Executive Summary

The extraction of DNA from the petrous bone, alongside the use of high throughput sequencing (HTS) coupled with single nucleotide polymorphisms (SNPs) represents, by far, the most efficient way to analyse degraded DNA and identify the remains at the Mother & Baby Homes.

Despite significant advances in analysing DNA and the establishment of a forensic DNA Database that utilises Short Tandem Repeat (STR) profiling, Forensic Science Ireland (FSI) does not have the expertise to carry out SNP analysis. This is not particularly unusual as STR profiling is the standard analysis used for criminal investigations.

However, despite the establishment of an Agency to co-ordinate the operation of identifying the remains at a putative site, there are provisions within the Bill that anchor the FSI in the sampling, analysis and matching of the DNA. Essentially, there are fundamental differences between STR forensics and HTS coupled with SNP analysis. The way DNA is processed and matched with relatives differs between the two techniques.

Irish universities have academics with significant capabilities in SNP array analysis. This has already been shown with the identification of Thomas Kent using this advanced DNA technology. The Bill allows for the Agency to hire consultants. The Bill should extend the operational activities of such consultants to enable them to work in partnership with the FSI. This could be to sample DNA from the petrous bone from the remains, to allow for the processing of the extracted DNA using SNP arrays from any remains and to match the DNA. Currently the Bill mandates that FSI conducts searches of DNA profiles on the DNA (Historic Remains) Database in order to make a familial match. This is problematic as STR matches use different software to matches using SNP arrays.

Introduction

My background is in biotechnology, molecular pathology and law. I obtained a PhD at the University of Leicester, the institute where DNA profiling was invented. I also completed a postdoctoral fellowship at the Brigham & Women's Hospital at Harvard Medical School. Further, I completed a part-time law degree at Griffith College, where I helped to establish the Innocence Project. The latter investigates miscarriages of justice, mostly through the use of DNA technologies. I am familiar with both the technical and legal aspects of the forensic DNA Database in operation in this jurisdiction. I am also familiar with the advanced DNA technologies that are used in archaeological and paleogenomics fields. I currently work as a Technology Transfer Manager at University College Dublin (UCD).

Background

In broad terms it appears that the Bill can be delineated into provisions that (i) deal with allowing an authorised Agency to access sites for the purpose of excavation and exhumation of human remains; and (ii) the identification of those remains using DNA technology. For the purposes of this submission, I shall not refer to those provisions dealing with accessing the site. It is presumed that the complexity of the Bill in this regard revolves around the difficulties in carrying out criminal investigations into the religious orders who owned the sites where the remains where found. Various individuals and Amnesty International[1] have called for criminal investigations into the

[1] https://www.amnesty.ie/amnesty-calls-for-reparations-and-criminal-investigation-following-mother-and-baby-homes-report/

Mother and Baby Homes due to the high levels of infant mortality rates, the unauthorised vaccine trials and alleged abnormalities regarding the registering of birth and death certificates. Regardless, as stated, I am not going to address those provisions in the Bill.

The premise of this submission is that routine DNA forensic testing utilising STRs will fail to identify the vast majority of the remains at the Mother and Baby Homes, including Tuam. This was the central tenet of another submission that I, along with Professor Dan Bradley, Associate Professor Jens Carlsson and Professor David MacHugh made in 2018 ('the 2018 Report') when calls were being made for the public to make submissions regarding the Options and Appropriate Courses of Actions available to the Government at the site of the former Mother & Baby Home, Tuam, Co. Galway. I include that report at the end of this submission for the attention of the Committee again by way of reference.

The requests for public consultations in 2018 were partly made due to the findings of the Expert Technical Group (ETG) in the previous year. The ETG Report was sceptical about the ability to identify the remains at Tuam using STR DNA techniques. In our report in 2018 we indicated that the ETG appeared to ignore the potential of using HTS coupled with SNPs to analyse degraded DNA. We suggested a number of actions, including a pilot study to extract DNA from the petrous bone from twenty different juvenile skulls, along with HTS of the human remains and integration with single nucleotide polymorphism SNP DNA array from living people to identify the remains.

It is important to note the reason we felt compelled to write this report was due to the apparent chasm between the DNA techniques used in forensic science and archaeological and paleogenomics research. We felt that the advanced DNA techniques used in archaeological research were not considered in the ETG Report. This was also surprising because, as we noted in the 2018 Report, there had been a collaboration between UCD researchers, FSI and the Garda Technical Bureau in using HTS and DNA extracted from the petrous bone in order to identify the remains of Thomas Kent, one of the Irish rebellion leaders in 2016.

The use of SNP analysis to identify human relationships is now being advocated by a number of key opinion leaders in the field. In August 2020 Jianye Ge and Bruce Budowle at the Centre for Human Identification at the University of North Texas Health Science Center produced a paper where they urged forensic laboratories to adopt high-density SNP arrays for the identification of human remains[2]. The paper was featured in the high-profile media platform Genomeweb and in that piece Justine Petrone [3] highlighted a number of issues that are very pertinent for the context around the legacy reasons for the continued use of STR forensics. I would like to quote extensively from it.

Petrone revealed that:

> *"Researchers are now suggesting to take a page from consumer genomics and adopt array-based SNP genotyping or whole-genome sequencing data to more reliably determine relationship."*

Quoting Ge:

> *"Theoretically, with so much SNP data, the accuracy of close relationship testing should approach 100 percent, which could rectify a serious concern of misidentifications, both*

[2] https://journals.plos.org/plosgenetics/article/metrics?id=10.1371/journal.pgen.1008929

[3] https://www.genomeweb.com/applied-markets/researchers-encourage-forensics-labs-adopt-new-genomic-technologies-relationship

related and unrelated, and third-degree or more distant relationships may be determined with high accuracy,"

Ge also indicated that it was time for forensic laboratories to move to new technologies. "*We encourage the whole community to consider shifting from STRs to SNPs*," he said.

Ge suggested that forensic genetics is traditionally more conservative when it comes to the adoption of technology, partly because the use of any technology for legal purposes must be highly vetted and validated. Further, another potential barrier to the uptake of new technologies is that the US Federal Bureau of Investigation's Combined DNA Index System (CODIS) contains STR marker information on roughly 15 million individuals. Indeed, as indicated earlier, STRs form the basis of identity for forensics across the globe, including Ireland and the UK.

Consumer genomics companies built their databases *de novo* on the basis of innovations from the life sciences industry, and these technologies have been adapted and improved by researchers in the archaeological and paleogenomics fields. In contrast, the forensic genetics community has both an entrenched method and a large database centred on it. For this reason, laboratories continue to churn out STRs while complimenting the results with SNP arrays or sequencing as supplementary tests. "In criminal cases, we still need to have the STR results," noted Ge. "CODIS only accepts STRs."

This assertion that SNP arrays would be significantly more effective at identifying the remains than STR forensics has consequences for certain provisions in the Bill. Essentially, the techniques used to process the DNA and match these sequences with relatives are different. With this context I will proceed to comment on specific provisions within the Certain Institutions Burials Bill and how they could be amended to accommodate SNP arrays.

Comments on the Bill

The Agency

According to Head 3 (5) an order will be brought delineating the boundaries of the site and the duration for which the Agency will operate *inter alia*. The existence of the Agency therefore appears associated with an individual “prescribed” site. Head 22 determines that “each Agency” established under the General Scheme shall prepare an annual report relating to the performance of the preceding year.

Technically, according to Head 51 DNA can be extracted from potential family members at the “Agency office” by an authorised person. However, Head 48 provides that the FSI will store all biological material and confidential information collected by the Agency and Head 51 (3) provides that samples from the bodies at the sites may be taken by a “member of staff of FSI”. Further, the DNA (Historic Remains) Database System is to be maintained by the FSI. It would appear, therefore that the Agency could be described as a temporary affiliate of the FSI.

Criminal Investigations

The Bill allows for the potential of criminal investigations. Head 6 provides that the Government will not make an order if there is evidence of “violent or unnatural circumstances” or there is an ongoing Garda investigation. Head 32 deals with the “Suspension of certain functions and information to be available to assist criminal investigations” and indicates that an Inspector or higher-ranking Garda may request the Agency to suspend works relating to excavation or exhumation on “the grounds that a criminal investigation relating to person(s) buried at a site is being conducted and the Agency shall comply with the request”.

The question then arises as to how this process is triggered. There is a focus in the Bill on the employees of the Agency being empowered to extract DNA for example. There is no mention of forensic anthropologists, although the Agency is also empowered to hire consultants or specialists. One would not be able to tell whether there has been "violent or unnatural circumstances" from a DNA profile. Rather, forensic anthropologists would need to ascertain whether there is trauma or bone malformation. This needs to be explicitly stated in the Bill. Otherwise, it is difficult to envisage how an actual criminal investigation would be triggered.

DNA Profiling and Identification

There is evidence that the Department of Children and Youth Affairs has taken on board some recommendations of the 2018 Report. According to the Bill a "DNA Profile",

> in relation to a person, means information comprising a set of identification characterisations of the DNA derived from an examination and analysis of a sample of biological material that is clearly identifiable as relating to the person and that is capable of comparison with similar information derived from an examination and analysis of another sample of biological material for the purpose of analysis.

According to the Criminal Justice (Forensic Evidence and DNA Database System) Act 2014,

> A "DNA profile", in relation to a person, means information comprising a set of identification characteristics of the non-coding part of DNA derived from an examination and analysis of a sample of biological material that is clearly identifiable as relating to the person and that is capable of comparison with similar information derived from an examination and analysis of another sample of biological material for the purpose of determining whether or not that other sample could relate to that person;

The latter is a relatively standard definition for DNA profiles for criminal justice databases in other jurisdictions and the key words relate to "non-coding". The Short Tandem Repeat (STR) markers were partly developed because they do not span across genes – the coding elements of the genome. In contrast a standard SNP analysis spans across the whole genome, including the coding elements. The Bill is silent with regard to the use of non-coding DNA.

The Bill dictates that a pilot programme should be used to evaluate whether DNA can be generated from the remains at any site. This is sensible and was also recommended by the 2018 Report. This section (Head 47) is silent as to who carries this out or how the DNA should be extracted. However, Head 51, which provides for the taking of samples from the bodies in the remains after a positive pilot programme, determines that "the samples may be taken by a member of staff of FSI or another person prescribed for that purpose". The 2018 Report indicated that using the petrous bone was the preferred method of extraction of DNA from the remains. Indeed, this is a technique now used by DNA anthropologists to extract ancient DNA. The technique could be taught to FSI scientists if they are unfamiliar with it.

In Head 52 (3) it is prescribed that "the Director of the Agency may request FSI to conduct a search of DNA profiles on the DNA (Historic Remains) Database in order to make a familial match". This is a concern as if HTS integrated with SNP analysis is used (as the 2018 Report suggested it should be), the techniques used for familial matching are different to standard STR analysis matching. In short, the FSI would not have the skill sets to carry this out.

Head 53 determines who may participate in the Identification Programme and 53(4) explicitly prescribes the nature of the relativity. As one would expect first order relatives (parents, siblings and

children) are included, along with a specific type of second order relative, a half-sibling. It does not make sense to exclude other second order relatives, including grandchildren, uncles, aunts, nephews and nieces. Indeed, the DNA from the niece of Thomas Kent was matched by Jens Carlsson's team to identify him using SNP analysis.

There appears to be an unnecessary haste to destroy the biological samples and delete the DNA profiles from both the remains and potential family members. Head 60 prescribes that samples taken from potential family members will be destroyed no later than the expiration of 3 months after certain events, including the generation of a DNA profile. It is difficult to understand the reasoning for this, especially if the appropriate informed consent is obtained from the potential family members. Head 65 provides for the destruction of samples taken from the bodies of the remains when an identification programme has concluded without a familial match being made and it is not considered possible to achieve a familial match. The lack of a match may be rectified by new technology in the future and it does not make sense to destroy the samples from remains, especially when they are unidentified.

DNA Reconciliation Projects

Alondra Nelson published a book in 2016 entitled 'The Social Life of DNA'.[4] On the 15th of January 2021 Nelson was appointed by the then President-elect Joseph Biden to the position of Deputy Director for Science and Society in the Office of Science and Technology Policy (OSTP).[5] Her book explores the potential of DNA technology to assist individuals in tracing their ancestry and, more importantly perhaps, to enable so-called 'Reconciliation Projects'.

> "*With Reconciliation Projects, DNA analysis is incorporated into attempts to reunite formerly opposed parties or formerly united ones (rejoining broken ties within a family, a community, a nation-state, or a diaspora); to uncover biographical or historical information that has been lost to the march of time; or to adjudicate contentious issues."*[6]

The endeavour to identify the remains at Mother & Baby Homes represents a significant opportunity for the State to engage in an historic 'Reconciliation Project'.

In her chapter on 'Reconciliation Projects' Nelson wrote:

> "*There comes a time in the life of every community when it must look humbly and seriously into its past in order to provide the best possible foundation for moving into a future based on healing and hope."*[7]

I would like to focus on three words here – "seriously", "healing" and "hope". In particular, the Committee needs to ask itself whether it is serious about identifying the remains in the Mother & Baby Homes. The ETG was correct in asserting that STR analysis was unlikely to be successful in identifying the remains at Tuam. However, the ETG overlooked the potential of HTS coupled with SNP array analysis. The State has used these advanced DNA technologies before to identify the remains of Thomas Kent. The horrors unearthed at Tuam, represent, I would argue, one of the most appalling occurrences since the foundation of the State. The people of Ireland have been shocked by the revelations that children's remains were dumped in a sewage tank. The Bill needs to act as an enabler to offer hope that these remains can be identified and buried in a dignified manner. This can only be done using SNP array analysis.

[4] Alondra Nelson, The Social Life of DNA (Boston, USA: Beacon Press, 2016).
[5] https://www.ias.edu/news/2021/nelson-ostp-appointment
[6] Alondra Nelson, The Social Life of DNA (Boston, USA: Beacon Press, 2016), p8-9.
[7] Alondra Nelson, The Social Life of DNA (Boston, USA: Beacon Press, 2016), p27.

Recommendations for the Bill

(1) Amend the Bill explicitly such that external consultants can work with the FSI to extract DNA from the remains;
(2) Amend the Bill explicitly such that external consultants can work with the FSI to analyse the DNA;
(3) Amend the Bill explicitly such that external consultants can work with the FSI to match the DNA; essentially the DNA (Historic Remains) Database System should not be based on STR matching;
(4) Amend the Bill such that aside from half-siblings other second order relatives, including grandchildren, uncles, aunts, nephews and nieces may participate in the Identification Programme;
(5) Amend the Bill such that provided appropriate informed consent is obtained the samples and profiles of potential family members without any matches would be held for an agreed longer period than the Bill provides;
(6) Amend the Bill such that any samples or profiles from the remains that are not matched are held indefinitely.

Consultation on the Options and Appropriate Courses of Actions available to Government at the Site of the former Mother & Baby Home, Tuam, Co. Galway, Ireland.

Professor Dan Bradley, Dr Jens Carlsson, Dr Stephen Donoghue, Professor David MacHugh.

Summary

A pilot study to extract DNA from the petrous bone from twenty different juvenile skulls would demonstrate the feasibility of the extraction technique.

Extraction from the petrous bone of individual skulls is relatively unobtrusive and the risk to destruction of the remains is minimal. Further, this technique is unlikely to have the problems associated with comingling as the DNA will be extracted from individual skulls.

High throughput sequencing (HTS) of human remains and integration with single nucleotide polymorphism (SNP) DNA array from living people has been extremely successful in genetic identification and analysis of archaeological materials; however, this approach is not pursued by the Expert Technical Group (ETG) as a viable option for identifying the remains at Tuam.

The costs associated with this pilot study are marginal and likely to be significantly cheaper than the figures quoted in the ETG Report.

ETG Report and Focus on STR Analysis

An Expert Technical Group (ETG) was commissioned on the 1st of June last year by Minister Zappone to examine the juvenile remains discovered at Tuam. The final report of the Group was presented to the Minister on the 19th of October 2017 and published by the Cabinet on the 12th of December 2017. The ETG report distinguished five options to deal with the site, ranging from memorialisation to forensic excavation of the total available area (ETG Report, i-ii). With regard to what is termed Humanitarian Forensic Action, essentially the use of forensic science to recover and identify individuals in mass death scenarios, the report identified a number of factors for consideration. These include the collection of witness testimony, further testing and evaluation of specific areas and a full forensic anthropological analysis. However, foremost in the report was an "assessment of [the] application of DNA technologies" (ETG Report, iii) to identify the remains.

Rather surprisingly, concerning the use of DNA for the identification of the remains, the tone adopted by the ETG is decidedly circumspect. The issue is "complex" and "individual identification of remains here is unlikely without further significant investigation" (ETG Report, ii). The report suggests that the comingled state of the remains makes identification "particularly challenging" (ETG Report, ii). There is a "risk of destruction to human remains" that raises ethical issues (ETG Report, ii). Following the publication of the ETG Report the Minister is quoted as saying that the "particular and complex circumstances of the situation in Tuam [are] unprecedented from a technical perspective" (Department of Children and Youth Affairs website).

The leading expert employed by the ETG to review the potential of DNA forensics to identify the remains is Dr Tim Clayton, formerly a senior scientist with the Forensic Sciences Service (FSS) in the UK. There is substantial experience in the UK in use of DNA forensics for criminal investigations. In 1995 the UK National DNA Database (NDNAD) was launched and somewhat controversially the profiles of over 5 million UK citizens are currently stored on the NDNAD (National DNA Database Strategy Board, Annual Report 2015/16). In contrast, in 2014, twenty years after the UK legislation was enacted, the Irish Dáil passed the Criminal Justice (Forensic Evidence and DNA Database

System). Last year the first report documenting the number and types of DNA profiles on the Irish DNA Database was published by the newly created Forensic Science Ireland (FSI) authority. The report indicated over 9,000 profiles were maintained on the Irish DNA Database (Forensic Science Ireland, 2016 Report).

Similar to other jurisdictions, with the establishment of the Irish DNA Database the FSI adopted standard Short Tandem Repeat (STR) analysis as its standard. STR analysis is used to compare specific regions (of circa 200 to 500 nucleotides), or loci, on DNA from two or more samples. The technique was developed in the 1990s after it was observed that the human genome contained numerous repeated DNA sequences. These genetic 'markers' are categorised according to which chromosome and in which region of the chromosome they are located. The number of repeat units making up the overall length of the repeat region facilitates identification of individual profiles and with enough STR markers can provide a unique genetic profile for an individual person, with the exception of monozygotic (identical) twins.

The Irish DNA Database System consists of both a criminal database (the "investigation division") and a missing/unknown persons database (the "identification division") (Criminal Justice (Forensic Evidence and DNA Database System) Act 2014). The legislation allows for the taking of a sample from an "unknown deceased person" under section 50 of the Act. The relevant Coroner of the region may authorise the taking of the sample from the deceased person. Further, section 48 allows for the taking of a sample from a "person who is a relative by blood of a missing person…for the purpose of generating a DNA profile in respect of the person concerned to be entered into the missing and unknown persons index of the DNA Database System to assist with finding or identifying the missing person". Thus, so-called familial profiling may be utilised to investigate a genetic link between a deceased person and a family member (Maguire *et al*. 2014). Finally, according to section 68, DNA profiles entered into the missing and unknown persons index of the DNA Database System may be compared with other DNA profiles in the identification division and DNA profiles in the investigation division.

On the face of it, the State has access to the tools to identify the remains at Tuam using DNA forensic techniques. However, the ETG report documents a number of potential problems in relation to the use of DNA technology at the site. First, mention is made of the destructive process associated with DNA extraction. It details the powdering of a complete tooth, or approximately 1g of bone, in liquid nitrogen, followed by treatment with various chemicals (ETG Report, p27). The report confirms that "testing skeletal remains is time-consuming and technically difficult as specialist DNA extraction techniques have to be applied". The ETG Report alludes to the problems associated with DNA degradation as a consequence of age and the environment. Further, one of the major problems identified by the ETG Report relates to the comingled nature of the bones and the difficulty in separating these remains. Finally, the Report also refers to the potential difficulty of collecting ante-mortem samples and establishing the genetic relationship of those to the deceased.

High Throughput Sequencing and SNP Arrays

It must be stated that the authors feel that many of the problems articulated in the ETG Report with regards to the use of DNA forensics at the Tuam site are overstated. In particular, the emphasis and focus solely on STR analysis as the only means to confirm identity is surprising: "While both Y-DNA and mtDNA may indicate relatedness, STR is the method which can provide identification" (ETG Report, p29). The ETG appear to ignore the potential of using high throughput sequencing (HTS) coupled with single nucleotide polymorphisms (SNPs) to analyse degraded DNA.

SNPs represent the simplest form of genetic variation; they arise as "DNA letter spelling" variations (usually two different variants) of one single DNA base-pair in the double-stranded DNA that makes

up individual human chromosomes. It is known that approximately 90 per cent of the genetic variation that exists among humans is a consequence of SNPs. Until the early years of this century, STR genetic markers (microsatellites) were the most widely used genetic markers for population genetic and relatedness analysis. However, rapid advances in genetic technologies, including the development of high-throughput sequencing (HTS) and the invention of relatively cheap SNP arrays or "chips" has dramatically changed the genetics landscape. HTS and SNP arrays are now overwhelmingly the tools of choice for assessing genetic variability in humans and other species. We can now routinely use many thousands to millions of SNPs for population genetics and genomic relatedness analyses with unprecedented statistical power, but at a relatively low cost.

DNA degradation is a serious issue for both STR and SNP array analysis and depends on a number of factors, including age, the environment, the source of DNA and the extraction method. However, HTS allows for the retrieval of vast amount of SNP data and allows for a greater likelihood of identification. A variety of studies have reviewed the potential of using SNPs in criminal forensic DNA databases (ENFSI DNA Database Management, 2017). However, it is highly unlikely that the STRs will be replaced by SNPs. First, legal certainty and precedence dictate that there is a reluctance to adopt a new type of genetic marker that would presented as standard evidence before the courts. Secondly, there would be a significant economic cost for countries with established and mature DNA databases to adopt a new genetic marker. Profiles utilising SNP markers would need to be generated from the original genetic material, including crime scenes and so-called reference samples from individual subjects. In essence, there is a need in mature DNA databases to maintain a legacy from information gleaned from the profiles generated (Butler 2015).

The ETG Report refers to best international practices for various forensic techniques. It references the European Network of Forensic Science Institutes (ENFSI) and the various working groups within that organisation to improve forensic science through the exchange of information and research and development. The DNA Database Management Review and Recommendations report from the ENFSI DNA Working Group recognised the potential for the use of mini-STR and SNP kits to obtain DNA profiles from degraded DNA (ENFSI DNA Database Management, 2017, p42). However, as noted above, the legacy problems of comparing those profiles generated with a different technology to the reference subject DNA profile that was generated with the standard STR technology was emphasised.

Nevertheless, these information legacy issues have little relevance for the identification of these remains in Tuam. As mentioned earlier, the Irish DNA Database utilises STRs and we would suggest that the technology associated with STR profiling and the existing profiles on the Irish DNA Database are likely to be redundant for the purposes of identification. In principle, there is no reason why advanced techniques should not be used for identification of the remains; for example, SNP data generated using HTS of the Tuam remains could be integrated with high-density SNP array data obtained from living people. This approach would provide tens of thousands, if not hundreds of thousands of binary genetic markers for highly accurate genetic identification and matching to putative living relatives.

Regarding the source, traditionally, forensic laboratories have used the femur as a target for securing DNA. Intuitively, this makes sense as the femur is the largest bone in the body. However, it is not the densest bone and HTS of ancient DNA (extracted from human remains that are several thousands of years old) has repeatedly shown that the petrous bone in the temporal bone, which is the densest bone in the body, generates higher quantity and quality DNA than femur or teeth. Using the petrous bone would not only be the preferred method for securing DNA, but also for minimising further damages to the human remains as the bone is accessed from the inside of the skull with a very small area affected. Further, while the remains at the Tuam site are comingled to a lesser or

higher degree, the use of the petrous bone would allow for ensuring that one individual is analysed at a time and the containment of DNA within the petrous bone ensures minimal DNA contamination between individual children.

The power of these advanced techniques was vividly illustrated recently when the Taoiseach's Office requested the assistance of one of the authors in identifying the remains of the Irish Easter Rising Rebel Thomas Kent. In a collaborative project involving personnel from An Garda Síochána, FSI and University College Dublin, SNP analysis using HTS was successfully implemented in confirming the identity of Thomas Kent. The remains of an individual believed to be Thomas Kent had been buried for 99 years. Extraction from the petrous bone yielded significant quantities of DNA and by employing HTS to detect SNP variants and comparing those to SNP variants from living relatives of Thomas Kent it was possible to confirm the expected relatedness with the living relatives.
The methods used to confirm the identity of Thomas Kent would be particularly useful for DNA analyses of the remains at the Tuam site. While the petrous bone is the most likely skeletal element to yield DNA, it should be emphasised that there is no guarantee that DNA can be secured from all individuals. Further, to successfully establish the identity of the remains it is crucial that living relatives contribute their DNA to be compared to the DNA from the remains. However, successful identification does not necessarily rely on first-degree relatives (parents or siblings) as even more distant relations can be used (in the case of Thomas Kent, the DNA from his nieces was used to confirm the identity). The costs for such analyses would be significantly less than the figures quoted in the ETG Report.

To that end, we have produced a Technical Annex to this document detailing the methodologies that would allow genetic identification of juvenile remains at Tuam. The Annex outlines the background and context around HTS methods and SNP arrays and the extraction procedures from the petrous bones of juveniles. It also details how libraries would be constructed using DNA extracted from the individual remains to generate SNP data that could be integrated and compared with SNP array data from living people (or from deceased people with stored biological material, e.g. archived medical samples). These genome-wide SNP data would then be used to establish a genetic relationship between children interred at Tuam and putative living relatives.

The authors disagree with the pessimistic and guarded tone adopted in the ETG Report regarding the potential to identify the remains at Tuam using DNA technology. There are challenges, but we would suggest that the expertise exists within Irish research institutions to overcome them. However, we do acknowledge that the ETG Report is accurate in its findings that any extraction and analysis of DNA from these remains would need to be carried out using quality procedures. The identification of Thomas Kent, at the request of the Taoiseach's office, using the outlined extraction methods, coupled with HTS and SNP analysis, further confirms the validity of the technique and the tremendous know how in this space in Irish universities alone.

References

Criminal Justice (Forensic Evidence and DNA Database System) Act 2014, http://www.irishstatutebook.ie/eli/2014/act/11/section/67/enacted/en/html
Department of Children and Youth Affairs website, accessed 12th March 2018 https://www.dcya.gov.ie/viewdoc.asp?DocID=4515
ENFSI DNA Database Management, Review and Recommendations, ENFSI DNA Working Group April 2017, p42.
Expert Technical Group Report, "Options and Appropriate Courses of Action available to Government at the site of the former Mother and Baby Home, Tuam, Co. Galway", December 2017.
Forensic Science Ireland, Annual Report 2016, p31. http://forensicscience.ie/documents/FSI%20Annual%20Report%202016.pdf

Maguire, C.N., et al. (2014) Familial searching: a specialist forensic DNA profiling service utilising the National DNA Database to identify unknown offenders via their relatives- the UK experience. *Forensic Sci Int Genet* **8**, 1-9.
National DNA Database Strategy Board, Annual Report 2015/16, p7. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/594185/58714_Un-Num_Nat_DNA_DB_Accessible.pdf

Technical annex

Methodologies for accurate genetic identification of juvenile remains at the site of the former Mother and Baby Home, Tuam, Co. Galway.

1. Background and context

During the 15 years since the international Human Genome Project was completed (Collins *et al.* 2003), basic and applied genetics research has been revolutionised by the development of powerful new technologies for determining the complete DNA sequence of complex animal and plant genomes (Goodwin *et al.* 2016; Levy & Myers 2016; Mardis 2017). These methods, generally termed 'high-throughput sequencing' (HTS), are rapid, accurate and extremely cost-effective compared to older techniques that were developed in the 1970s and used for initial sequencing of the two first reference human genomes (Lander *et al.* 2001; Venter *et al.* 2001).

Within the last decade, HTS technologies have also proven remarkably useful for retrieval of high-quality genomic information from archaeological material and have galvanised the new fields of human and animal paleogenomics (Der Sarkissian *et al.* 2015; Orlando *et al.* 2015; Leonardi *et al.* 2017; MacHugh *et al.* 2017). Application of HTS to DNA extracted using skeletal material from humans who lived thousands of years ago has produced fundamental new insights into human evolution, biogeography and prehistory, particularly in Europe (Jones *et al.* 2015; Mathieson *et al.* 2015; Cassidy *et al.* 2016; Fu *et al.* 2016; Lazaridis *et al.* 2016; Jones *et al.* 2017; Lazaridis *et al.* 2017; Lipson *et al.* 2017; Martiniano *et al.* 2017; Mathieson *et al.* 2018; Olalde *et al.* 2018).

A key breakthrough that has facilitated large-scale genomic analyses of these ancient humans, was work by Irish researchers based at UCD and TCD showing that the inner part of the petrous bone is an excellent source of human DNA (Gamba *et al.* 2014; Pinhasi *et al.* 2015). The petrous bone is located in the skull, and as the densest skeletal element in mammals, it provides exceptionally high quantities of DNA suitable for generation of whole-genome sequence information—even from archaeological material. This observation has recently been supported by research published in the forensic science literature demonstrating that the petrous bone is the best source of DNA for genetic identification of individuals from both mediaeval archaeological sites (Pilli *et al.* 2018) and recent criminal investigations (Kulstein *et al.* 2018).

The importance of HTS methods for genetic identification of recently interred human skeletal remains is also increasingly being recognised by working forensic scientists. In particular, they highlight the importance of using hundreds or thousands of simple binary genetic markers (single-nucleotide polymorphisms—SNPs) that can be readily accessed using HTS methods and that provide genetic information for high-resolution genetic identification, biological sex assignment, determination of ethnicity, and for evaluating physical characteristics such as eye, skin and hair pigmentation (Børsting & Morling 2015; Butler 2015; Alvarez-Cubero *et al.* 2017; Budowle *et al.* 2017). In this regard, UCD researchers, in collaboration with Forensic Science Ireland and the Garda Technical Bureau, have recently used HTS and DNA extracted from the petrous bone of Thomas Kent, an Irish revolutionary who was executed after the 1916 Easter Rising. Analysis of Thomas Kent's genome-wide SNP data subsequently demonstrated that he had the expected genetic relationship to two living second-degree relatives (Fernandes *et al.* 2017).

In the context of the scientific developments described above, we propose that DNA extraction from petrous bones coupled with HTS and routine SNP array technologies can provide a robust platform for individualising the juvenile remains interred at the Tuam Mother and Baby Home and for

genetically matching these deceased children with putative first-, second- or third-degree relatives. An outline of how this could be done on a pilot scale and as a proof-of-principle is provided below.

2. Extraction of endogenous genomic DNA from juvenile petrous bones

Recently published research work has shown that it is possible to generate whole-genome sequence information using HTS with a petrous bone from a human infant that lived approximately 11,500 years ago (Moreno-Mayar *et al.* 2018). Consequently, we are confident that HTS methods could be used to generate whole-genome sequence and SNP information with petrous bones from the remains interred at Tuam. We therefore propose that DNA be extracted from one osseous inner ear element of the petrous part of the temporal bone sampled from 20 different juvenile individuals at Tuam. This skeletal element provides the highest yield of endogenous DNA (Pinhasi *et al.* 2015) and DNA from 20 individual children would provide sufficient biological and technical replication to demonstrate the feasibility of the methods proposed here. In addition, sampling DNA from one of the two osseous inner ear elements (left or right) from juvenile skulls would automatically individualise remains from the children examined for this pilot study. It would also be minimally destructive to the physical integrity of the skeletal remains (Sirak *et al.* 2017).

DNA would be extracted using quality forensic science procedures in dedicated clean room facilities using established laboratory methods for petrous bone powder routinely implemented by archaeological genomics researchers in Ireland and elsewhere (MacHugh *et al.* 2000; Jones *et al.* 2017; Lipson *et al.* 2017; Martiniano *et al.* 2017; Mathieson *et al.* 2018; Olalde *et al.* 2018). It is important to note that very small quantities of petrous bone powder are required for DNA extraction (100–300 mg). Standard precautions to avoid contamination when working with archaeological DNA would be implemented, including wearing coveralls, mask, hair cover, shoe covers and double gloves.

3. High-throughput sequencing (HTS) of petrous bone genomic DNA samples

Multiplexed (index DNA sequence-barcoded) libraries for HTS of DNA from individual juvenile petrous bones would be constructed using a protocol suitable appropriate for fragmented and damaged genomic DNA. These techniques would be based on routine published methods developed by archaeological genomics researchers in Ireland and elsewhere (Meyer & Kircher 2010; Jones *et al.* 2017; Martiniano *et al.* 2017). DNA libraries would be initially screened and evaluated using a medium-throughput HTS platform (e.g. Illumina MiSeq™ or MiniSeq™). Following this, libraries would be sequenced using a high-throughput HTS platform (e.g. Illumina HiSeq™ 4000, HiSeq™ X or NovaSeq™ 6000). Raw sequence read processing, quality filtering and mapping to the human genome (nuclear and mitochondrial) would be performed using established bioinformatics methods (Gamba *et al.* 2014; Jones *et al.* 2015; Jones *et al.* 2017; Martiniano *et al.* 2017) Sequencing depth of individual HTS libraries would be calibrated to obtain at least 20× nucleotide coverage for each individual child's genome.

4. Genomic profiling of biological sex, ethnicity and physical characteristics in deceased children

Biological sex would be determined by examining the ratio of Y chromosome reads to reads aligning to both sex chromosomes (X and Y) (Skoglund *et al.* 2013). If deemed necessary, individual ethnicity at continental and intracontinental levels could be evaluated using publically available genome-wide SNP array data sets from modern European and global human populations and bioinformatics and statistical techniques developed by researchers in Ireland and elsewhere (Novembre *et al.* 2008; Leslie *et al.* 2015; Byrne *et al.* 2018). A particularly valuable resource would be the recently published *Irish DNA Atlas* (Gilbert *et al.* 2017). There is also the potential to infer physical traits with a significant genetic component (mature adult height, eye, skin and hair pigmentation) using

functional population genomics methods that have been widely applied in modern and ancient human DNA samples (Fortes *et al.* 2013; Gamba *et al.* 2014; Maronas *et al.* 2015; Walsh & Kayser 2016; Peltzer *et al.* 2018). For example, Irish researchers have recently leveraged extensive information on the genetic architecture of human height to predict adult stature in prehistoric and Bronze Age Iberian people (Martiniano *et al.* 2017).

5. **Using genome-wide SNP data for analyses of genetic relationship among children interred at Tuam and putative living relatives**

For the purposes of a pilot study, it would be relatively straightforward to use genome-wide SNP information generated from HTS data to infer genetic relationship among the deceased children. This approach has been widely applied in modern and ancient human DNA samples (Dou *et al.* 2017; Knipper *et al.* 2017; Martin *et al.* 2017; Theunert *et al.* 2017; Mo *et al.* 2018). For example, using thousands of shared SNPs, it would be possible to establish first-degree (e.g. siblings), second-degree (e.g. half-siblings, uncle/aunt–niece/nephew) or third-degree relationships (e.g. first-cousins) among the deceased children interred at the Tuam Mother and Baby Home. Finally, although it would be beyond the scope of a pilot study, genetic relationship to putative living relatives could be readily determined through integration of high-density SNP array data generated from genomic DNA purified from blood, cheek swab or saliva samples. The required data could be generated very cost-effectively from putative living relatives using technological solutions provided by direct-to-consumer genomics and genetic ancestry services (e.g. 23andMe, Inc. – www.23andme.com; Ancestry International DNA, LLC. – www.ancestry.co.uk; MyHeritage Ltd. – www.myheritage.com). Note: it would also be possible to determine genetic relationship to deceased people with appropriate stored biological samples (e.g. archived medical samples).

Scientific references

Alvarez-Cubero M.J., et al. (2017) Next generation sequencing: an application in forensic sciences? *Ann. Hum. Biol.* **44**, 581-92.

Børsting C. & Morling N. (2015) Next generation sequencing and its applications in forensic genetics. *Forensic Sci Int Genet* **18**, 78-89.

Budowle B., et al. (2017) Increasing the reach of forensic genetics with massively parallel sequencing. *Forensic Sci Med Pathol* **13**, 342-9.

Butler J.M. (2015) The future of forensic DNA analysis. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **370**.

Byrne R.P., et al. (2018) Insular Celtic population structure and genomic footprints of migration. *PLoS Genet.* **14**, e1007152.

Cassidy L.M., et al. (2016) Neolithic and Bronze Age migration to Ireland and establishment of the insular Atlantic genome. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 368-73.

Collins F.S., et al. (2003) The Human Genome Project: lessons from large-scale biology. *Science* **300**, 286-90.

Der Sarkissian C., et al. (2015) Ancient genomics. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **370**, 20130387.

Dou J., et al. (2017) Estimation of kinship coefficient in structured and admixed populations using sparse sequencing data. *PLoS Genet.* **13**, e1007021.

Fernandes D., et al. (2017) The identification of a 1916 Irish rebel: new approach for estimating relatedness from low coverage homozygous genomes. *Sci. Rep.* **7**, 41529.

Fortes G.G., et al. (2013) Phenotypes from ancient DNA: approaches, insights and prospects. *Bioessays* **35**, 690-5.

Fu Q., et al. (2016) The genetic history of Ice Age Europe. *Nature* **534**, 200-5.

Gamba C., et al. (2014) Genome flux and stasis in a five millennium transect of European prehistory. *Nat. Commun.* **5**, 5257.

Gilbert E., et al. (2017) The Irish DNA Atlas: revealing fine-scale population structure and history within Ireland. *Sci. Rep.* **7**, 17199.

Goodwin S., et al. (2016) Coming of age: ten years of next-generation sequencing technologies. *Nat. Rev. Genet.* **17**, 333-51.
Jones E.R., et al. (2015) Upper Palaeolithic genomes reveal deep roots of modern Eurasians. *Nat. Commun.* **6**, 8912.
Jones E.R., et al. (2017) The Neolithic Transition in the Baltic Was Not Driven by Admixture with Early European Farmers. *Curr. Biol.* **27**, 576-82.
Knipper C., et al. (2017) Female exogamy and gene pool diversification at the transition from the Final Neolithic to the Early Bronze Age in central Europe. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 10083-8.
Kulstein G., et al. (2018) As solid as a rock-comparison of CE- and MPS-based analyses of the petrosal bone as a source of DNA for forensic identification of challenging cranial bones. *Int. J. Legal Med.* **132**, 13-24.
Lander E.S., et al. (2001) Initial sequencing and analysis of the human genome. *Nature* **409**, 860-921.
Lazaridis I., et al. (2017) Genetic origins of the Minoans and Mycenaeans. *Nature* **548**, 214-8.
Lazaridis I., et al. (2016) Genomic insights into the origin of farming in the ancient Near East. *Nature* **536**, 419-24.
Leonardi M., et al. (2017) Evolutionary Patterns and Processes: Lessons from Ancient DNA. *Syst. Biol.* **66**, e1-e29.
Leslie S., et al. (2015) The fine-scale genetic structure of the British population. *Nature* **519**, 309-14.
Levy S.E. & Myers R.M. (2016) Advancements in Next-Generation Sequencing. *Annu. Rev. Genomics Hum. Genet.* **17**, 95-115.
Lipson M., et al. (2017) Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature* **551**, 368-72.
MacHugh D.E., et al. (2000) The extraction and analysis of ancient DNA from bone and teeth: a survey of current methodologies. *Ancient Biomolecules* **3**, 81-102.
MacHugh D.E., et al. (2017) Taming the past: ancient DNA and the study of animal domestication. *Annu. Rev. Anim. Biosci.* **5**, 329-51.
Mardis E.R. (2017) DNA sequencing technologies: 2006-2016. *Nat. Protoc.* **12**, 213-8.
Maronas O., et al. (2015) The genetics of skin, hair, and eye color variation and its relevance to forensic pigmentation predictive tests. *Forensic Sci Rev* **27**, 13-40.
Martin M.D., et al. (2017) Determination of genetic relatedness from low-coverage human genome sequences using pedigree simulations. *Mol. Ecol.* **26**, 4145-57.
Martiniano R., et al. (2017) The population genomics of archaeological transition in west Iberia: Investigation of ancient substructure using imputation and haplotype-based methods. *PLoS Genet.* **13**, e1006852.
Mathieson I., et al. (2018) The genomic history of southeastern Europe. *Nature* **555**, 197-203.
Mathieson I., et al. (2015) Genome-wide patterns of selection in 230 ancient Eurasians. *Nature* **528**, 499-503.
Meyer M. & Kircher M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, pdb.prot5448.
Mo S.K., et al. (2018) A 472-SNP panel for pairwise kinship testing of second-degree relatives. *Forensic Sci Int Genet* **34**, 178-85.
Moreno-Mayar J.V., et al. (2018) Terminal Pleistocene Alaskan genome reveals first founding population of Native Americans. *Nature* **553**, 203-7.
Novembre J., et al. (2008) Genes mirror geography within Europe. *Nature* **456**, 98-101.
Olalde I., et al. (2018) The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature* **555**, 190-6.
Orlando L., et al. (2015) Reconstructing ancient genomes and epigenomes. *Nat. Rev. Genet.* **16**, 395-408.
Peltzer A., et al. (2018) Inferring genetic origins and phenotypic traits of George Bahr, the architect of the Dresden Frauenkirche. *Sci. Rep.* **8**, 2115.

Pilli E., et al. (2018) Neither femur nor tooth: Petrous bone for identifying archaeological bone samples via forensic approach. *Forensic Sci. Int.* **283**, 144-9.
Pinhasi R., et al. (2015) Optimal ancient DNA yields from the inner ear part of the human petrous bone. *PLoS ONE* **10**, e0129102.
Sirak K.A., et al. (2017) A minimally-invasive method for sampling human petrous bones from the cranial base for ancient DNA analysis. *Biotechniques* **62**, 283-9.
Skoglund P., et al. (2013) Accurate sex identification of ancient human remains using DNA shotgun sequencing. *J. Archaeol. Sci.* **40**, 4477-82.
Theunert C., et al. (2017) Joint Estimation of Relatedness Coefficients and Allele Frequencies from Ancient Samples. *Genetics* **206**, 1025-35.
Venter J.C., et al. (2001) The sequence of the human genome. *Science* **291**, 1304-51.
Walsh S. & Kayser M. (2016) A practical guide to the HIrisPlex System: simultaneous prediction of eye and hair color from DNA. *Methods Mol. Biol.* **1420**, 213-31.