

# AmtDB: a database of ancient human mitochondrial genomes

Edvard Ehler<sup>1,2,\*</sup>, Jiří Novotný<sup>1,3</sup>, Anna Juras<sup>2</sup>, Maciej Chyleński<sup>4</sup>, Ondřej Moravčík<sup>1</sup> and Jan Pačes<sup>1,3,\*</sup>

<sup>1</sup>Institute of Molecular Genetics of the ASCR, Vídeňská 1083, 142 20 Prague 4, Czech Republic, <sup>2</sup>Department of Human Evolutionary Biology, Institute of Anthropology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61-614 Poznań, Poland, <sup>3</sup>Department of Informatics and Chemistry, Faculty of Chemical Technology, University of Chemistry and Technology, Technická 5, 166 28 Prague 6, Dejvice, Czech Republic and <sup>4</sup>Institute of Archaeology, Faculty of History, Adam Mickiewicz University in Poznań, Umultowska 89D, 61-614 Poznań, Poland

Received August 03, 2018; Revised September 04, 2018; Editorial Decision September 07, 2018; Accepted September 21, 2018

## ABSTRACT

**Ancient mitochondrial DNA is used for tracing human past demographic events due to its population-level variability. The number of published ancient mitochondrial genomes has increased in recent years, alongside with the development of high-throughput sequencing and capture enrichment methods. Here, we present AmtDB, the first database of ancient human mitochondrial genomes. Release version contains 1107 hand-curated ancient samples, freely accessible for download, together with the individual descriptors, including geographic location, radiocarbon dating, and archaeological culture affiliation. The database also features an interactive map for sample location visualization. AmtDB is a key platform for ancient population genetic studies and is available at <https://amtdb.org>.**

## INTRODUCTION

Ancient DNA (aDNA) is a genetic material obtained from ancient specimens, and unlike modern DNA, undergoes fragmentation and post-mortem damages caused mainly by environmental factors (1). Ancient DNA studies, conducted in the last 30 years, have confirmed that while maintaining appropriate procedures, we are able to recover genetic material from ancient specimens. Until recently, the majority of human aDNA studies were focused mainly on mitochondrial DNA (mtDNA) thanks to the fact that mtDNA is present in cells in a higher copy number than the nuclear genome, and therefore it is often the only genetic marker that can be recovered from poorly preserved samples. Due to its maternal inheritance, high mutation rate, absence of recombination and population-level variability, it is a useful tool for reconstructing the past demographic events (2).

Despite the long-standing interest in ancient mtDNA, it was only in the past few years, when a high number of complete mt genomes were made available, alongside with the development of the high-throughput sequencing, often combined with the capture enrichment methods.

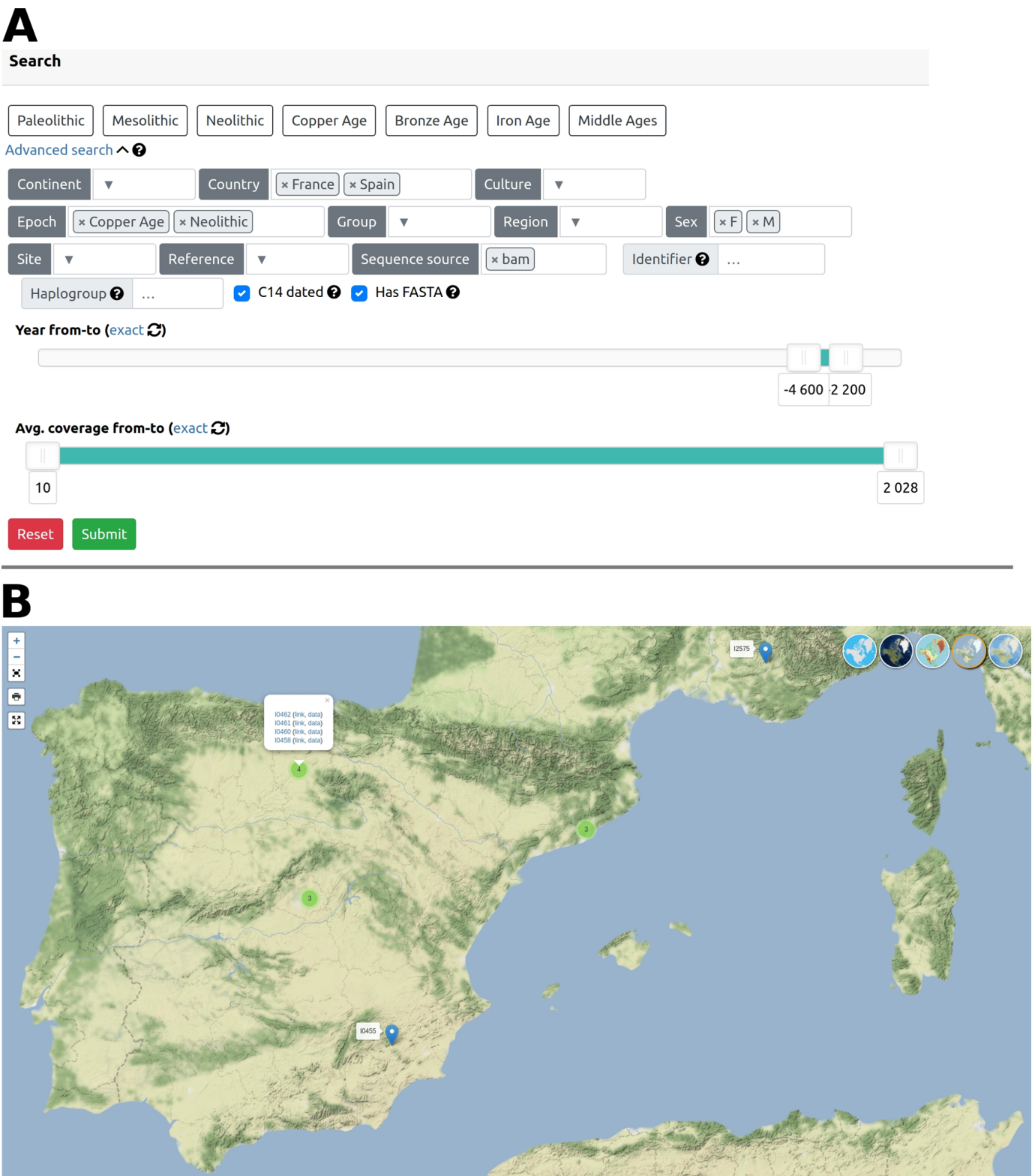
Mitochondrial DNA, often as a part of nuclear genome studies, was used to reconstruct demographic events that took place in pre-LGM (Last Glacial Maximum) and post-LGM era in Europe (3,4), to trace demographic changes that shaped past and modern populations mtDNA variation (5–13), including the influence of Neolithization process (14–23), and Steppe migrations (24–26). Moreover, mtDNA was used in several kinship studies as a molecular marker which excludes direct maternal kinship between ancient individuals (27–31).

Although there are currently available modern mtDNA databases, e.g. EMPOP (32), MITOMAP (33), HmtDB (34), and mtDB (35), there is no database that would be dedicated specifically to ancient mt genomes. A database concentrated primarily on ancient DNA is the Online Ancient Genome Repository (<https://www.oagr.org.au>). OAGR is the database primarily for samples generated (or collaborated on) by the Australian Centre for Ancient DNA, University of Adelaide, and includes both human SNP markers data and microbiome data. Our AmtDB is filling this gap by consistent way of mapping the published aDNA samples from different sources, and providing the associated meta-data in standard, uniform, easily-downloadable-and-usable way, together with the mt genomes sequences and links to other resources. While our primary focus lies on ancient mtDNA, the metadata itself can be easily used in ancient genomic, archaeological or anthropological studies.

## Database overview and functionality

The AmtDB database, as of initial version v1.000, contains 1107 samples. For 887 of these samples we provide the full

\*To whom correspondence should be addressed. Tel: +420 296 443 446; Email: edvard.ehler@img.cas.cz  
Correspondence may also be addressed to Jan Pačes. Email: jan.paces@img.cas.cz



**Figure 1.** AmtDB (<https://amtdb.org>) advanced search overview. **(A)** The database was filtered for Neolithic and Copper Age samples from France and Spain. These samples also have following attributes: known sex ('F' or 'M'), age between 4600 and 2200 BCE, complete mtDNA available, mt reconstructed (sequence source) from BAM files with average coverage at least 10, and are radiocarbon dated. **(B)** Visualization of the search results on a map, showing unclustered and clustered samples (clustering can be toggled on and off). Tooltip with sample links can be displayed for each sample or cluster (here shown for four samples cluster from Arroyal I site in Burgos, Spain).

mt sequences in FASTA format. For all samples, we offer metadata in form of additional descriptors. Although we utilize custom scripts for semi-automated data retrieval, all provided data are hand-curated and checked.

Authors of the aDNA studies usually provide the mtDNA sequences in three different ways, or in any combination of thereof:

1. As complete mtDNA sequences deposited in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) database (labeled as *fasta*).
2. As results from high-throughput sequencing, in the form of SAM/BAM files deposited in an appropriate database, i.e. European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) or Sequence Read Archive (<https://www.ncbi.nlm.nih.gov/sra>) (labeled as *bam*).
3. In haplotype format, i.e. list of changed position in comparison to rCRS (36) or RSRS (37) (labeled as *reconstructed*).

In case of available FASTA from GenBank, we provide this sequence in the database. Otherwise, we either reconstruct the mt sequence from the haplotype using the Haplosearch (<https://haplosearch.com>) tool (38), or preferably reconstruct the mt sequence from provided SAM/BAM files, merging multiple files (per individual) with the use of SAMtools (39). The bioinformatics pipeline in this procedure includes mapping the merged reads as single-end reads against the rCRS with BWA software (40) and collapsing duplicate sequence reads with identical start and end coordinates using FilterUniqueSAMCons.py script (41). Consensus sequences are built using ANGSD toolkit (42). In these samples we also display the average sequence depth (coverage). User can filter all samples according to the mt sequence source discussed above (*fasta*, *bam*, *reconstructed*).

More details about mtDNA reconstruction pipeline can be found in our previous publication (15) and in AmtDB documentation (<https://amtdb.org/help>).

Besides the mt sequences, the AmtDB contains additional information about the samples, the metadata. The samples can be selected and browsed based on primary ID and alternative ID(s), several geographic location descriptors, latitude and longitude in decimal degrees, archaeological site, or group of archaeological cultural background descriptors. We also supply a comment column, which may contain additional info for the sample, usually information about relationship, uncertainties, or important notes that do not fit into other category and might be valuable for researchers. Biological variables include sex, mt haplogroup, Y chromosomal haplogroup, and Y chromosomal haplotype. For sample age related information, we use calibrated BCE or CE ((Before) Common Era) dates wherever possible. For the radiocarbon dated samples, we provide the precise min. and max. values of the 95.4% probability interval for calibrated (B)CE date, uncalibrated BP (Before Present) age, and radiocarbon laboratory and sample code. For samples that are not directly  $^{14}\text{C}$  dated, but other samples from the same layer are, we provide calibrated (B)CE age of the layer. For samples, that were dated only according to the material culture associated with the sample, we use uncali-

brated (B)CE age. Our database search engine allows to filter  $^{14}\text{C}$  dated samples only. For each sample we also provide publication reference, DOI based reference link and link to sample (mt) sequence depository.

Focal point of our simple, clear and user-friendly interface with advanced search options (Figure 1A) is the visualization of the filtered samples on an interactive world map (Figure 1B). Samples on the map can be clustered together by their distance and smaller clusters are created when the map is zoomed in. Tooltip with sample links appears when cluster is right clicked. Maps are available in several graphical overlays (political, physical, satellite or blind map), and are ready for download together with all provided sequences and metadata, without registration.

## CONCLUSION

The database is currently in initial operational capability phase, v1.000, and will get 2–3 major updates per year, concentrating on adding more published samples into the database. We believe the community of ancient human populations researchers will find AmtDB useful, as to our best knowledge, there is no comparable database in terms of usability and data content.

## DATA AVAILABILITY

The Ancient human mitochondrial genomes database can be found at <https://amtdb.org>.

## FUNDING

Ministry of Education, Youth and Sports of the Czech Republic [ELIXIR-CZ project LM2015047, part of the international ELIXIR infrastructure, under the Projects CESNET, LM2015042]; Polish National Science Center [2014/12/W/NZ2/00466]. Funding for open access charge: Ministry of Education, Youth and Sports of the Czech Republic.

*Conflict of interest statement.* None declared.

## REFERENCES

1. Pääbo, S. (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 1939–1943.
2. Ramakrishnan, U. and Hadly, E.A. (2009) Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies. *Mol. Ecol.*, **18**, 1310–1330.
3. Posth, C., Renaud, G., Mittnik, A., Drucker, D.G., Rougier, H., Cupillard, C., Valentin, F., Thevenet, C., Furtwängler, A., Wißing, C. *et al.* (2016) Pleistocene mitochondrial genomes suggest a single major dispersal of non-africans and a late glacial population turnover in Europe. *Curr. Biol.*, **26**, 827–833.
4. Fu, Q., Posth, C., Hajdinjak, M., Petr, M., Mallick, S., Fernandes, D., Furtwängler, A., Haak, W., Meyer, M., Mittnik, A. *et al.* (2016) The genetic history of Ice Age Europe. *Nature*, **534**, 200–205.
5. Brandt, G., Haak, W., Adler, C.J., Roth, C., Szecsenyi-Nagy, A., Karimnia, S., Moller-Rieker, S., Meller, H., Ganslmeier, R., Friederich, S. *et al.* (2013) Ancient DNA reveals key stages in the formation of central European mitochondrial genetic diversity. *Science*, **342**, 257–261.
6. Brotherton, P., Haak, W., Templeton, J., Brandt, G., Soubrier, J., Jane Adler, C., Richards, S.M., Der Sarkissian, C., Ganslmeier, R., Friederich, S. *et al.* (2013) Neolithic mitochondrial haplogroup H



- genomes and the genetic origins of Europeans. *Nat. Commun.*, **4**, 1764.
7. Gallego-Llorente, M., Connell, S., Jones, E.R., Merrett, D.C., Jeon, Y., Eriksson, A., Siska, V., Gamba, C., Meiklejohn, C., Beyer, R. *et al.* (2016) The genetics of an early Neolithic pastoralist from the Zagros, Iran. *Sci. Rep.*, **6**, 31326.
  8. Kılınç, G.M., Omrak, A., Özer, F., Günther, T., Büyükkarakaya, A.M., Bıçakçı, E., Baird, D., Dönertaş, H.M., Ghalichi, A., Yaka, R. *et al.* (2016) The demographic development of the first farmers in Anatolia. *Curr. Biol.*, **26**, 2659–2666.
  9. Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D.C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K. *et al.* (2016) Genomic insights into the origin of farming in the ancient Near East. *Nature*, **536**, 419–424.
  10. Omrak, A., Günther, T., Valdiosera, C., Svensson, E.M., Malmström, H., Kiesewetter, H., Aylward, W., Storå, J., Jakobsson, M. and Götherström, A. (2016) Genomic evidence establishes anatolia as the source of the European neolithic gene pool. *Curr. Biol.*, **26**, 270–275.
  11. Haber, M., Doumet-Serhal, C., Scheib, C., Xue, Y., Danecsek, P., Mezzavilla, M., Youhanna, S., Martiniano, R., Prado-Martinez, J., Szpak, M. *et al.* (2017) Continuity and admixture in the last five millennia of lebanese history from ancient canaanite and Present-Day lebanese genome sequences. *Am. J. Hum. Genet.*, **101**, 274–282.
  12. Mathieson, I., Alpaslan-Roodenberg, S., Posth, C., Szécsényi-Nagy, A., Rohland, N., Mallick, S., Olalde, I., Broomandkhoshbacht, N., Candilio, F., Cheronet, O. *et al.* (2018) The genomic history of southeastern Europe. *Nature*, **555**, 197–203.
  13. Olalde, I., Brace, S., Allentoft, M.E., Armit, J., Kristiansen, K., Booth, T., Rohland, N., Mallick, S., Szécsényi-Nagy, A., Mittnik, A. *et al.* (2018) The Beaker phenomenon and the genomic transformation of northwest Europe. *Nature*, **555**, 190–196.
  14. Haak, W., Balanovsky, O., Sanchez, J.J., Koshel, S., Zaporozhchenko, V., Adler, C.J., Der Sarkissian, C.S.I., Brandt, G., Schwarz, C., Nicklisch, N. *et al.* (2010) Ancient DNA from European early neolithic farmers reveals their near eastern affinities. *PLoS Biol.*, **8**, e1000536.
  15. Chyleński, M., Juras, A., Ehler, E., Malmström, H., Piontek, J., Jakobsson, M., Marciniak, A. and Dabert, M. (2017) Late Danubian mitochondrial genomes shed light into the Neolithisation of Central Europe in the 5th millennium BC. *BMC Evol. Biol.*, **17**, 80.
  16. Skoglund, P., Malmström, H., Raghavan, M., Stora, J., Hall, P., Willerslev, E., Gilbert, M.T.P., Götherström, A. and Jakobsson, M. (2012) Origins and genetic legacy of neolithic farmers and Hunter-Gatherers in Europe. *Science*, **336**, 466–469.
  17. Skoglund, P., Malmström, H., Omrak, A., Raghavan, M., Valdiosera, C., Günther, T., Hall, P., Tambets, K., Parik, J., Sjögren, K.-G. *et al.* (2014) Genomic diversity and admixture differs for Stone-Age scandinavian foragers and farmers. *Science*, **344**, 747–750.
  18. Bollongino, R., Nehlich, O., Richards, M.P., Orschiedt, J., Thomas, M.G., Sell, C., Fajkošová, Z., Powell, A. and Burger, J. (2013) 2000 years of parallel societies in stone age central europe. *Science*, **342**, 479–481.
  19. Gamba, C., Jones, E.R., Teasdale, M.D., McLaughlin, R.L., Gonzalez-Fortes, G., Mattiangeli, V., Domboróczki, L., Kövári, I., Pap, I., Anders, A. *et al.* (2014) Genome flux and stasis in a five millennium transect of European prehistory. *Nat. Commun.*, **5**, 5257.
  20. Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P.H., Schraiber, J.G., Castellano, S., Lipson, M. *et al.* (2014) Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature*, **513**, 409–413.
  21. Lipson, M., Szécsényi-Nagy, A., Mallick, S., Pósa, A., Stégmár, B., Keerl, V., Rohland, N., Stewardson, K., Ferry, M., Michel, M. *et al.* (2017) Parallel palaeogenomic transects reveal complex genetic history of early European farmers. *Nature*, **551**, 368–372.
  22. Saag, L., Varul, L., Scheib, C.L., Stenderup, J., Allentoft, M.E., Saag, L., Pagani, L., Reidla, M., Tambets, K., Metspalu, E. *et al.* (2017) Extensive farming in estonia started through a Sex-Biased migration from the steppe. *Curr. Biol.*, **27**, 2185–2193.
  23. Mittnik, A., Wang, C.-C., Pfrenkle, S., Daubaras, M., Zariņa, G., Hallgren, F., Allmäe, R., Khartanovich, V., Moiseyev, V., Tõrv, M. *et al.* (2018) The genetic prehistory of the Baltic Sea region. *Nat. Commun.*, **9**, 442.
  24. Allentoft, M.E., Sikora, M., Sjögren, K.-G., Rasmussen, S., Rasmussen, M., Stenderup, J., Damgaard, P.B., Schroeder, H., Ahlström, T., Vinner, L. *et al.* (2015) Population genomics of Bronze Age Eurasia. *Nature*, **522**, 167–172.
  25. Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G., Nordenfelt, S., Harney, E., Stewardson, K. *et al.* (2015) Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature*, **522**, 207–211.
  26. De Barros Damgaard, P., Marchi, N., Rasmussen, S., Peyrot, M., Renaud, G., Korneliussen, T., Moreno-Mayar, J.V., Pedersen, M.W., Goldberg, A., Usmanova, E. *et al.* (2018) 137 ancient human genomes from across the Eurasian steppes. *Nature*, **557**, 369–374.
  27. Lee, E.J., Renneberg, R., Harder, M., Krause-Kyora, B., Rinne, C., Mueller, J., Nebel, A. and von Wurmb-Schwark, N. (2014) Collective burials among agro-pastoral societies in later Neolithic Germany: perspectives from ancient DNA. *J. Archaeol. Sci.*, **51**, 174–180.
  28. Juras, A., Chyleński, M., Krenz-Niedbala, M., Malmström, H., Ehler, E., Pospieszny, Ł., Łukasik, S., Bednarczyk, J., Piontek, J., Jakobsson, M. *et al.* (2017) Investigating kinship of Neolithic post-LBK human remains from Krusza Zamkowa, Poland using ancient DNA. *Forensic Sci. Int. Genet.*, **26**, 30–39.
  29. Haak, W., Brandt, G., de Jong, H.N., Meyer, C., Ganslmeier, R., Heyd, V., Hawkesworth, C., Pike, A.W.G., Meller, H. and Alt, K.W. (2008) Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 18226–18231.
  30. Naumann, E., Krzewińska, M., Götherström, A. and Eriksson, G. (2014) Slaves as burial gifts in Viking Age Norway? Evidence from stable isotope and ancient DNA analyses. *J. Archaeol. Sci.*, **41**, 533–540.
  31. Malmström, H., Vretemark, M., Tillmar, A., Durling, M.B., Skoglund, P., Gilbert, M.T.P., Willerslev, E., Holmlund, G. and Götherström, A. (2012) Finding the founder of Stockholm – A kinship study based on Y-chromosomal, autosomal and mitochondrial DNA. *Ann. Anat. - Anat. Anzeiger*, **194**, 138–145.
  32. Parson, W. and Dür, A. (2007) EMPOP – a forensic mtDNA database. *Forensic Sci. Int. Genet.*, **1**, 88–92.
  33. Lott, M.T., Leipzig, J.N., Derbeneva, O., Michael Xie, H., Chalkia, D., Sarmady, M., Procaccio, V. and Wallace, D.C. (2013) MtDNA variation and analysis using Mitomap and Mitomaster. *Curr. Protoc. Bioinform.*, **44**, 1.23.1–1.23.26.
  34. Clima, R., Preste, R., Calabrese, C., Diroma, M.A., Santorsola, M., Scioscia, G., Simone, D., Shen, L., Gasparre, G. and Attimonelli, M. (2017) HmtDB 2016: data update, a better performing query system and human mitochondrial DNA haplogroup predictor. *Nucleic Acids Res.*, **45**, D698–D706.
  35. Ingman, M. and Gyllenstein, U. (2006) mtDB: Human Mitochondrial Genome Database, a resource for population genetics and medical sciences. *Nucleic Acids Res.*, **34**, D749–D751.
  36. Andrews, R.M., Kubacka, I., Chinnery, P.F., Lightowlers, R.N., Turnbull, D.M. and Howell, N. (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. *Nat. Genet.*, **23**, 147–147.
  37. Behar, D.M., van Oven, M., Rosset, S., Metspalu, M., Loogväli, E.-L., Silva, N.M., Kivisild, T., Torroni, A. and Villems, R. (2012) A ‘Copernican’ reassessment of the human mitochondrial DNA tree from its root. *Am. J. Hum. Genet.*, **90**, 675–684.
  38. Fregel, R. and Delgado, S. (2011) HaploSearch: A tool for haplotype-sequence two-way transformation. *Mitochondrion*, **11**, 366–367.
  39. Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009) The sequence Alignment/Map format and SAMtools. *Bioinformatics*, **25**, 2078–2079.
  40. Li, H. and Durbin, R. (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, **25**, 1754–1760.
  41. Meyer, M. and Kircher, M. (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.*, **2010**, pdb.prot5448.
  42. Korneliussen, T.S., Albrechtsen, A. and Nielsen, R. (2014) ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics*, **15**, 356.