Traditional and Rapid DNA Approaches to bone sample processing with Applied Biosystems™ Workflows

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INTRODUCTION

Bones are one of the most difficult sample types encountered in forensic laboratories. DNA is often degraded and obtained in low quantity, making it difficult to obtain interpretable STR profiles. Also, given that bone samples are often related to disaster victim identification (DVI), mass fatalities or missing persons, the age and condition of bones present sample processing challenges for these human remains. This work presents two approaches to obtaining discriminating short tandem repeat (STR) profiles from a diverse set of bone samples.

A traditional capillary electrophoresis (CE) workflow, incorporating a simplified DNA extraction using the Applied Biosystems PrepFiler™ *Express* BTA Forensic DNA Extraction Kit and the SeqStudio™ Genetic Analyzer, is one option available to forensic DNA laboratories aiming to recover suitable results from bone samples of varying quality. With this workflow, an analyst can generate results from a batch of ~12 samples in under three days.

With the evolution of Rapid DNA technology, a streamlined sample-to-answer approach to forensic bone sample identification gives forensic laboratories and law enforcement the ability to analyze skeletal remains at the point of action in a fully automated workflow. The Applied Biosystems RapidINTEL™ Sample Cartridge Kit run on the RapidHIT™ ID System allows for analysis of samples when time to result is critical and/or a fully equipped forensic laboratory is unavailable, as may be the case in many DVI and other missing persons scenarios.

MATERIALS AND METHODS

Samples and Bone Preparation

Eighteen bones were provided to Thermo Fisher Scientific by the Institute of Legal Medicine, University of Mainz, Germany (ILM Mainz) and processed at the Thermo Fisher laboratory in Darmstadt, Germany. The bones ranged from fresh or high quality (e.g., a few days old with limited exposure to environmental insults) to mid or low quality (e.g., aged, partially decomposed or placed in formic acid) (Table 1).

Sample ID	Bone	Condition
M-1 to M-4	Femurs	Skeletonized
M-5	Tibia	1-2 days aged, no extreme exposure
M-6	Femur	14 days aged, no extreme exposure
M-7	Femur	1-2 days in formic acid, decomposed
M-8	Femur	56 days aged, corpse in water, decomposed
M-9, M-12, M-14, M-16	Skull	Skeletonized
M-10, M-11	Rib	1-2 days aged, no extreme exposure
M-13	Femur	1-2 days aged, corpse in water
M-15, M-17	Femur	1-2 days aged, corpse burned
M-18	Femur	11 days aged, decomposed in home

Table 1: Sample list including bone types and information on age and condition of bone, if available.

After removing soft tissue or debris adhering to the bones, the samples were processed as follows:

1. A 6 mm steel drill was pressed at a 180° angle onto the surface of the bone, and drilled into the bone 2–6 cm. A Bosch Professional Akku Drill GSR 12V-15 was used.
2. Bone chips sticking to the drill were collected using a single-use scalpel and placed into a tube. The sample was placed at -20C° prior to processing.
3. 50–60 mg of bone chips were removed from the tube using a swab or single-use tweezers and either placed directly into the RapidINTEL Sample Cartridge or a PrepFiler lysate tube.

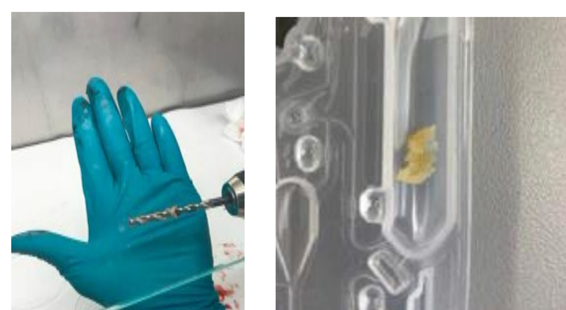

Figure 1: Image from the bone sampling process on the left and 55.5 mg of bone chips placed directly in the RapidINTEL Sample Cartridge on the right.

Rapid Workflow

Samples were run directly on the RapidHIT ID System v1.1.3 with RapidINTEL Cartridges (GlobalFiler™ Express chemistry). Secondary analysis was performed using the guidelines for allele interpretation outlined in the RapidINTEL Sample Cartridge for Blood and Saliva Samples Validation User Bulletin (Publication Number MAN0018979 Rev A.0) with GeneMarker™ HID v2.9.5.

CE Workflow

Two batches of bone samples were extracted and purified with PrepFiler *Express* BTA Forensic DNA Extraction chemistry run on the AutoMate *Express*™ Forensic DNA Extraction System.

The samples were quantified on a 7500 Real-Time PCR system with the Quantifiler™ Trio DNA Quantification Kit and amplified on a Veriti thermal cycler with the GlobalFiler™ PCR Amplification Kit. Samples were run on the SeqStudio™ Genetic Analyzer for HID and data was analyzed with GeneMapper ID-X v1.6. Recommended protocols from the applicable User Guides were followed.

RESULTS

Rapid Workflow

Sixteen of the eighteen samples passed primary analysis and two of the samples failed to generate a DNA profile from the instrument. Of the sixteen samples which passed primary analysis, full single source profiles were confirmed for five samples (~31%). One of these samples (M-13) passed primary analysis without the need for manual interpretation or secondary review.

All eight of the mid to high quality samples provided discriminating profiles. In addition to the five samples generating full profiles, two samples provided at least 73% allele recovery. One femur (M-15) generated a profile from at least two individuals with all markers containing data suitable for further interpretation; however, secondary analysis was not performed.

Table 2 summarizes the primary analysis allele recovery rate (the percentage of markers that meet all system thresholds) and secondary analysis allele recovery rate (the percentage of markers that can be confidently called or confirmed with manual review) for single source samples passing primary analysis. The "Quality" column offers a subjective assessment of the bone based on age, condition, and environmental information.

Two samples failed to pass primary analysis with failing size quality (M-2, M-7). There was no evidence of failure caused by the instrument or consumables; therefore, the failures may have been unique to the samples themselves.

Sample ID	Quality	Primary Analysis (percentage of unflagged markers)	Secondary Analysis (percentage of markers confirmed after review)	Final result
M-5	High	91%	100%	
M-6		50%	86%	
M-10		95%	100%	
M-11		95%	100%	
M-17		95%	100%	
M-13	Mid	100%	N/A	
M-18		4.5%	73%	
M-1	Low	0	0	
M-3		0	0	
M-4		0	0	
M-9		0	0	
M-12		0	0	
M-14		0	0	
M-16		0	0	
M-8		0	0	

Table 2: Marker recovery rate for single source samples passing primary analysis. Final results are displayed as follows: a full single source profile with all markers either meeting system thresholds or confirmed during secondary analysis (green), a partial single source profile with at least one marker with an allele in the stochastic range or low peak height ratio between sister alleles (yellow), or no peaks detected (red).

CE Workflow

Quantifiler Trio results show those samples assessed as low quality yielded low or zero DNA quantities. In all cases, 500 pg or 15 µL of sample extract was added to the GlobalFiler amplification reaction.

All eighteen samples passed sizing quality; full single source profiles were confirmed for eleven samples (~61%). Partial profiles were obtained for an additional five samples; the quantification results indicated that the input for these samples was within stochastic range and allele or marker drop out was evident. Sample M-15 resulted in a mixture profile from at least two individuals.

Sample ID	Quality	Quantifiler Trio Small Autosomal Results	Final Result
M-5	High	33.94 ng/µL	
M-6		6.2 ng/µL	
M-10		31.78 ng/µL	
M-11		6.88 ng/µL	
M-17		5.83 ng/µL	
M-15		3.96 ng/µL	Mixture
M-13	Mid	36.31 ng/µL	
M-18		5.60 ng/µL	
M-1	Low	0.02 ng/µL	
M-2		>0.01ng/µL	
M-3		>0.01ng/µL	
M-4		>0.01ng/µL	
M-9		0.00 ng/µL	
M-12		0.12 ng/µL	
M-14		>0.01 ng/µL	
M-16		0.04 ng/µL	
M-7		0.01 ng/µL	
M-8		0.02 ng/µL	

Table 3: Quantification and CE-STR results. Final results are displayed as follows: a full single source profile at a 50 RFU peak amplitude threshold (green), partial single source profile (yellow), or no peaks detected (red).

Comparison

When comparing the steps from bone sample preparation to the generation of raw data files, there is significantly more manual interaction during the traditional CE workflow. Lysis to capillary electrophoresis and data collection are all automated on the RapidHIT ID System; whereas several instruments, kits, consumables, and hands-on time are needed to process the samples traditionally. If processing a single sample, the CE workflow takes about 4.5x longer with more waste in reagents in relation to the Rapid workflow. However, if a laboratory batches bone samples the traditional method can become more efficient unless the laboratory has more than one RapidHIT instrument.

The Rapid and CE workflow generated interpretable profiles from mid to high quality bone samples. Bones that were of low quality fared better in the CE workflow. Full profiles were generated from four bones in the traditional workflow that generated no results in the rapid workflow (M-1, M-8, M-12, and M-16). Partial profiles were obtained traditionally from five samples which resulted in no profile otherwise (M-2, M-3, M-4, M-7, and M-14).

In the CE workflow, ~94% of samples generated results that could be used for reference comparison or database search; whereas, in the Rapid workflow ~44% generated similar results.

Sample ID	Quality	CE Result	Rapid Result
M-5	High		
M-6			
M-10			
M-11			
M-17			
M-15		Mixture	Mixture
M-13	Mid		
M-18			
M-1	Low		
M-2			
M-3			
M-4			
M-9			
M-12			
M-14			
M-16			
M-7			
M-8			

Table 5: CE and Rapid final result comparison.

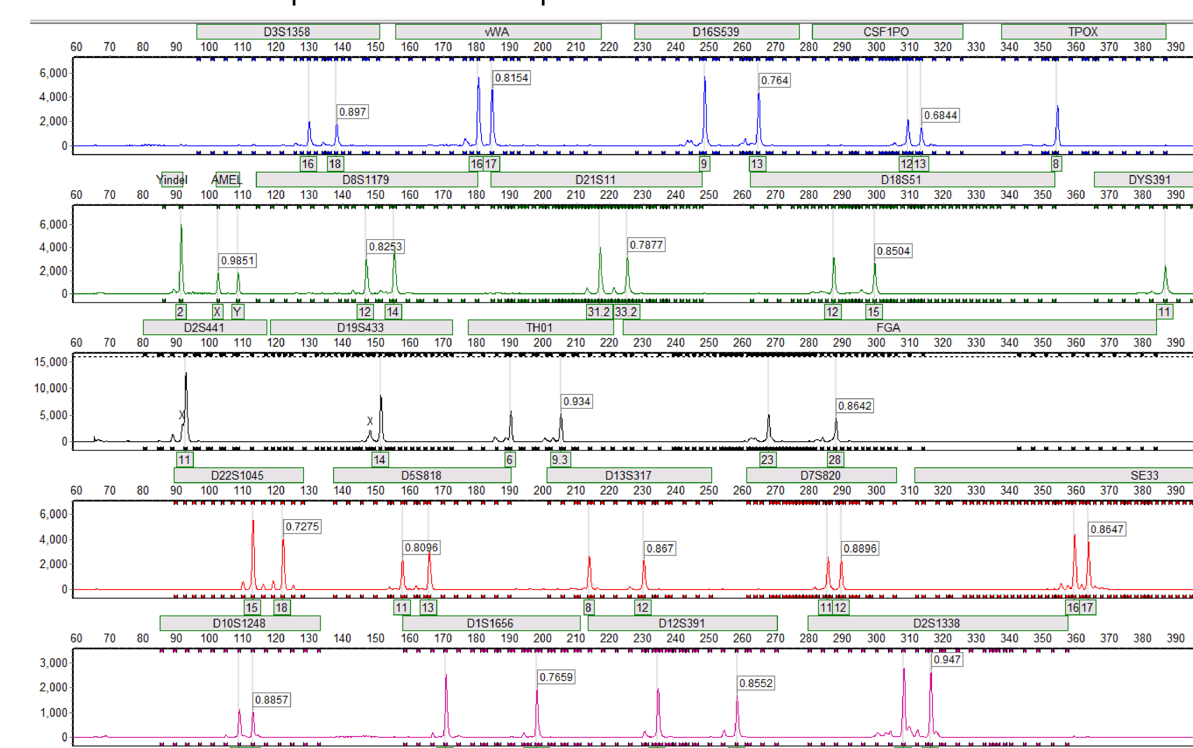

Figure 2: Electropherogram from sample M-5 which generated a full profile in both the Rapid (shown) and CE workflows. The sample is a tibia bone aged only 1-2 days with no exposure to extreme environmental conditions.

CONCLUSIONS

At missing persons, mass fatalities, and other disaster scenes, case circumstances can dictate the preferred sample processing workflow.

Skeletal remains discovered in remote geographic locations that require a fast time to result may benefit from the automated Rapid DNA platform. Likewise, mid to high quality bone samples are amenable to the Rapid DNA workflow using the sample preparation protocol described herein. Given the discrimination potential of the GFE chemistry, partial and full profiles are sufficient for database search or reference comparison with the goal of conclusive identification.

With lower quality samples, such as remains exposed to extreme environmental conditions and/or mature in age, a traditional capillary electrophoresis workflow may be required to generate results sufficient for search or comparison.

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