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USER II treatment of ancient DNA extracts V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Enzymatic removal of uracil bases from ancient DNA fragments. "*Antarctic Thermolabile Uracil DNA glycosylase (UDG) catalyzes the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease III breaks the phosphodiester backbone at the 3′and 5′ sides of the abasic site. In addition to generating a different a 3′-terminus than USER Enzyme (a 3′-phospho-α, β-unsaturated aldehyde versus the 3′phosphate left by USER Enzyme, **NEB #M5505**), Thermolabile USER II Enzyme (**NEB #M5508**) can also be completely heat inactivated after 10 minutes at 65°C.*" - https://www.neb.com/en/products/m5508-thermolabile-user-ii-enzyme?srsltid=AfmBOooXGOM1bvBPvVpj3P3CCKKOiRKUrQ-PfReg2m_cax4zrM3j8pN4.

Protocol materials

- Thermolabile USER II Enzyme - 250 units **New England Biolabs Catalog #**M5508L
- Buffer PE **Qiagen Catalog #**19065
- Buffer EB **Qiagen Catalog #**19086
- 1.5 mL LoBind tubes **Eppendorf Catalog #**022431021
- Monarch DNA Cleanup Columns (5ug) - 100 columns **New England Biolabs Catalog #**T1034L

Before start

- UV-treat buffers (modified Buffer PB, Buffer PE, and Buffer EB/EBT) before starting for 10 min in a UV box.
- Do not UV Eppendorf/PCR tubes.
- PE buffer: Add Molecular Grade Ethanol as indicated on the bottle before use.
- Clean all surfaces with 5% bleach solution followed by 70% ethanol before and after use.
- Clean all equipment with 70% ethanol before and after use.

Buffer preparation

1 See following protocol for buffer preparation protocols.

Protocol

NAME

Ancient DNA Extraction from Bones and Teeth

CREATED BY

Vanssy Li

Preview

Required buffers for this protocol:

- Buffer PE
- Modified Buffer PB
- Buffer EB - if using LoBind tubes
- Buffer EBT - if not using LoBind tubes (Buffer EB + Tween-20)

Incubation with USER II

2 In a PCR tube, add 4.8 µL

Thermolabile USER II Enzyme - 250 units **New England Biolabs Catalog #**M5508L

to 27.2 µL DNA extract, for a final reaction volume of 32 µL.

2.1 Optional: Or add 2.4 µL USER II enzyme to 13.6 µL DNA extract, for a final reaction volume of 16 µL, if you want to keep some of the DNA extract as untreated.

3 In a thermocycler, incubate the reaction for 3 hours at 37°C then cool down to 10°C. Can be left overnight at 10°C.

Clean-up

4 Clean up using

Monarch DNA Cleanup Columns (5ug) - 100 columns **New England Biolabs Catalog #**T1034L

Note

- Do not touch the film of the spin column when adding liquids.
- Pay attention when adding small volume of liquid. Make sure the liquid do not attach on the wall.
- As of 31 December 2024 the "Monarch DNA Cleanup Columns (5ug) New England Biolabs Catalog #T1024L" have been discontinued and replaced by "Monarch Spin Columns S1A and Tubes New England Biolabs Catalog #T2037L".

4.1 Add 450 µl modified Buffer PB to the spin column.

4.2 Add the entire USER II-DNA reaction (32 or 16 µL) and mix by pipetting or inversion.

4.3 Centrifuge at 8,000 RPM in a benchtop centrifuge for 1 min, discard flow through.

4.4 Add 650 µl Buffer PE **Qiagen Catalog #**19065 to the spin column.

4.5 Centrifuge at 8,000 RPM for 1 min, discard flow through.

4.6 Centrifuge at max speed for 1 min to dry the column.

4.7 Place the spin column in a fresh 1.5 mL Eppendorf tube. Use 1.5 mL LoBind tubes **Eppendorf Catalog #**022431021, if available, but if not available, then use EBT buffer instead of EB buffer for the final elution (Step 4.8).

4.8 Add 20 µL Buffer EB **Qiagen Catalog #**19086 to the spin column. If not using LoBind tubes, add 20 uL EBT buffer instead.

4.9 Incubate for 5 mins at room temperature to allow the DNA to elute from the column and dissolve in the buffer.

4.10 Centrifuge at max speed for 1 min.

4.11 Repeat Steps 4.8-4.10.

4.12 Discard the spin column and store the eluted DNA at -20ºC.

Protocol references

Hofreiter, M., Jaenicke, V., Serre, D., Haeseler, A. v., & Pääbo, S. (2001). DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Research, 29(23), 4793-4799. https://doi.org/10.1093/nar/29.23.4793