# As solid as a rock – the petrous bone as a source of DNA for the comparison of CE- and MPS-based forensic identification of challenging cranial bones

**Galina Kulstein[1], Thorsten Hadrys[2], Keith Elliott[3], Miro Vranes[4] and Peter Wiegand[1]**

[1] Institute of Legal Medicine, DNA department, University Hospital of Ulm, Germany, [2] Institute of Forensic Sciences, DNA department, Bavarian State Criminal Police Office, Maillingerstr. 15, 80636 Munich, Germany, [3] QIAGEN Ltd, Skelton House, Lloyd Street North, Manchester, M15 6SH, UK, [4] QIAGEN GmbH, 1 QIAGEN Strasse, Hilden, D-40724 Germany

## Introduction

Short tandem repeat (STR) typing from skeletal remains is a very challenging task. Numerous abiotic (temperature and humidity at provenance and storage period) and biotic (e.g. microorganisms) factors can impair the analysis either by degradation or contamination of endogenous DNA, or by inhibition of the amplification [1-2]. Therefore, sample selection is a critical step. Processing partial or singular skeletal elements, it is favorable to select bone areas where DNA preservation is comparably higher [3-5]. Especially cranial bones (that are composed of multiple parts) are often accidentally discovered during criminal investigations.

## Aim

In this examination, we evaluated the potential of the petrous bone for identification of human skeletal remains in forensic case work. Material from different sections of eight unknown cranial bones and – where available – additionally other skeletal elements, collected at the DNA department of the Institute of Legal Medicine in Ulm, Germany from 2010 to 2017 were processed with an optimized DNA extraction, quantification and STR typing strategy and compared to massively parallel sequencing (MPS) analysis.

## Material

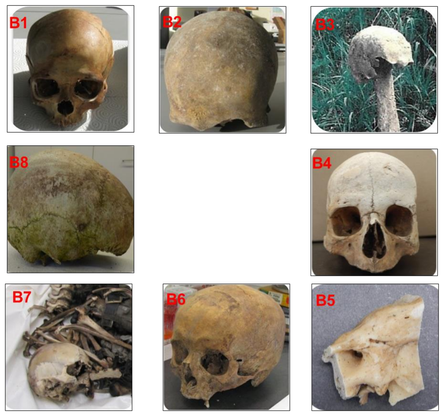


**Figure 1:** Overview of skull specimens.

## Methods

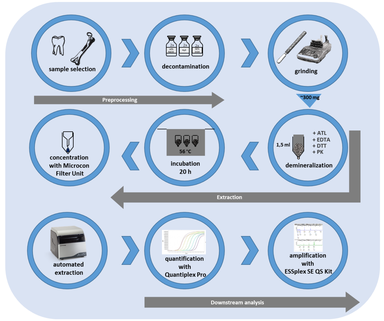


**Figure 2:** Strategy for DNA analysis of skeletal remains: Decontamination was performed according to Huet et al. [6]. Bones were ground in contamination-free disposable grinding chambers. 300 mg bone powder was used for DNA isolation and purification as described in Kulstein et al. [7]. DNA was quantified with the Quantiplex pro Kit (Qiagen) according to the manufacturer's recommendations and amplified with the Investigator ESSplex QS kit (Qiagen). Detection was conducted on an ABI PRISM 3130 Genetic Analyzer.

## Results: Petrous bone

| | Specimen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | |
| Case | DNA amount [pg/µl] | Alleles | Report able | DNA amount [pg/µl] | Alleles | Report able | DNA amount [pg/µl] | DI | Alleles | Report able |
| B1 | - | - | ☐ | <5 | 6/24ª | ☐ | 2 | 6 | 28/34 | ☒ |
| B2 | - | - | ☐ | <5 | 16/24ª | ☒ | 3 | 7.1 | 31/34 | ☒ |
| B3 | - | - | ☐ | - | - | ☐ | 4 | 4.7 | 34/34 | ☒ |
| B4 | - | - | ☐ | 1.7 | 0/34 | ☐ | 7.8 | 24.93 | 31/34 | ☒ |
| B5 | - | - | ☐ | - | - | ☐ | 11.3 | 7.46 | 34/34 | ☒ |
| B6 | 5.2* | 34/34 | ☒ | - | - | ☐ | 19 | 7.2 | 34/34 | ☒ |
| B7 | 56.9† | 34/34 | ☒ | - | - | ☐ | 13.5 | 4.21 | 34/34 | ☒ |
| B8 | 6.7† | 19/34 | ☒ | - | - | ☐ | 46.1 | 8.8 | 34/34 | ☒ |

† femur * molar ¤ analysis with in-house kit P11

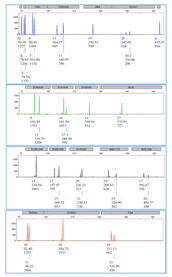

**Table 1:** DNA levels of bone samples were low. The degradation indices (DI) were elevated, indicating that DNA of the bone samples was degraded. Inhibition indices were not increased. Because DNA amounts were low, maximum input was used for subsequent STR amplification.

**Figure 3:** STR profiling with the Investigator ESSplex SE QS resulted in reportable profiles for all cases. 'Ski-slope' profiles were observed and showed that the DNA of the samples – as indicated by the increased DI – was degraded. This exemplar shows the results of case B5.

## Results: CE versus MPS

**Table 2**: Overview of the amount of analyzed markers with capillary electrophoresis (CE) and MPS. Altogether, the Illumina marker sets consists of 229 markers including Amelogenin, 27 autosomal STRs, 24 Y-STRs, 7 X-STRs, 54 biogeographical ancestry SNPs, 94 iSNPs and 22 phenotype-informative SNPs. CE assay consisted of 16 markers and Amelogenin.

| | | CE | | MPS | | | | |
|---|---|---|---|---|---|---|---|---|
| Case | Gender | Autosomal STRs+ Amel* | Autosomal STRs+ Amel* | Y-STRS | X-STRs | iSNPs | Hair/eye SNPs | Biogeographical SNPs |
| B1 | female | 14 (88.2%) | 16 (100%) | ND | 6 (85.7%) | 90 (95.7%) | 22 (100%) | 54 (100%) |
| B2 | male | 16 (100%) | 14 (87.5%) | 16 (66.7%) | 5 (71.4%) | 84 (89.4%) | 22 (100%) | 53 (98.1%) |
| B3 | male | 16 (100%) | 15 (93.8%) | 17 (70.8%) | 5 (71.4%) | 90 (95.7%) | 22 (100%) | 49 (90.7%) |
| B4 | male | 16 (100%) | 15 (93.8%) | 21 (87.5%) | 6 (85.7%) | 93 (98.9%) | 22 (100%) | 54 (100%) |
| B5 | male | 16 (100%) | 16 (100%) | 24 (100%) | 6 (85.7%) | 93 (98.9%) | 22 (100%) | 54 (100%) |
| B6 | male | 16 (100%) | 14 (87.5%) | 15 (62.5) | 5 (71.4%) | 64 (68.1%) | 12 (54.5%) | 40 (74.1%) |
| B7 | Male | 16 (100%) | 16 (100%) | 24 (100%) | 7 (100%) | 92 (97.9%) | 22 (100%) | 53 (98.1%) |
| B8 | female | 16 (100%) | 16 (100%) | ND | 5 (71.4%) | 69 (73.4%) | 21 (95.5%) | 38 (70.4%) |

* Loci typed

## Results: MPS

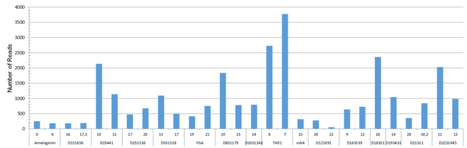


**Figure 4:** Autosomal allele calls between MPS and CE were mostly concordant. This example is from case B5. MPS provided additional allele calls where CE showed allelic dropout and vice versa (see also table 2). Some inconsistencies were observed due to stutter evaluation or to dropout (e.g. for D12S391).

**Table 3**: Prediction of the biogeographical ancestry assigned all analyzed individuals to European or Ad Mixed American populations. Results of hair and eye color prediction were assessed for all but two samples by the UAS. Case B6 and B8 were evaluated by the HIrisPlex eye and hair color DNA phenotyping webtool, which is available publicly via http://hirisplex.erasmusmc.nl/#, [8-9].

| Case | Hair color | | | | Eye color | | | Biogeographical ancestry |
|---|---|---|---|---|---|---|---|---|
| | Brown | Red | Black | Blond | Intermediate | Brown | Blue | |
| B1 | 0.59 | 0.00 | 0.20 | 0.21 | 0.05 | 0.95 | 0.00 | Ad Mixed American |
| B2 | 0.42 | 0.00 | 0.16 | 0.43 | 0.17 | 0.50 | 0.33 | European/ Ad Mixed American |
| B3 | 0.50 | 0.00 | 0.04 | 0.46 | 0.16 | 0.70 | 0.14 | European/ Ad Mixed American |
| B4 | 0.33 | 0.06 | 0.08 | 0.56 | 0.16 | 0.70 | 0.14 | Ad Mixed American |
| B5 | 0.37 | 0.08 | 0.14 | 0.42 | 0.14 | 0.79 | 0.08 | European |
| B6* | 0.22 (0.012) | 0.01 (0.022) | 0.02 (0.014) | 0.74 (0.015) | 0.06 (0.029) | 0.04 (0.005) | 0.91 (0.01) | European |
| B7 | 0.48 | 0.04 | 0.30 | 0.18 | 0.21 | 0.63 | 0.16 | European |
| B8* | 0.14 (0.002) | 0.02 (0.014) | 0.05 | 0.80 (0.005) | 0.08 | 0.06 | 0.86 | European |

## Conclusions

- ✓ Petrous bone is suitable for the analysis of challening bones samples
- ✓ Quantiplex Pro allows accurate quantification in low-template samples like bones
- ✓ Innovative degradation index shows if degradation occurred in samples
- ✓ Investigator ESSplex SE QS amplifies reproducible profiles with high sensitivity especially for polymorphic SE33
- ✓ MPS is a promising platform due to simultaneous analysis of multiple types of DNA markers that allow to evolve from 'passive comparison' into the 'active search'

**References**

[1] Kemp BM, Smith DG (2005) Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 154:53-61.
[2] Alaeddini R (2012) Forensic implications of PCR inhibition – a review. Forensic Sci Int Genet 6:297-305.
[3] Rohland N, Hofreiter M (2007) Ancient DNA extraction from bones and teeth. Nat Protoc 2:1756-1762.
[4] Parsons TJ, Weedn VW (1997) Preservation and recovery of DNA in postmortem specimens and trace samples. In: Haglund WD, Sorg MS, (ed.) Forensic taphonomy: the postmortem fate of human remains. Boca Raton, FL: CRC Press, 109-38
[5] Keyser-Tracqui C, Ludes B (2005) Methods for the study of ancient DNA. Methods Mol Biol 297:253-264
[6] Huel R, Amory S, Bilić A, Vidović S, Jasaragić E, Parsons TJ (2012): DNA Extraction from Aged Skeletal Samples for STR Typing by Capillary Electrophoresis. In: Antonio Alonso (ed) DNA electrophoresis Protocols for Forensic Genetics, Methods in Molecular Biology, vol.830, Springer Science+Business Media, LLC.
[7] Kulstein G, Hadrys T, Wiegand P (2017) As solid as a rock – comparison of CE- and MPS-based analyses of the petrosal bone as a source of DNA for forensic identification of challenging cranial bones, Int J Legal Med. Doi: 10.1007/s00414-017-1653-z
[8] Walsh S, Liu F, Wollstein A, Kovatsi L, Ralf A, Kosiniak-Kamysz A, Branicki W, Kayser M (2013) The HIrisPlex system for simultaneous prediction of hair and eye color from DNA. Forensic Sci Int Genet 7:98–115.
[9] Walsh S, Chaitanya L, Clarisse L, Wirken L, Draus-Barini J, Kovatsi L, Maeda H, Ishikawa T, Sijen T, de Knijff P, Branicki W, Liu F, Kayser M (2014) Developmental validation of the HIrisPlex system: DNA-based eye and hair colour prediction for forensic and anthropological usage. Forensic Sci Int Genet 9:150-161.

## Contact

Galina.Kulstein@uniklinik-ulm.de

## Acknowledgments

We kindly thank Gaby Kottmair for the helping hand with the bone preparation and Angelika Fürst for the preparation and accomplishment of the MPS analyses.