# .janno file details

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# 1 Background

The .janno file columns are specified in the Poseidon package specification here. The following documentation includes additional background information for many of the variables. This should make it more easy to compile the necessary information for both published and unpublished data. The .pdf version of the latest version of this document is available here.

## 2 Identifiers

The Poseidon\_ID column represents each sample with an ideally world-wide unique identifier string often equal to the identifier used in the respective accompanying publication. There is no central authority to issue these identifiers, so it remains in the hand of the authors to avoid duplication. We're aware of this inconsistency and hope the aDNA community will eventually come together to establish a mechanism to ensure uniqueness of identifiers. If there are multiple samples from one individual, then they have to be clearly distinguished with relevant suffixes added to the Poseidon\_ID. Poseidon\_IDs are also employed in the genetic data files in a Poseidon package and therefore have to adhere to certain constraints.

The column Alternative\_IDs provides a way to list other IDs used for the respective individual. These might for example be names used in different publications or popular names like "Iceman", "Ötzi", "Girl of the Uchter Moor", "Tollund Man", etc.. The Relation\_\* columns described below allow to more precisely express the relationship type "identical" among samples in a Poseidon package.

The Collection\_ID column stores an additional, secondary identifier as it is often provided by collaboration partners (archaeologists, museums, collections) that provide specimen for archaeogenetic research. These identifiers might have a very heterogenous structure and may not be unique across different projects or institutions. The Collection\_ID column is therefore a free form text field.

The Group\_Name column contains one or multiple group or population names for each individual, separated by; The first entry must be identical to the one used in the genotype data for the respective sample in a Poseidon package. Assigning group and population names is a hard problem in archeogenetics (Eisenmann et al. 2018), so the .janno file allows for more than one identifier.

# 3 Relations among samples/individuals

To systematically document biological relationships uncovered among samples/individuals in one or multiple Poseidon datasets (e.g. with software like READ (Kuhn et al. 2018) or BREADR (Rohrlach et al. 2023), the .janno file can be fit with a set of columns featuring the Relation\_\* prefix. They together should be capable to encode all kinds of pairwise, biological relationships an individual might have.

Relation\_To is a string list column (so: multiple values are possible if separated by ;) that stores the Poseidon\_IDs of other samples/individuals to which the current individual has some relationship.

Relation\_Degree stores a formal description of the closeness of this relationship as measured purely from aDNA data. It is therefore also a list column that can hold the following values for each relationship:

- identical: The two samples are from the same individual or from identical twins
- first: The two individuals are closely related a first degree relationship (e.g. siblings, parent-offspring)
- second: A second degree relationship (e.g. cousins, grandparent to grandchild)
- thirdToFifth: A third to fifth degree relationship (e.g. great-grandparent to great-grandchild)
- sixthToTenth: A sixth to tenth degree relationship
- unrelated: Unrelated this is the default state among all individuals, which does not have to be expressed explicitly. This category will therefore probably never be used
- other: Any other kind of relationship not covered by the aforementioned categories

For each entry in Relation\_To there must be a corresponding entry in Relation\_Degree.

Relation\_Type allows to add more verbose details about the relationship type, if it was possible to reconstruct that from the archaeological or historical context. Because there are too many possible permutations, there is no pre-defined set of values for what can and cannot be entered here. It is advisable, though, to stick to a general scheme like the following, which describes a given relationship from the point of view of the current individual:

- father\_of: This individual is likely the father of the partner individual
- grandchild\_of: This individual is likely the grandchild of the partner individual
- mother\_or\_daughter\_of: This individual is likely either the mother or daughter of the partner individual (which might be unclear, in case of imprecise archaeological dating)
- . . .

Unlike Relation\_Degree, Relation\_Type can be left empty even if there are entries in Relation\_To. But if it is filled, then the number of values must be equal to the number of entries in both Relation\_To and Relation\_Degree.

The Relation\_Note column allows to add free-text information about the relationships of this individual. This might also include information about the method used to infer the degree and type.

# 4 Spatial position

The .janno file contains five columns to describe the spatial origin of an individual sample: Country, Location, Site and finally Latitude and Longitude.

The Country column should contain a present-day political country name following the English short name in ISO 3166.

The Country\_ISO column should contain the present-day political country of origin of the sample, expressed in codes using the standard ISO 3166-1 alpha-2, like "AR" for Argentina or "NO" for Norway.

The Location column allows for free form text entry and can contain further, unspecified location information. This might be the name of an administrative or geographic region, or an arbitrary unit of reference like a mountain, lake or city close to the point of discory of the respective sample.

The Site column should contain a site name, ideally in the latin alphabet and ideally the name that is commonly used in publications.

The Latitude and Longitude column should contain geographic coordinates (WGS84) in decimal degrees (DD) with a precision of not more than five places after the decimal point. This yields a precision of about 1.1132m at the equator which is sufficient to describe the position of an archaeological site. Coordinates in other formats like for example Degrees Minutes Seconds (DMS) or in completely different coordinate reference systems should be transformed. There exist many open source software solutions to do that, most based on the PROJ library e.g. the The World Coordinate Converter.

## 5 Temporal position

The temporal position of a sample is encoded with seven different columns in the .janno file: Date\_C14\_Labnr, Date\_C14\_Uncal\_BP, Date\_C14\_Uncal\_BP\_Err, Date\_BC\_AD\_Median, Date\_BC\_AD\_Start, Date\_BC\_AD\_Stop, Date\_Type

#### 5.1 General structure

The Date\_Type column handles the general distinction between the most common forms of age information:

- modern: Applies to present day reference samples, so not ancient DNA.
- C14: Applies if there is a set of radiocarbon dates explicitly listed in the columns Date\_C14\_Labnr, Date\_C14\_Uncal\_BP and Date\_C14\_Uncal\_BP\_Err whose post-calibration probability distribution is a meaningful prior for the individual's year of death. The dates do not always have to be directly from the individual's tissue, but they should be immediately relevant for their year of death (e.g. a date from a grain kernel recovered from the individual's grave).
- contextual: Applies in all other cases if the columns Date\_BC\_AD\_Median, Date\_BC\_AD\_Start, Date\_BC\_AD\_Stop can be filled. This includes age attribution based on the archaeologically determined stratigraphy or typological information. contextual should also be chosen if the sample is dated very indirectly with radiocarbon dating (e.g. radiocarbon dates from other, unrelated features of the same site) or dated with other physical or chemical dating methods (e.g. dendrochronology or optically stimulated luminescence).

So Date\_C14\_Labnr, Date\_C14\_Uncal\_BP and Date\_C14\_Uncal\_BP\_Err only go along with Date\_Type = C14, whereas Date\_BC\_AD\_Median, Date\_BC\_AD\_Start, Date\_BC\_AD\_Stop complement both Date\_Type = C14 and Date\_Type = contextual. Radiocarbon dates that only serve as secondary evidence for a contextual dating should not be reported in Date\_C14\_Labnr, Date\_C14\_Uncal\_BP and Date\_C14\_Uncal\_BP\_Err.

#### 5.2 The columns in detail

Each radiocarbon date has a unique identifier: the "lab number". It consists of a lab code issued by the journal Radiocarbon for each laboratory and a serial number. This lab number makes the date well identifiable and should be reported in Date\_C14\_Labnr with the lab code separated from the serial number with a minus symbol.

The uncalibrated radiocarbon measurement can be described by a Gaussian distribution with mean and standard deviation. So the column Date\_C14\_Uncal\_BP holds the mean of that distribution in years before present (BP) as usually reported by radiocarbon laboratories. The age is always a positive integer value

starting from a zero that corresponds to 1950 AD. The column Date\_C14\_Uncal\_BP\_Err holds the respective standard deviation for each date in years. This should be the 1-sigma distance, so that the probability that the actual uncalibrated age of the measured sample is within the Date\_C14\_Uncal\_BP\_Err range is about 68%.

Date\_C14\_Labnr, Date\_C14\_Uncal\_BP and Date\_C14\_Uncal\_BP\_Err each can hold multiple values separated by ; to allow for multiple radiocarbon dates for each aDNA sample. With multiple values the number and order of values in the columns must be equal.

In the columns Date\_BC\_AD\_Median, Date\_BC\_AD\_Start, Date\_BC\_AD\_Stop ages are reported in years BC and AD, so in relation to the zero point of the Gregorian calender. BC dates are represented with negative, AD with positive integer values.

- If radiocarbon dates are available (Date\_Type = C14): Date\_BC\_AD\_Median should report the median age after calibration. With multiple dates this can be determined either with sum calibration or more complex (e.g. bayesian) age modelling. Date\_BC\_AD\_Start and Date\_BC\_AD\_Stop should report the starting/ending age of a 95% probability window around the age median.
- If only contextual (e.g. from archaeological typology) age information is available (Date\_Type = contextual): Date\_BC\_AD\_Start and Date\_BC\_AD\_Stop should simply report the approximate starting and end date determined by the respective source of scientific authority (e.g. an archaeologist knowledgable about the relevant typological sequences). In this case Date\_BC\_AD\_Median should be calculated as the mean of Date\_BC\_AD\_Start and Date\_BC\_AD\_Stop rounded to an integer value.
- If the sample is a modern reference sample (Date\_Type = modern): Date\_BC\_AD\_Median, Date\_BC\_AD\_Start, Date\_BC\_AD\_Stop should all be set to the value 2000, for 2000AD.

The column Date\_Note allows to add arbitrary free-text information about the dating of a sample.

## 6 Genetic summary data

## 6.1 Individual properties

The Genetic\_Sex column should encode the biological sex as determined from the DNA read distribution on the X and Y chromosome. It only allows for the entries

- F: female
- M: male
- U: unknown

This limitation stems from the genotype data formats by Plink and the Eigensoft software package. Edge cases (e.g. XXY, XYY, X0, ...) can not be expressed with this format and should be reported as U with an additional comment in the free text Note field. Genetic sex determination for ancient DNA can be performed for example with Sex.DetERRmine (Lamnidis et al. 2018).

The MT\_Haplogroup column is meant to store the human mitochondrial DNA haplogroup for the respective individual in a simple string. The entry can be arbitrarily precise. A software tool to determine the MT haplogroup is for example Haplogrep (Schönherr et al. 2023).

The Y\_Haplogroup column holds the respective human Y-chromosome DNA haplogroup in a simple string. The notation should follow a syntax with the main branch + the most terminal derived Y-SNP separated with a minus symbol (e.g. R1b-P312).

#### 6.2 Library properties

The Source\_Tissue column documents the skeletal, soft tissue or other elements from which source material for DNA library preparation have been extracted. If multiple libraries have been taken from different elements, these can be listed separated by ;. Specific bone names should be reported with an underscore (e.g. bone\_phalanx, tooth\_molar).

The Nr\_Libraries column holds a simple integer value of the number of libraries that have been prepared for an individual.

The Capture\_Type column specifies the general pre-sequencing preparation methods that have been applied to the library. See Knapp/Hofreiter 2010 for a review of the different techniques (not including younger developments). This field can hold one of multiple different values, but also multiple of these separated by; if different methods have been applied for different libraries.

- Shotgun: Sequencing without any enrichment (whole genome sequencing, screening etc.)
- 1240k: Target enrichment with hybridization capture optimised for sequences covering the 1240k SNP array (Fu et al. 2015, Haak et al. 2015, Mathieson et al. 2015)
- ArborComplete, ArborPrimePlus, ArborAncestralPlus: Target enrichment with hybridization capture as provided by Arbor Biosciences in three different kits branded myBaits Expert Human Affinities
- TwistAncientDNA: Target enrichment with hybridization capture as provided by Twist Bioscience (Rohland et al. 2018)
- OtherCapture: Target enrichment with hybridization capture for any other set of sequences
- ReferenceGenome: Modern reference genomes where aDNA fragmentation is not an issue and other sample preparation techniques apply

The UDG column documents if the libraries for the respective individual went through UDG (USER enzyme) treatment. This wet lab protocol step removes molecular damage in the form of deaminated cytosines characteristic of ancient DNA.

- minus: A protocol without UDG treatment (e.g. Aaron/Neumann/Brandt et al. 2020a)
- half: A protocol with UDG-half treatment (e.g. Aaron/Neumann/Brandt et al. 2020b)
- plus: A protocol with UDG-full treatment (e.g. Aaron/Neumann/Brandt et al. 2020c)
- mixed: Multiple later merged libraries went through different UDG treatment approaches

The Library\_Names column should contain the names for the library as used in the publication.

The Library\_Built column describes the library preparation method regarding single- or double-stranded protocols. See e.g. Gansauge/Meyer 2013 for more information.

- ds: Double-stranded library preparation
- ss: Single-stranded library preparation
- mixed: If multiple libraries with different strandedness were used. See also the Sequencing Source File in the Poseidon package as a way to provide details.

The Genotype\_Ploidy column stores a characteristic of the aDNA data treatment. Humans have two complete sets of chromosomes in their cells and hence are diploid organisms. For many computational aDNA applications it is more practical, though, to work with pseudo-haploid data, so data were only one read per position is selected by a random sampling process.

- diploid: No random read selection
- haploid: Random read selection to produce pseudo-haploid data

The column Data\_Preparation\_Pipeline\_URL should finally store an URL that links to a complete and human-readable description of the computational pipeline (for example a specific configuration for nf-core/eager Fellows Yates et al. 2021) by which the sample data was processed.

### 6.3 Data yield

The Endogenous column holds the percentage of mapped reads over the total amount of reads that went into the mapping pipeline. That boils down to the DNA percentage of the library that matches the (human) reference. It should be determined from Shotgun libraries (so before any hybridization capture), not on target and without any quality filtering. In case of multiple libraries only the highest value should be reported. The % endogenous DNA can be calculated for example with the endorS.py script.

The Nr SNPs column gives the number of SNPs reported in the genotype data files for this individual.

The Coverage\_on\_Target\_SNPs column reports the mean SNP coverage on the target SNP array (e.g. 1240K) for the merged libraries of this sample. To calculate the coverage it is necessary to determine which SNPs are covered how many times by the mapped reads. Individual SNPs might be covered multiple times, whereas others may not be covered at all by the highly deteriorated ancient DNA. The coverage for each SNP is therefore a number between 0 and n. The statistic can be determined for example with the QualiMap (Okonechnikov/Conesa/García-Alcalde 2016) software package. In case of multiple libraries, the coverage can be given as a mean across all of them.

### 6.4 Data quality

The Damage column contains the % damage on the first position of the 5' end for the main Shotgun library used for sequencing or capture. This is an important statistic to verify the age of ancient DNA. In case of multiple libraries you should report a value from the merged read alignment.

#### 6.4.1 Contamination

Contamination of ancient DNA with foreign reads is a major challenge for archaeogenetics. There exist multiple competing ideas, algorithms and software tools to estimate the degree of contamination for individual samples (e.g. ANGSD (Korneliussen/Albrechtsen/Nielsen 2014), contamLD (Nakatsuka et al. 2020) or hapCon (Huang/Ringbauer 2022)), with some methods only applicable under certain circumstances (e.g. popular X-chromosome based approaches only work on male individuals). Also the results of different methods tend to differ both in the degree of contamination they estimate and in the way the output is usually encoded. To cover the multitude of methods in this domain, and to make the results representable in the .janno file, we offer the Contamination\_\* column family.

Contamination is a list column to represent the different contamination values estimated for a sample with one or multiple software tools. As usual multiple values are separated by ;.

Contamination\_Err is another list column to store the respective (standard) error term for the values in Contamination.

Some tools for contamination estimation do not return a mean plus a standard error. ContamMix, for example, yields a 95% confidence interval instead, to better represent assymetric output distributions. Contamination and Contamination\_Err can not represent this. We suggest to derive a mean and a standard error from these alternative outputs. The latter can be calculated as the largest distance from the mean to the limits of the confidence interval.

Contamination\_Meas finally is the third necessary list column, which contextualizes the values in Contamination and Contamination\_Err. Each measure in these columns has to be accompanied by the software and software version used to calculate it. The individual entries might e.g. look like this:

- ANGSD v0.935
- hapCon v0.4a1
- custom script

This setup has the consequence that the columns Contamination, Contamination\_Err, Contamination\_Meas always have to have the same number of ;-separated values.

The Contamination\_Note column is a free text field to add additional information about the contamination estimates, e.g. which parameters where used with the respective software tools.

#### 7 Context information

The Genetic\_Source\_Accession\_IDs column was introduced to link the derived genotype data in Poseidon with the raw sequencing data typically uploaded to archives like the ENA (Burgin 2022) or SRA (Katz 2021). There projects or even individual samples are given clear identifiers: Accession IDs. This janno column is supposed to store one or multiple of these Accessions IDs for each individual/sample in Poseidon. If multiple

are entered, then they should be arranged by descending specificity from left to right (e.g. project id > sample id > sequencing run id).

The Primary\_Contact column is a free form text field that stores the name of the main or the corresponding author of the respective paper for published data.

The Publication column holds either the value unpublished for (yet) unpublished samples or – for published data – one or multiple citation-keys of the form Author Journal Year without any spaces or special characters. These keys have to be identical to the Bib TeX citation-keys identifying the respective entries in the .bib file of the package. Bib TeX is a file format to store bibliographic information, where each entry (article, book, website, ...) is defined by a series of parameters (authors, year of publication, journal, ...). Here's an example .bib file with two entries for Cassidy et al. 2015 and Feldman et al. 2019:

```
@article{CassidyPNAS2015,
    doi = {10.1073/pnas.1518445113},
    url = {https://doi.org/10.1073%2Fpnas.1518445113},
    year = 2015,
    month = {dec},
    publisher = {Proceedings of the National Academy of Sciences},
    volume = {113},
    number = \{2\},
    pages = \{368--373\},
    author = {Lara M. Cassidy and Rui Martiniano and Eileen M. Murphy and
              Matthew D. Teasdale and James Mallory and Barrie Hartwell
              and Daniel G. Bradley},
    title = {Neolithic and Bronze Age migration to Ireland and establishment
             of the insular Atlantic genome},
    journal = {Proceedings of the National Academy of Sciences}
}
@article{FeldmanScienceAdvances2019,
    doi = {10.1126/sciadv.aax0061},
    url = {https://doi.org/10.1126%2Fsciadv.aax0061},
    year = 2019,
    month = {jul},
    publisher = {American Association for the Advancement of Science ({AAAS})},
    volume = \{5\},
    number = \{7\},
    pages = \{eaax0061\},
    author = {Michal Feldman and Daniel M. Master and Raffaela A. Bianco and
              Marta Burri and Philipp W. Stockhammer and Alissa Mittnik and
              Adam J. Aja and Choongwon Jeong and Johannes Krause},
    title = {Ancient {DNA} sheds light on the genetic origins of early Iron Age
             Philistines},
    journal = {Science Advances}
}
```

The string CassidyPNAS2015 is the citation-key of the first entry. To cite both publications in the Publication column, one would enter CassidyPNAS2015;FeldmanScienceAdvances2019.

When creating a new Poseidon package the .bib file should be filled together with the Publication column. One of the most simple ways to obtain the BibTeX entries may be to request them with the doi from the doi2bib wep app. It could be necessary to adjust the result manually, though. The citation-key, for example, has to be replaced by the one used in the Publication column.

The Note column is a free form text field that can contain small amounts of additional information that is not yet expressed in a more systematic form in the the other .janno file columns.

The Keywords column was introduced to allow for tagging individuals with arbitrary keywords. This should simplify sorting and filtering in personal Poseidon package repositories. Each keyword is a string and multiple keywords can be separated with ;.