Investigating the use of the MiSeq FGx^{TM} platform for highly degraded DNA samples for the identification of human remains

PSG3: Increase Wellness, Safety and Tackle Social Ills

Year-end report | 31 January 2020

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2. ABSTRACT

The abstract should include information about the value of the project to knowledge generation in universities and its linkages to the priorities of the WCG, as well as the progress made to date.

Forensic mortuaries in South Africa and worldwide are faced with the ever-increasing challenge of managing unidentified bodies. Unidentified human remains pose a serious burden on society and governing bodies. The state's Forensic Pathology Services are straining under an ever-increasing caseload, which includes bodies that are decomposed, burnt, or skeletonised. The loss of physical features and fingerprints makes identifying these individuals particularly difficult. The financial burden on government is also felt since the storage and subsequent disposal takes up space and incurs costs. Therefore, better methods in identifying these individuals is required to ensure unknown bodies are restored to their families, to improve service delivery, and to reduce financial burdens felt by the Western Cape Government.

This multi-phased project aims to improve methods of identification human identification using DNA analysis in the context of bodies which are no longer physically recognisable (Ethics approval ref: 222/2019). Consequently, the project focused on samples such as teeth and nails, which are more resistant to decomposition than soft tissues. However, due to these properties, obtaining usable DNA from hard tissue is particularly challenging. This CHEC-funded project focused on the first phase of this larger study, which aimed to optimise DNA extraction methods from control teeth (n=52) and nails (n=25).

The standard DNA extraction method (QIAamp DNA Investigator kit), currently used by the South African Police Service, was initially tested to generate baseline results. Adjustments were made to this baseline method which significantly improved DNA recovery and integrity for both sample types. Additionally, the phenol chloroform method was assessed on teeth, which improved DNA recovery but not DNA profiling success. The respective modified QIAamp DNA Investigator methods were partially successful on forensic casework teeth and consistently successful on nails from deceased individuals. These protocols have been translated into standard operating procedures which will be included in the internal validation study scheduled for 2020. Further work is required to optimise DNA extraction from bone, as well as to investigate next generation sequencing methods to generate DNA profiles on highly degraded forensic samples (*e.g.* bone and teeth) which are typically encountered in skeletonised human remains.

The outcomes of this ongoing project have the potential to addresses capacity and quality aspects of service delivery of the Forensic Pathology Services in the local Cape Town by improving methods of identification. This will, in turn, help address the burden communities feel when loved ones go missing and their bodies remain unidentified and never returned to them. This project was aligned with the outcomes stipulated in PSG3.

3. INTRODUCTION AND PROJECT AIMS / QUESTIONS

Reference to the objectives and outcomes specified in the funding proposal should be included in this section. This section should just summarise the information provided in the original proposal.

Introduction

A serious problem faced in the South African Forensic Pathology Services (FPS) is the identification of unknown human remains. These individuals often remain unidentified due to the absence of identity documents, or in some cases, loss of physical features as a result of prolonged post-mortem intervals, or due to external forces (e.g. fire).

The burden of unidentified human remains is not surprising, given in 2017/18 alone over one million serious crimes were reported in South Africa, of which approximately 20 000 were murders (South African Police, 2018). In many cases the remains of unidentified individuals undergo pauper burials, or are cremated and scattered over memorial ground, or in worse cases, onto landfills. In addition to the ethical and moral issues surrounding unidentified human remains, failure to identify these individuals leads to storage issues and places a financial burden on the State.

Deoxyribose nucleic acid (DNA) has proven helpful in facilitating the identification process. However, in certain circumstances obtaining DNA of a sufficient quantity and quality to generate a forensic DNA profile is challenging, and in some cases, impossible. In particular, degraded samples pose an additional challenge, as extensive DNA degradation has often occurred post-mortem due to exposure to the elements or high temperatures.

Current technologies used to generate DNA profiles for identification purposes are frequently unsuccessful in cases of prolonged and complicated post-mortem intervals, thus, leading to further delays in the investigation process. With constant technological advancements in the field of genetics, such as next generation sequencing, improvements in success rates for highly degraded samples are being made.

Aim and objectives

The overall aim of this on-going research project is to optimise a DNA analysis workflow for highly degraded human remains for identification purposes. This aim has been divided into parts, whereby the **aim for 2019 was to optimise the DNA extraction step on hard tissue control samples for human identification applications**. The DNA extraction step is the first step in a series of methods to ultimately generate a DNA profile, the latter which is used for human identification.

The objectives were to:

1. Identify tissue samples that would be available as a source of DNA in decomposed, burnt, or skeletonised human remains by a retrospective analysis of past cases.
2. Extract DNA from the fresh sample types using different extraction kits and identify the best performing method.
3. Assess DNA for quantity and quality and determine the best performing extraction method.

4. RESEARCH APPROACH AND METHODS

A short summary of the approach and methods used should be included, but detailed descriptions of highly technical processes may be omitted.

Approval
Ethics approval was obtained for this study (HREC: 222/2019).

Retrospective review
The first objective was to identify tissue samples that would be available as a source of DNA in human remains. To this end, a retrospective analysis of the UCT Forensic Human Skeletal Repository was done. This entailed the analysis of each of the 150 cases present in the repository and the recording if bones, teeth and other tissue samples were present.

Selection of sample type
Based on the findings from the retrospective analysis, cranial bone (n = 95 cases) and teeth samples (n = 75 cases) were identified as being the most common sample types present. Therefore, DNA analyses should focus on these sample types. However, since optimisation experiments require large amounts of samples to test for several variables, and that fresh samples of cranial bones are not easily obtainable from donors, teeth were prioritised for this study. In addition, previous research in my research group showed that nails were present in many of the unidentified remains at Salt River Mortuary (Reid *et al.,* 2020). Therefore, teeth and nails were included as sample types in this study.

Overview of optimisation approach
The standard DNA extraction method (QIAamp DNA Investigator kit), currently used by the Forensic Science Laboratory (FSL) at the South African Police Service (SAPS), was initially tested on teeth and nails to generate baseline results. This will be referred to as method 1. After DNA was extracted, DNA concentration, DNA degradation and DNA profiling success were measured using standard forensic genetics methods. Based on these results, adjustments to the method were applied. In the case of teeth, a modified phenol chloroform method was also assessed, which will be referred to as method 2 (Pagan *et al*., 2010). After each adjustment, DNA metrics were assessed and used to inform the next step.

Samples and procedure (summary)
A total of 52 teeth from three donors were used in the optimisation phase where method 1 (no adjustments) and method 2 were applied. A further six teeth from a forensic case example were used for assessment of the best method in an authentic context. Additionally, four teeth from a single donor were used to test further adjustments on method 1 in attempts to improve results. These adjustments included: (i) the cooling method utilised prior to homogenisation, (ii) the tooth powder input amount, (iii) the revolution per minute (rpm) used during incubation and (iv) the use of a modified demineralisation buffer. The best combination of variables was assessed on a forensic case example, where a tooth and bone sample were available.

For nails, 25 nails from five donors were used in the optimisation phase where method 1 was applied, initially without adjustments and then with the following adjustments: (i) the weight of input nail and (ii) the revolution per minute (rpm) used during incubation. The best two combinations of variables were each assessed on nail clippings from six deceased individuals.

5. CONCLUSIONS AND RECOMMENDATIONS FOR FOLLOW-UP ACTION

This is the most critical aspect of the report in that it needs to synthesise the findings, draw conclusions and suggest follow-up actions, e.g. for further research, policy development and implementation.

Results

Teeth

DNA was extracted from 52 teeth using the QIAamp® kit (method 1) and the modified phenol-chloroform method (method 2). The results showed that the average DNA yield was significantly higher using method 2 compared to method 1 (Figure 1a). DNA was also significantly less degraded when method 2 was used (Figure 1b).

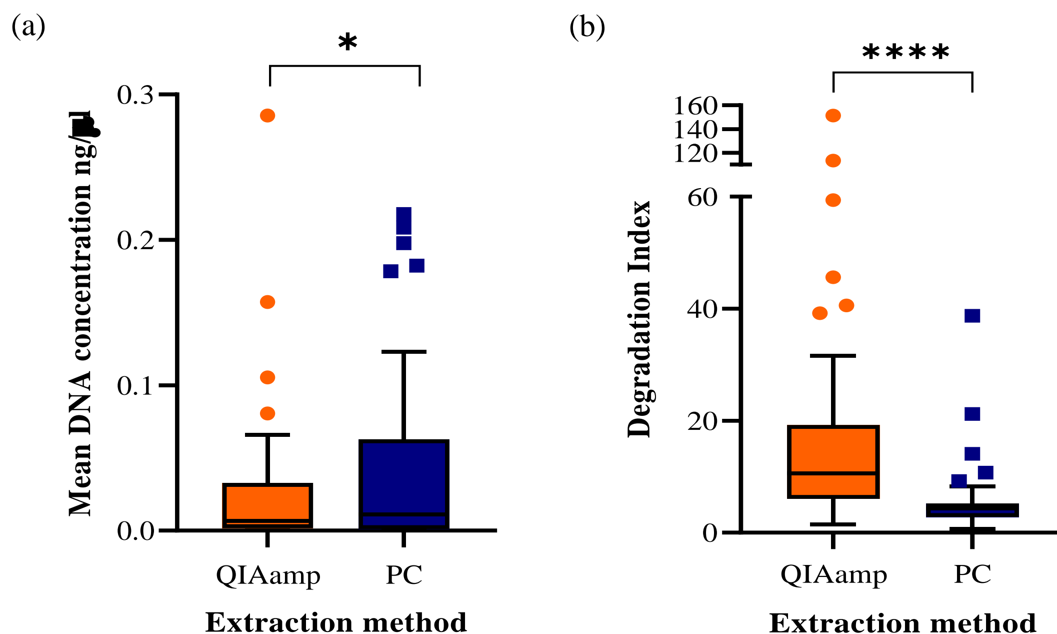

Figure 1: Box plots comparing (a) the mean DNA recovery and (b) the Degradation Index of the two extraction methods. Circles and squares indicate outliers; PC = Phenol chloroform; * ($p < 0.05$) and **** ($p < 0.0001$).

Despite method 2 producing significantly better DNA concentrations and less degraded DNA, there was no statistically significant difference in DNA profiling success: 33 % full profiles for method 1 and 41 % full profiles for method 2. When method 2 was applied to six teeth from a forensic case, none of the teeth produced full DNA profiles, but 3 of the teeth produced partial DNA profiles. These results show that method 2 is an improvement, but there was still scope to develop the workflow further.

Therefore, method 1 was investigated further on control teeth (n = 4), and included four additional adjustments. The results showed that teeth should undergo flash freezing with liquid nitrogen prior to homogenisation, 0.05 g (a decreased input amount) should then be incubated with an adjusted demineralisation buffer at a decreased rpm of 450. These adjustments substantially improved DNA concentration and reduced DNA degradation compared to the baseline method 1 procedure. When applied to teeth and bone from a forensic case, partial DNA profiles were generated from all samples. The modified method 1 seemed to perform best overall.

Nails

The baseline protocol indicates that 5 mg of nail must be incubated for 16 hours in the first step of the DNA extraction procedure. Using this procedure, 80% of samples yielded full DNA profiles, while 20% yielded partial DNA profiles (Table 1). In attempts to improve this, 10 mg of nail was incubated at 16 hours which yielded 100% full DNA profiles. However, 10 mg of nail might not always be available in forensic investigations, so 2 mg of nail was also investigated, both with 16 hours incubation and 2 hours incubation. Full DNA profiles were generated from all the samples when 2 mg of nail was used. Therefore, with the motivation to use as little sample as possible, the 2 mg of nail protocol was applied to nails from six deceased individuals, and both incubation times were tested. Full profiles were obtained from all 6 samples (100%) when 2 mg of nail was incubated for 16 hours, whereas full profiles were obtained from 5 out of 6 samples (83.3%) when 2 hours incubation was done. Therefore, the 2 mg of nail combined with 16 hours incubation was selected as the best protocol.

Table 1: Percentages of full DNA profiles when different weights of nails and different incubations were applied, to nails from living and deceased individuals.

	Incubation time	Nail input weight	Full profiles	Comments
Living	16 hours	5 mg	80%	Baseline protocol
		10 mg	100%	Increased sample might not always be available
		2 mg	100%	Selected for testing on deceased individuals
	2 hours	2 mg	100%	
Deceased	16 hours	2 mg	100%	Selected protocol
	2 hours	2 mg	83.3%	-

Outcomes

These results have been translated into standard operating procedures:

1. DNA extraction from hard tissues using the QIAamp® DNA Investigator kit
2. DNA extraction from nails using the QIAamp® DNA Investigator kit

Risk assessments have also been done and documented.

Both of these standard operating procedures will be included in the internal validation study scheduled for 2020. After internal validation, these procedures can be standardly used in Forensic Pathology Services to extract DNA from teeth and nails. We anticipate that this will be especially beneficial for the new Observatory Forensic Pathology Institute, which is due to open later this year.

Conclusion

The aim and objectives of this study were met. DNA extraction methods were optimised for teeth and nails, which are both common biological sample types available in unidentified human remains. The variables adjusted in this study significantly improved DNA recovery and DNA profiling success.

For teeth, the phenol chloroform method as well as the modified QIAamp® DNA Investigator kit were able to recover DNA with significantly higher concentrations and less degradation than the baseline

method. However, the modified QIAamp® DNA Investigator kit performed better on casework samples. Due to the nature of skeletonised human remains, forensic samples are typically degraded, and this was the reason why full DNA profiles were not obtained. Therefore, future work to improve DNA profiling step is needed. This was anticipated and is accounted for the overall study, which aims to use next generation sequencing techniques in the future, to assess this. However, the substantial improvements in DNA recovery have contributed immensely to the improvement of the overall workflow and has been translated into a standard operating procedure.

For nails, improvements were made to the baseline protocol, which led to the consistent generation of full DNA profiles from nails from both living and deceased individuals. This study was the first to use as little as 2 mg of nail and still generate full DNA profiles. The use of such small amounts of nail is also beneficial in a forensic context, where samples are typically limited.

In the future, the DNA extraction procedure needs to be optimised and assessed for bone samples. Additionally, next generation sequencing methods need to be investigated for the analysis of highly degraded DNA samples, which are typically encountered in teeth and bone samples from skeletonised human remains.

6. BUDGET

Please reflect the actual spend to date against the budget included in the original proposal

	Description of budget line item	Amount requested	Amount spent
a.	**DNA extraction method 1:** Qiagen Investigator QIAmp DNA extraction kit (50 samples); R8000 per kit x 3	R 24 000	R23 708.40
b.	**DNA extraction method 2:** Reagents for manual phenol chloroform DNA extraction	R 10 000	R4 675.24
c.	Absolute ethanol (500ml): R1000 per bottle x 2	R 2 000	R963.70
d.	Quantifiler Trio kit (400 reactions): R14 000 per kit x 1	R 14 000	R21 135.57
e.			
f.			
	TOTAL	**R50 000**	**R50 482.91**

Line item b (the reagents for DNA extraction method 2) was also funded by internal UCT funds which supported a Master's project (additional funds are not reflected here).