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Setting the stage – Building and working in an ancient DNA laboratory

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SUMMARY

With the introduction of next generation high throughput sequencing in 2005 and the resulting revolution in genetics, ancient DNA research has rapidly developed from an interesting but marginal field within evolutionary biology into one that can contribute significantly to our understanding of evolution in general and the development of our own species in particular. While the amount of sequence data available from ancient human, other animal and plant remains has increased dramatically over the past five years, some key limitations of ancient DNA research remain. Most notably, reduction of contamination and the authentication of results are of utmost importance. A number of studies have addressed different aspects of sampling, DNA extraction and DNA manipulation in order to establish protocols that most efficiently generate reproducible and authentic results. As increasing numbers of researchers from different backgrounds become interested in using ancient DNA technology to address key questions, the need for practical guidelines on how to construct and use an ancient DNA facility arises. The aim of this article is therefore to provide practical tips for building a state-of-the-art ancient DNA facility. It is intended to help researchers new to the field of ancient DNA research generally, and those considering the application of next generation sequencing, in their planning process.

1. Introduction

The development of next generation sequencing (NGS) has revolutionized ancient DNA (aDNA) research like almost no other field of genetics. Within a few months of the introduction of NGS in 2005 (Margulies et al., 2005), Poinar et al. (2006) published 13 million bp from the nuclear genome of the extinct woolly mammoth. When compared with the 27,000 bp of cave bear sequence (Noonan et al., 2005) that represented the largest nuclear data set available from an extinct species in the pre-NGS era, the data set obtained by Poinar et al. (2006) represented a 480-fold increase. NGS development has so far resulted in the publication of low coverage draft nuclear genomes of the woolly mammoth (0.8-fold, Miller et al., 2008), the Neanderthal (1-fold, Green et al., 2010) a new hominin dubbed Denisovans (1.9-fold, Reich et al., 2010) and a high-quality 20-fold coverage nuclear genome of a 4000-year-old Palaeo-Eskimo (Rasmussen et al., 2010). To date, very few studies investigating functional genetics from ancient samples have been conducted and all of these have used conventional cloning and Sanger sequencing (Campbell et al., 2010; Krause et al., 2007; Lalueza-Fox et al., 2007; Römpler et al., 2006). The capability to sequence entire genomes from ancient samples now makes it feasible to obtain a large amount of functionally informative nuclear data from subfossil remains and, as a result, opens up huge potential for future ancient DNA studies.

As is now widely known, ancient DNA extracts are usually characterised by a low endogenous molecule number as well as short and chemically altered molecules (Pääbo et al., 2004). As a result of these characteristics, issues of contamination from numerous sources have always been present. In past cases where particularly astonishing findings were reported, such as the recovery of DNA from dinosaur remains (for example Woodward et al., 1994), it has generally been determined that contamination, rather than endogenous DNA sequences, explained the results (Pääbo et al., 2004). The "dinosaur" DNA sequenced by Woodward et al. (1994) for example was later suggested to be of human origin, representing a mitochondrial insertion in the nuclear human genome (Hedges and Schweitzer, 1995; Allard et al., 1995; Henikoff, 1995; Zischler et al., 1995). As a result of these contamination issues, a number of authenticity criteria for ancient DNA sequence data (including Sanger and NGS data) have been suggested, including the use of a dedicated ancient DNA clean room facility for all pre-amplification work with ancient DNA (Cooper and Poinar, 2000; Green et al., 2009; Pääbo et al., 2004). The importance of using such a clean room was demonstrated in 2006, when two studies focusing on nuclear DNA from the same Neanderthal sample produced inconsistent results (Green et al., 2006; Noonan et al., 2006; Wall and Kim,

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2007). Later studies showed that the two extracts, which were produced under stringent ancient DNA protocols in a state-of-the-art clean room facility, left the facility with very low levels of modern human contamination. However, at least one of those extracts (Green et al., 2006) was contaminated with modern human DNA in the subsequent library preparation for NGS, which was conducted in a different, non-clean room, laboratory (Green et al., 2009).

The development of NGS has provided a new set of tools to identify and even avoid modern contamination in ancient DNA experiments. It allows sequencing of the very short molecules which are characteristic of ancient DNA and which are generally too short to be amplified by the polymerase chain reaction (PCR). NGS thereby increases the number of endogenous ancient molecules accessible for sequencing and reduces the risk of favoring long molecules originating from modern contaminants (Krause et al., 2010). Because many individual molecules covering a site of interest can be sequenced, NGS also allows easier identification of contaminating molecules. However, NGS also introduces new types of contamination that pose significant additional challenges for ancient DNA studies. For example, using traditional PCR and Sanger sequencing, a target region is amplified and either cloned or directly sequenced. These amplified PCR products could, theoretically, get back into the dedicated ancient DNA facility and be incorporated in later reactions which can cause erroneous results, but at least there is some knowledge of and control over what has been amplified; it is generally known what species or loci have been studied in a laboratory. This is not the case for NGS. An ancient DNA extract can contain a large amount of exogenous DNA from numerous environmental sources, including bacteria and fungi, many of which are unknown to science. Noonan et al. (2005), for example, analysed metagenomic libraries constructed with unamplified DNA extracted from skeletal remains of two 40,000-year-old extinct cave bears and found that up to 66% of the sequences obtained did not produce any matches when compared to all entries on GenBank. In one library, only 1% could be identified as being of carnivore origin. Similarly low levels of endogenous DNA were obtained from Neanderthal samples (Green et al., 2010). The ancient extract can also contain significant amounts of contaminating human DNA, especially if the sample was collected and handled without DNA studies in mind, as is common for most museum specimens. Miller et al. (2009) for example found that up to 8.9% of the total reads produced from 454 high throughput sequencing of a museum preserved, 104-year-old, dry skin sample of a Tasmanian tiger were of human origin. When producing a sequencing library for NGS, a large number of exogenous DNA molecules in the ancient DNA extract will be ligated to universal NGS adaptors. The library is then amplified in the post-PCR laboratory, producing millions of adaptor-ligated copies of *all* of the extracted DNA, including that of all of the unknown organisms and potentially a significant amount of exogenous human DNA. As PCR products are suitable for airborne distribution, these adaptor-ligated copies could easily be distributed throughout the post-PCR laboratory, adding to all of the other amplified PCR products being generated in other (NGS) experiments. If any one of those molecules makes its way back to the ancient DNA facility, the laboratory will be contaminated with ready-to-sequence human, bacterial, or other DNA that may be of interest in future aDNA studies. This will be unidentifiable contamination representing organisms that may never have been worked on previously in the respective laboratory. This is particularly critical when using NGS for determining the composition of ancient or even modern environmental samples, for example. Thus all of the issues of between-laboratory contamination control precautions, as well as the pre-laboratory contamination precautions, are even more important than they were in pre-NGS days.

As more and more researchers become interested in ancient DNA studies there is an increased need for suitable facilities in which to conduct these studies. Authenticity requirements for ancient DNA data have been proposed previously (Cooper and Poinar, 2000; Pääbo et al., 2004; Green et al., 2009) and guidelines for work with ancient DNA always include the requirement of a dedicated, isolated laboratory environment (Cooper and Poinar, 2000). But what does this actually mean? Over time, a set of guidelines has evolved that define a suitable ancient DNA work space, however many of these guidelines have been established as a result of personal and often unpublished experiences of various researchers. In this review we focus on the logistical and spatial requirements for setting up an ancient DNA facility, with the aim of providing a guideline for research groups that want to commit to a serious ancient DNA program and have the opportunity and support to construct such a facility.

2. Technical requirements for an ancient DNA facility

Contaminating DNA can be introduced to an experiment in multiple ways, including through contaminated reagents or samples and through carry-over of DNA and PCR amplification products from previous experiments (Champlot et al., 2010). Carry-over of PCR products in particular is one of the main sources of contamination of ancient DNA extracts (Cooper and Poinar, 2000; Pääbo et al., 2004). PCR products can exist in millions of copies in each post-PCR laboratory and can be distributed through the air or attached to clothing or shoes (Champlot et al., 2010). These products are likely to include copies of specific target regions that are to be amplified from ancient DNA extracts. Studies have shown that from a few microliters of wash water used to clean a post-PCR laboratory, numerous genomic regions from a number of species worked on in the laboratory could be amplified (Hummel, 2003 and references therein). Thus one of the main challenges in the design of an ancient DNA facility is to keep contaminating DNA, and particularly previously amplified PCR products, out. Standard precautions to achieve this include:

2.1. Location of facility

Spatial isolation of the ancient DNA facility from the post-PCR laboratory is essential. Many established ancient DNA research groups go as far as to have the ancient DNA facility in a separate building from any post-PCR laboratories. No dedicated ancient DNA facility should ever have been used as a post-PCR laboratory at any time in the past.

Separate access to the ancient DNA facility is ideal. It is established practice to only access the aDNA facility first thing in the day – before one has entered any post-PCR laboratory. Movement should always be unidirectional – from the ancient lab to the post-PCR rooms. Entry into the aDNA facility after having been in the post-PCR laboratory on the same day increases the risk of carrying PCR products from the latter to the former laboratory and for this reason access to the aDNA facility should never be through the post-PCR laboratory.

2.2. Design

The concept of spatial separation of working steps is not only useful between the ancient DNA laboratory and post-PCR lab, it also helps to reduce cross contamination between experiments in the ancient DNA facility itself. Ideally, different steps can be conducted in different rooms and/or in dedicated hoods. While the setup may vary between different ancient DNA facilities depending on the space available, we recommend a minimum of three separate rooms. This allows for the separation of three major working areas allowing for specific activities:

Box 1: Example setup of a three-room ancient DNA facility.
The complete facility has a positive pressure system accompanied by a HEPA air filter system and is air conditioned. Every room is fitted with UVC light sources that can be switched on and off from outside the respective room. The facility consists of three consecutive rooms, i.e. room 2 can only be reached through room 1 and room 3 can only be reached through room 2.
Room 1:
The room has an entry area for changing into suitable clean room clothing (see main text). It has storage facilities for consumables and samples, and can also be equipped with a fridge and freezer for temporary storage of inwards goods. It has bench space for UV irradiation of samples and consumables, and also for all pre-extraction handling of samples, such as photographing, measuring and cataloguing. It has a UV-proof cupboard for computer and other UV-sensitive equipment needed in this room. All surfaces are UVC and bleach resistant.
Room 2:
This room is fitted with a fume hood that can be fully closed and has internal UVC light and power sockets. The room is fitted with UVC light and has bench space for handling samples. Further important equipment includes a fine scale for weighing of samples and a dentist drill or similar tools for cutting and drilling samples as well as mortars and pestles or other equipment for grinding samples. As sawdust can occur when cutting samples, the room should be fitted with a water source for cleaning purposes.
Room 3:
This is the core of the ancient DNA facility. The room is fitted with at least two individual enclosed workstations or PCR hoods: one for DNA extraction and manipulation, and one entirely DNA-free for PCR setup. The hoods should have internal UVC sources and can be Class II biosafety cabinets. The DNA extraction hood should contain a small table centrifuge. Essential equipment in this room includes most standard molecular biology laboratory gear such as centrifuges (with rotors for plates as well as 1.5 mL reaction tubes, and, depending on the volume of the extractions performed, potentially also for up to 50 mL tubes), heating block, incubator, vortex, rocker, scales, fridge and freezer. If NGS is being undertaken, perhaps surprisingly for an ancient lab, the room needs to be equipped with a thermal cycler for library preparation because the adaptor ligation process includes a number of enzymatic reactions with a range of different temperature requirements. For obvious reasons it is essential that this machine has never been previously used for PCR and is never used for PCR while in the clean room. Other useful equipment includes a dishwasher run with ultrapure water (for glassware used in the clean room), and a microwave.

Room 1: Changing into dedicated clean room clothing (such as coveralls, hairnet, facemask, laboratory shoes, double gloves), storage of consumables, pre-extraction processing of potentially contaminated museum samples, ultraviolet (UV) C irradiation of samples and consumables.
Room 2: Ideally fitted with a fume hood (with internal UVC fitted) for cutting bone samples. As this can be a dusty process, separating it from the subsequent DNA extraction and manipulation steps reduces the risk of cross contamination between samples.
Room 3: DNA extraction and manipulation and PCR setup, ideally in separate hoods fitted with internal UVC.

Spatial limitations may require alternative arrangements. In Box 1 we describe an example setup for a three-room ancient DNA facility.

2.3. Access

To reduce the risk of introducing contamination, aDNA research groups often practice a limited access policy. Access to the ancient DNA facility should be limited to trained personnel and maintenance staff who understand the protocols for reducing potential contamination. For this reason some aDNA facilities are fitted with windows to allow guests to view work being undertaken in the laboratory. Such windows also can be an additional safety feature for staff working alone in the laboratory. It is also important to consider access of support staff such as cleaning crews and repair workers who, with their equipment, may have been working in, or passing through, post-PCR laboratories.

2.4. Consumables and equipment

As PCR products are ubiquitous in post-PCR laboratories it is important to make sure that no consumables or equipment for the ancient DNA facility have been sourced from post-PCR laboratories. In addition to standard molecular laboratory equipment, a few more items are advantageous in the aDNA facility. To keep levels of environmental DNA low, the aDNA facility can be UV irradiated when it is not in use. For this purpose UVC light ($\lambda = 254$ nm) is often used and overhead UV lighting of the lab is ideal. A further strategy to reduce the levels of environmental DNA is regular bleaching of all surfaces (see for example Champlot et al., 2010). It should be noted that both bleach and UVC can be damaging to some surfaces, a factor that should be taken into account when building the aDNA facility; use bleach- and UVC- resistant materials wherever possible. Further useful features are a positive pressure systems and HEPA-filtered air conditioning. Dedicated laminar flow hoods and fume hoods for DNA extraction and manipulation further reduce the risk of contaminating the experiment.

2.5. Laboratory protocol

Laboratory protocols vary and depend on the organisms on which research is being undertaken. For example if all precautions to prevent PCR product carry-over are taken, a researcher is less likely to introduce relevant contamination when extracting DNA from a mammoth bone than when DNA from subfossil human remains is extracted. This is not only valid when human DNA is targeted but in particular also when human associated bacterial DNA is investigated. However, for NGS studies even non-target DNA can become a nuisance as it will get sequenced along with the target DNA and reduce the sequence reads on target. It is therefore recommended to reduce the amount of DNA in the ancient DNA facility as much as possible. Dedicated clean room clothing such as full body coveralls (as are routinely used in forensic work) can help achieve this goal. Wearing face masks, face shields and hairnets further reduces the amount of DNA shed by the researcher. Dedicated clean room shoes are useful to reduce carry-over contamination, and as ancient DNA work requires regular changing of gloves, wearing two pairs of gloves will prevent the exposure of skin when changing gloves.

3. Conclusion

The development of NGS technology has created tremendous new opportunities for ancient DNA research. As a result, an increasing number of researchers are establishing ancient DNA facilities. This provides us with an opportunity as a research community to step back and rethink current practices, both to ensure that requirements are satisfactory for existing techniques and, perhaps more importantly, also for developing technologies such as NGS. Setting up an ancient DNA facility is no small feat; it

requires time, money and institutional support. Our aim is for this review to provide guidelines and considerations that will assist new researchers in the field or those wanting to upgrade their facilities. We also suggest that modern DNA labs using NGS for environmental sequencing might want to consider some of the issues raised here as contamination is a problem not limited to ancient DNA alone.

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References

Allard, M.W., Young, D., Huyen, Y., 1995. Detecting dinosaur DNA. Science 268, 1192.

Campbell, K.L., Roberts, J.E.E., Watson, L.N., Stetefeld, J., Sloan, A.M., Signore, A.V., Howatt, J.W., Tame, J.R.H., Rohland, N., Shen, T.J., Austin, J.J., Hofreiter, M., Ho, C., Weber, R.E., Cooper, A., 2010. Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance. Nature Genetics 42, 536–591.

Champlot, S., Berthelot, C., Pruvost, M., Bennett, E.A., Grange, T., Geigl, E.M., 2010. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. PLoS ONE, 5.

Cooper, A., Poinar, H.N., 2000. Ancient DNA: do it right or not at all. Science 289, 1139.

Green, R.E., Krause, J., Ptak, S.E., Briggs, A.W., Ronan, M.T., Simons, J.F., Du, L., Egholm, M., Rothberg, J.M., Paunovic, M., Pääbo, S., 2006. Analysis of one million base pairs of Neanderthal DNA. Nature 444, 330–336.

Green, R.E., Briggs, A.W., Krause, J., Prüfer, K., Burbano, H.A., Siebauer, M., Lachmann, M., Pääbo, S., 2009. The Neandertal genome and ancient DNA authenticity. EMBO Journal 28, 2494–2502.

Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W.W., Fritz, M.H.Y., Hansen, N.F., Durand, E.Y., Malaspinas, A.S., Jensen, J.D., Marques-Bonet, T., Alkan, C., Prüfer, K., Meyer, M., Burbano, H.A., Good, J.M., Schultz, R., Aximu-Petri, A., Butthof, A., Höber, B., Höffner, B., Siegemund, M., Weihmann, A., Nusbaum, C., Lander, E.S., Russ, C., Novod, N., Affourtit, J., Egholm, M., Verna, C., Rudan, P., Brajković, D., Kućan, Ž., Gušić, I., Doronichev, V.B., Golovanova, L.V., Lalueza-Fox, C., de la Rasilla, M., Fortea, J., Rosas, A., Schmitz, R.W., Johnson, P.L.F., Eichler, E.E., Falush, D., Birney, E., Mullikin, J.C., Slatkin, M., Nielsen, R., Kelso, J., Lachmann, M., Reich, D., Pääbo, S., 2010. A draft sequence of the neandertal genome. Science 328, 710–722.

Hedges, S.B., Schweitzer, M.H., 1995. Detecting dinosaur DNA. Science 268, 1191–1192.

Henikoff, S., 1995. Detecting dinosaur DNA. Science 268, 1192.

Hummel, S., 2003. Ancient DNA Typing: Methods, Strategies, and Applications. Springer Verlag, Berlin/Heidelberg/New York.

Krause, J., Briggs, A.W., Kircher, M., Maricic, T., Zwyns, N., Derevianko, A., Pääbo, S., 2010. A complete mtDNA genome of an early modern human from Kostenki, Russia. Current Biology 20, 231–236.

Krause, J., Lalueza-Fox, C., Orlando, L., Enard, W., Green, R.E., Burbano, H.A., Hublin, J.J., Hänni, C., Fortea, J., de la Rasilla, M., Bertranpetit, J., Rosas, A., Pääbo, S., 2007. The derived FOXP2 variant of modern humans was shared with Neandertals. Current Biology 17, 1908–1912.

Lalueza-Fox, C., Römpler, H., Caramelli, D., Staubert, C., Catalano, G., Hughes, D., Rohland, N., Pilli, E., Longo, L., Condemi, S., de la Rasilla, M., Fortea, J., Rosas, A., Stoneking, M., Schöneberg, T., Bertranpetit, J., Hofreiter, M., 2007. A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. Science 318, 1453–1455.

Margulies, M., Egholm, M., Altman, W.E., Attiya, S., Bader, J.S., Bemben, L.A., Berka, J., Braverman, M.S., Chen, Y.J., Chen, Z.T., Dewell, S.B., Du, L., Fierro, J.M., Gomes, X.V., Godwin, B.C., He, W., Helgesen, S., Ho, C.H., Irzyk, G.P., Jando, S.C., Alenquer, M.L.I., Jarvie, T.P., Jirage, K.B., Kim, J.B., Knight, J.R., Lanza, J.R., Leamon, J.H., Lefkowitz, S.M., Lei, M., Li, J., Lohman, K.L., Lu, H., Makhijani, V.B., McDade, K.E., McKenna, M.P., Myers, E.W., Nickerson, E., Nobile, J.R., Plant, R., Puc, B.P., Ronan, M.T., Roth, G.T., Sarkis, G.J., Simons, J.F., Simpson, J.W., Srinivasan, M., Tartaro, K.R., Tomasz, A., Vogt, K.A., Volkmer, G.A., Wang, S.H., Wang, Y., Weiner, M.P., Yu, P.G., Begley, R.F., Rothberg, J.M., 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature 437, 376–380.

Miller, W., Drautz, D.I., Ratan, A., Pusey, B., Qi, J., Lesk, A.M., Tomsho, L.P., Packard, M.D., Zhao, F.Q., Sher, A., Tikhonov, A., Raney, B., Patterson, N., Lindblad-Toh, K., Lander, E.S., Knight, J.R., Irzyk, G.P., Fredrikson, K.M., Harkins, T.T., Sheridan, S., Pringle, T., Schuster, S.C., 2008. Sequencing the nuclear genome of the extinct woolly mammoth. Nature 456, 387–390.

Miller, W., Drautz, D.I., Janecka, J.E., Lesk, A.M., Ratan, A., Tomsho, L.P., Packard, M., Zhang, Y., McClellan, L.R., Qi, J., Zhao, F., Gilbert, M.T.P., Dalén, L., Arsuaga, J.L., Ericson, P.G.P., Huson, D.H., Helgen, K.M., Murphy, W.J., Götherström, A., Schuster, S.C., 2009. The mitochondrial genome sequence of the Tasmanian tiger (Thylacinus cynocephalus). Genome Research 19, 213–220.

Noonan, J.P., Hofreiter, M., Smith, D., Priest, J.R., Rohland, N., Rabeder, G., Krause, J., Detter, J.C., Pääbo, S., Rubin, E.M., 2005. Genomic sequencing of Pleistocene cave bears. Science 309, 597–600.

Noonan, J.P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Platt, D., Pääbo, S., Pritchard, J.K., Rubin, E.M., 2006. Sequencing and analysis of Neanderthal genomic DNA. Science 314, 1113–1118.

Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2004. Genetic analyses from ancient DNA. Annual Review of Genetics 38, 645–679.

Poinar, H.N., Schwarz, C., Qi, J., Shapiro, B., MacPhee, R.D.E., Buigues, B., Tikhonov, A., Huson, D.H., Tomsho, L.P., Auch, A., Rampp, M., Miller, W., Schuster, S.C., 2006. Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. Science 311, 392–394.

Rasmussen, M., Li, Y.R., Lindgreen, S., Pedersen, J.S., Albrechtsen, A., Moltke, I., Metspalu, M., Metspalu, E., Kivisild, T., Gupta, R., Bertalan, M., Nielsen, K., Gilbert, M.T.P., Wang, Y., Raghavan, M., Campos, P.F., Kamp, H.M., Wilson, A.S., Gledhill, A., Tridico, S., Bunce, M., Lorenzen, E.D., Binladen, J., Guo, X.S., Zhao, J., Zhang, X.Q., Zhang, H., Li, Z., Chen, M.F., Orlando, L., Kristiansen, K., Bak, M., Tommerup, N., Bendixen, C., Pierre, T.L., Gronnow, B., Meldgaard, M., Andreasen, C., Fedorova, S.A., Osipova, L.P., Higham, T.F.G., Ramsey, C.B., Hansen, T.V.O., Nielsen, F.C., Crawford, M.H., Brunak, S., Sicheritz-Ponten, T., Villems, R., Nielsen, R., Krogh, A., Wang, J., Willerslev, E., 2010. Ancient human genome sequence of an extinct Palaeo-Eskimo. Nature 463, 757–762.

Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L.F., Maricic, T., Good, J.M., Marques-Bonet, T., Alkan, C., Fu, Q.M., Mallick, S., Li, H., Meyer, M., Eichler, E.E., Stoneking, M., Richards, M., Talamo, S., Shunkov, M.V., Derevianko, A.P., Hublin, J.J., Kelso, J., Slatkin, M., Pääbo, S., 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature 468, 1053–1060.

Römpler, H., Rohland, N., Lalueza-Fox, C., Willerslev, E., Kuznetsova, T., Rabeder, G., Bertranpetit, J., Schöneberg, T., Hofreiter, M., 2006. Nuclear gene indicates coat-color polymorphism in mammoths. Science 313, 62–162.

Wall, J.D., Kim, S.K., 2007. Inconsistencies in Neanderthal genomic DNA sequences. PLoS Genetics 3, 1862–1866.

Woodward, S.R., Weyand, N.J., Bunnell, M., 1994. DNA sequences from Cretaceous period bone fragments. Science 266, 1229–1232.

Zischler, H., Höss, M., Handt, O., von Haeseler, A., van der Kuyl, A.C., Goudsmit, J., Pääbo, S., 1995. Detecting dinosaur DNA. Science 268, 1192–1193.