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Degenerate Oligonucleotide-Primed PCR (DOP-PCR) and Massively Parallel Sequencing (MPS): Strategies for Improved Analyses of Degraded DNA from Human Skeletal Remains

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Forensic and ancient DNA samples often are damaged and in limited quantity as a result of exposure to harsh conditions. Several strategies exist as potential approaches to overcome challenges posed by degraded and low copy templates. One is a PCR-based whole genome amplification method called degenerate-oligonucleotide-primed PCR (DOP-PCR). The efficacy of four modified versions of the original DOP-PCR primer was assessed. The primers resulted in improved STR profiles from contemporary human skeletal remains, American Civil War era bone samples, and skeletal remains of WWII soldiers over those obtained by routine STR typing.

Another strategy, one that greatly increases the amount of data that can be gleaned from a sample, is massively parallel sequencing. Although the paramount objective of DNA testing of unidentified skeletal remains is positive identification, cases involving historical or archaeological bones often lack reference samples for comparison. MPS offers an opportunity to provide additional biometric data in such cases. MPS was used to analyze 140-year-old human skeletal remains from an historical town of the American Old West. Given that the remains were in an unmarked grave and no records existed regarding the identity of the individual, officials requested the analysis of DNA markers that could help predict the individual's biogeographic ancestry and external physical traits. Results were obtained for 25/26 Y-STRs, 34/34 Y SNPs, 165/165 AIMS, 28/28 phenotype-informative SNPs, 102/102 human identity SNPs, 27/29 autosomal STRs (plus amelogenin), 4/8 X-STRs, and 11 regions of the mtDNA genome. This study is among the first to genetically characterize historical human remains with forensic genetic marker kits specifically designed for MPS. It demonstrates the potential of MPS to analyze old skeletal samples and to provide substantially more genetic information from the same initial quantities of DNA compared to traditional CE-based analyses.

Autosomal and Y-STR Analysis of Degraded DNA from the 120-Year-Old Skeletal Remains of Ezekiel Harper

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The 120-year-old skeletal remains of Confederate Civil War soldier Captain Ezekiel "Zeke" Harper were exhumed by court order in January 2011 for DNA analysis. The goal of the DNA testing was to support or refute whether Captain Harper had fathered a son (Earl J. Maxwell) with his Native American maid prior to his murder in 1892. Bones with adequate structural integrity (left tibia, right tibia, right femur, mandible, four teeth) were retrieved from the burial site and sent to the Institute of Applied Genetics in Fort Worth, Texas for analysis. Given the age and condition of the remains, three different extraction methods were used to maximize the probability of DNA recovery. The majority of the DNA isolates from over fifty separate bone sections yielded partial autosomal STR genotypes and partial Y-STR haplotypes. After comparing the partial results for concordance, consensus profiles were generated for comparison to reference samples from alleged family members. Considering the genetic recombination that occurs in autosomal DNA over the generations within a family, Y-STR analysis was determined to be the most appropriate and informative approach for determining potential kinship. Two of Earl J. Maxwell's grandsons submitted buccal samples for comparison. The Y-STR haplotypes obtained from both of these reference samples were identical to each other and to the alleles in Ezekiel Harper's consensus profile at all 17 loci examined. This Y-STR haplotype was not found in either of two major Y-STR population databases (U.S. Y-STR database and YHRD). The fact that the Y-STR haplotype obtained from Ezekiel's skeletal remains and Earl's grandsons is not found in either population database demonstrates its rarity and further supports a paternal lineage relationship among them. Results of the genetic analyses are consistent with the hypothesis that Earl J. Maxwell is the son of Ezekiel Harper.

Characterization of Unidentified 140-Year-Old Human Skeletal Remains using Massively Parallel DNA Sequencing

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Although the paramount objective of forensic DNA testing of unidentified human remains is positive identification, cases involving historical or archaeological skeletal remains often lack reference samples for comparison. Massively parallel sequencing (MPS) offers an opportunity to provide biometric data in such cases. In this study, MPS was used to provide identity marker information for 140-year-old human skeletal remains from Deadwood, South Dakota, a famous town of the American Old West. Given that the remains were in an unmarked grave and no records existed regarding the identity of the individual, city officials requested the analysis of DNA markers that could help predict the individual's biogeographic ancestry and external physical traits. Results were obtained for 25/26 Y-STRs, 34/34 Y SNPs, 165/165 ancestry-informative SNPs, 28/28 phenotype-informative SNPs, 102/102 human identity SNPs, 27/29 autosomal STRs (plus amelogenin), and 4/8 X-STRs (as well as eleven regions of mtDNA). The Y-chromosome (Y-STR, Y-SNP) and mtDNA profiles of the unidentified skeletal remains are consistent with the R1b and H1 haplogroups, respectively. Both of these haplogroups are the most common haplogroups in Western Europe. Ancestry-informative SNP analysis also supported a European background. The genetic results are consistent with the findings of a previous anthropological report which determined that the remains belong to a male of European ancestry (Caucasian). Phenotype-informative SNP data provided strong support that the individual had light red hair and brown eyes. This study is among the first to genetically characterize historical human remains with forensic genetic marker kits specifically designed for MPS. It demonstrates the potential of MPS to analyze old skeletal samples and to provide substantially more genetic information from the same initial quantities of DNA as that of CE-based analyses.

Optimising DNA Identification of Human Skeletal Remains

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DNA based human identification is fundamental for facilitating both criminal and coronial investigations, in particular disaster victim identification, missing persons investigations and repatriation of war dead. Standard DNA analysis (For example STR profiling) of unknown samples can be compared to known data on databases to establish a match. However, many real-world human identification cases involve trace and/or degraded samples with low quantity and quality DNA thus limiting the application of standard approaches. As a result, alternative methods are needed to identify an individual or provide forensic intelligence (e.g. biogeographical ancestry or physical appearance) to narrow the pool of potential matches and provide information about an unknown individual. However, such analyses consume the often-limited DNA samples and require specialised expertise and equipment, increasing cost and time.

To maximise the chance of obtaining informative results, quickly and efficiently, we have developed two quantitative PCR assays to determine the mitochondrial (mtDNA) and nuclear DNA (nDNA) content within degraded samples. The mtDNA quantification assay simultaneously targets three human mtDNA fragments of different lengths (85, 190 and 455 bp) providing an indication of the DNA quantity and quality available. The inclusion of an internal positive control in this assay also gives an indication of the presence of inhibitors. Our nDNA assay quantifies an 80bp fragment of the Amelogenin gene allowing quantification of nDNA as well as gender assignment using high-resolution melt analysis. These results direct our choice of the subsequent analyses performed to obtain an informative result upon the first attempt. We discuss the application of these assays to forensic and ancient human skeletal remains and highlight the value of the information provided by these assays to downstream analysis results.

Ancient DNA Analysis of an Irish Medieval Population

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The archaeological excavation of a medieval cemetery in North West Ireland led to the recovery of the largest collection of human remains from a burial ground in Ireland to date. The collection included a substantial number of juvenile remains. In order to enhance the interpretation of the assemblage and give a more complete picture of the population, a molecular sexing tool was developed. Sex identification of human remains is generally assigned using the morphology of the skeleton or on some occasions using associated grave goods. However in instances when an assemblage contains immature or fragmentary material, an alternative and reliable means of sexing these individuals is required. Ancient DNA analysis has proven itself to be such a reliable alternative. In this study the reliability and reproducibility of two PCR based sexing methods were evaluated first on 38 adults of known sex to determine the accuracy of these methods for sexing individuals from the Ballyhanna assemblage. Using real time PCR and STR profiling systems, a reliable and unambiguous sexing system was developed. The reproducibility of the amplified samples meant that the methods were valid and subsequently could be used to determine juvenile sex.

Comparison of DNA Extraction Methods for Compromised Skeletal Remains – a Case Study

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A compromised bone sample was received at the New South Wales Forensic & Analytical Science Service (NSW FASS) for identification purposes. The bone was believed to have been exposed to salt water; however the extent of exposure as well as the age of the bone were unknown. Previous attempts at nuclear DNA typing had also been unsuccessful. Extracting DNA from skeletal remains at NSW FASS is typically performed using an optimised PrepFiler BTA™ manual lysis procedure, followed by automated extraction on the Automate Express™ Forensic DNA Extraction System. Routine processing of the bone sample using this DNA extraction method, followed by mitochondrial DNA sequencing (hypervariable regions 1 and 2) generated incomplete results. Alternative DNA extraction methods involving total demineralisation and purification using silica columns (QIAquick® PCR Purification Kit or QIAamp® DNA Investigator Kit) were trialled and yielded improved results for both mitochondrial and nuclear DNA typing. A comparison of the results obtained with each extraction method will be discussed. The results from this case study will assist with future method development for the identification of compromised skeletal remains at NSW FASS.

Identification of Gyula Ágner a hero of the World War II based on classical anthropological and mitochondrial DNA results

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Gyula Ágner was a Royal Hungarian First Lieutenant during the World War II and died 30 years old in a mine shrapnel injury in 27th of April, 1944 in Luczky, Ukraine. In October, 2014 the Hungarian Ministry of Defence made an investigation in Luczky and exhumated the skeleton, then transported the remains of the deceased to Budapest, Hungary. They asked the Institute of Forensic Medicine, Network of Forensic Science Institutes (Budapest, Hungary) to identify the remains. Classical anthropological methods were used to predict morphological gender, age at death and height, furthermore metrical and pathological characters were analysed as well. Maternal relatedness was examined between the living niece of the deceased (his sister's daughter) and the bone remains. DNA was isolated from the leftward femur using EZ1 DNA Investigator Kit and EZ1 Advanced Instrument (Qiagen®). Hypervariable regions of the mtDNA control region (HV1, HV2 and HV3) were amplified by Qiagen® Multiplex PCR Kit in different monoplex reactions. F15971/H16401/L48/H408/F403/R599 primers were used in HV1-HV2-HV3 PCR and BigDye sequencing reactions. The results of the anthropological and genetical analysis supported the hypothesis that the bone remains belong to Gyula Ágner.

Dynasty and population of the Piast state in view of the integrated historical, anthropological and genomic studies

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There are no controversies among the historians that the process of establishment of the Piast state constitutes the initial phase of the Polish statehood formation. It is also generally accepted that our knowledge about this process is very limited due to the lack of reliable written and material sources. The substantial progress that has been recently made in ancient DNA (aDNA) sequencing gives us a unique chance to overcome this situation.
The main objective of the project is to answer several questions, important for the Polish and European history, and related to the following issues: (i) the origin of the population inhabiting the region between Oder and Vistula rivers in the time of the Piast state formation – verification of hypothesis concerning settlement continuation or discontinuation from the Roman Iron Age until the 12th century; (ii) genetic, morphological and cultural homogeneity of the 10-12th-century Wielkopolska population – verification of several hypotheses concerning relations of historical Wielkopolska population with the neighboring populations; (iii) local or foreign origin of the Piast Dynasty – verification of the hypotheses concerning the origin of the Piast family; (iv) local or foreign origin of the early Piast state's elites and their comprehensive analysis with reference to historical data – verification of the hypotheses concerning the involvement of immigrants in the process of elite formation in the early Piast state.
To achieve the main goals of the project, we plan multidimensional and interdisciplinary (historical, archeological, anthropological, genetic and genomic) studies of the populations inhabiting the territory of the present-day Wielkopolska in the time ranging from the Roman Iron Age up to the Early Middle Ages. We plan to achieve the above-mentioned objectives with the use of standard methods of physical anthropology and modern bioarcheological and bioinformatic techniques, including the aDNA analysis by NGS.

Mediaeval Population in the Centre and Country. Archaeology, Bioarchaeology and Genetics of Cemeteries of Prague Castle and Bohemia

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The project deals with population inhabiting during Middle Ages PragueCastle and its immediate hinterland and population in rural areas of eastern and central Bohemia. The starting pint will be archaeologically investigated burial grounds. The analysis will combine archaeological methods (chronology, social stratification), methods of bioarchaeology (demographic characteristic, physical activities, nutrition, biological kinship, geographic origin) and methods of archeogenetics (familiar relationship between skeletal remains, geographic origin). The analysis will identify the basic characteristics of the population living in the Middle Ages at Prague Castle, with an expected high proportion of the non-domestic origin and with higher proportion of people with high social status. The mainly local origin of buried will be confirmed in rural cemeteries.

Pushing the limits of DNA analyses from fossil bone

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Forensic DNA analyses have to fulfil strict requirements since their results need to stand scrutiny in court. Ancient DNA analyses, while dealing with similar problems, are free of these constraints, allowing researchers to push the limits of any available approach. During the last 10 years, research on ancient DNA has literally been turned upside down. Originally restricted to small fragments of mitochondrial DNA, new DNA sequencing technologies and other developments now allow obtaining complete paleogenomes from ancient DNA at an increasing pace. While initially, simply the availability and continued development of next generation sequencing has been driving this process, more recently accessory steps such as optimized DNA extraction and library construction approaches have become more important in accessing DNA from samples at the limit of DNA preservation, including middle Pleistocene samples and samples from subtropical and tropical regions. I will discuss the latest developments that have allowed accessing DNA from such borderline samples and also give an outlook in which areas further progress might be possible to ultimately analyze DNA up to the limits of biomolecular preservation.

The "Roots Project". Rapid Analysis of mtDNA and Y-DNA from Bone in Tracing of Displaced Human Lineages

Bruce A. Jackson, Guiseppe Sena, Stacy Okada, Christine Houth, and Ondine Gossner

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The “Roots Project” utilizes mitochondrial DNA (mtDNA) and Y-chromosome DNA (Y-DNA) to trace the maternal and paternal lineages, respectively of Americans. Although the project primarily traces the lineages of African-Americans whose descendants were victims of the trans-Atlantic slave trade we have recently begun to apply our technology to relink Jewish-Americans who had been separated from European family members by the Holocaust. In both cases we have expanded our research to include the analysis of bones from historical burial sites, which allows the closing of DNA lineages gaps due to death and migration.
To these ends we have developed a rapid DNA extraction method for bone that produces analyzable DNA samples in 90 minutes. This methodology is linked to an 8-node dual-cure Linux Beowulf cluster that is named “Cerberus” that allows the rapid (pico-second) sequence analysis of mtDNA and Y-DNA extracted from pristine tissue and bone samples, resulting in prompt and highly accurate haplotype and haplogroup assignments.

DNA identification of the victims of the Malaysia Airlines flight MH17 crash

Presenting Author: KAL Arnoud
A DNA identification effort of this scale can only be realized with the aid of many individuals. This includes personnel from the following agencies: Netherlands Forensic Institute, Dutch DVI team (LTFO), Forensic Laboratory for DNA Investigation (FLDO, Leiden), Bode Cellmark Forensics, LGC Forensics and International Commission on Missing Persons (ICMP).

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On the 17th of July 2014, Malaysia Airlines flight MH17 from Amsterdam to Kuala Lumpur crashed in the Ukraine resulting in 298 victims. The DNA identification process met several challenges: 1) close biological relationships between victims; 2) highly fragmented human remains due to explosions and fall from 10.000 m; 3) severely burned human remains due to fires at crash sites; 4) an open population crash as it involved a populated area with an armed civil conflict, which invoked identification of all human remains.
The NFI received 8000 post mortem samples (bone, bone marrow, tooth, tissue) of 4900 human remains and 850 ante mortem samples comprising 350 personal victim items (toothbrushes, razors, clothes), 300 reference buccal swabs from relatives of victims and 200 reference DNA profiles. The AM samples were used to generate pedigrees for most of the 298 victims. A QiaAmp-based DNA extraction protocol was used as standard method for Freezer/Mill-prepared bone powder. Approximately 20% of the human remains required additional techniques to obtain DNA profile such as demineralisation of bone powder, microcon purification, ethanol precipitation, mini-amplicon STR profiling and/or sensitized DNA typing methods (increased cycle number or enhanced CE injection settings). Three foreign laboratories (ICMP, Bode Cellmark Forensics, LGC Forensic) assisted with DNA profiling. The Bonaparte DVI software (www.bonaparte-dvi.com) that is based on Bayesian networks was used for the screening and matching.
For 98,5% of the 4900 human remains usable DNA profiles were obtained. These DNA profiles were matched to 296 of the 298 missing victims.

Identification of WWI Commonwealth Soldiers

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DNA Identification of WWI soldiers from Fromelles, Northern France, has been studied by LGC over the past 6 years. The Battle of Fromelles took place on the 19th-20th July 1916 and it is estimated that more than 4,500 Commonwealth soldiers were killed or missing in action. 250 body remains were discovered at the mass grave at Fromelles and were excavated over a 6 month period. Working with the Commonwealth War Graves Commission and Oxford Archaeology LGC has been using modified extraction protocols to obtain DNA for identification in comparison with known relatives who were thought to have died during this battle. The poster covers a number of challenges observed in the DNA examination and DNA processing of these archaeological samples.

DNA identification from different bone types

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In many forensic genetic investigations, for human identification, only material for analysis are human bones what is quite demanding, as opposed to samples of blood or saliva. In bone samples there is presence of many potential inhibitors and small amount of high quality DNA, so bones are the most chalenging biological samples for STR analysis. In this study we present succes rate of DNA profiles from different bone samples, not older than 1 year. In DNA lab of MOI femur is the most commonly used for identification, but, it often happens that rib and elbow or flat bones, like bones of skull, are material for analysis. In our laboratory we analyzed 45 samples of femur, skull, rib and elbow. All samples were exposed to the same procedure of washing, extraction (QIAamp DNA Mini Kit), quantification (Qantifiler Human DNA Quantification Kit), STR amplification (AmpFlSTR Identifiler Plus Kit and AmpFlSTR NGM Kit) and detection by capillary electrophoresis on ABI 3130 XL. Succes rate of total number of samples is 95,55%, and according to the bone type, results are: 97,14 % femur, 100% skull, 75% rib and 100% of elbow.

Investigating specialist DNA techniques for improving DNA identification success rates from compromised human remains

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The Specialist DNA Laboratory at the New South Wales Forensic & Analytical Science Service has been investigating the use of specialised DNA techniques to complement the routine identification of compromised human remains. The casework investigation of a number of compromised bone samples will be explored to highlight the utility of these techniques, including a total demineralisation and silica-based DNA extraction and purification method, the Quantifiler® Trio Kit's sample quality index, mitochondrial DNA mini primer sets and the use of massively parallel sequencing targeting single nucleotide polymorphism marker panels, an indel marker and the whole mitochondrial DNA genome. Specifically, the SNPforID 34-Plex, HIrisPlex and the Amelogenin marker were sequenced using the Ion PGM System™, in an attempt to infer intelligence information such as the ancestry, phenotype (hair and eye colour) and gender of the bone donors. In addition, the Early Access HID-Ion AmpliSeq™ Mitochondrial Tiling Path Panel was evaluated to assess if whole mitochondrial genome sequence information could be obtained from compromised bone samples. The utility of these various techniques and their potential implementation into routine forensic casework in the Specialist DNA Laboratory will be discussed.