

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. [For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

▶ Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to determine ancient DNA sample size a priori; all ancient samples that provided authentic DNA were included. Modern DNA sampling focused on multiple locations in the island of Malakula, where much of the ancient material originated, and voluntary participants provided island-wide coverage for population genetic analyses.

2. Data exclusions

Describe any data exclusions.

aDNA samples were variously excluded from different analyses on the basis of contamination estimates or sequence coverage, as detailed in the Results and Methods sections.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

The established wet and dry lab methodological pipeline, including the use of publicly available software, is clearly described in the Methods (and Results) section. Each sample is analyzed for up to 1,24 Million markers across the human genome that represent an internal replication of the findings.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

n/a, no randomization was necessary.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

n/a, no blinding was necessary.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- A statement indicating how many times each experiment was replicated
- The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- Test values indicating whether an effect is present
Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
- Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

All software used in this study are publicly available and referenced in the main and methods text:

```
bcl2fastq v2.17.1.14
dnaclust v3.0.0
EAGER v1.92.44
AdapterRemoval v2
BWA
samtools
pmdtools v0.60
pileupCaller
DamageProfiler
ANGSD
schmutzi
smartpca v13050
ADMIXTURE v1.3.0
qpDstats v711
qpWave v400
qpAdm v610
qpGraph v5211
ALDER
CircularMapper
HaploGrep2
R
Python
```

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

n/a, no unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

n/a, no antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

n/a, no eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

n/a, no eukaryotic cell lines were used

c. Report whether the cell lines were tested for mycoplasma contamination.

n/a, no eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

n/a, no commonly misidentified cell lines were used.

► **Animals and human research participants**Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

n/a, no animals were used.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Modern DNA data was generated from saliva samples collected from adult participants in 5 communities across the islands of Malakula and Efate in Vanuatu. All participants gave informed consent. All fieldwork was carried out in accordance with the research permissions obtained from the Vanuatu Kaljoral Senta and the ethical approval granted by the Ethik-Kommission der Friedrich-Schiller-Universität in Jena, Germany.