

Supplementary information

Section 1: Description of sites and burials

Abbreviations: MK= Malmö Museum; LUHM= Lund University Historical Museum; BM= Blekinge Museum; NM= Danish National Museum; ÖM= Östergötland Museum. The reference to the museums indicate the repositories for the remains.

Scanian sample

Malmö, Bronsyxan (MK)

Excavated in 1970 by Malmö Museum division of Archaeology due to exploitation of a new area around the Fosie church.

The grave measured 2.85x1.5 m and was oriented N-S, had a stone packing and a probable wooden lid. The individual was buried in crouching position with the head towards N, facing E. **Finds:** Battle axe type E:1, Hollow-edged flint axe and perforated boar tusk, vessel type J (at head), 4 flint blades, 2 flint scrapers and 2 flint flakes.

References:

Håkansson, H., Johanson, G. & Salomonsson, B. (1973). Ett gravfynd från Kv Bronsyxan, Malmö. *Elbogen* 1972. p. 135–163.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Degeberga 26:3 (LUHM)

Excavated by Sydsvensk Arkeologi in 2013 as part of the research project *Degebergas förkristna kultplats* (Eng. The pre-Christian ritual place of Degeberga (our translation)). The grave was probably a boat construction (canoe) with dimensions around 3.25x0.6 m, oriented E-W. The pit was circulated by furrows and post holes, interpreted as a superstructure. It is noteworthy that the individuals are buried with the heads towards each other; feet towards each other are more common and usually reflect a male and a female.

Individual 1: Adolescent. Buried in right-sided hocker (crouching position), head to W facing S.

Finds: 1 fragment of antler pick-axe and hollow-edged flint axe behind head. Pottery type F, G or H in front of torso. 2 retouched flint blades at tibiae.

Individual 2: Adolescent. Buried in left-sided hocker (crouching position), head to E facing S.

Finds: Pottery type F, G or H by tibiae, pottery G:2 west of skeleton by feet. 2 bone/antler pendants with unclear position (found when sieving).

Additional bone awl interpreted as related to individual 2.

References:

Björk, T. & Wickberg, Y. (2014). *Degeberga 26:3. Degeberga socken. Arkeologisk undersökning 2013. Rapport 2014:60*. Sydsvensk Arkeologi.

Dösemarken (MK 321)

Excavated by Malmö Kulturmiljö in 2006 due to planned exploitation of the area. The grave was oriented N-S and measured 1.9x1.2 m. The individual was buried in crouching position with the head towards N, facing E. Stone packing. **Finds:** Bone needle. Finds of eleven pottery sherds and 13 flint flakes in the grave filling were not interpreted as grave goods.

References:

Ifverson, P. (2007). Arkeologisk förundersökning 2006. Dösemarken. Fastigheten Limhamn 155:355. Hyllie socken i Malmö stad, Skåne län. Enheten för Arkeologi Rapport 2007:014. Malmö, Malmö Kulturmiljö.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Fredriksberg (LUHM 25481)

The two graves were found at the Fredriksberg farm when digging for gravel and was excavated by Lund University Historical Museum archaeologist J E Forssander in the late 1920s.

Malmer grave 71: Grave oriented N-S with a stone packing. The individual was buried in crouching position with the head to the S facing E. **Finds:** Vessel type J:3, 1 flint blade, 2 flint scrapers, 2 bone needles, bird radius (swan?). The grave is exhibited as a preparation in the Lund University Historical Museum.

Malmer, grave 72: The grave was oriented E-W without stone packing. The individual was buried in crouching position with the head towards W. Later 14C-analyses confirms a later date of LNII. No finds.

References:

Forssander, J-E. (1929). *Berättelse över undersökning av enmansgrav från stenåldern påträffad å Fredriksbergs gårds ägor, Oxie sn och hd, Malmöhus län*. Archival document. Malmö Museum.

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund: Univ. Bonn

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Ingelstorp 31:6 (LUHM)

The grave was excavated by professor Märta Strömberg and colleagues within the research project about the parish of Hagestad, Scania, spanning the years 1969 and 1974-1977. Grave 1 was oriented N-S, had the dimensions of 3.3 x 2.6 m, and was covered by a stone packing. The skeleton was poorly preserved, but the individual was buried in crouching position with the head to N facing E.

Finds: hollow-edged flint axe, thin bladed flint axe, hollow-edged chisel, flint blades, flint flakes, amber beads, red-coloured lump/stone, two ceramic vessels type H:2, and ribs from (probably) cattle.

References:

Strömberg, M. (1982). *Ingelstorp: zur Siedlungsentwicklung eines südschwedischen Dorfes*. Bonn: Habelt.

Kastanjegården (MK)

The site was excavated by Malmö Museum in autumn 1973 and led by archaeologist Bengt Salomonsson in relation to the development of a new area for houses in south of Fosie church outside of the town Malmö. The cemetery was oriented N-S and was approximately 17 m long. The graves were aligned in N-S in a linear cemetery common in the BAC.

Four graves:

Grave 105: 3 individuals in rectangular wooden construction, c 2.5x1.4 m large, oriented N-S with a stone packing. 1 adult female N-S, head S facing E. At the head a 5-7 year old child with the head to the south. At the feet a child of same age with the head to the north. **Finds:** quern stone in stone filling, parts of 2 vessels, 7 amber beads, 1 thin bladed flint axe, 4 flint blades, 2 flint scrapers, 5 flint flakes 3 with retouch, single ceramic fragments (J:3 and adJ).

Grave 106: C. 2.5x1.7 m large, orientation NE-SW with a stone packing. teeth from 8-10 y o child. Head NO facing SW. In filling: quernstone fragments and a knapping stone. 23 amber beads/fragments, vessel J:2, thick necked hollow-edged flint axe and 1 flint blade. 1 C/D arrowhead and 3 flint blade and 4 flint flakes together at feet (west).

Grave 108. Oriented N-S, c. 2.5x1.5 m large with a stone packing. Teeth from teenager. Probably wooden construction of 1.9*0.6 m. The individual was probably oriented N-S head N facing E. **Finds:** Battle axe E:1 (at head), hollow-edged flint axe. 2 flint blades, 2 flint flakes.

Grave 109. Oriented E-W, c. 2.5x2.1 m large with a stone packing. Teeth from child aged 4-6 years, head to the east. **Finds:** 3 amber beads, 1 thin bladed hollow-edged flint axe. Fragment of fire damaged hollow-edged flint axe, 2 flint blades, vessel J:2 (upside down and with flint scraper inside).

References:

Winge, G. (1976). *Gravfältet vid Kastanjegården*. Malmöfynd nr 3. Malmö, Malmö Museum.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Kjylbjersbacken (LUHM 27499)

Malmer grave field 90. Excavated by Lund University Historical Museum archaeologist Folke Hansen, on several occasions during the years 1934-1935. The excavation followed reports of destroyed prehistoric graves due to gravel digging. Twelve graves with single and multiple inhumations were excavated. Hansen also excavated an additional grave in 1944 after new reports of finds of human bones. The cemetery spans from late MNB to the Early Bronze Age.

Grave 3 (grave 2 in original documentation): The grave was oriented NNW-SSE with a stone packing. The individual was buried on the back with the arms and legs bent with the head towards NNW. No finds.

Grave 4: The grave was oriented NNE-SSW and covered by a cairn. The individual was buried in outstretched position with bent arms and the head towards NNE. **Finds:** a small flint point/dagger.

Grave 6: The grave was oriented E-W and covered by a cairn. The individual was buried in outstretched position with bent arms and the head towards E. **Finds:** a bone awl.

Grave 7: The grave was oriented NNE-SSW, no stone packing. The individual was buried in somewhat crouching position with the head towards SSW. No finds.

Grave 8: The grave was oriented N-S and covered by a cairn. The individual was buried in crouching position on the right side with the head towards S, facing E. No finds.

Graves 9-11: Three individuals inhumed in the same grave. The grave was oriented E-W and covered by a cairn. No finds.

Grave 9 was buried in outstretched position with bent arms, the head towards E. This is the oldest burial.

Grave 10 was a child buried directly on top of grave 9. The lower extremities were bent, and the head positioned towards W.

Grave 11 was also a child burial. The individual was buried in outstretched position with the head towards W.

Finds: A bone awl and a flint blade was recovered and interpreted as belonging to grave 10.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund: Univ.. Bonn

Tornberg, A. (2018). *Health, cattle and ploughs – Bioarchaeological consequences of the Secondary Products Revolution in southern Sweden, 2300-1100 BCE*. (Diss.), Lund University.

Lilla Bedinge 56 (LUHM 27496)

The cemetery of Lilla Bedinge was excavated by Lund University Historical Museum archaeologist, Folke Hansen, in the 1930s. The cemetery contained inhumation graves from the BAC, Late Neolithic, and Early Bronze Age, as well as cremation urn graves from the Late Bronze Age. The cremation graves were concentrated in the northern part of the cemetery, but in direct vicinity to the inhumation graves.

Grave 47(child; primary; cr 4): Boat shaped stone construction, oriented NNE-SSW. The individuals buried in outstretched positions with heads towards SSW. (5 individuals according to Malmer). The crania were positioned higher stratigraphically than the primary burial. The child was found just outside the construction. **Finds:** Red-brown lump of color, whetstone fragment in the stone construction.

Only cr4 of BAC date, Primary burial of LN/EBA date and the child EBA. The stratigraphical distortion of the 14C dates might reflect that earlier BAC burials were disturbed in the construction of the primary burial but were redeposited in the covering cairn.

Grave 49: Grave with multiple inhumations of at least 3 adults and 2 children. The grave was oriented NE-SW with the adults buried in sitting position with crossed legs after each other, facing NW. **Finds:** Bone awl.

Grave 52: Double grave with stone and wooden constructions. The grave was oriented NE-SW. *NE skeleton:* positioned on the left side with the head towards NE. *SW skeleton:* positioned on the right side with the head towards SW. **Finds:** Pottery: G:3, H:2, H:2; Flint: thick bladed (hollow edged?); 106 amber beads, copper earring, comb-stamp in bone, 4 flint blades, sheep bone.

Grave 53: Funnel shaped stone and wooden constructions, oriented NNW-SSE. The individual positioned on the left side with the head towards NNW. **Finds:** Thick-bladed flint axe, antler pick-axe, 3 flint blades, bone from golden eagle and sheep

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund : Univ.. Bonn

During, E. (1988). En osteologisk undersökning av tre skelettgravar från Bedingegravfältet. In: *Stridsyxekultur i Sydskandinavien. Rapport från det andra nordiska symposiet om Stridsyxetid i Sydskandinavien*. Larsson, L. (Ed.). Lund University. Institute of Archaeology.

Norra Hyllievång (MK 350:432)

The area was excavated by Malmö Kulturmiljö in 2006 and consisted of two cemeteries, a northern and a southern. The southern cemetery included two BAC burials and a plausible BAC burial, while the northern cemetery was of Late Neolithic date.

Grave 1: The grave was oriented N-S and measured 2.6x1.3 m with a stone packing. The individual was buried in crouching position with the head towards N, facing E. **Finds:** Thick necked hollow-edged flint axe, thin-bladed hollow-edged flint axe (head region), vessel type H (feet), filling: stone which transferred red color, flint blade, flint scraper.

Grave 2: The grave was oriented N-S and measured 3x1.4 m with a stone packing. A mandible was recovered in the N, suggesting that the individual was buried with the head to N. **Finds:** Hollow-edged flint axe, hollow-edged flint chisel, thin bladed flint axe, pottery type H and a vessel of unknown type, amber beads, 2 flint blades, and 10 flint arrowheads type D (plausibly organized in a quiver).

References:

Lindhé, E. & Grehn, F. (2008). *Arkeologisk slutundersökning 2006. Norra Hyllievång. Hyllie 155:91, 165:61. Hyllie socken i Malmö stad, Skåne län*. Enheten för Arkeologi Rapport 2008:001. Malmö, Malmö Kulturmiljö.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellaneneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Oxie 7 (LUHM 27857)

Malmer grave 73. A c. 1 m shallow pit with an individual in crouching position, oriented E-W, head towards W. No stone construction, no finds.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund : Univ.. Bonn.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellaneneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Skepparslöv (LUHM 20804)

Malmer grave 77. Child burial. No stone construction. **Finds:** Pottery G:2, N; thin bladed chisel, 3 bone rings, 1 flint blade, small fragments of animal bones.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund : Univ.. Bonn

Snorthög (LUHM 28424)

At the site of Snorthög a Late Bronze Age mound with cremations covered a flat cemetery with ten graves from the transition between MNB/LNI-LNII. The site was excavated by Lund University Historical Museum archaeologist Bror-Magnus Vifot in 1938. The flat burials were visible as dark colourations in rectangular and oval shapes. Only one grave, grave I, was covered by a small cairn. Grave X is an infant burial kept as a preparation at the LUHM, which has disabled analysis and radiocarbon dating.

Grave II: The grave was oriented SW-NE. The individual was buried in crouching position on the left side with the head NE. **Finds:** Rust-colored lump, flint arrow-head.

Grave III: The grave was oriented SE-NW. The individual was buried in crouching position on the left side with the head towards NW. Probable wooden construction. No finds.

Grave IV: The grave was oriented WSW-ENE. The individual was buried on the left side with the head towards WSW. **Finds:** Under the chest a dagger blade.

Grave V: The grave was oriented NW-SE. The individual was buried in crouching position on the left side with the head towards SE. **Finds:** Arrowhead of flint.

Grave VIII: The grave was oriented NW-SE. The individual was buried in crouching position on the left side with the head towards SE. **Finds:** Pottery and flint dagger blade.

Grave IX: The grave was oriented SW-NE. The individual was buried on the right side with the head towards SW. No finds.

References:

Tornberg, A. (2018). *Health, cattle and ploughs – Bioarchaeological consequences of the Secondary Products Revolution in southern Sweden, 2300-1100 BCE*. (Diss.), Lund University.

Solnäs (LUHM)

Excavated by Lund University Historical Museum archaeologist Folke Hansen in 1929 due to reports of disturbed graves following gravel digging at the Solnäs farm.

Grave 7: Orientation N-S, buried in crouching position on the right side with the head towards north. **Finds:** Flint dagger/flint spearhead under femur/pelvis – n.b. point broken in ancient times towards pelvis.

Grave 8: Child burial found directly underneath grave 7. Outstretched position. The lower legs missing, possibly disturbed when burying the individual in grave 7.

References:

Hansen, F. (1929). Arkeologisk rapport, LUHM dnr. 2695/28 & 1527/29.

Svågertorp (MK)

Excavated by Malmö Kulturmiljö in year 2000. The grave was oval in shape, oriented N-S, and measured 3x1.6 m. Stone packing. The individual was buried in crouching position with the head towards N facing E. **Finds:** Vessel J:2 at feet. Asymmetric ceramic bead. Head: Battleaxe E:1, hollow-edged flint axe, 3 flint blades. A drilled fossil as well as unworked fossils and seashells.

References:

Koch, H. & Tuominen, K. (2008). *Arkeologisk slutundersökning 2000. Svågertorps industriområde. Delområde K, P & S. Bunkeflo socken i Malmö stad, Skåne län*. Enheten för Arkeologi Rapport 2006:080. Malmö, Malmö Kulturmiljö.

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Tygelsjö (LUHM 5083)

Malmer grave 88. Find of a skull cap by the naturalist Sven Nilsson in the 19th Century. Grave with stone construction. The skull cap was penetrated by an antler pickaxe still embedded in the skull. Later analyses by Tornberg (unpublished) suggest that the individual suffered from an additional perimortem injury. The skull is dated to 2465-2200 BC (LuS 11847) by Tornberg and Jacobsson (2018).

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund : Univ.. Bonn

Brink, K. (2009). *I palissadernas tid: om stolphål och skärvor och sociala relationer under yngre mellanneolitikum*. Diss. Lund: Lund University, 2009. Malmö.

Tornberg, A. & Jacobsson, L. (2018). Care and consequences of traumatic brain injury in Neolithic Sweden: A case study of ante mortem skull trauma and brain injury addressed through the bioarchaeology of care. *International Journal of Osteoarcheology*. 28:188–198.

Vellinge, Skolan (LUHM 30187)

Excavated by the Swedish National Heritage Board division of Archaeological Investigations in 1990. The grave was oriented N-S and included remains from three individuals: a primary burial and teeth from an adolescent and a molar from an adult. Original osteological analysis by Caroline Ahlström Arcini.

Primary burial: The individual was buried in crouching position on the right side with the head to the south facing east.

Secondary burials: teeth, skull fragments, and foot bones from an adolescent. A molar with occlusal wear from an adult.

References:

Söderberg, B. (1993). *Skåne, Vellinge socken, Kv. Skolan RAÄ 17 1990 Arkeologisk för- och slutundersökning inom bytomt*. Rapport Riksantikvarieämbetet UV-Syd.

Viby (LUHM 88068)

Malmer mentions two graves, but the site includes three individuals.

Malmer grave 26 (I): funnel-shaped grave, oriented N-S with wooden construction. The individual buried on the left side with the head to the north facing east. **Finds:** amber bead, small animal bone fragments, a flint blade.

Malmer grave 27 (II): Oriented N-S with a stone packing. No finds.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund : Univ.. Bonn

Åraslöv 6 (LUHM 26761)

Malmer grave 68 (I): Oriented E-W with the head to the W. **Finds:** thin-bladed flint axe, antler pick-axe, small animal bone fragments, flint blade.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund: Univ. Bonn

Östra Torp 4 (LUHM 28423)

Malmer grave 107. Excavated by the Lund University Historical Museum archaeologist Bror-Magnus Vifot in 1939 after it was found and reported by the landowner. The grave was partly destroyed when Vifot arrived but measured approximately 2.2 metres and was covered with a cairn. Only the lower limbs were still in situ while the rest of the skeleton was found in commingled state in the disturbed grave filling. The bones in situ suggest that the individual was buried in crouching position, oriented NNE-SSW on the left side with the head towards NNE. **Finds:** Hollow-edged flint axe, 3 flint blades and 8 undecorated fragments of pottery.

References:

Malmer, M.P. (1962). *Jungneolithische Studien*. Diss. Lund: Univ. Bonn

Tornberg, A. & Jacobsson, L. (2018). Care and consequences of traumatic brain injury in Neolithic Sweden: A case study of ante mortem skull trauma and brain injury addressed through the bioarchaeology of care. *International Journal of Osteoarchaeology*. 28:188–198.

[Other Swedish samples](#)

Region Blekinge – Norjeskogen (BM)

The excavated grave was part of a large projects due to the construction of a new road. The excavations were done in collaboration between Blekinge Museum, Arkeologerna, Sydsvensk Arkeologi AB, Kalmar County Museum, and Småland's Museum in 2011-2013. The cemetery contained three BAC graves, of which one probably was a double burial, oriented N-S, with the individuals buried with their heads in each direction. None of the other two graves however contained human remains, which is why only the northernmost of the graves is included and described further here.

The grave was oriented E-W and measured 2.8x1.4 m with a stone packing. The grave was complex and cut through a Neolithic cultural layer. The individual was positioned in crouching position with the head to the west. The individual was mostly cremated with varying degrees of heat exposure. Parts of the body is missing. The interpretation is that that the individual was cremated in another site and then placed in the grave in relatively well anatomical order. **Finds:** pottery type N, burnt hollow-edged flint axe, amber and bone beads, decorated bone pendant, a flint blade, burnt hazelnut shells, and whetstone fragments. Most artefacts were burnt, but some of the amber beads were not.

Reference:

Rudebeck, E. 2021. Norjeskogen och neolitikum i Blakinge och Sydöstra Götaland. In: *Att leva vid Vesan. Arkeologi längs nya väg E22 i västra Blekinge*. Rudebeck, E. & Anglert, M. (eds.). Blekinge museum. 179-302.

Region Östergötland – Bergsvägen (ÖM)

The grave was found in 1953 during construction work for a new road at Bergsvägen in Linköping. The grave is a double burial of a male and a female as well as remains from a dog. Bones of an infant was also found in association with the grave but has turned out to be of later date (Malmström, Helena, forthcoming).

The grave was oriented E-W with the female buried on the left side with the head towards W, and the male buried on the right side with the head to the east. Both individuals were buried in crouching position and the remains of the dog, which evidence a perimortem injury to the skull, was positioned behind the back of the female. **Finds associated with the female:** Flint chisel, flint and stone axe, antler dagger, two ceramic vessels, beaver mandible, copper spirals, amber beads, bone awl, and a flint blade. **Finds associated with the male:** Battle axe, flint knife, and a spear-thrower.

[Danish sample](#)

Jutland, Gjerrild parish – Gjerrild (NM 704/56)

The Gjerrild stone cist was excavated in 1956 by National Museum curator C.L. Vebæk, assisted by two local helpers after human remains had been discovered in 1955. Finds of large stones had already been reported in the late 19th Century. The grave is situated in an area with high abundance of high-quality flint, which plausibly has made the area attractive to all different Middle Neolithic groups that inhabited the area, namely Pitted Ware Culture, Funnelbeaker Culture, and Single Grave Culture. The stone cist of Bøstrup type is characteristic for Late Single Grave Culture of NE Jutland and was found centrally in a mound. The cist was oriented roughly N-S and measured c. 2.6-3x1.7-1.8 m. The grave contained the remains of at least 14 individuals: 8 adults and 6 children.

Skeletons 6 and 7, both adult males, were buried in outstretched position beside each other with their heads in SW facing SE. **Skeleton 5**, a male child, was buried in crouching position on the left side with the head towards S. The individual was positioned on top of the lower legs of Skeleton 6. **Skeleton 8** is the well-preserved remains of a male child positioned in outstretched position along the E long side of the cist.

A concentration of bones, with some separations of individuals, were found in the NW corner of the cist and are referred to as “skeletal group”.

References:

Vebæk, C.L., NM & Balslev Jørgensen, J. 1956. Archaeological report. Panum.

Egkjord, A. F. H., Margaryan, A., Fischer, A., Sjögren, K. G., Price, T. D., Johannsen, N. N.,... & Allentoft, M. E. (2021). Genomic Steppe Ancestry in Skeletons from the Neolithic Single Grave Culture in Denmark. *Plos one*, 16(1), e0244872.

Section 2: DNA laboratory protocol

All sampling and DNA analysis was performed by the SciLife Laboratory in Uppsala, Sweden.

Sampling and DNA Extraction

Prior to sampling, the sample was decontaminated first by UV-irradiation (6 J/cm² at 254 nm) of each surface for 20 min and the exterior surface was removed mechanically using a Dremel tool. The sample was wiped with 0.5% sodium hypochlorite solution and UV-irradiated Milli-Q® water using sterile cotton swabs, and subjected to UV-irradiation again for each surface. A small sub-sample, weighing 50 - 150 mg was drilled from each sample using a Dremel tool fitted with a 2mm drillbit. DNA was extracted following a modified silica-based method, where 1 M urea was used in place of sodium dodecyl sulphate in the extraction buffer^{2,3}. For every 10 samples, one extraction blank was included as the negative control and carried along throughout the whole process. Briefly, the sub-sample was first pre-treated with 1 ml of 0.5 M EDTA (Invitrogen) and incubated at 37 °C for 30 min. The EDTA solution was removed and the sub-sample was digested with 1 ml of extraction buffer (0.44 M EDTA/ 1 M urea) containing 0.25 mg/ml proteinase K (Sigma-Aldrich) and incubated with rotation at 37 °C for 22 h, second incubation at 55 °C for another 6.5 h, followed by third incubation at 37 °C for 17 h. Supernatant was collected from the digested sub-sample and concentrated using an Amicon Ultra-4 filter unit (Millipore). The DNA extract was purified with MinElute PCR purification kit (QIAGEN) according manufacturer's instructions and eluted in a total volume of 110 µl EB buffer.

DNA Library Construction

Double-stranded blunt-end library for next generation sequencing was prepared from 20 µl of DNA extract according to protocol with some modifications⁴. MinElute PCR purification kit was used to clean up enzymatic reactions instead of SPRI beads. For every 10 samples, one extraction blank and one library blank (using water) were included as negative controls and 5 (8) carried along throughout the whole process till the quality control step. The DNA library was quantified by real-time quantitative PCR (qPCR) in a 25-µl reaction using Maxima SYBR green master mix (Thermo Fisher Scientific), 200 nM IS7 primer and 200 nM IS8 primer, to determine the number of indexing cycles. Dual-indexing PCR amplification of the DNA library was performed, in duplicates, in a 50-µl reaction using 6 µl DNA library, 5 U AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific), 1' GeneAmp Gold buffer (Thermo Fisher Scientific), 2.5 mM MgCl₂, 250 µM of each dNTP, 200 nM P7-indexing primer and 200 nM P5-indexing primer^{4–6}. The PCR reaction was performed at 94 °C for 10 min, 11 - 16 cycles of (94 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s), and 72 °C for 10 min. The duplicates of the indexed DNA library, i.e. sequencing library, were pooled and purified with Mag-Bind® TotalPure NGS (Omega). Quality and quantification of the sequencing library was analysed by the BioAnalyzer 2100 (Agilent).

Sequencing

Fifteen dual-indexed sequencing libraries from this project were pooled (27 libraries in total) in equimolar ratios and sequenced on a lane of an Illumina NovaSeq S4 flow cell using v1.5 chemistry and 150 bp paired-end reads, at SciLifeLab National Genomics Infrastructure in Stockholm.