

Jul 09, 2024

Chromatographic separation of strontium in archaeological cremated remains for Thermal Ionisation Mass Spectrometry (TIMS) analysis

DOI

dx.doi.org/10.17504/protocols.io.j8nlk8d5xl5r/v1

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Protocol Citation: Maura De Coster, Lisette M. Kootker 2024. Chromatographic separation of strontium in archaeological cremated remains for Thermal Ionisation Mass Spectrometry (TIMS) analysis. [protocols.io](#)

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Protocol status: Working

We use this protocol and it is working

Created: June 18, 2024

Last Modified: July 09, 2024

Protocol Integer ID: 101997

Keywords: archaeology, strontium, isotope, TIMS, cremations, calcined bones

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Abstract

This protocol describes in great detail all the steps that must be taken for strontium isotope analysis on archaeological cremated remains, from the receipt of the samples to the deposition of the data generated by Thermal Ionisation Mass Spectrometry (TIMS).

Image Attribution

All images by Lisette M. Kootker

Protocol materials

 Acetic acid (glacial 100%, Suprapur for trace analysis, Supelco) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1.00066.1000**

Step 1

Safety warnings



HNO₃ safety sheet



null.pdf 91KB

HCl safety sheet



HYDROCHLORIC-ACID-NF-FCC-21... 65KB

Cleaning and leaching

- 1 Mechanically clean the cremated bone samples using a handheld drill (e.g., PROXXON or Dremel) to remove the outer layer of the bone, so all soil residue is removed. If present, also remove all of the trabecular bone (but see De Coster et al. 2024 paragraph 4.1).

33m

Next, following the protocol described in Snoeck et al., 2015,2018 transfer the cleaned bone fragments to glass vials and leach them with circa  1.0 mL (10:1 ratio)  1 Molarity (M)

 Acetic acid (glacial 100%, Suprapur for trace analysis, Supelco) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1.00066.1000**

ultrasonically for  00:03:00 -  00:10:00, followed by two Milli-Q™ rinses, and a

 00:10:00 Milli-Q™ ultrasonic wash. Repeat these steps until the Milli-Q™ in the glass vials are clear or show a white cloudy color. Dry the samples on a hotplate at  50 °C

 Overnight .



Colored liquid indicates the presence of contaminating materials. The process is repeated using Milli-Q water until the liquid is clear. Photo: L.M. Kootker

CITATION

Maura De Coster, Saskia Ammer, Tim Laning, Lisette M. Kootker (2024). The Relevance of Sr–O–C Isotope Analysis on Burnt Human Skeletal Remains in Archeological and Forensic Contexts: A Review and Future Directions. WIRE Forensic Science.

LINK

<https://doi.org/10.1002/wfs.2.1524>

CITATION

Snoeck C, Lee-Thorp J, Schulting R, de Jong J, Debouge W, Mattielli N (2015). Calcined bone provides a reliable substrate for strontium isotope ratios as shown by an enrichment experiment..

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<https://doi.org/10.1038/s41598-018-28969-8>

- 1.1 If *Pars petrosae* were sampled, cut them midmodiolarly using a **Buehler IsoMet 1000 precision saw** (Veselka et al. 2021). Locate the otic capsule and subsample in acid-cleaned Eppendorf® tubes approximately  10 mg of bone powder with a handheld drill (e.g.,

PROXXON or Dremel) and transfer the samples to a clean laboratory. Here, follow the protocol described in step 1.

CITATION

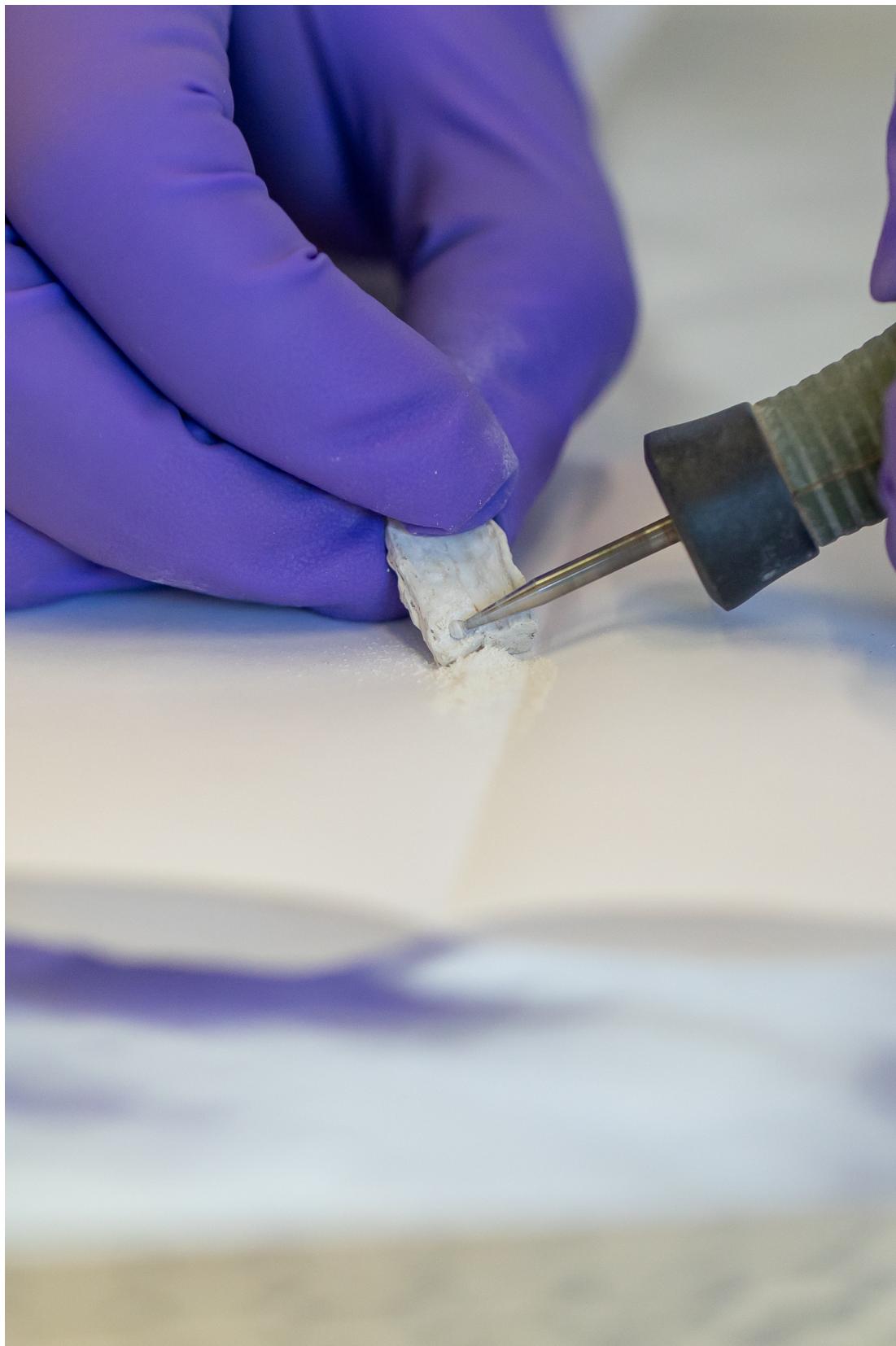
Veselka B, Locher H, de Groot JCMJ, Davies GR, Snoeck C, Kootker LM (2021). Strontium isotope ratios related to childhood mobility: Revisiting sampling strategies of the calcined human pars petrosa ossis temporalis..

LINK

<https://doi.org/10.1002/rcm.9038>

Subsampling

- 2 After drying the samples on the hotplates the samples, subsample ca.  10-20 mg using a handheld drill (e.g., PROXXON or Dremel) equipped with a diamond tipped ball burr. The bone should be fully white after the mechanical cleaning. Any grey or black bones are not suitable for Sr isotope analysis. Therefore, exclude the discolored samples from further analysis. However, if only parts of the bone are not fully calcined/white, specifically target the white surfaces.



After the samples have been dried on a hotplate or in an oven, the sample is powdered. Include only fully white bone for analysis. Sample around any discolored surfaces. Photo: L.M. Kootker

Sample dissolution

- 3 Weigh 2-10 mg of cremated bone powder into an acid-cleaned Eppendorf® centrifuge tube. Note down the weights in gram. Next, add $600 \mu\text{L}$ of pro-analysis quality 3M HNO_3 . Bone powder will dissolve within seconds in Room temperature . If small fragments of bone are sampled, place the Eppendorf® tube in an ultrasonic bath for ca. $00:10:00$ to allow all enamel to dissolve. Next, centrifuge the samples for $00:03:00$ at $12000 \text{ rpm, Room temperature}$.

13m

Chromatographic separation

- 4 Separate Sr from the matrix using in-house made Sr columns made from 1.5 ml pipette tips and using a 3.5 mm PE frit (Angst and Pfister, $h = 2 \text{ mm}$, porosity $35 \mu\text{m}$).

The columns are stored in pipette tip boxes in $\pm 3\text{M HCl}$. Take out a column with a plastic tweezer, tap the HCL out of the pipette tip and rinse 3 times with Milli-Q. Place the column in the rack and carefully fill with Milli-Q. Add $80 \mu\text{L}$ Sr resin ($120 \mu\text{L}$ in slurry (0.2M HNO_3 , Eichrom Technologies, 100–150 μm mesh).



In-house made column filled with 80 µL Sr resin, Vrije Universiteit Amsterdam. Photo: L.M. Kootker

Clean the columns using the following steps:

1 CV (column volume) 3M HNO₃

1 CV Milli-Q

1 CV 3M HNO₃

1 CV Milli-Q

Condition the columns by adding  500 µL 3M HNO₃.

Next, load  500 µL of sample. The remaining 100 µL can be used for concentration measurements. Use a new, acid-cleaned pipette tip for every sample (see 4.2). Once the samples dripped through the columns, wash the samples twice with  900 µL 3M HNO₃. Replace the waste beakers containing the pre-fraction with acid-cleaned 5 or 7 ml PFA (Savillex) beakers (see 4.1). To elute the Sr, wash with  900 µL Milli-Q. Add 0.07 to 0.11 gram (1-3 drops) of ⁸⁴Sr spike to the blank(s). Add 1 drop 0.5% H₃PO₄ to the samples (and blanks and in-house standard if applicable).

Close the beakers and transfer them to a hotplate. Place the beakers and the caps on a hotplate at $\text{120 } ^\circ\text{C}$ overnight. Once dry, nitrate with 4-6 drops of 14M (concentrated) HNO_3 . Dry the samples (and blanks and in-house standards if applicable) at $\text{120 } ^\circ\text{C}$.

- 4.1 Cleaning Teflon® PFA (high-purity Perfluoroalkoxy resin) laboratory equipment: sub-boil in pro-analysis quality 3M HNO_3 and 6-7M HCl for 2 hours each in a fume cupboard. Rinse 3 times with Milli-Q between the baths. Add ca. 3 ml 6-7M HCl, close the caps and leave on a hotplate at $\text{120 } ^\circ\text{C}$ for 2-5 days. Discard the acid, rinse 2 times with Milli-Q and store in a clean box.
- 4.2 Cleaning pipette tips: Fill a pipette box with pipette tips, leaving one slot empty. Fill the box with 500 ml of approximately 3M HCl and let it stand for about 5-10 days at Room temperature . Remove the acid and rinse three times with Milli-Q. Place on a hotplate at $\text{60 } ^\circ\text{C}$ to dry.

TIMS preparation

- 5 Add $2 \mu\text{L}$ 10% HNO_3 to the dried samples. Place the outgassed single annealed rhenium filaments in the dedicated holders and increase the current to 1.5 mA. Create little 'dams' with parafilm. Reduce the current to 0 mA. Pipette $2 \mu\text{L}$ of TaCl_5 and $1 \mu\text{L}$ of sample (50%, to allow for a rerun if needed) between the parafilm dams on the filament. Dry slowly at 1.0 mA. Once dry, gradually increase the current to 1.3 mA until the samples turn black, then to 1.6 mA to burn away the parafilm. Increase further to approximately 1.8-2.0 mA until the samples glow. Once they are bright red, immediately reduce the current to 0 mA and load the sample onto the turret.

Use a new, acid-cleaned pipette tip (see 4.2) for every sample.

Upload data

- 6 Upload the $^{87}\text{Sr}/^{86}\text{Sr}$ data as a dataset on IsoArch.eu. IsoArch is an open and collaborative database of georeferenced isotopic measures of bioarcheological samples from all time periods and all around the world. The IsoArch initiative supports the CARE principles. In parallel, IsoArch has adopted the FAIR practices to ensure that datasets are readily discoverable and compatible within the IsoArch database.

Dataset

IsoArch

isoarch.eu

NAME

LINK

CITATION

Plomp E, Stantis C, James HF, Cheung C, Snoeck C, Kootker L, Kharobi A, Borges C, Moreiras Reynaga DK, Pospieszny Ł, Fulminante F, Stevens R, Alaica AK, Becker A, de Rochefort X, Salesse K (2022). The IsoArch initiative: Working towards an open and collaborative isotope data culture in bioarchaeology..

LINK

<https://doi.org/10.1016/j.dib.2022.108595>

Citations

Step 1

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Step 6

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