The Effect of Using an Extra PCR Cycle with GlobalFiler® when Amplifying Skeletal Samples

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ABSTRACT

Skeletal samples often yield low-template and/or degraded DNA that can result in incomplete short tandem repeat (STR) profiles. Common strategies, such as increasing the number of PCR cycles, have been used to overcome this challenge.

In this study, various bone and tooth samples (N=24) were harvested from cremated, embalmed, buried, or decomposed human remains to compare the effects of increasing the number of PCR amplifications from 29 to 30 using the GlobalFiler® PCR Amplification kit (ThermoFisher Scientific). DNA was extracted from 100 mg of bone powder by following the recommended bone protocols using the QIAamp® DNA Investigator kit (Qiagen) or the PrepFiler® BTA Forensic DNA Extraction kit (ThermoFisher Scientific).

Overall, this research has shown that regardless of the DNA extraction method used, consistently more alleles were recovered from bone and tooth samples with the addition of an extra PCR cycle using the Globalfiler® PCR Amplification kit with minimal adverse STR artifacts.

INTRODUCTION

Using STRs to identify human remains has its challenges. Bone and tooth samples often contain low amounts of DNA and depending on the time and conditions to which they were exposed, are often highly degraded and/or show PCR inhibition (1). When DNA degrades, it breaks into increasingly smaller fragments which may lead to amplification failure of the longer STR markers (> 250 bp) (2, 3).

In addition, the low amount of DNA template (< 100 pg) available for amplification often negatively affects STR results due to stochastic effects such as allele and/or locus dropout or drop-in, allele imbalance, and increased stutter (3-5). The most common approach to improving STR results from low template samples is to increase the number of PCR cycles (low copy number typing). However, this method may also result in more complicated STR profiles requiring more careful interpretation (3-5).

RESULTS AND DISCUSSION

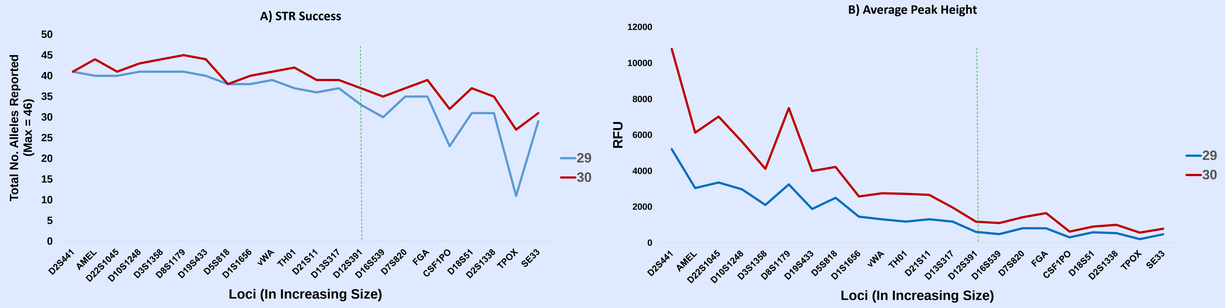

Figure 2 – Comparison of STR profile quality between 29 and 30 PCR cycles based on (A) total number of alleles reported; (B) average peak heights. Dotted green line indicates ~250 bp in length.

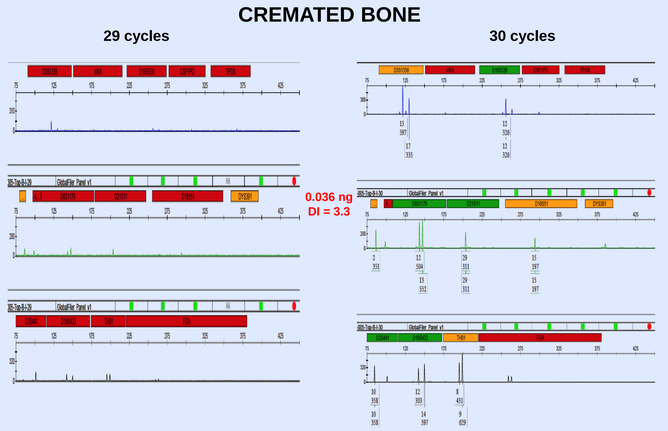

Figure 3 – Examples of STR profiles using GlobalFiler® with 29 and 30 PCR cycles.

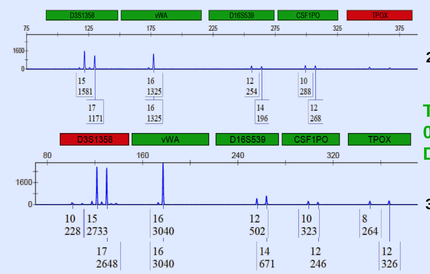

Figure 4 – Example of a drop-in event. Drop-in was observed in 14% of the amplifications.

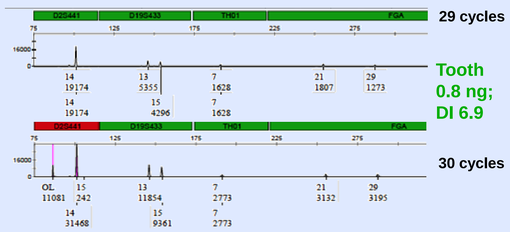

Figure 5 – Example of a pull-up event. Pull-up was observed only once in 48 amplifications.

MATERIALS AND METHODS

Sample Preparation:

- 9 bone and 3 tooth samples were collected from cadavers that were cremated, buried, embalmed, or decomposed for up to 18 months (Fig. 1).
- Bone surface was sanded (Dremel) and cut into chips (~0.5mm x 0.5 mm).
- Bone chips were cleaned with a series of 5 min washes (15% bleach, dH_2O, 100% ethanol) and dried overnight.
- Chips were powdered in a 6700 SPEX liquid nitrogen freezer mill.

Figure 1 – A representation of skeletal samples used in this project.

- Bone powder (100 mg) was extracted using the QIAamp® DNA Investigator kit (Qiagen) and the PrepFiler® BTA Forensic DNA Extraction kit (ThermoFisher Scientific).
- DNA was quantified using the QuantiFiler® Trio DNA Quantification kit (ThermoFisher Scientific).
- STR profiles were generated using the GlobalFiler® PCR Amplification kit (ThermoFisher Scientific) using 29 or 30 PCR cycles.

CONCLUSIONS

- Significantly more DNA was obtained when samples were extracted using the PrepFiler® BTA Forensic DNA kit ($p < 0.01$).

With an increase in cycle number from 29 to 30 using Globalfiler®:

- A significant increase in the number of alleles detected and the peak heights ($p < 0.05$) (Fig. 2 and 3).
- No significant difference ($p > 0.05$) in heterozygote peak height balance was observed (data not shown).
- An increase in STR artifacts was observed.
- Elevated stutter peaks were observed with both 29 and 30 cycles but was not found to be statistically significant.
- Seven drop-in alleles (Fig. 4), six off-ladder peaks, and one event of pull-up (Fig. 5) was observed in 48 amplifications. These STR artifacts were only observed in samples amplified with 30 cycles.

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