STUDY OF DNA DEGRADATION IN TIME-BOUND BONE POWDER SPECIMEN

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ABSTRACT

Bone specimen has been commonly encountered in Forensic DNA analysis for body identification process in cases such as homicide or missing persons. Routinely, bone specimen received could have been exposed to the environment for such a period of time or they were recovered at a later stage since the occurrence of incident. DNA is well persevered in -20ºC. However, many variables can affect the quality and quantity of DNA presence in bone specimen which include environmental conditions such as temperature, humidity, bacterial activity and degradation process. Eight bone specimens powder from completed casework for the past 10 years which were kept for training purposes in -20ºC were re-analyzed to obtain the desired DNA profile. Comparison between the initial DNA profiles with the re-analyzed DNA profile were made to distinguish any significance differences or findings. 75% of the powdered bone specimen showed no significant difference between the initial and re-analyzed DNA profile. This indicated that the DNA in the powdered bone specimen kept at -20ºC is well preserved and stable. However, we found that two powdered bone specimens (kept for 5 and 7 years) demonstrated loss of alleles in few loci suggesting that DNA degradation could still have taken place over the time or the homogeneity of the powdered bone specimen affecting the DNA yield.

MATERIAL & METHOD

- Eight known casework bone powder samples (Table 1) were selected and subjected to re-extraction after a period of storage time (Table 2) in -20ºC freezer.
- DNA isolation from the bone powder samples were carried out using the PrepFiler BTA™ Forensic DNA Extraction Kits with Automate Express™ Forensic DNA Extraction System due to its robustness with simple set up and operation[6].
- DNA amplification was carried out using AmpFISTR® Identifiler® Plus PCR Amplification Kit and electrophoresed by Genetic Analyzer 3500xl.

RESULTS

Results obtained from this study were simplified as below:

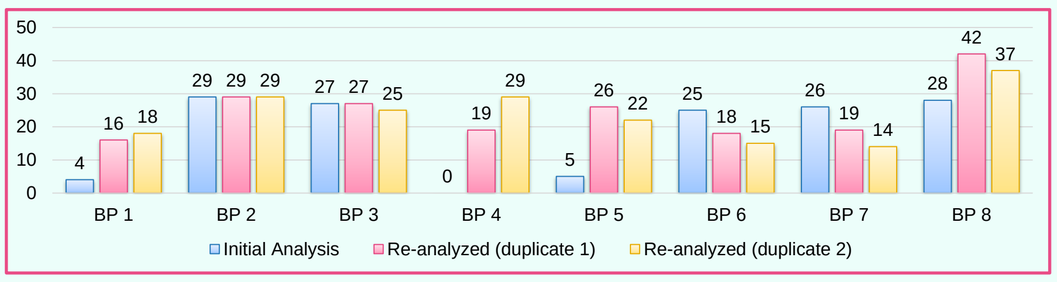


Figure 1: Comparison of number of alleles obtained in each samples. Initial analysis are the first analysis done to the samples in each casework analysis while re-analyzed (duplicate 1 & 2) are the analysis done during this study.

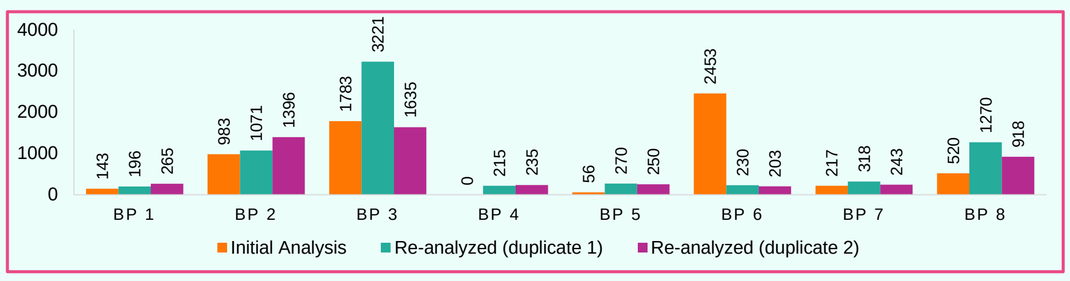
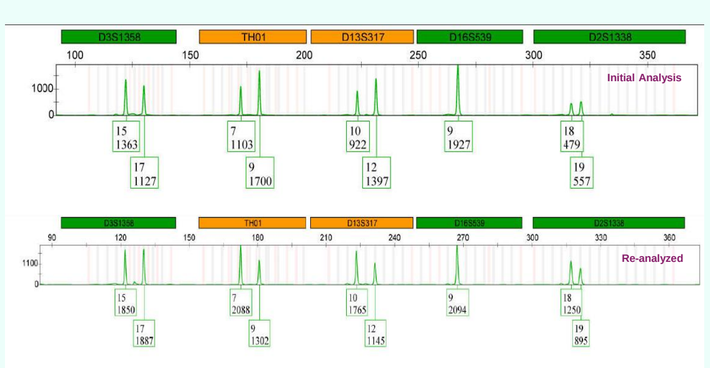
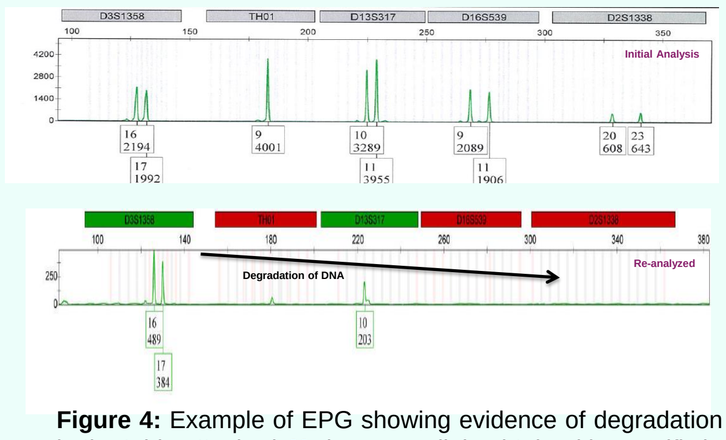


Figure 2: Comparison of allele's peak height average in each samples.



LOCUS	BP 2		
	INITIAL	DUP 1	DUP 2
D8S1179	13,14	13,14	13,14
D21S11	30	30	30
D7S820	8,11	8,11	8,11
CSF1PO	9,10	9,10	9,10
D3S1358	15,17	15,17	15,17
THO1	7,9	7,9	7,9
D13S317	10,12	10,12	10,12
D16S539	9	9	9
D2S1338	18,19	18,19	18,19
D19S433	13	13	13
vWA	14,17	14,17	14,17
TPOX	8,11	8,11	8,11
D18S51	14,15	14,15	14,15
AMEL	XY	XY	XY
D5S818	7,12	7,12	7,12
FGA	18,23.2	18,23.2	18,23.2

Figure 3: Example of EPG with consistent allele obtained in initial analysis and re-analyzed samples. Full DNA profile are as in the table attached.



LOCUS	BP 6		
	INITIAL	DUP 1	DUP 2
D8S1179	11,12	11,12	11
D21S11	30,32.2	32.2	32.2
D7S820	11	X	X
CSF1PO	12	X	X
D3S1358	16,17	16,17	16,17
THO1	9	9	X
D13S317	10,11	10	10
D16S539	9,11	X	X
D2S1338	X	X	X
D19S433	14,14.2	14	14,14.2
vWA	14,17	14,17	14
TPOX	8	8	8
D18S51	15	15	X
AMEL	XY	XY	XY
D5S818	11,13	11,13	11,13
FGA	22,24	22,24	20,22

Figure 4: Example of EPG showing evidence of degradation of DNA. Full DNA profile are as in the table attached. **X** shows no allele obtained in specific locus.

INTRODUCTION

DNA profiling has become the main method for forensic human identification in the last two decades with extensive developments in the analysis of human skeletal remains. Human skeletal remains or bones are often encountered in forensic DNA analysis for body identification process in cases such as homicide or missing persons. Hard structure of bones preserves DNA within its physiological space comparatively well and for a longer time[1]. It is known that compared to soft tissues that may rapidly decomposed, bones can withstand environmental conditions including temperature and humidity[2] and viable DNA fragments can be recovered from bones up to several thousand years[3,4].

In Reference Centre of Forensic DNA Analysis Southern Region, Department of Chemistry Malaysia Johor State, quite a number of unidentified body cases involving skeletal remains were analysed involving those that could have been exposed to the environment for such a period of time or were recovered at a later stage after the incident. Prevailing environment factors such as temperature, humidity, bacterial activity and degradation process could attribute to the differences in quality and quantity of DNA available in the skeletal remains obtained[5]. This study was carried out to demonstrate the effect of time in degradation of DNA in bone powder specimen that had been stored in the laboratory for a different period of time to evaluate the robustness of PrepFiler BTA™ Forensic DNA Extraction Kits.

SAMPLE ID	SOURCE	YEAR ACQUIRED
BP 1	Skull	2020
BP 2	Femur	2019
BP 3	Phalanges	2018
BP 4	Femur	2018
BP 5	Femur	2017
BP 6	Femur	2016
BP 7	Skull	2014
BP 8	Femur	2014

Table 1: Samples used in the study

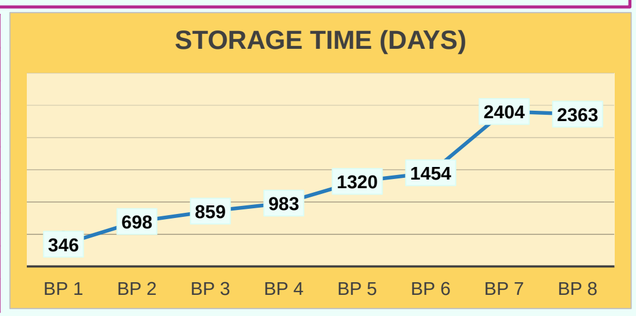


Table 2: Storage time of each samples

DISCUSSION

The variables in this study were successfully analysed and interpreted. Based on the results obtained (Figure 1), two specimens (BP 2 and BP 3) demonstrated similar results between the initial extraction and re-analysis where same number of alleles were obtained in each STR locus. Average peak heights for each allele are also increased even though the specimens were stored up to 859 days. While three specimens (BP 1, BP 4 and BP 5) showed an increase numbers of allele obtained in the re-analysis process despite the difference of storage time for each specimens. Incomplete cell lysis in extraction incubation time of the initial extraction of these three specimens could explain why more alleles were obtained in these specimens during the re-analysis process.

Based on the peak heights evaluation (Figure 2), two specimens (BP 6 and BP 7) showed evidence of DNA degradation occurred since the storage time is up to 2404 days. Significant drop of average peak heights was observed in BP 6 from 2453 rfu to 230 and 203 rfu in the re-analyzed specimens. This finding showed that DNA quality and quantity may be affected by longer storage time of bone specimens. Mixed DNA profile was developed from specimen BP 8 which demonstrated different number of alleles recovered suggesting possible DNA contamination to the powdered bone specimen. Powdery substances are prone to electrostatic charge[7] that might be the source of contamination. Analysis of bone specimen using the PrepFiler BTA™ Forensic DNA Extraction Kits is superior to the time consuming organic extraction process with less inhibition due to its use of magnetic beads[8] where the protocols were optimized for extracting DNA from compromised bone specimens.

CONCLUSION

In conclusion, DNA in powdered bone specimens is still stable and can be well preserved in -20ºC over a period of time. However, degradation could still take place within the sample. The homogeneity of the powdered bone specimen used in the analysis is important to ensure maximum DNA recovery for the samples.

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ACKNOWLEDGEMENT

We would like to thank Zalinda Binti Sabran for her technical assistance during this study.