



Dec 02, 2024

Neutralisation acid etching of enamel for proteomic amelogenin sex estimation

DOI

<https://dx.doi.org/10.17504/protocols.io.14egn6qr6l5d/v1>

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Protocol Citation: Charlotte Blacka 2024. Neutralisation acid etching of enamel for proteomic amelogenin sex estimation. [protocols.io https://dx.doi.org/10.17504/protocols.io.14egn6qr6l5d/v1](https://dx.doi.org/10.17504/protocols.io.14egn6qr6l5d/v1)

Manuscript citation:

Blacka, C, Dowle, A, Lisowski, M, Alexander, M, Hendy, J, Penkman, K and Mosely, J. 2024. "Rapid Amelogenin Sex Estimation of Human and Cattle Remains Using Untargeted Evosep-timsTOF Mass Spectrometry." *Authorea Inc.* <https://doi.org/10.22541/au.172599306.61984507/v1>.



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Protocol status: Working

We use this protocol and it's working

Created: September 20, 2024

Last Modified: December 02, 2024

Protocol Integer ID: 108103

Keywords: Amelogenin, Palaeoproteomics, Proteomics, Acid Etching, Enamel, Sex Estimation, Sex Determination, enamel for proteomic amelogenin sex estimation minimally, proteomic amelogenin sex estimation minimally, rapid sampling of enamel protein, enamel protein, sex estimation via mass spectrometric analysis, dimorphic amelogenin protein, etching method, sex estimation, acid etch, enamel, neutralisation acid, mass spectrometric analysis

Funders Acknowledgements:

Natural Environment Research Council

Grant ID: NE/S00713X/1

Abstract

Minimally invasive and rapid sampling of enamel proteins for sex estimation via mass spectrometric analysis of sexually dimorphic amelogenin proteins is becoming increasingly important in bioarchaeological studies. Here, an acid etching method followed by neutralisation by base addition to the acid etch, and subsequent Evotip clean-up, is outlined.

Materials

(Optional) 3% H₂O₂

5% HCl

5% NH₄OH

0.2 mL PCR tube

LoBind tubes

Evotips



Acid Etch Preparation

- 1 If tooth is visibly dirty, it can be rinsed briefly with 3% H_2O_2 (v/v) in water. *
- 2 If there is no exposed enamel to sample, the tooth can be drilled, sanded or etched with 5% HCl for a few minutes to expose enamel for acid etch sampling. *
- 3 Pipette 60 μL of 5% HCl into the cap of a 0.2 mL PCR tube, forming a convex meniscus.
- 4 Place the exposed enamel of the tooth in contact with the 5% HCl (v/v) in water and maintain contact for 2 minutes. 2m
- 5 Close the cap of the 0.2 mL PCR tube to transfer the 5% HCl (v/v) in water into the tube, and pipette 45 μL of 5% NH_4OH (v/v) in water into the PCR tube. A white precipitate should form upon the addition of the NH_4OH .
- 6 Vortex/mix the sample for ~5 seconds. 5s
- 7 Centrifuge the sample at 13,250 rcf for 3 minutes. 3m
- 8 Transfer the supernatant to a 0.5 mL LoBind tube and store in the freezer until Evtip clean-up.

Evtip Clean-Up

6m 30s

- 9 Wash dry Evtips with 20 μL of solvent B (acetonitrile containing 0.1% (v/v) formic acid) and centrifuge for 60 seconds at 800 rcf. 1m
- 10 Soak the Evtips in 100 μL of 1-propanol until the tips are pale white (approximately 20 seconds). 20s
- 11 Equilibrate the Evtips with 20 μL of solvent A (aqueous 0.1% (v/v) formic acid) and centrifuge for 60 seconds at 800 rcf. 1m



- 12 Transfer the full acid etch supernatant to the Evotip and centrifuge for 60 seconds at 800 rcf. 1m
- 13 Wash the tips with 50 μ L of solvent A and centrifuge for 60 seconds at 800 rcf. 1m
- 14 Repeat step 13 twice more. 2m
- 15 Transfer 100 μ L of solvent A to the tips and centrifuge for 10 seconds at 800 rcf, ensuring the tips stay wet. 10s
- 16 Load the Evotips onto the Evosep LC.

Protocol references

Blacka, C, Dowle, A, Lisowski, M, Alexander, M, Hendy, J, Penkman, K and Mosely, J. 2024. "Rapid Amelogenin Sex Estimation of Human and Cattle Remains Using Untargeted Evosep-timsTOF Mass Spectrometry." *Authorea Inc.* <https://doi.org/10.22541/au.172599306.61984507/v1>.

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