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Sheep and Goat Skulls from Follobanen Bispegata Oslo, Norway Ancient DNA Sampling Report

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Picture on front page: Four horned sheep skull (SL 8424) from Follobanen after sampling. Photo: Agata Gondek.

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Caprine skulls from Follobanen Bispegata excavation in Oslo

Five partial caprine skulls from the excavation in Follobanen Bispegata in Oslo (Berge, Ødeby, Holmen, Derrick, & Helstad, In prep) were given to us for possible ancient DNA sampling as part of the project "The Horses and Sheep of the Vikings: Archaeogenomics of Domesticates in the North Atlantic Research". Only two of the skulls had a petrous bone which is preferred for sampling for ancient DNA as it often has excellent DNA preservation (Hansen et al., 2017; Pinhasi et al., 2015). Since the focus of the project was acquiring ancient DNA samples from sheep (*Ovis aries*) skulls from goats (*Capra hircus*, see below) were not sampled even if the petrous bone was present. Full information on the skulls can be found in Table 1.

Speciation of the skulls

Skulls were identified to species by zooarchaeologist Albína Hulda Pálsdóttir. Identification was done with the aid of reference manuals, Boessneck (1969) for skull structure and suture shape, and petrous bones were identified to species following Mallet & Guadelli (2013). Species identification based on petrous bone shape for sheep and goat is quite reliable when done by people with experience (pers. obs. Albína Hulda Pálsdóttir).

Table 1: Full information on all the skulls from Follobanen Bispegata considered for ancient DNA analysis.

Site	Layer	Date	Species	Element	Archaeological context	Approximate dates	Comment	Sampled for aDNA
Follobanen	16236	15.3.2018	Goat	Nearly complete skull with petrous bone.	Wasteheap, bygård	Late 1100 to early 1200	Brain case broken, possible butchery.	No, wrong species.
Follobanen	16004	13.3.2018	Goat	Nearly complete skull	Possible levelling layer under a building- secondary deposit	1200-1300	Horn cores removed, chopped through occipital region.	No, wrong species.
Follobanen	16004	13.3.2018	Goat	Partial skull, no petrous bone.	Possible levelling layer under a building- secondary deposit	1200-1300	Horn cores have been removed and the skull chopped through the brain case and the occipital region.	No, wrong species.
Follobanen	80236	Unknown	Sheep/goat	Partial skull, no petrous bone.	Built up layers (probably dumped- secondary deposit)	1100-1200	Possibly chopped in half. Very small skull.	No petrous bone present.
Follobanen	8424	5.10.2017	Sheep	Four horned skull	Refuse layers (possible primary refuse)	1150-1350	Top of the skull with the horn cores seems to have been chopped.	Yes, sheep and unusual trait, four horned.

Skulls from Follobanen not sampled for ancient DNA

Nearly complete goat skull from layer 16236

The goat (*Capra hircus*) skull from layer 16236 (Figure 1) is nearly complete. The skull can be clearly identified as goat based on the angle between the coronal and frontal sutrues following Boessneck (1969, pp. 332–333) (Figure 2) as well as on the shape of the petrous bone following Mallet & Guadelli (2013) (Figure 3).

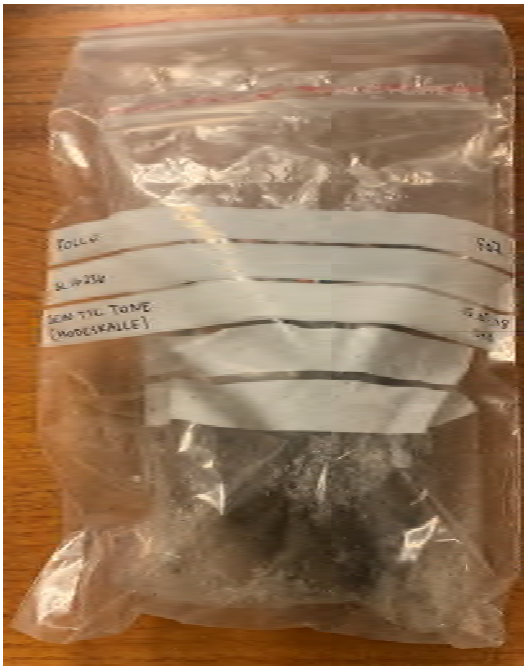

Figure 1: The goat skull from layer 16236 in the original bag. Photo: Albína Hulda Pálsdóttir.

Figure 2: Goat skull from layer 16236, the 90° angle between the coronal and frontal sutures which is charactersitic for goats (Boessneck, 1969, pp. 332–333) is clearly visible. Photo: Albína Hulda Pálsdóttir.

Figure 3: The goat skull from layer 16236 with the petrous bone visible. Photo: Albína Hulda Pálsdóttir.

Partial sheep/goat skull from layer 80236

The partial sheep/goat skull from layer 80236 (Figure 4) did not include the petrous bone and could not be clearly identified as sheep or goat. It has likely been chopped in half. It is possible the individual was still a juvenile as the skull was quite small and delicate. The individual was naturally polled.

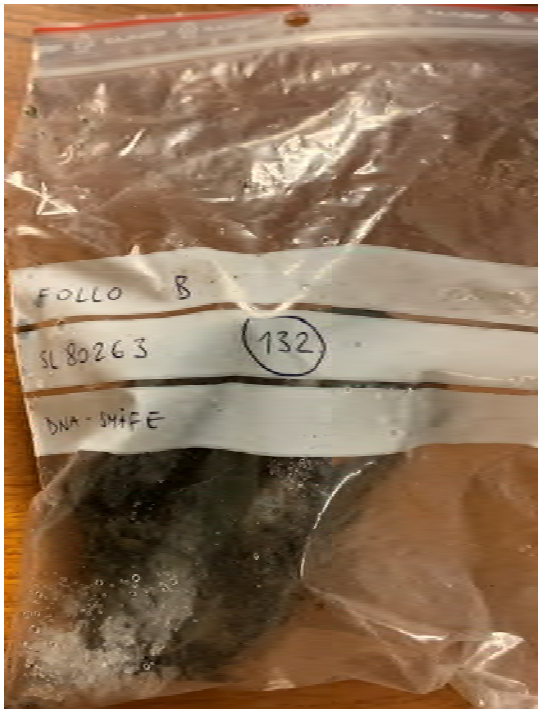

Figure 4: The sheep/goat skull from layer 80263 in the original bag. Photo: Albína Hulda Pálsdóttir.

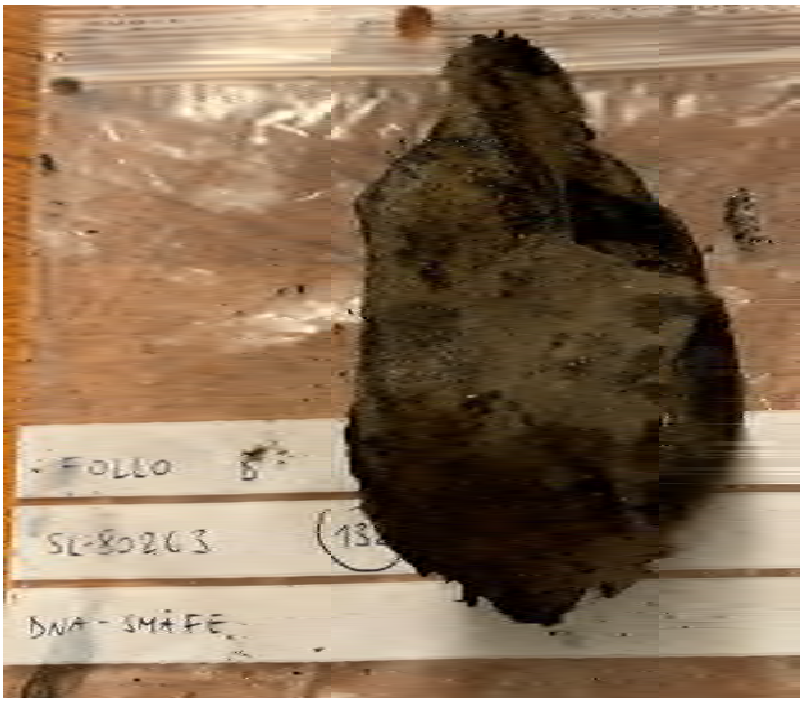

Figure 5: The sheep/goat skull from layer 80263 which was likely chopped in half. Photo: Albína Hulda Pálsdóttir.

The two skulls from layer 16004

There were two partial skulls from layer 16004, both from goats. One had the petrous bone intact but the other did not.

Goat skull with petrous bone from layer 16004

The first skull from layer 16004 (Figure 6) had a petrous bone and can be identified as a goat (*Capra hircus*) based on its shape (Figure 7) following Mallet & Guadelli (2013). The horn cores have been removed from the skull, presumably the horn was used for craft working (Figure 8). The skull has also been chopped through occipital region (Figure 12).

Figure 6: Goat (Capra hircus) skull with petrous bone from layer 16004 in original bag. Photo: Albína Hulda Pálsdóttir.

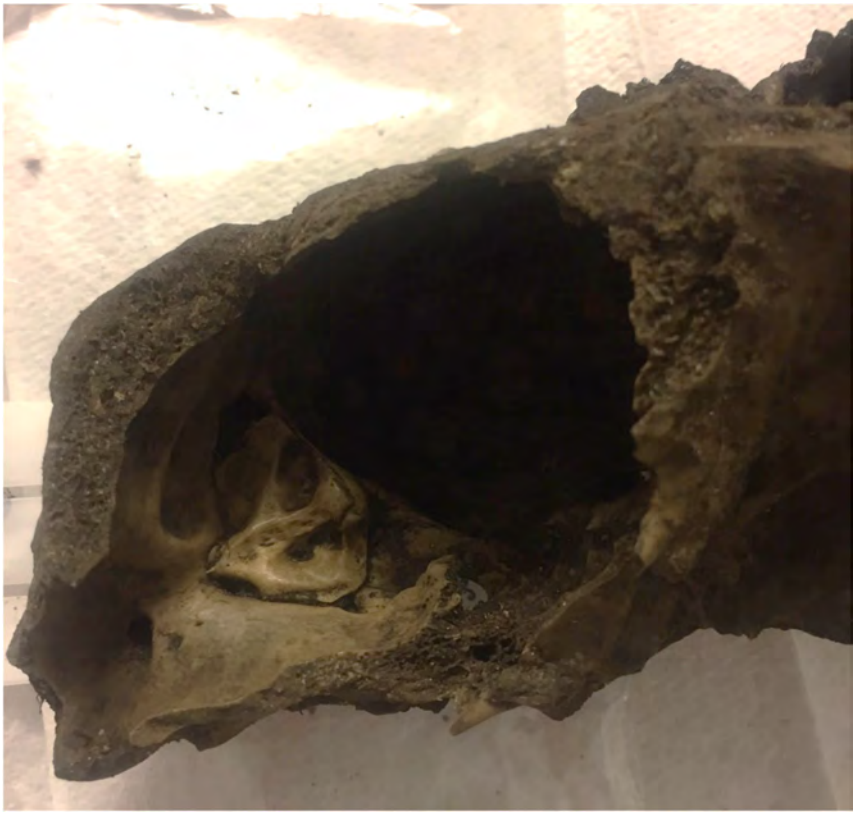

Figure 7: Goat skull from layer 16004 with petrous bone visible, the petrous bone can clearly be identified as goat (Capra hircus). Photo by Agata Gondek.

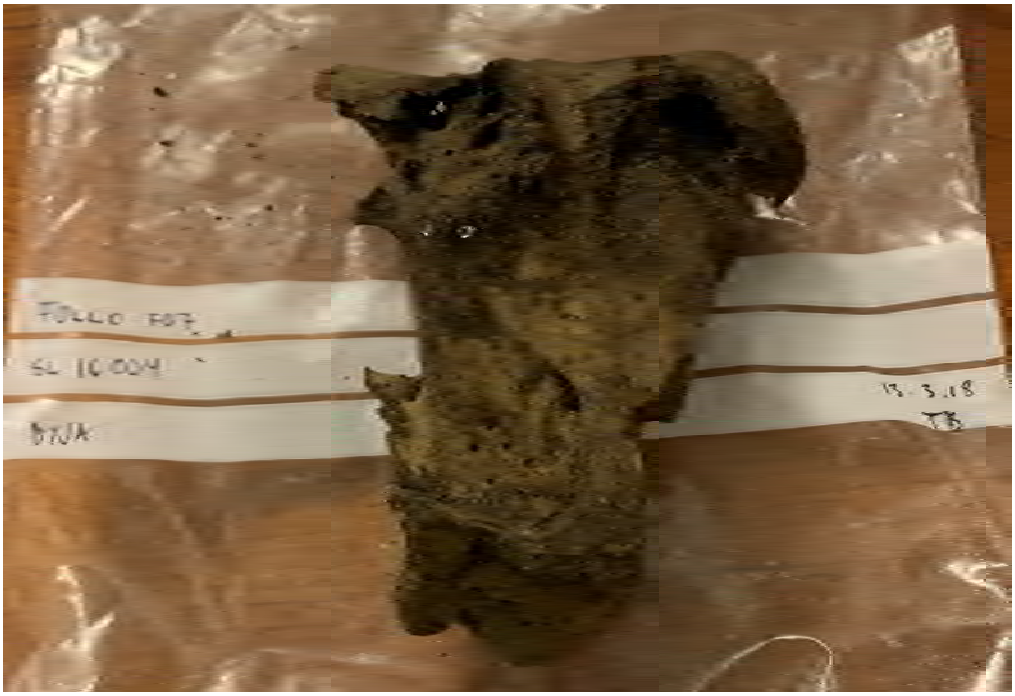

Figure 8: Goat (Capra hircus) skull with petrous bone from layer 16004, horn cores have been removed. Photo: Albína Hulda Pálsdóttir.

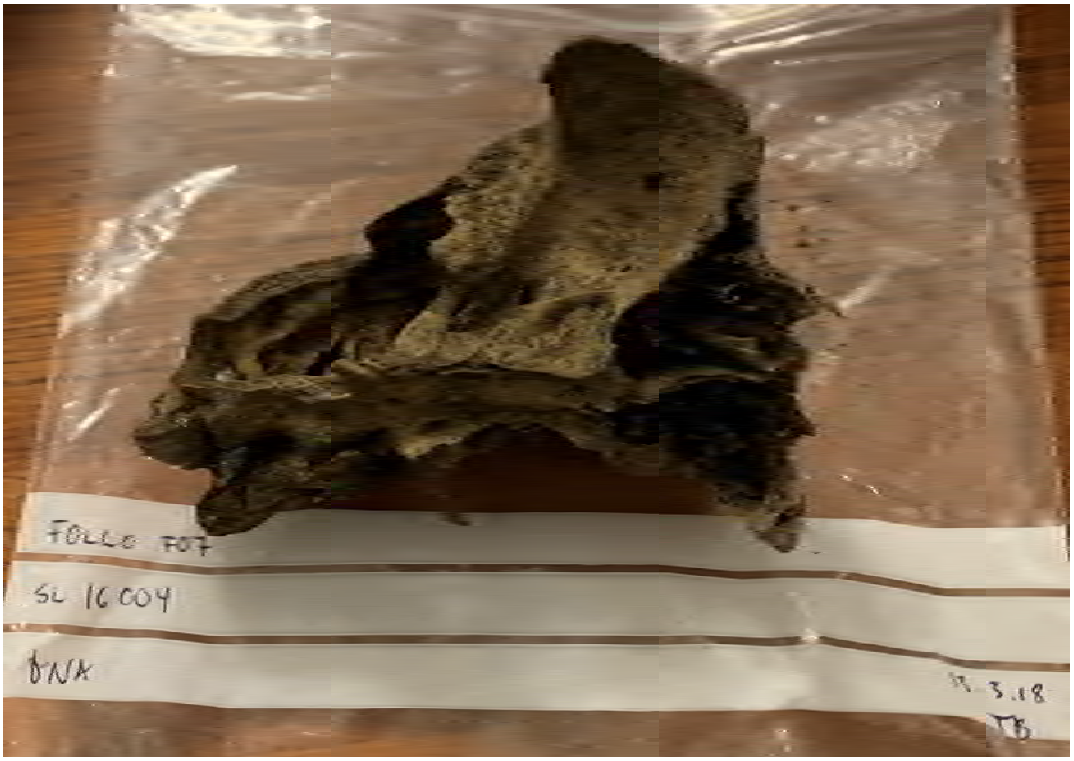

Figure 9: Goat (Capra hircus) skull with petrous bone from layer 16004 which has been chopped through the occipital region. Photo: Albína Hulda Pálsdóttir.

Goat skull without petrous bone from layer 16004

The second skull from layer 16004 (Figure 10) did not have a petrous bone. The skull can be identified as goat based on the cranial sutures (Boessneck, 1969). The horn cores seem to have been intentionally removed from the skull (Figure 11), possibly the horns were used as raw material from some craft work. The skull has clearly been chopped through both the occipital and brain case (Figure 12).

Figure 10: Goat (Capra hircus) skull from layer 16004 in original bag. Photo: Albína Hulda Pálsdóttir.

Figure 11: Goat (Capra hircus) skull from layer 16004 with horn cores removed, the 90° angle between the coronal and frontal sutures which is characersitic for goats (Boessneck, 1969, pp. 332–333) is clearly visible. Photo: Albína Hulda Pálsdóttir.

Figure 12: Goat (Capra hircus) skull from layer 16004 chopped through the braincase and occipital region. Photo: Albína Hulda Pálsdóttir.

VSH083 Four horned sheep skull sampled for ancient DNA

The only skull sampled for ancient DNA from the Follobanen Bispegata was the four horned sheep skull from layer 8242 (Figure 13). Unfortunately, only the very top part of the skull was present so there was no petrous bone to sample. Despite that we decided to attempt to cut a small sample from the base of one of the four horn cores to get an ancient DNA sample (Figure 17). Four horned skulls are rare in the archaeological record (Daróczi-Szabó & Daróczi-Szabó, 2018; Putelat, 2006) and it would have been very interesting to get ancient DNA from such an unusual individual.

The four horned skull seems to have been intentionally chopped to keep the four horns together and it seems likely that the skull was on display before being discarded. The skull is therefore a rather unusual artefact as well as an interesting zooarchaeological specimen.

Figure 13: Photo of the four horned sheep skull from layer 8424 with context information. Photo by: Agata Gondek.

Figure 14: Four horned sheep skull VSH083 before sampling. Top view. Photo by Giada Ferrari.

Figure 15: Four horned sheep skull VSH083 before sampling. Bottom view. Photo by Giada Ferrari.

Before sampling, the skull was photographed (Figure 14 and Figure 15) and measured following recommendations in Pálsdóttir et al. (2019). There are no measurement standards for four horned sheep skulls so the standard of von den Driesch (1976, pp. 27–30) for two horned cattle skulls was adapted (Figure 16 and Table 2). Measurements were done with digital callipers to the mm or with a tape measure.

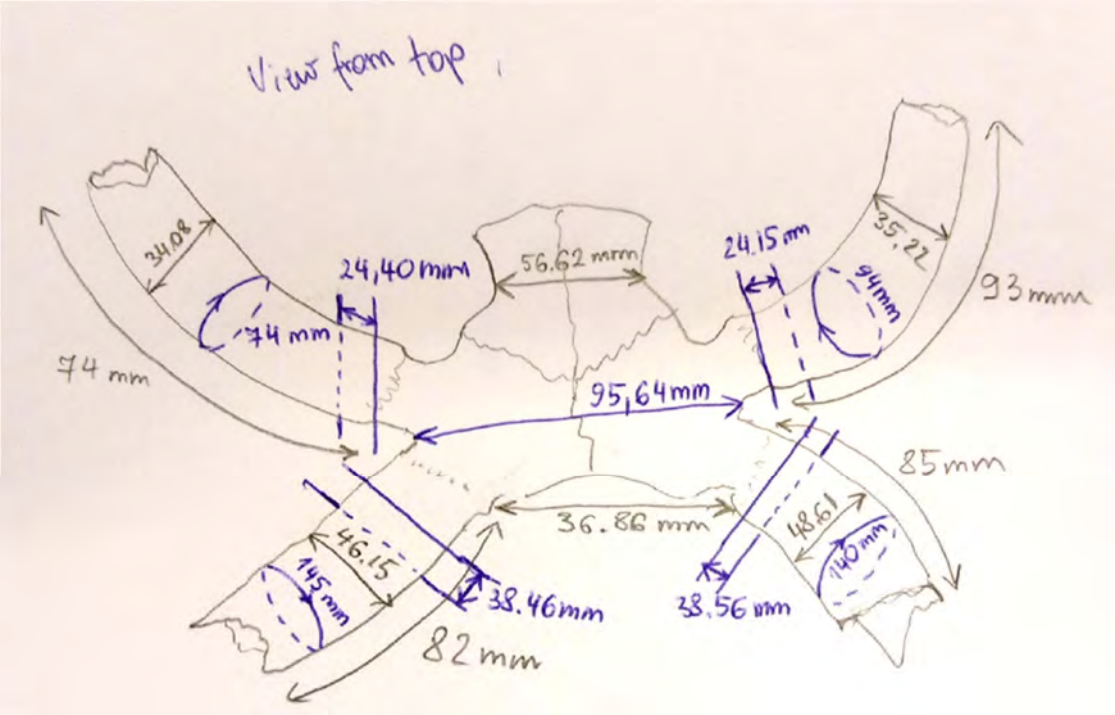

Figure 16: Measurments of the four horned sheep skull. Drawing by Agata Gondek.

Table 2: Measurements in mm of the four horned skull (VSH083) following von den Driesch (1976, pp. 27–30).

Measurement definition	Skull	Lower right horn core	Upper right horn core	Lower left horn core	Upper left horn core
30. Least occipital breath	56,6	-	-	-	-
31. Least breath between the bases of horn cores (in the middle)	95,6	-	-	-	-
31. Least breath between the bases of horn cores (anterior)	36,9				
44. Horn core basal circumference.	-	74,0	145,0	94,0	140,0
45. Greatest diameter of horn core base	-	34,1	46,2	35,2	48,6
46. Least diameter of horn core base	-	24,4	38,5	24,2	38,6
47. Length of the outer curvature of the horn core	-	74,0	82,0	93,0	85,0

The skull was also photographed after sampling (Figure 17).

Figure 17: Four horned sheep skull (VSH083) after sampling for aDNA. Scale 10 cm. Photo by Agata Gondek.

Laboratory methods

All DNA extraction and pre-PCR library protocols were performed in the dedicated ancient DNA laboratory at the Department of Biosciences, University of Oslo following strict aDNA precautions (Allentoft et al., 2012; Cooper & Poinar, 2000). Laboratory protocols, data processing and filtering are described in Star et al. (2018). In short, after UV expose on all sides, samples were milled using a custom designed stainless-steel mortar (Gondek, Boessenkool, & Star, 2018). Extraction used a combined bleach and pre-digestion protocol (Boessenkool et al., 2016). Specifically, bleach washes were performed in duplicate (150-200 mg of powder each) (Boessenkool et al., 2016), washed with H_2O and pre-digested, which was followed by an overnight, second digestion (Gamba et al., 2015). The two eluates were concentrated (Amicon-30kDA Centrifugal Filter Units) after the overnight digestion and combined, extracting DNA using Minelute (Qiagen) according to manufacturer's instructions. Using 60 µl pre-heated (60°C) EB buffer, DNA was eluted by incubating for 15 min at 37°C (Star et al., 2014). After this, blunt-end Illumina libraries were built (Meyer & Kircher, 2010; Schroeder et al., 2015) and library quality was assessed using a Bioanalyzer 2100 (Agilent).

Results of ancient DNA analysis of skull VSH083

Unfortunately, the DNA libraries from the four horned sheep skull (VSH083, 8424) did not yield sufficient DNA preservation for further analyses within this study (Figure 18). It would be possible to

amplify the sample to get a mitochondrial genome but there is very little geographical or temporal structure in the mitochondrial genomes of European sheep (Hiendleder, Lewalski, Wassmuth, & Janke, 1998; Niemi et al., 2013; Rannamäe et al., 2016; Tapio, 2006) so this would yield very little information. Therefore, it was decided not to process the sample further at this time.

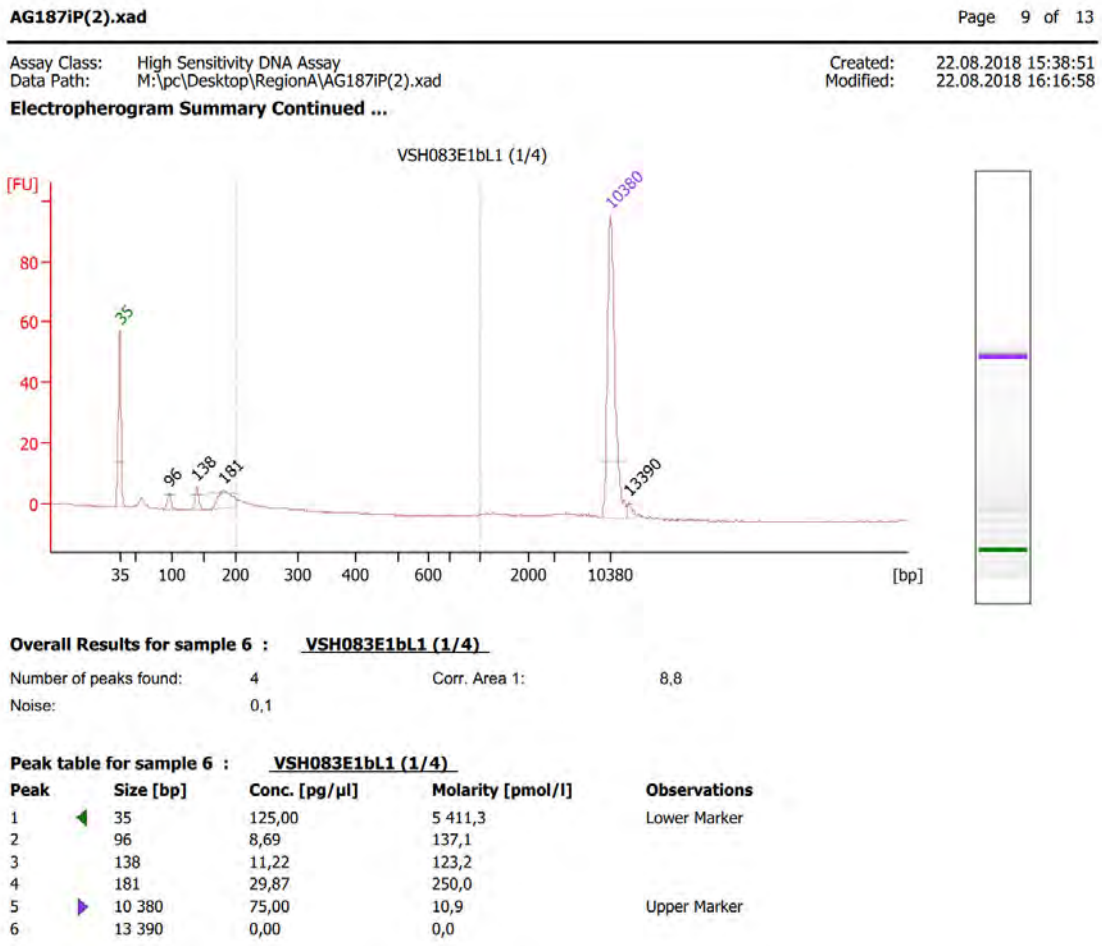

Figure 18: Results from Bioanalyzer 2100 (Agilent) for sample VSH083. The sample had little DNA and was therefore not selected for screening.

Discussion

This small sample of the archaeofauna from the Follobanen excavation is very interesting. The number of goat skulls was rather unexpected but would make sense as goats seem to have been preferred to sheep in urban areas as they can be milked for much longer periods of time and have more omnivorous diets than sheep and their horns and skins were popular raw material for craft working (e.g. Lie, 1988; Noddle, 1994; Salvagno & Albarella, 2019). The butchery patterns observed are also interesting and certainly show that there is lot of interesting information to be found in the archaeofauna from the site with traditional zooarchaeological analysis.

Return of samples

Following recommendations in Pálsdóttir et al. (2019) all unsampled bone and the four horned sheep skull that was sampled were returned to NIKU. Bone powder and DNA extracts and other sample products from sample VSH083 will be kept in the CEES, UiO, ancient DNA laboratory and will be accessible to other researchers once the current project is completed.

Funding

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