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Proteinase K solution for ancient DNA extraction

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Anna Schmidt¹, Sarah Nagel¹

¹Max Planck Institute for Evolutionary Anthropology

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Abstract

This document describes the preparation of proteinase K solution for ancient DNA extraction from bones, teeth, sediments and other materials, following the method described by Dabney et al. 2013 and refined by Rohland et al. 2018. Proteinase K is added to the extraction buffer stock solution (see separate document) on the day of DNA extraction.

References

- Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., & Meyer, M. (2013). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences of the United States of America*, 110(39), 15758-15763.
- Rohland, N., Glocke, I., Ayinuer-Petri, A., & Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nature Protocols*, 13, 2447-2461.

Note

This protocol describes the preparation of up to 50 ml Proteinase K solution (10 mg/ml) in units of 10 ml.

Materials

	Reagent//consumable	Supplier	Catalogue number
	Reagents		
	Proteinase K, recombinant, PCR grade, lyophilised, 100 mg	Sigma Aldrich	3115836001
	TE buffer (decontaminated)*\$	self	
	Consumables		
	50 ml Falcon tube with stand	Corning	210261
	1.5 ml LoBind Tubes (decontaminated)**	Eppendorf	VB-0285
	25 ml serological pipette	Corning BV	357525
	10 ml serological pipette	Corning BV	357551
	5 ml serological pipette	Corning BV	357543

*See document in the Appendix for preparation of TE buffer.

\$ See document in the Appendix for decontamination of reagents and buffers.

** See document in the Appendix for decontamination of material.

Safety information

[CAUTION: Proteinase K]

Proteinase K causes skin and eye irritation, respiratory irritation and may cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust/fume/gas/vapours/spray and always wear protective gloves and eye protection when working with this chemical.

Always refer to the Material Safety Data Sheet attached (MSDS) for detailed information.



Equipment

- Serological pipette controller (e.g. battery-powered pipetting aid for glass pipettes, cat. no. TC16.1)

Protocol

1. Add 4.5 ml TE buffer to a vial containing 100 mg Proteinase K. Mix thoroughly by vortexing, then let the solution sit for 10 minutes to ensure the powder dissolves completely.
2. Transfer complete volume to a fresh 50 ml Falcon tube, add 5.5 ml TE buffer and mix thoroughly.
3. Prepare 1.0 ml aliquots in 1.5 ml LoBind tubes and store them in the freezer until use. Shelf life is at least 1 year from preparation.

Note

[Documentation]

Note the lot numbers and the date and initials written on the reagents used for the preparation in Labfolder (orange fields).

Note

[Labeling]

Label the tubes with "Proteinase K, 10 mg/ml", date and the initials of the person who dissolved the enzyme.

Appendix



Document

NAME

TE buffer

CREATED BY

Anna Schmidt

Preview

Document

NAME

UV decontamination of materials

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Elena Essel

Preview

Document

NAME

UV decontamination of reagents/buffers

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Elena Essel

Preview



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