Title: Bioarchaeological and paleogenomic profiling of the unusual Neolithic burial from Grotta di Pietra Sant’Angelo (Calabria, Italy)

Responder: Francesco Fontani

Reply: SLBDS is what you need, that is the deep sequencing of the DNA extracted from the tooth of the individual, as specified in the Methods section. There is no assembly, the raw data has been mapped to the rCRS and processed to generate a consensus sequence (via Schmutzi). All of this is standard aDNA practice. We used HaploGrep2 to assign haplogroup, double-checking variants through visual inspection in IGV, and later annotated with MITOS for uploading on GenBank

Title: An infant burial from Arma Veirana in northwestern Italy provides insights into funerary practices and female personhood in early Mesolithic Europe

Responder: Mateja Hajdinjak

Reply: The BAM files with the suffix .mtDNA.bam were used for the mitochondrial DNA sequence reconstruction (see the attached screenshot from the ENA webpage). The libraries under the IDs of A30952 and A30953 correspond to the libraries made from the specimen, whereas the A30957 and A30960 correspond to the extraction and library negative controls, respectively. We used two different approaches for reconstruction of the mtDNA sequence, you can find the information under the Materials and Methods section of the paper (<https://www.nature.com/articles/s41598-021-02804-z#Sec6>, section "Reconstruction of the mitochondrial genome and phylogenetic analysis”). One is the software schmutzi (<https://genomebiology.biomedcentral.com/articles/10.1186/s13059-015-0776-0>), and the other is relatively straightforward - taking the fragments mapped across the mtDNA reference and calling a consensus base covered by at least (minimum of) three DNA fragments and where at least 67% of the DNA fragments agree, ie have the same base. This approach was first introduced and described in <https://www.nature.com/articles/nature12788>. Both approaches result in identical mtDNA consensus sequences.

Title: Initial Upper Palaeolithic Homo sapiens from Bacho Kiro Cave, Bulgaria

Responder: Mateja Hajdinjak

Reply: The BAM files corresponding to the Bacho Kiro Cave individuals are deposited in the ENA under the study ID PRJEB35466 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB35466>), and as it is detailed in the data availability statement of the paper: <https://www.nature.com/articles/s41586-020-2259-z#data-availability>. As for Arma Veirana, you can find the details on the mitochondrial sequence reconstruction in the supplementary information of the paper, page 23: <https://static-content.springer.com/esm/art%3A10.1038%2Fs41586-020-2259-z/MediaObjects/41586_2020_2259_MOESM1_ESM.pdf> For the information on which library IDs, ie which BAM files in ENA correspond to each individual/specimen, there is a detailed table on the page 25 of the supplementary information of the manuscript. All of our manuscripts usually contain the information on the data availability with the corresponding BAM files uploaded to the ENA, and the information on which library ID is which can be retrieved from either materials and methods or the supplementary information.

Title: Biomolecular insights into North African-related ancestry, mobility and diet in eleventh-century Al-Andalus

Responder: Marina Soares Da Silva

Reply: If I understood correctly, you’re interested in the modern Iberian mitogenome dataset, is that correct? If so, these were mapped with BWA-MEM, as integrated in the old version of EAGER (not the more recent nextflow implementation of eager2), and mapped against the rCRS reference sequence (NC\_012920). The processing is detailed in the Supplementary methods (see *Modern Iberian mtDNA dataset*)*.* For the ancient mtDNA, we used BWA with specific settings for aDNA mapping (also in the supplementary methods, in *Sequence data processing*). I think we only made the consensus FASTA files available for the modern mtDNA sequences, these are the ones on GenBank (accession codes: MZ920249 - MZ921390). The ENA files correspond to ancient DNA whole-genome sequencing RAW files. If you let us know which data you’re interested in, we can retrieve the relevant files for sharing.

Title: A draft human pangenome reference

Responder: Benedict Paten

Reply: See the "Cleaning Steps" in the methods for how we went about fishing out and deduplicating the mito contigs from the hifasm assemblies. Please see Genbank accessions for links to the underlying sequencing data - it is all in there. Adding Julian in case I'm missing something.