Technical Tips for Obtaining Reliable DNA Identification of Historic Human Remains

Dongya Y. Yang and Camilla F. Speller

ABSTRACT

DNA analysis has become an important tool for the identification of historic individuals. False results may occur if the historic sample becomes contaminated. Both historic archaeologists and DNA researchers are provided here with specific technical tips in order to obtain reliable DNA results: (1) historic bone samples should be clean collected for DNA analysis; (2) short, overlapping mitochondrial DNA fragments should be used for the historic bone samples, while longer mtDNA fragments should be targeted for the assumed living relatives; and (3) hair or cheek swab samples from the assumed relatives should be collected and analyzed only after the bone DNA analysis is completed.

Introduction

DNA analysis of skeletal remains is becoming an important tool for identification of historic individuals, thanks to the advent of forensic DNA technology and ancient DNA research (Jeffreys et al. 1992; Holland et al. 1993; Stone et al. 2001; Katzenberg et al. 2005). These techniques have made it possible to retrieve genetic information from skeletal remains in order to establish identity, which otherwise may not be achieved through conventional methods (Hagelberg et al. 1991; Kurosaki et al. 1993; Lleonart et al. 2000). The potential exists for false identification of the individual due to contamination of the sample (Wilson et al. 1993; Wilson et al. 1995). To minimize or detect contamination in the analysis of historic human remains, the potential problems encountered in DNA identifications are discussed and some effective technical tips for both historic archaeologists and DNA researchers are presented.

As a Forensic Identification

The use of DNA for identification of historic human remains is similar to forensic DNA individual identification, as both are dependent upon a match or mismatch for identification (Wilson et al. 1995). Nonetheless, there are some major differences between the two types of cases. Unlike modern forensic DNA analysis that can use multiple nuclear DNA markers for identification, historic DNA cases are usually restricted to analyzing mitochondrial DNA, which is more likely to survive degradation over time due to its high copy number per cell (O'Rourke et al. 2000; Kaestle and Horsburgh 2002). Since mtDNA serves as a single DNA marker, it has limited discrimination power. Due to its maternal inheritance pattern, however, it can be a benefit for identification of historic human remains through the comparison of mitochondrial sequences with those of living relatives (Gill et al. 1994).

As Ancient DNA Analysis

Unlike regular forensic DNA tests, DNA identification of historic human remains is more similar to an ancient DNA study, which faces the tremendous challenges of DNA degradation and contamination (O'Rourke et al. 2000; Hofreiter et al. 2001; Kaestle and Horsburgh 2002; Alonso et al. 2003). Although DNA degradation over time may cause failure of the DNA test, sample contamination is more troublesome since it may lead to false identification of the remains.

Contamination derives from the fact that a highly sensitive PCR amplification technique is required to amplify minute amounts of preserved ancient DNA molecules for the analysis, while DNA shed from people who have handled and analyzed the remains can easily outnumber the authentic ancient DNA molecules (Yang et al. 2003). Potential

TECHNICAL BRIEFS IN HISTORICAL ARCHAEOLOGY, 2006, 1: 11–15

contamination of the historic samples must be effectively controlled, and the research protocols must be carefully designed in order to detect possible contamination.

Uniqueness of Historic Human DNA

Human skeletal remains that are submitted for DNA identification are often assigned a tentative identity based on historic records and archaeological investigations. The DNA test will largely be used to confirm or refute the assumed identification, albeit DNA analysis also can be used to identify the population background of unknown human remains (Handt et al. 1994; Monsalve et al. 1996; Stone and Stoneking 1999). With the exception of some extremely special circumstances where biological samples of the assumed individuals include premortem hair samples or other biotissues (Stone et al. 2001), historic DNA profiles are obtained from skeletal remains and then subsequently compared with profiles from assumed genetic living relatives. For famous figures in history, pervasive genealogical records make the search for relatives comparatively simple, although the complete assurance of genetic relatedness can be an issue, whether modern or historical (Dawid and Mortera 1998; Gornik et al. 2002). In the identification of the Ekaterinburg remains as potential members of the Romanov Imperial family of Russia, mtDNA reference sequences were easily obtained from Empress Alexandra's grandnephew Prince Phillip of Great Britain (Gill et al. 1994) and Grand Duke Georgij, the Tsar's brother (Ivanov et al. 1996).

However, in the case of less well-known individuals, the involvement of historic archaeologists is critical since they are able to examine the reliability of historic records and potentially provide a presumed identity. For example, in the case involving an infant burial in an historical church cemetery in Cochrane, Alberta, archaeological and historical information was obtained through the discovery and excavation of the cemetery, analysis of associated historical artifacts, and examination of archival records and newspapers (Katzenberg et al. 2005). This process proved crucial in determining a presumptive identification for the five-month-old child whose identity was later confirmed through molecular techniques (Katzenberg et al. 2005).

The identification of an individual is based on a match or a mismatch of DNA profiles between the remains and the assumed living relatives. A mismatch can immediately refute the assumed identity, while a match of a rare mtDNA profile can be a good indicator of positive identification (Wilson et al. 1995). Due to the dual challenges of degradation and contamination in ancient DNA studies, all analyses can potentially lead to a false match or false mismatch, resulting in misidentification. A false mismatch occurs if contaminant DNA instead of authentic bone DNA is analyzed; a false match can appear when DNA samples from historic remains and living relatives are contaminated by the same source, or when one sample contaminates the other.

In general, analyzing historic DNA has certain advantages over other types of ancient DNA. The relatively recent nature of historic DNA makes it more likely to yield adequate amounts of the DNA template for analysis, and associated historical records can provide an independent source of information for cross reference.

The key to success of historic DNA analyses is the elimination of possible contamination. Based on several published case studies, including one in which the senior author was involved, technical steps for researchers can be defined in order to prevent this problem (Katzenberg et al. 2005).

Collection of Bone Samples and Reference DNA Samples

Human remains should be clean-excavated using the same protocols as would be used for other types of ancient DNA analysis (Yang and Watt 2005). In general, a couple of small bone samples should be adequate for analysis. For bone samples collected without using clean-excavation techniques, larger bone samples should be submitted. This allows for removal of the outer bone surface layer for acquisition of clean samples without contamination. In addition, to effectively monitor for contamination, archaeologists and others who might have contacted the bone samples should also submit a sample of their own DNA for comparison.

Extreme care should be taken when collecting and handling reference DNA samples from assumed living relatives. To control contamination, the reference DNA should be collected after the bone samples have been processed and DNA-profiled. Reference hair or cheek swab samples

should not be stored with the skeletal samples; preferably, they should be separately and directly submitted to the DNA lab by the living relatives.

Special Procedures for DNA Lab Analysis

In a DNA lab, false matches or mismatches caused by contamination are major concerns. Within the field of ancient DNA, these problems have received a lot of attention, and DNA researchers can refer to published protocols to conduct their studies and to authenticate DNA results (Montiel et al. 2001; Poinar 2003; Pääbo et al. 2004). For DNA analysis of historic human remains, a "unique" false match can be caused by cross-sample contamination of bone DNA and reference DNA within the lab setting. This contamination cannot be easily detected should it occur, but it can be prevented if proper measures are carried out.

The most effective way to avoid sample contamination and a false match problem is through the physical or geographic separation of the analysis of the ancient and modern samples. In reality, the involvement of two research labs or groups to analyze one individual might not always be feasible, in particular in those less "famous" cases. As an alternative, simple and effective ways to avoid contamination can be using temporal separation of the analyses of two types of DNA samples (Capelli et al. 2003, Katzenberg et al. 2005) and PCR amplification of differently sized DNA fragments for both samples (Katzenberg et al. 2005). DNA researchers should always analyze the historic bone samples first, targeting the first and second hypervariable segments (HV1 and HV2) of the mitochondrial control region (Anderson et al. 1981). Shorter DNA fragments from the bone DNA samples should be attempted, using overlapping PCR techniques to generate longer DNA fragments (Stone and Stoneking 1998). Once analysis of the bone DNA is complete, much longer DNA fragments should be used to amplify the potential relative's reference DNA sample as well as the reference samples contributed by the archaeologist and lab members. In this way, previously amplified PCR products from the bone DNA samples cannot contaminate the modern reference DNA samples since they are too short to be amplified in a PCR reaction designed for a much longer sequence. If analysis is temporally separated, the modern reference DNA samples cannot become a contaminant source for the bone DNA sample.

It should be pointed out that profile matching becomes complicated when the shared mtDNA sequence is common to a given community, which occurs in relatively closed communities where individuals potentially share the same maternally inherited mtDNA sequence (Kaestle and Horsburgh 2002). Mitochondrial DNA markers are more useful when applied to highly dynamic and admixed human populations. The information about the population's history of movement and admixture derived from historical and archaeological research can be crucial to a successful identification (Nasidze et al. 2005).

In order to obtain a more precise DNA profile and probable identification, analysis of nuclear DNA may be attempted. If the historic skeletal remains are only a few hundred year old, the possibility exists that single-copy nuclear DNA may be preserved. For example, the Y chromosome STR marker could be used to trace paternal lineages for a male individual (Hummel and Herrmann 1994). When both mtDNA and Y-STR are incorporated into the analysis and the results indicate the same lineages, a more confident positive identification can be made.

Conclusions

DNA analysis is a very powerful tool for individual identification of historic remains. The high risk of contamination that can readily lead to false identification can be effectively addressed through active involvement of historic archaeologists by selecting appropriate bone samples for analysis and through cross-referencing of reliable historic information. For DNA researchers, the risk for a false match between the remains and reference DNA samples can be overcome by temporally separating the analysis of the ancient and modern DNA samples and targeting DNA fragments of different lengths.

ACKNOWLEDGMENTS

Dongya Yang would like to thank Annie Katzenberg for inviting him to conduct DNA analysis on the historic infant remains from Alberta, Canada, which gave him his first hands-on experience and allowed him to appreciate the challenges in DNA analysis of historic human remains. This study is supported in part by a SSHRC research grant given to Yang.

REFERENCES

Alonso, Antonio, Cristina Albarran, Pablo Martin, Pilar Garcia, Oscar Garcia, Concepcion de la Rua, Ainhoa Alzualde, Lourdes Fernandez de Simon, Manuel Sancho, and Jose Fernandez Piqueras
2003 Multiplex-PCR of Short Amplicons for mtDNA Sequencing from Ancient DNA. *International Congress Series* 1239:585–588.

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young
1981 Sequence and Organization of the Human Mitochondrial Genome. *Nature* 290(5806):457–465.

Capelli, C., F. Tschentscher, and V. L. Pascali
2003 "Ancient" Protocols for the Crime Scene? Similarities and Differences between Forensic Genetics and Ancient DNA Anaysis. *Forensic Science International* 131(1):59-64.

Dawid, A. P., and J. Mortera
1998 Forensic Identification with Imperfect Evidence. *Biometrika* 85(4):835–849.

Gill, P., P. L. Ivanov, C. Kimpton, R. Piercy, N. Benson, G. Tully, I. Evett, E. Hagelberg, and K. Sullivan
1994 Identification of the Remains of the Romanov Family by DNA Analysis. *Nature Genetics* 6(2):130–135.

Gornik, Ivan , Mladen Marcikic, Milovan Kubat, Dragan Primorac, and Gordan Lauc
2002 The Identification of War Victims by Reverse Paternity is Associated with Significant Risks of False Inclusion. *International Journal of Legal Medicine* 116(9):255–257.

Hagelberg, Erika, Ian C. Gray, and Alec J. Jeffreys
1991 Identification of the Skeletal Remains of a Murder Victim by DNA Analysis. *Nature* 352(6334):427–429.

Handt, Oliva, Martin Richards, Marion Trommsdorff, Christian Kilger, Jaana Simanainen, Oleg Georgiev, Karin Bauer, Anne Stone, Robert E. M. Hedges, Walter Schaffner, Gerd Utermann, Brian Sykes, and Svante Pääbo
1994 Molecular-Genetic Analyses of the Tyrolean Ice Man. *Science* 264(5166):1775–1778.

Hofreiter, Michael, David Serre, Hendrik N. Poinar, Melanie Kuch, and Svante Pääbo
2001 Ancient DNA. *Nature Review Genetics* 2(5):353–359.

Holland, M. M., D. L. Fisher, L. G. Mitchell, W. C. Rodriquez, J. J. Canik, C. R. Merril, and V. W. Weedn
1993 Mitochondrial DNA Sequence Analysis of Human Skeletal Remains: Identification of Remains from the Vietnam War. *Journal of Forensic Sciences* 38(3):542–543.

Hummel, Susanne, and Bernd Herrmann
1994 Y-Chromosomal DNA from Ancient Bones. In *Ancient DNA*, B. Herrmann and S. Hummel, editors, pp. 205–210. Springer-Verlag Inc., New York, NY.

Ivanov, Pavel L., Mark J. Wadhams, Rhonda K. Roby, Mitchell M. Holland, Victor W. Weedn, and Thomas J. Parsons
1996 Mitochondrial DNA Sequence Heteroplasmy in the Grand Duke of Russia Georgij Romanov Establishes the Authenticity of the Remains of Tsar Nicholas II. *Nature Genetics* 12(4):417–420.

Jeffreys, Alec J., Maxine J. Allen, Erika Hagelberg, and Andreas Sonnberg
1992 Identification of the Skeletal Remains of Mengele, Josef, by DNA Analysis. *Forensic Science International* 56(1):65–76.

Kaestle, Frederika A., and K. Ann Horsburgh
2002 Ancient DNA in Anthropology: Methods, Applications, and Ethics. *Yearbook of Physical Anthropology,* Vol. 45, pp. 92–130.

Katzenberg, M. A., G. Oetelaar, J. Oetelaar, C. Fitzgerald, Dongya Y. Yang, and Shelley R. Saunders
2005 Identification of Historical Human Skeletal Remains: A Case Study Using Skeletal and Dental Age, History, and DNA. *International Journal of Osteoarchaeology* 14(1):61–72.

Kurosaki, K., T. Matsushita, and S. Ueda
1993 Individual DNA Identification from Ancient Human Remains. *American Journal of Human Genetics* 53(3):638–643.

Lleonart, R., E. Riego, M. V. S. de la Pena, K. Bacallao, F. Amaro, M. Santiesteban, M. Blanco, H. Currenti, A. Puentes, F. Rolo, L. Herrera, and J. de la Fuente
2000 Forensic Identification of Skeletal Remains from Members of Ernesto Che Guevara's Guerrillas in Bolivia Based on DNA Typing. *International Journal of Legal Medicine* 113(2):98–101.

Monsalve, M. V., F. Cardenas, F. Guhl, A. D. Delaney, and D. V. Devine
1996 Phylogenetic Analysis of mtDNA Lineages in South American Mummies. *Annals of Human Genetics* 60(4):293–303.

Montiel, Rafael, Assumpcio Malgosa, and Paolo Francalacci
2001 Authenticating Ancient Human Mitochondrial DNA. *Human Biology* 73(5):689–713.

Nasidze, Ivan, Dominique Quinque, Isabelle Dupanloup, Richard Cordaux, Lyudmila Kokshunova, and Mark Stoneking
2005 Genetic Evidence for the Mongolian Ancestry of Kalmyks. *American Journal of Physical Anthropology* 128(4):846-854.

O'Rourke, Dennis H., M. Geoffrey Hayes, and Shawn W. Carlyle
2000 Ancient DNA Studies in Physical Anthropology. *Annual Review of Anthropology* 29:217–242.

Pääbo, Svante, Hendrik Poinar, David Serre, Viviane Jaenicke-Despres, Juliane Hebler, Nadin Rohland, Melanie Kuch, Johannes Krause, Linda Vigilant, and Michael Hofreiter
2004 Genetic Analyses from Ancient DNA. *Annual Review of Genetics* 38:645–679.

Poinar, Hendrik N.
2003 The Top 10 List: Criteria of Authenticity for DNA from Ancient and Forensic Samples. *International Congress Series* 1239:575–579.

Stone, Anne, James E. Starrs, and Mark Stoneking
2001 Mitochondrial DNA Analysis of the Presumptive Remains of Jesse James. *Journal of Forensic Sciences* 46(1):173–176.

Stone, Anne C., and Mark Stoneking
1998 MtDNA Analysis of a Prehistoric Oneota Population: Implications for the Peopling of the New World. *American Journal of Human Genetics* 62:1153–1170.

1999 Analysis of Ancient DNA from a Prehistoric Amerindian Cemetery. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 354(1379):153–159.

Wilson, Mark R., Joseph A. Dizinno, Deborah Polanskey, Jeri Replogle, and Bruce Budowle
1995 Validation of Mitochondrial-DNA Sequencing for Forensic Casework Analysis. *International Journal of Legal Medicine* 108(2):68–74.

Wilson, Mark R., Mark Stoneking, Mitchell M. Holland, Joseph A. Dizinno, and Bruce Budowle
1993 Guidelines for the Use of Mitochondrial-DNA Sequencing in Forensic-Science. *Crime Laboratory Digest* 20(4):68–77.

Yang, Dongya Y., Barry Eng, and Shelley R. Saunders
2003 Hypersensitive PCR, Ancient Human mtDNA, and Contamination. *Human Biology* 75(3):355–64.

Yang, Dongya Y., and Kathy Watt
2005 Contamination Controls When Preparing Archaeological Remains for Ancient DNA Analysis. *Journal of Archaeological Science* 32(3):331–336.

Dongya Y. Yang and Camilla F. Speller
Ancient DNA Laboratory,
Department of Archaeology,
Simon Fraser University
Burnaby, BC V5A 1S6, Canada
<donyang@sfu.ca>