Get more answers from challenging bone samples using the latest tools in forensic DNA analysis

In this application note, we demonstrate:

- Useful short tandem repeat (STR) profiles from highly degraded samples analyzed with Applied Biosystems™ GlobalFiler™ and NGM Detect™ PCR Amplification Kits
- Simplified DNA extraction from challenging bone samples using the Applied Biosystems™ PrepFiler™ BTA Forensic DNA Extraction Kit
- Robust quality and quantity assessment of DNA from bone using the Applied Biosystems™ Quantifiler™ Trio DNA Quantification Kit, to enable decisions that deliver improved allele recovery

Get more answers from challenging samples: obtain quality STR profiles from bone samples

Bone samples are one of the most difficult sample types encountered in forensic laboratories. The extracted DNA is often degraded and obtained in low quantity, making it difficult to obtain usable STR profiles. Also, the age, amount, and condition of bone samples present challenges for processing. Older bones (>20 years) are problematic, typically with low DNA yield, but the storage condition of even contemporary bones (<20 years) can present challenges depending on the crime scene condition and environmental exposure of the bones.

Extraction of DNA from a bone sample in a lab is extremely laborious. Nonstandard physical and chemical extraction techniques can be used to improve the DNA quantity and quality. These protocols start by physically obtaining a bone sample by saw-cutting and/or pulverization, followed by labor-intensive demineralization, and finally DNA extraction with hazardous chemicals. The journey from bone sample to interpretable DNA results is difficult, and is compounded by the challenges presented when analyzing the data from these compromised low copy number samples. Labs are now able to process samples with an improved likelihood of achieving results, with easy-to-use tools and a reduction in workflow steps.

In this study, we present data from three forensic laboratories to demonstrate: high-quality DNA results from bones when using the PrepFiler BTA Forensic DNA Extraction Kit; the value of the Quantifiler Trio DNA Quantification Kit; and high sensitivity when amplifying highly compromised or low copy number samples with either the GlobalFiler PCR Amplification Kit or NGM Detect PCR Amplification Kit.

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Materials and methods

Three forensic laboratories that process bone samples participated in this study. These test sites employed commonly used extraction methods for a variety of bone samples, as shown in Table 1. Post-extraction, all samples were quantified and, when possible, 1 ng of DNA was added to each of the GlobalFiler reactions and 500 pg of DNA was added to each of the NGM Detect reactions. For the majority of samples, less than the recommended quantity of DNA was available to be added. In these cases, the full 15 µL sample volume was added to the amplification reaction. Samples were amplified using both 29 and 30 PCR cycles for the GlobalFiler kit and 30 PCR cycles for the NGM Detect kit. All test sites used Applied Biosystems™ 3500 or 3500xL Genetic Analyzers and the recommended run parameters for these instruments.

DNA quantification and results

To improve confidence in obtaining optimal STR allele recovery downstream from both contemporary and older bone samples, the test sites used Applied Biosystems™ Quantifiler™ HP or Quantifiler Trio DNA Quantification Kits to assess the results of their DNA extraction. The Quantifiler Trio and HP kits enable efficient and accurate quantification of human DNA, and analysis includes a quality index to indicate the presence of degraded DNA along with PCR inhibitors. This is especially important for bone samples, due to the high probability of degradation in this sample type, and to assess potential inhibitors that may have been coextracted and could impact the quality of the STR result. In addition, the Quantifiler Trio kit determines the quantity of male DNA present in samples. These results help guide the selection of the most appropriate STR kits to help maximize the success of analysis.

As shown in Figure 1, the majority of the bone samples had very low DNA quantities. The Quantifiler Trio and HP assays are designed for multicopy loci to provide increased sensitivity and reliable quantification down to 5 pg/µL.

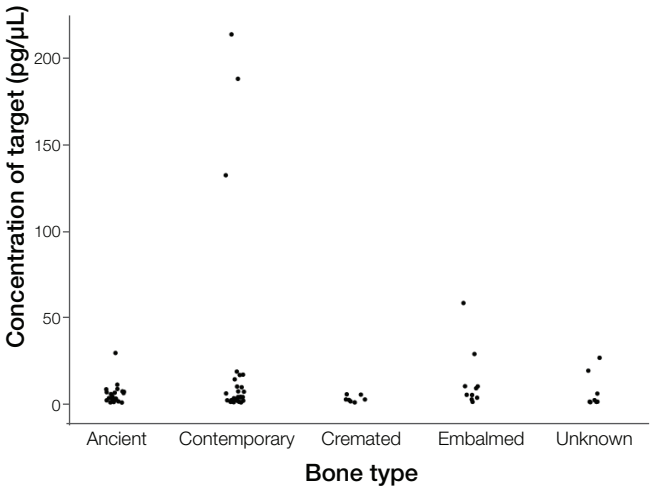

Figure 1. Bone sample analysis using the Quantifiler Trio kit. With the exception of three of the contemporary bone samples, less than 67 pg/µL of DNA (needed for 1 ng DNA input) was added to the GlobalFiler reaction for these challenging samples.

Table 1. Bone types and extraction methods included in the study.

Site	No. of bone samples	Bone types	Extraction methods
1	41	Femur, tibia	Modified organic, Hi-Flow™ DNA purification spin columns (Protein Ark), AFDIL
2	24	Cremated vertebral arch, embalmed femur, embalmed tooth, tibia, femur	QIAamp™ DNA Investigator Kit (Qiagen), PrepFiler BTA kit
3	11	Femur, tibia, humerus, shaft of a long bone	QIAamp DNA Investigator Kit, organic

As shown in Table 2, many of the bone samples yielded degraded DNA. As expected, this resulted in a "degradation slope" or "ski slope" (with allele dropout for larger loci) for many of the samples. One example is demonstrated in Figure 2.

Here we discuss ways to recover the maximum number of alleles with information obtained from the Quantifiler Trio or HP kit. Forensic scientists may use the degradation index (DI) as an indicator that more DNA will likely be required to recover the larger loci. If the DNA concentration is so low that additional sample volume cannot be added, an additional PCR cycle can be used. Another approach is to perform two PCR amplifications with two different kits—for example, Applied Biosystems™ NGM SElect™ and NGM Detect PCR Amplification Kits, or NGM Detect and GlobalFiler PCR Amplification Kits—to generate a composite profile from the two results. These kit combinations have been designed with common loci but contain different primer sequences and marker positions that allow for recovery of alleles from more markers when used in a dual amplification strategy. The GlobalFiler and NGM Detect PCR Amplification Kits contain 10 and 7 mini-STRs, respectively. Together the two kits provide 13 distinct mini-STRs.

Table 2. Degradation index (DI) summary of extracted samples.

Sample type	DI: 1–4	D1: 5–10	D1: >10
Cremated	22	3	2
Contemporary	23	5	3
Embalmed	4	6	0
Older	16	3	2
Unknown	4	4	0
Total	48	21	7

STR analysis and results

The GlobalFiler and NGM Detect PCR Amplification Kits are designed to provide maximum information recovery from difficult sample types by the inclusion of mini-STRs (amplicon sizes of less than 230 bp), a highly robust master mix, and the option to add up to 15 µL of sample input. The newly developed NGM Detect assay has an additional feature—the internal quality control (IQC). The IQC is a tool to assess the PCR reaction: in conjunction with the STR marker data properties, researchers can infer whether a sample is showing signs of degradation or inhibition. While these features can be used to help improve the resulting DNA profiles, there are still cases where the DNA input is too low or the DNA is too degraded to provide useful information. Laboratories are looking for solutions to optimize information recovery with this difficult sample type.

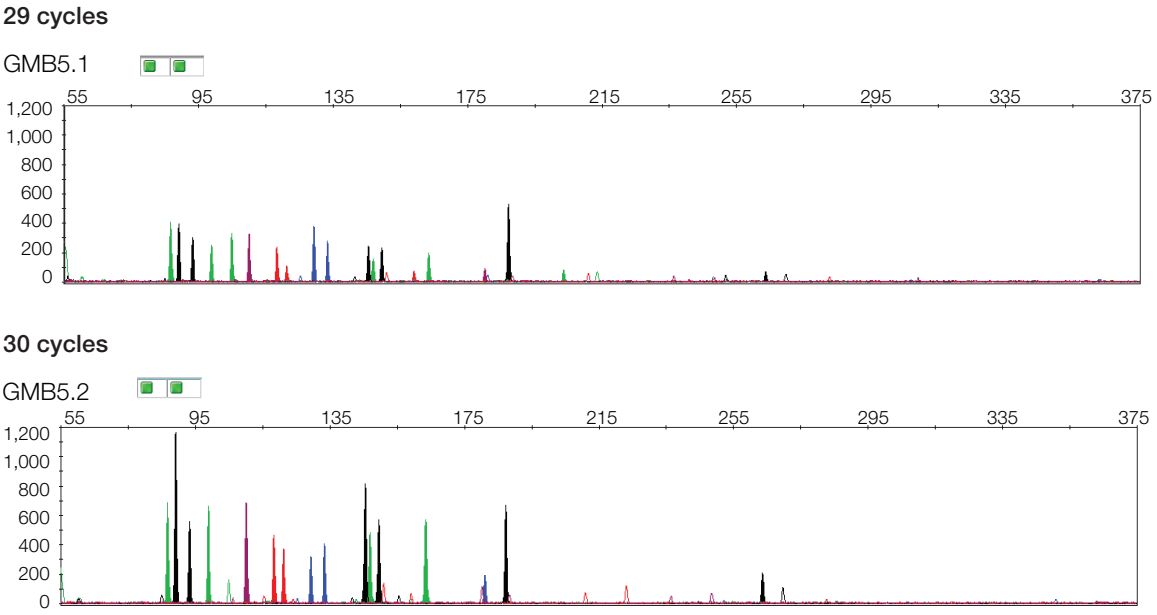

Figure 2. Improved GlobalFiler amplification performance with one additional PCR cycle. This aged femur bone sample, demonstrating a typical degradation slope, was purified by organic extraction and analyzed on the 3500 Genetic Analyzer. The Quantifiler HP assay was used to determine DNA input of 120 pg with a DI of 4.

This study compared the effect of adding an additional PCR cycle to the GlobalFiler PCR protocol. Internal studies showed that increasing the number of PCR cycles to 30 increased the amount of useful information recovered while having minimal impact on background signal and PCR artifacts (data not shown). All three forensic laboratories tested the impact of using the GlobalFiler assay with 30 PCR cycles with their bone samples; Figure 2 shows a representative sample of this comparision. Additionally, one laboratory processed the same sample set with the NGM Detect assay (Table 3).

Table 3. Number of alleles called (including amelogenin) for each sample when analyzed with the GlobalFiler assay with 29 and 30 PCR cycles, and the NGM Detect assay with 30 cycles. The maximum possible numbers of alleles for the GlobalFiler and NGM Detect assays are 46 and 35, respectively.

Sample no.	Skeletal element	Treatment	DI	DNA (ng)	GlobalFiler assay, 29 cycles: no. of alleles called	GlobalFiler assay, 30 cycles: no. of alleles called	NGM Detect assay, 30 cycles: no. of alleles called
1	Tibia	Saltwater submersion	NA	0.08	21	32	27
2	Tooth	Thermally degraded	0.72	0.07	4	10	15
3	Tibia	Skeletonized remains	1.17	0.05	30	35	31
4	Femur	Skeletonized remains	8.15	0.09	2	11	11
5	Tooth	Thermally degraded	NA	0.05	2	11	16
6	Femur	Active decomposition	2.25	0.11	33	36	31
7	Femur	Embalmed	3.13	0.10	9	25	24
8	Vertebral arch	Cremated	5.36	0.11	6	28	31
9	Femur	Active decomposition	2.24	0.12	21	41	34
10	Femur	Unknown	4.66	0.14	30	30	30
11	Vertebral arch	Cremated	6.71	0.50	24	28	27
12	Humerus	Embalmed	NA	0.24	12	11	17
13	Femur	Buried	6.71	0.14	0	0	0
14	Tooth	Thermally degraded	16.11	0.17	10	18	22
15	Tooth	Thermally degraded	204.06	0.15	5	7	14
16	Tooth	Embalmed	9.90	0.13	14	25	25
17	Tooth	Skeletonized remains	5.62	0.39	38	39	29
18	Foot phalanx	Embalmed	5.47	0.48	31	37	29
19	Hand phalanx	Embalmed	23.79	0.48	18	26	24
20	Vertebral arch	Cremated	4.97	0.34	39	44	35
21	Tibia	Skeletonized remains	25.14	0.50	0	0	0
22	Tooth	Skeletonized remains	4.20	0.42	36	39	34
23	Femur	Buried	6.68	0.42	34	38	35
24	Tooth	Embalmed	7.29	0.41	38	41	32

As shown in Figure 3, the average increase in peak height (PH) due to an additional PCR cycle for the 5 largest STR markers is greater than 100 RFU. This makes analysis more straightforward because the peaks are increased relative to the background noise. Bones that are older than 20 years showed greater increases in peak height with addition of one PCR cycle when compared with fresh bones.

As shown in Figure 4, 3–6 additional alleles were typically recovered with 30 PCR cycles, when compared to 29 PCR cycles, in all sample types analyzed.

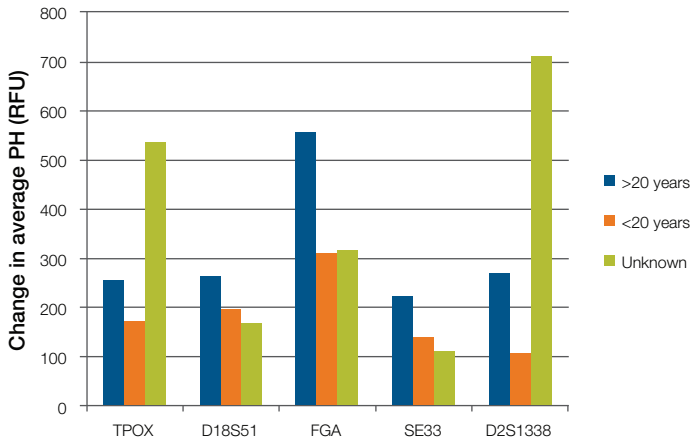

Figure 3. Increase in average peak height (PH) for five of the largest GlobalFiler STR markers, with one additional PCR cycle (30 vs. 29 cycles). Only native bone samples are shown in the graph (no embalmed or cremated samples).

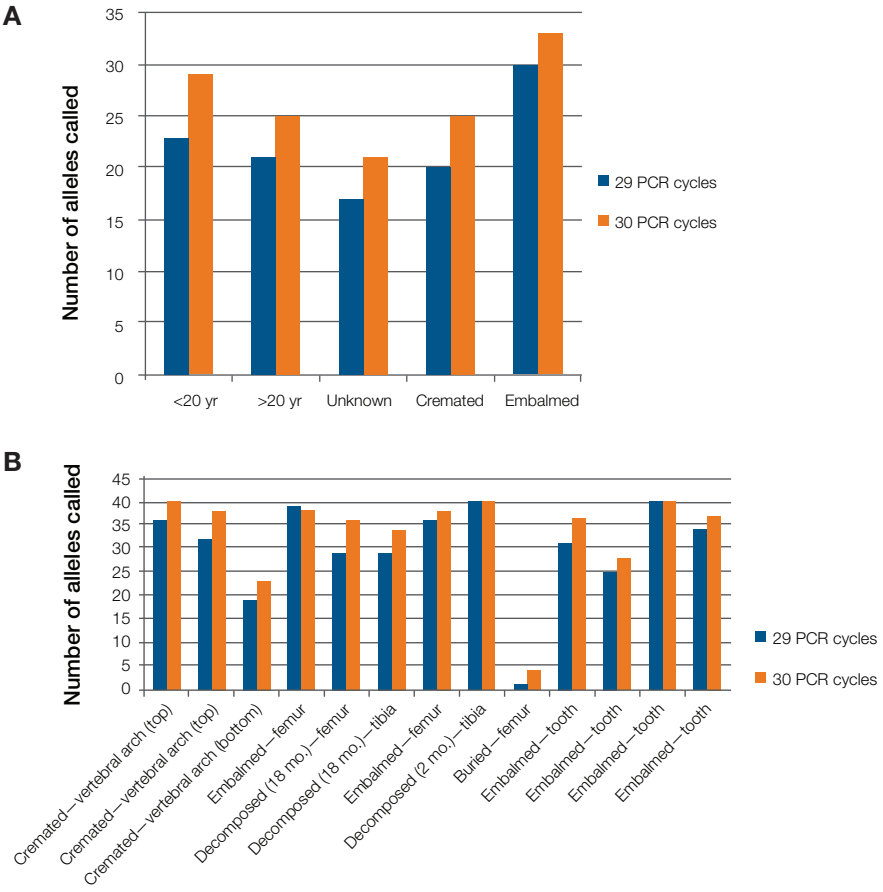

Figure 4. The mean number of alleles (excluding amelogenin) called for each sample type used in the GlobalFiler study and the increase in number of alleles called with one additional PCR cycle. (A) The samples were extracted with the methods described in Table 1. **(B)** Samples were prepared using the PrepFiler BTA DNA Extraction Kit.

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When compromised samples were analyzed with a dual amplification strategy using GlobalFiler and NGM Detect PCR Amplification Kits, the number of alleles called increased by 20–50% (Table 4).

Conclusions

Bone samples present significant challenges to forensic DNA analysis, in terms of both quantity and quality of the DNA. Both aspects have the potential to reduce the number of alleles recovered, resulting in an incomplete and unusable STR profile. Quantifiler HP and Quantifiler Trio DNA Quantification Kits were used to assess the quality and quantity of the extracted DNA, and provide guidance on amplification kits and strategies to maximize allele and marker recovery. When using the GlobalFiler PCR Amplification Kit, DNA analysts can increase the success rate of allele recovery by 10–26% by adding an additional PCR cycle (from 29 to 30). Lastly, a dual amplification strategy may be used to recover up to 50% more markers, especially if the DI (>5) indicates a more degraded sample quality.

Table 4. Gain of marker recovery from compromised sample, using both GlobalFiler and NGM Detect PCR Amplification Kits.

Sample no.	Sample type	Markers recovered with GlobalFiler kit (30 cycles)	Markers recovered with NGM Detect kit	Markers recovered using both STR assays
2	Thermally degraded tooth	**Total 7**: D3, Y-indel, D21, D2, D19, SE33, D10	**Total 6**: D2, SE33, D16, D18, D3, FGA	**Total 10**: D2, D3, D6, D10 D18, D19, D21, FGA, SE33, Y-indel
4	Decomposed femur	**Total 7**: D10, D22, D2, Y-indel, Amel, D8, D3	**Total 7**: Y-indel, Amel, FGA, D16, D18, D8, D2	**Total 10**: D2, D3, D8, D10, D16, D18, D22, Y-indel, Amel, FGA
19	Embalmed hand phalanx	**Total 14**: D3, vWA, Y-indel, Amel, D8, D21, D2, D19, D22, D5, D7, D10, D1, D12	**Total 13**: D2, SE33, D16, D18, THO1, D3, FGA, Y-indel, Amel, vWA, D21, D8, D19	**Total 19**: D1, D2, D3, D5, D7, D8, D10, D12, D16, D18, D19, D21, D22, Y-indel, Amel, vWA, FGA, SE33, THO1

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