates during the PCR (Table 1).

## Discussion

Generally, concordances between mini- and conventional STR kits reported in previous studies have been very high in the case of modern human samples. Hill et al. (13) observed a 99.7% concordance in the combined use of the MiniFiler™ and Identifiler™ kits with various human population groups. Alenizi et al. (20) also showed a 99.88% of concordance between the same kits for a Kuwaiti population.

However, we also believe that the differences in loci profiles obtained in our study pose no challenge to the authenticity of MiniFiler™ and Identifiler™ analyses, given that most of them were likely to have been caused by potential allelic drop-outs in the Identifiler™ kit assay (Table 2). Otherwise, the differences seem to be due mainly to the superior success rate of MiniFiler™ kit analysis with degraded or inhibited aDNA that was not easily identified in the conventional Identifiler™ kit assay.

Over the past decades, there have been a number of STR genotyping studies on human remains found in archaeological fields. Most of them have used conventional multiplex STR kits such as AmpFℓSTR® Profiler Plus™, AmpFℓSTR® Identifiler™, or Powerplex® ES (1,4,6–12). However, autosomal STR analysis of aDNA samples with conventional STR kits remains a challenge, owing to the poorer preservation status of such samples. MiniSTR kits, therefore, could be useful for determination of larger-sized STR loci profiles that cannot easily be obtained with conventional STR kits. In this regard, recent report of Vanek et al. (30), in which study

TABLE 1—*Autosomal short tandem repeat analyses repeated 10 times (i.e., 5 for MiniFiler™ and 5 for Identifiler™) for each individual sample.*

| Kit | Exp. | D8 S1179 | D21 S11 | D7 S820 | CSF1PO | D3 S1358 | TH 01 | D13 S317 | D16 S539 | D2 S1338 | D19 S433 | vWA | TPOX | D18 S51 | D5 S818 | FGA | Quantifiler (pg/uL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SN 1-32 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 28,– | – | 11, – | | | 12, – | 11, – | **25, –** | | | | – | | – | 3.39 |
| | 2 | | 28,30.2 | – | 11, – | | | 12, – | 11,12 | 17,25 | | | | **23, –** | | **24, –** | 3.39 |
| | 3 | | 28,30.2 | 10,11 | 11,12 | | | 12, – | – | 17,25 | | | | 13,23 | | 19,24 | 10.4 |
| | 4 | | 28,30.2 | 10,11 | 11,12 | | | 12, – | – | 17,25 | | | | 13,23 | | 19,24 | 10.4 |
| | 5 | | 28,30.2 | 10,11 | 11,12 | | | 12, – | – | 17, – | | | | 13,23 | | 19,24 | 10.4 |
| | Cons | | 28,30.2 | 10,11 | 11,12 | | | 12, – | – | 17,25 | | | | 13,23 | | 19,24 | |
| ID | 1 | – | 28, – | – | – | – | – | – | – | – | – | 17, – | 8, – | – | – | – | 3.39 |
| | 2 | – | 28, – | – | – | – | – | – | – | – | – | 17, – | 8, – | – | – | – | 3.39 |
| | 3 | 11,15 | 28,30.2 | 10,11 | 11, – | 16,17 | 9, – | – | 11,12 | 25, – | 13,15 | 17,18 | 8, – | 13,23 | 12,13 | 19,24 | 10.4 |
| | 4 | 11,15 | 28,30.2 | 10,11 | 11,12 | 16,17 | 9, – | 12, – | 11,12 | 17,25 | 13,15 | 17, – | 8, – | 13, – | 12,13 | – | 10.4 |
| | 5 | 11, – | 28,30.2 | 10, – | 11,12 | 16,17 | 9, – | 12, – | 11,12 | 17, – | 13,15 | 17,18 | 8, – | 13,23 | 12, – | – | 10.4 |
| | Cons | 11, – | 28,30.2 | 10, – | 11, – | 16,17 | 9, – | – | 11,12 | – | 13,15 | 17, – | 8, – | 13, – | 12, – | – | |
| MF+ID | | 11, – | 28,30.2 | 10,11 | 11,12 | 16,17 | 9, – | 12, – | 11,12 | 17,25 | 13,15 | 17, – | 8, – | 13,23 | 12, – | 19,24 | |
| SN 4-18-1 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 31, – | 11, – | 9,11 | | | 10, – | – | – | | | | – | | – | 20.2 |
| | 2 | | 31, – | 11, – | 9,11 | | | 10,13 | 10,11 | 20,24 | | | | 15,16 | | 24,28 | 20.2 |
| | 3 | | 31, – | 11, – | 9,11 | | | 10,13 | – | **24, –** | | | | 15,16 | | – | 20.2 |
| | 4 | | 31, – | – | 9,11 | | | 10,13 | – | 20,24 | | | | **16, –** | | 24,28 | 23.1 |
| | 5 | | 31, – | 11, – | **11, –** | | | 10,13 | 10, – | 20,24 | | | | 15,16 | | 24,28 | 23.1 |
| | Cons | | 31, – | 11, – | 9,11 | | | 10,13 | – | 20,24 | | | | 15,16 | | 24,28 | |
| ID | 1 | 14, – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 20.2 |
| | 2 | 13, – | – | – | – | – | 9, – | – | 10, – | – | – | 17,18 | – | – | 9, – | – | 20.2 |
| | 3 | – | – | – | – | – | – | – | – | – | – | 17,18 | – | – | – | – | 20.2 |
| | 4 | 14, – | 31, – | – | – | 15, – | 9, – | 10, – | 11, – | – | 16.2, – | 17,18 | 8, – | 15, – | 9,13 | 24, – | 23.1 |
| | 5 | 14, – | 31, – | – | – | 15, – | 9, – | 10, – | 11, – | – | 14,16.2 | 17,18 | 9, – | – | 13, – | – | 23.1 |
| | Cons | 14, – | – | – | – | – | 9, – | – | – | – | – | 17,18 | – | – | – | – | |
| MF+ID | | 14, – | 31, – | 11, – | 9,11 | – | 9, – | 10,13 | – | 20,24 | – | 17,18 | – | 15,16 | – | 24,28 | |
| SN 4-18-2 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 29,31 | 10,12 | 10,12 | | | – | 10,11 | **24, –** | | | | – | | – | 12.8 |
| | 2 | | 29,31 | 10, – | 10, – | | | 11, – | 10,11 | **24, –** | | | | – | | 26, – | 12.8 |
| | 3 | | 29,31 | 10, – | 10,12 | | | 11, – | 10,11 | 17, – | | | | – | | 26, – | 12.8 |
| | 4 | | 29,31 | 10,12 | 10,12 | | | 11, – | 10,11 | 17,24 | | | | 14,15 | | 21,26 | 12.6 |
| | 5 | | 29,31 | 10,12 | 10,12 | | | 11, – | 10,11 | 17,24 | | | | 14,15 | | 21,26 | 12.6 |
| | Cons | | 29,31 | 10,12 | 10,12 | | | 11, – | 10,11 | 17,24 | | | | – | | 26, – | |
| ID | 1 | 10, – | 29, – | 10, – | – | 17, – | 6, – | 11, – | 11, – | – | 14, – | 14, – | 8, – | – | 9,11 | – | 12.8 |
| | 2 | 10, – | 29, – | – | OL | 17,18 | 6, – | – | – | – | 14, – | 14, – | – | 14, – | **11, –** | – | 12.8 |
| | 3 | 10,11 | **31, –** | – | 12, – | 17,18 | 6, – | – | – | – | 14, – | 14, – | – | – | – | – | 12.8 |
| | 4 | 10,11 | 29,31 | – | – | – | 6, – | – | 10,11 | – | 14, – | 14, – | 8,11 | – | 9,11 | – | 12.6 |
| | 5 | 10,11 | 29,31 | – | – | 17,18 | 6, – | 11, – | 10,11 | – | 14, – | 14, – | 8,11 | – | 9,11 | 21, – | 12.6 |
| | Cons | 10,11 | 29,31 | – | – | 17,18 | 6, – | – | 11, – | – | 14, – | 14, – | 8, – | – | 9,11 | – | |
| MF+ID | | 10,11 | 29,31 | 10,12 | 10,12 | 17,18 | 6, – | 11, – | 10,11 | 17,24 | 14, – | 14, – | 8, – | – | 9,11 | 26, – | |
| SN 4-25-2 | | | | | | | | | | | | | | | | | |
| MF | 1 | | – | 11, – | 11, – | | | 11, – | – | – | | | | – | | – | 3.47 |
| | 2 | | – | 11, – | 11,13 | | | 11, – | 9, – | 20, – | | | | 13,15 | | 22, – | 3.47 |
| | 3 | | 29,31 | – | 11, – | | | 11, – | – | 24, – | | | | 15, – | | 22, – | 3.47 |
| | 4 | | – | – | – | | | 11, – | 8, – | – | | | | – | | – | 17.2 |
| | 5 | | – | – | – | | | – | – | – | | | | – | | – | 17.2 |
| | Cons | | – | – | 11, – | | | 11, – | – | – | | | | – | | – | |
| ID | 1 | 13,14 | – | 10, – | – | 16, – | – | – | – | – | – | 18, – | – | – | – | – | 3.47 |
| | 2 | – | 29,OL | – | 11, – | – | – | – | – | – | 15, – | 15, – | – | – | – | – | 3.47 |

*continued*

TABLE 1— *Continued.*

| Kit | Exp. | D8 S1179 | D21 S11 | D7 S820 | CSF1PO | D3 S1358 | TH 01 | D13 S317 | D16 S539 | D2 S1338 | D19 S433 | vWA | TPOX | D18 S51 | D5 S818 | FGA | Quantifiler (pg/uL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | – | – | 11, – | – | 16, – | – | – | – | – | 15, – | – | 11, – | – | – | – | 3.47 | |
| 4 | – | – | – | – | – | – | – | – | – | – | – | – | 15, – | – | – | 17.2 | |
| | 5 | – | – | – | – | – | – | – | – | – | – | 18, – | – | – | – | – | 17.2 |
| | Cons | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | |
| MF+ID | | – | – | – | 11, – | – | – | 11, – | – | – | – | – | – | – | – | – | |
| SH 2-10-1 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 28,31 | 11,12 | 11,13 | | | 10,12 | 11,12 | 17,23 | | | | 16, – | | 22,24 | 12 |
| | 2 | | 28,31 | 11,12 | 11,13 | | | 10,12 | – | 17,23 | | | | 16, – | | 22,24 | 12 |
| | 3 | | 28,31 | 11,12 | 11,13 | | | 10,12 | 11,12 | 17,23 | | | | 16, – | | 22,24 | 12 |
| | 4 | | 28, – | 11,12 | 11,13 | | | 10,12 | 11,12 | **23, –** | | | | 16, – | | 22,24 | 10.2 |
| | 5 | | 28,31 | 11,12 | 11,13 | | | 10,12 | 11,12 | 17,23 | | | | 16, – | | 22,24 | 10.2 |
| | Cons | | 28,31 | 11,12 | 11,13 | | | 10,12 | 11,12 | 17,23 | | | | 16, – | | 22,24 | |
| ID | 1 | 11, – | 28,31 | 11,12 | – | 15, – | 7,9 | 10,12 | 11,12 | – | 13, – | 14,18 | 8,11 | 16, – | 12,13 | 22,24 | 12 |
| | 2 | 11, – | 28,31 | 11, – | 11,13 | 15, – | 7,9 | 10,12 | 11,12 | – | 13, – | 14,18 | 8,11 | 15,16 | 12,13 | 22,24 | 12 |
| | 3 | 11, – | – | – | – | – | – | – | – | – | – | 14, – | – | – | – | – | 12 |
| | 4 | 11, – | 28, – | – | – | 15, – | 7, – | **12, –** | 11,12 | – | – | 14, – | – | – | **13, –** | – | 10.2 |
| | 5 | 11, – | – | 11,12 | – | 15, – | 7,9 | 10,12 | 11,12 | – | 13, – | **18, –** | – | 16, – | 12, – | – | 10.2 |
| | Cons | 11, – | 28, – | 11, – | – | 15, – | 7,9 | 10,12 | 11,12 | – | 13, – | 14,18 | – | 16, – | 12,13 | – | |
| MF+ID | | 11, – | 28,31 | 11,12 | 11,13 | 15, – | 7,9 | 10,12 | 11,12 | 17,23 | 13, – | 14,18 | – | 16, – | 12,13 | 22,24 | |
| EP 1-50-2 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 29,30 | – | 10, – | | | 9, – | 9,10 | 20, – | | | | 13,17 | | – | 0.56 |
| | 2 | | 29, – | 11, – | 10,12 | | | 9, – | 9, – | 17,20 | | | | 17, – | | – | 0.56 |
| | 3 | | 29, – | – | 10,12 | | | 9, – | – | 17,20 | | | | 13, – | | – | 0.56 |
| | 4 | | – | – | – | | | 9, – | – | – | | | | 17, – | | – | 0.32 |
| | 5 | | – | – | – | | | – | 9, – | – | | | | – | | – | 0.32 |
| | Cons | | 29, – | – | 10, – | | | 9, – | 9, – | 20, – | | | | 17, – | | – | |
| ID | 1 | – | 30, – | – | – | 16, – | – | – | 9, – | 17, – | 13,15 | 16, – | – | – | – | – | 0.56 |
| | 2 | – | 30,OL | – | – | – | – | – | – | – | – | 16, – | – | – | – | – | 0.56 |
| | 3 | – | – | – | – | – | – | – | – | – | 13, – | – | – | – | – | – | 0.56 |
| | 4 | – | – | – | – | – | – | – | – | – | 14, – | – | – | – | – | – | 0.32 |
| | 5 | 13, – | – | – | – | – | 6,9 | – | – | – | – | 18, – | 8, – | – | – | – | 0.32 |
| | Cons | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | |
| MF+ID | | – | 29, – | – | 10, – | – | – | 9, – | 9, – | 20, – | – | – | – | 17, – | – | – | |
| EP 1-188-1 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 31.2,32 | 12, – | 10,11 | | | 10,11 | 12, – | 18,22 | | | | 15,19 | | 21,23 | 30.2 |
| | 2 | | 31.2, – | 12, – | 10,11 | | | 10,11 | 12, – | 18,22 | | | | 15,19 | | 23, – | 30.2 |
| | 3 | | 31.2,32 | 12, – | 10,11 | | | 10,11 | 12, – | 18,22 | | | | 15,19 | | – | 22.1 |
| | 4 | | 31.2,32 | – | 10,11 | | | 10,11 | – | 18,22 | | | | 15,19 | | – | 22.1 |
| | 5 | | – | – | 10,11 | | | 10,11 | – | 18,22 | | | | 15,19 | | – | 22.1 |
| | Cons | | 31.2,32 | 12, – | 10,11 | | | 10,11 | 12, – | 18,22 | | | | 15,19 | | – | |
| ID | 1 | 11 | 32, – | 12, – | 10, – | 15,16 | 7,8 | 11, – | – | 18, – | 13,15.2 | 14,16 | 8, – | 19, – | 11,12 | 21, – | 30.2 |
| | 2 | 11,13 | – | 12, – | 10, – | 16, – | 7, – | – | 12, – | – | 13,15.2 | 14,16 | 8, – | – | 12, – | – | 30.2 |
| | 3 | **13, –** | – | – | – | – | – | – | – | – | – | 14, – | – | – | – | – | 22.1 |
| | 4 | 11,13 | – | – | – | – | – | – | – | 22, – | – | 14,16 | 8, – | – | – | – | 22.1 |
| | 5 | 11,13 | 31.2, – | 12, – | 10, – | 15,16 | 7,8 | 10,11 | 12, – | 18, – | 13,15.2 | 14,16 | 8, – | 15,19 | 11,12 | 21,23 | 22.1 |
| | Cons | 11,13 | – | 12, – | 10, – | 16, – | 7, – | – | – | – | 13,15.2 | 14,16 | 8, – | – | 12, – | – | |
| MF+ID | | 11,13 | 31.2,32 | 12, – | 10,11 | 16, – | 7, – | 10,11 | 12, – | 18,22 | 13,15.2 | 14,16 | 8, – | 15,19 | 12, – | – | |
| EP 1-188-2 | | | | | | | | | | | | | | | | | |
| MF | 1 | | 30, – | 11, – | – | | | 9,12 | 9, – | 22, – | | | | – | | 19,22 | 3.09 |
| | 2 | | 29, – | 11, – | 12, – | | | 9,12 | 9, – | 22, – | | | | **16, –** | | 22, – | 3.09 |
| | 3 | | – | – | 12,13 | | | 9,12 | – | 22, – | | | | 14,16 | | – | 15.1 |
| | 4 | | – | – | 12,13 | | | 9,12 | – | 22, – | | | | 14,16 | | – | 15.1 |

*continued*

TABLE 1— *Continued.*

| Kit | Exp. | D8 S1179 | D21 S11 | D7 S820 | CSF1PO | D3 S1358 | TH 01 | D13 S317 | D16 S539 | D2 S1338 | D19 S433 | vWA | TPOX | D18 S51 | D5 S818 | FGA | Quantifiler (pg/uL) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | | – | – | 12,13 | | | 9,12 | – | 22, – | | | | 14,16 | | – | 15.1 | | |
| Cons | | – | – | 12,13 | | | 9,12 | – | 22, – | | | | 14,16 | | – | | | |
| ID | 1 | 13, – | – | – | – | | – | – | – | – | 13, – | – | – | 16, – | 13, – | – | 3.09 | |
| | 2 | 12, – | – | – | – | 13, – | – | – | – | – | – | 15.2, – | – | – | – | 13, – | – | 3.09 |
| | 3 | 12,13 | – | – | – | – | – | – | – | – | – | – | 17, – | 8, – | – | – | – | 15.1 |
| | 4 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 15.1 |
| | 5 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 15.1 |
| | Cons | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | |
| MF+ID | | – | – | – | 12,13 | – | – | 9,12 | – | 22, – | – | – | – | 14,16 | – | – | | |

MF, MiniFiler™; ID, Identifiler™; Cons, consensus profile obtained from either MiniFiler™ or Identifiler™ kit analyses (allele profiles repeated three of five repetitions were considered authentic); MF+ID, consensus profile obtained from combined use of MiniFiler™ and Identifiler™ kit analyses; OL, off-ladder. Boldface heterozygous loci showed the drop-outs in small-sized alleles, possibly by processing a small number of starting DNA templates during PCR.

TABLE 2—*Loci profiles between MiniFiler™ and Identifiler™ kit analysis.*

| | Sample | Locus | MiniFiler™ | Identifiler™ |
|---|---|---|---|---|
| 1 | SN1-32 | D7S820 | 10,11 | 10,– |
| 2 | SN1-32 | CSF1PO | 11,12 | 11,– |
| 3 | SN1-32 | D13S317 | 12,– | – |
| 4 | SN1-32 | D16S539 | – | 11,12 |
| 5 | SN1-32 | D2S1338 | 17,25 | – |
| 6 | SN1-32 | D18S51 | 13,23 | 13,– |
| 7 | SN1-32 | FGA | 19,24 | – |
| 8 | SN4-18-1 | D21S11 | 31,– | – |
| 9 | SN4-18-1 | D7S820 | 11,– | – |
| 10 | SN4-18-1 | CSF1PO | 9,11 | – |
| 11 | SN4-18-1 | D13S317 | 10,13 | – |
| 12 | SN4-18-1 | D2S1338 | 20,24 | – |
| 13 | SN4-18-1 | D18S51 | 15,16 | – |
| 14 | SN4-18-1 | FGA | 24,28 | – |
| 15 | SN4-18-2 | D7S820 | 10,12 | – |
| 16 | SN4-18-2 | CSF1PO | 10,12 | – |
| 17 | SN4-18-2 | D13S317 | 11,– | – |
| 18 | SN4-18-2 | D16S539 | 10,11 | 11,– |
| 19 | SN4-18-2 | D2S1338 | 17,24 | – |
| 20 | SN4-18-2 | FGA | 26,– | – |
| 21 | SN4-25-2 | CSF1PO | 11,– | – |
| 22 | SN4-25-2 | D13S317 | 11,– | – |
| 23 | SH2-10-1 | D21S11 | 28,31 | 28,– |
| 24 | SH2-10-1 | D7S820 | 11,12 | 11,– |
| 25 | SH2-10-1 | CSF1PO | 11,13 | – |
| 26 | SH2-10-1 | D2S1338 | 17,23 | – |
| 27 | SH2-10-1 | FGA | 22,24 | – |
| 28 | EP1-50-2 | D21S11 | 29,– | – |
| 29 | EP1-50-2 | CSF1PO | 10,– | – |
| 30 | EP1-50-2 | D13S317 | 9,– | – |
| 31 | EP1-50-2 | D16S539 | 9,– | – |
| 32 | EP1-50-2 | D2S1338 | 20,– | – |
| 33 | EP1-50-2 | D18S51 | 17,– | – |
| 34 | EP1-188-1 | D21S11 | 31.2,32 | – |
| 35 | EP1-188-1 | CSF1PO | 10,11 | 10,– |
| 36 | EP1-188-1 | D13S317 | 10,11 | – |
| 37 | EP1-188-1 | D16S539 | 12,– | – |
| 38 | EP1-188-1 | D2S1338 | 18,22 | – |
| 39 | EP1-188-1 | D18S51 | 15,19 | – |
| 40 | EP1-188-2 | CSF1PO | 12,13 | – |
| 41 | EP1-188-2 | D13S317 | 9,12 | – |
| 42 | EP1-188-2 | D2S1338 | 22,– | – |
| 43 | EP1-188-2 | D18S51 | 14,16 | – |

autosomal DNA profiles were successfully determined by use of coupled MiniFiler™ and Identifiler™ STR kit assays, commands researchers' attention.

The present study showed how combined mini- and conventional STR kit analyses could be beneficial for aDNA samples obtained from the archaeological field in Korea. Whereas in the Identifilier™ kit assay, 21.87% (14 of 64) of the larger-sized loci (i.e., D7S820, D13S317, D16S539, D21S11, D2S1338, D18S51, CSF1PO, and FGA) showed consensus profiles, the proportion was 75% (48 of 64) in the MiniFiler™ kit assay, for the same loci. As for the range of the number of loci where consensus profiles were obtained, we observed a superior performance for the MiniFiler™ assay as well: 2–8 loci per sample (average = 6 ± 2), compared with only 0–5 per sample (average = 1.8 ± 2.2) in the Identifiler™ assay for the same large loci. In the combined MiniFiler™ and Identifiler™ analyses performed on the ancient samples, the number of consensus 15-STR locus profiles was as high as 79 of 120 loci (65.83%), a much better result than was attained with the Identifiler™ kit alone (44/120 loci, 36.7%).

Considering this evidence of the technical benefits of combined MiniFiler™ and Identifiler™ kit analyses in multiplex STR genotyping of aDNA samples, it is recommendable for aDNA experts to strongly consider MiniFiler™ kit analysis as an effective complementary to conventional STR kit analysis.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Data S1.** The criteria of authentication followed by our laboratory during ancient DNA work.

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