Application Note

Automated purification of DNA from bones of a Bronze Age family using the BioRobot® EZ1® workstation

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The BioRobot EZ1 workstation was used with the EZ1 DNA Tissue Kit and the EZ1 DNA Forensic Card to successfully purify DNA from 3000-year-old bones. STR analysis and sequencing of mitochondrial DNA established genetic relationships within a Bronze Age family.

Introduction

DNA testing has added a new and valuable tool for anthropologists investigating ancient samples. Since DNA is a relatively stable macromolecule, it can survive for thousands of years, with varying degrees of degradation, depending on the storage conditions.

An archeological find at Lichtenstein cave, near Dorste, Lower Saxony, Germany uncovered bronze artifacts and the remains of approximately 40 people from the Bronze Age. The bones had been relatively well preserved for approximately 3000 years due to the consistent cool temperature and inaccessibility of the cave, which is very narrow with a low ceiling. Many of the bones were covered with gypsum sinter, a type of calcium phosphate from saturated water that dripped in the cave. This gypsum sinter layer was undisturbed, indicating that nobody had entered the cave for approximately 3000 years.

Bronze Age human remains are seldom found in central Europe since cremation was commonly practiced. When the site was first excavated in the 1980s, it was thought to represent a site of human sacrifice. However, lack of signs of violence on the bones plus the mixture of different genders and a wide range of ages at death suggest a different explanation. DNA analysis indicates that the remains form a extended family clan, covering at least 3 generations. This suggests that the site is actually a family burial chamber. Analysis of further finds of this sort may lead to a re-evaluation of the frequency of human sacrifice in Bronze Age Europe and alternative burial practices.

We have established the first prehistoric family tree based on DNA analysis of 3 generations in the Lichtenstein cave. In this report, we present genotyping data for 3 individuals comprising one family from the Lichtenstein cave, with automated purification of DNA from Bronze Age bones using the BioRobot EZ1 workstation.

Materials and Methods

Bones were recovered from Lichtenstein cave, near Dorste, Lower Saxony, Germany. This archeological find dates from the Bronze Age, approximately 3000 years old. The cave temperature remains steady at 6–8°C, and many bones were covered with gypsum sinter (pH 8). Following removal, bones were stored at –20°C. In this report, bones from 3 individuals, Do 1482, Do 3706, and Do 3709, were analyzed.

Three samples were extracted from each individual: one each from the femur, skull (pars petrosae), and teeth. Surfaces of the bones and teeth were removed and discarded. The remaining bone was ground to a fine powder, and 150–200 mg bone powder was added to 600–700 µl 0.5 M EDTA and incubated at 37°C for 24 hours. Proteinase K digestion was carried out at 56°C for 2 hours using 20 µl QIAGEN® Proteinase K. The digest mixture was centrifuged in a microcentrifuge at 6000 rpm for 4 minutes, and DNA was purified on the BioRobot EZ1 workstation from 200 µl of the supernatant using the EZ1 DNA Tissue Kit and the trace protocol on the EZ1 DNA Forensic Card.* Purified DNA was eluted in 50 µl water. (Contact QIAGEN for a detailed protocol.) STR analysis was carried out using 3–10 µl of eluate in a 25 µl reaction volume for 35–40 cycles. Amplification was carried out on the ABI PRISM® 373 Genetic Analyzer using an octaplex system of our own design, the Amp*Fl*STR® Profiler Plus® PCR Amplification Kit (Applied Biosystems), or the PowerPlex® Y System (Promega).

For sequencing of mitochondrial DNA, 339 bp and 321 bp regions of the hypervariable regions HVR1 and HVR2 were amplified in 35 PCR cycles. The PCR products were cleaned up using the MinElute® PCR Purification Kit and sequenced on the ABI PRISM 310 DNA Sequencer.

Results and discussion

DNA was successfully purified from 3000-year-old bones for genotyping by STR analysis. Genetic fingerprinting enabled determination of familial relationships among the individuals tested. STR results, using an octaplex assay, established the identification of a mother (Do 3706), father (Do 1482), and female child (Do 3709) (Figure 1, Table 1).

STR analysis using the Amp*Fl*STR Profiler Plus PCR Amplification Kit confirmed the octaplex results and provided information about 3 additional markers (Figure 2, Table 1).

Table 1. Genotyping Results for a Bronze Age Family

		STR markers										
Subhead	**Typing assay**	**Amelo-genin**	**D3S1358**	**VWA**	**FGA**	**D8S1179**	**D21S11**	**D18S51**	**D5S818**	**D13S317**	**D7S820**	**CSF1PO**
Do 1482 (father)	Octaplex	XY	16/18	17/19	21/22	n.a.	30.2/32.2	n.a.	11/12	12	n.a.	11/13
	Profiler Plus	XY	16/18	17/19	21/22	13	30.2	15/17	11/12	12	8/11	n.a.
Do 3709 (child)	Octaplex	X	16/18	17	21/23	n.a.	29/32.2	n.a.	11/12	9/12	n.a.	10/13
	Profiler Plus	X	16/18	17	21/23	12/13	29/32.2	16/17	11/12	9/12	8/10	n.a.
Do 3706 (mother)	Octaplex	X	18	17/19	21/23	n.a.	28/29	n.a.	12	8/9	n.a.	10/11
	Profiler Plus	X	18	(17)	21/23	12/15	(28)	(16)	12	–/–	(10)	n.a.

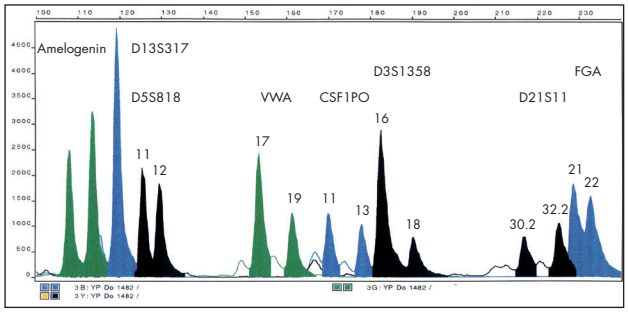
Summary of STR data in Figures 1 and 2. Numbers in parentheses are uncertain, due to low peak heights (DNA from individual Do 3706 had suffered more degradation than the other 2 samples).

n.a.: not analyzed.

* Editor's note: QIAGEN has subsequently released the EZ1 DNA Investigator Kit and Card, optimized for a wide range of forensic applications. The new kit and card provide more efficient yields and improved performance in downstream STR analysis. See ordering information at end of article.

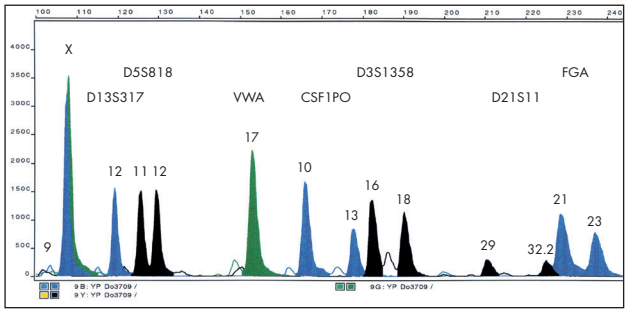
Ⓐ STR Analysis of DNA from Ancient Bones of a Bronze Age Family

Do 1482 (father)



Ⓑ

Do 3709 (child)



Ⓒ

Do 3706 (mother)

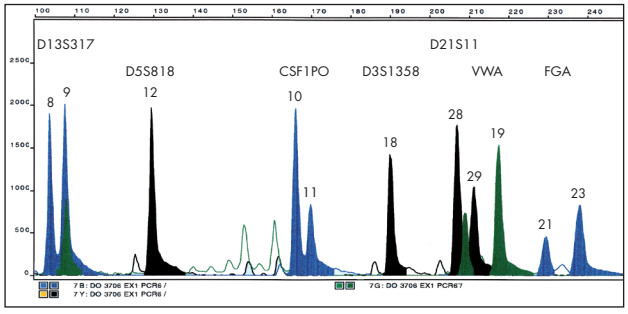


Figure 1. DNA was purified from 3000-year-old bones using the BioRobot EZ1 system. Genetic fingerprinting was carried out using an in-house octaplex PCR assay with DNA from individual **A** Do 1482 (father), **B** individual Do 3709 (child), and **C** individual Do 3706 (mother). For the last sample, an early version of the octaplex assay, with different primers for VWA, was used. Therefore the VWA peaks are at a different position. The analysis shows the familial relationship of the 3 individuals tested.

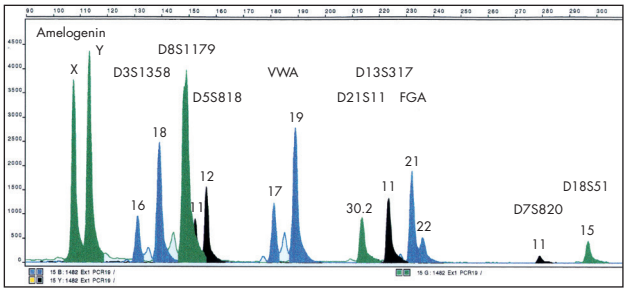
STR analysis using the Amp*Fl*STR Profiler Plus PCR Amplification Kit confirmed the octaplex results and provided information about 3 additional markers (Figure 2, Table 1)

Amplification of a region of mitochondrial DNA, followed by sequencing, enabled identification of haplogroups for the 3 individuals. Comparison of polymorphic sites indicated that both mother and child are of haplogroup T, consistent with the maternity of the child (Figure 3).

STR analysis of the Y chromosome of the father provided information for 12 additional STR markers (Figure 4). This information will prove valuable for identification of male relatives of Do 1482 within the archeological site. These results are part of ongoing project to establish the genetic relationship of 40 individuals from the Lichtenstein cave. The family analyzed here form part of an extended family clan, with 4 or 5 generations, interred in the Lichtenstein cave.

Ⓐ STR Analysis Using the Amp*Fl*STR Profiler Plus Kit

Do 1482 (father)



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Do 3709 (child)

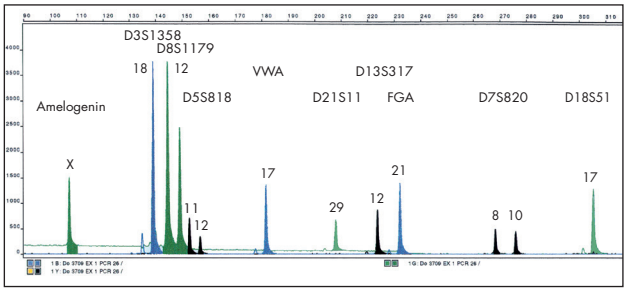

Figure 2. DNA was purified from 3000-year-old bones using the BioRobot EZ1 system. Genetic fingerprinting was carried out using the Amp*Fl*STR Profiler Plus PCR Amplification Kit with DNA from individual Do 1482 (father) and individual Do 3709 (child). DNA from individual Do 3706 (mother) had suffered more degradation than the other 2 samples, and peak heights were low, but still mostly readable (not shown, see Table 1). These results confirm the octaplex results and provide information about 3 additional markers.

Conclusions

- DNA from 3000-year-old bones was successfully purified using the BioRobot EZ1 system. The DNA was well suited for PCR amplification and STR analysis.
- Genotyping of DNA from the bones enabled identification of familial relationships between the individuals. The Bronze Age family analyzed in this report form part of an extended family clan. This suggests that the Lichtenstein cave formed a family burial site and not a site of human sacrifice, as previously assumed.

Sequencing of Mitochondrial DNA from Ancient Bones

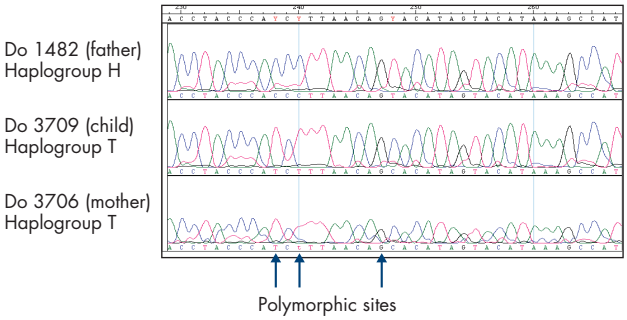

Figure 3. DNA was purified from 3000-year-old bones using the BioRobot EZ1 system. The HVR1 region of the mitochondrial genome was amplified by PCR and sequenced. Comparison of polymorphic sites indicate that both mother and child are of haplogroup T, consistent with the maternity of the child.

STR Haplotype of Y Chromosome

Do 1482 (father)

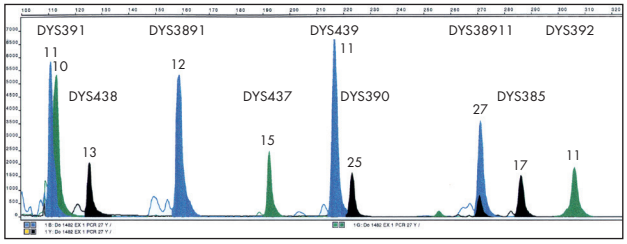

Figure 4. DNA was purified from bones of individual Do 1482 (father) using the BioRobot EZ1 system. STR analysis of the Y chromosome was carried out using the PowerPlex Y System.

Ordering Information

Product	Contents	Cat. no.
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1-year warranty on parts and labor*	9000705
EZ1 DNA Forensic Card	Preprogrammed card for BioRobot EZ1 Forensic protocols	9015864
EZ1 DNA Tissue Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffer G2, Proteinase K	953034
QIAGEN Proteinase K (2 ml)	2 ml (>600 mAU/ml, solution)	19131
Related products		
TissueLyser	Universal laboratory mixer-mill disruptor	85210† 85200‡ 85220§
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985
EZ1 DNA Investigator Card	Preprogrammed card for BioRobot EZ1 DNA Investigator protocols	9016387
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges, Disposable Tip Holders, Disposable Filter-Tips, Sample Tubes, Elution Tubes, Buffers and Reagents; includes Certificate of Analysis	952034

* Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

† North America. ‡ Japan. § Rest of world.

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