DNA Analysis on a Viking-age boat grave from Sala hytta Västmanland, grave A2

Picture from the excavation site by Oscar Almgren 1907 (Almgren 1907:11)

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Abstract
Viking-age boat grave burials are a less common but still repeatedly used way to bury the dead during the late Iron Age. Boat burials are exceptional in many aspects, not only due to placing the individual in a boat with numerous burial gifts including animals, but also by burying the individual without prior cremation, a common practice during the Iron Age. The aim of this thesis is to genetically analyse inhumation boat graves and compare the genetic composition of the ancient individuals with modern populations through population genetic analyses. This will highlight these particular human remains in a mobility context. A total of 11 individuals was analysed, but only one yielded enough DNA for further statistical analyses. This one individual proved genetically exceptionally well preserved. The results clearly show that the individual (a female) has a genetic affinity to populations in northern Europe. However, the results do not discriminate between modern Baltic/Scandinavian populations, depending on the statistical test.

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1. Introduction

The Viking-age is the last part of the Iron Age in Scandinavia (although considered the Early Medieval period on continental Europe) and dates from the late 750 CE to the 1050 CE. A time often associated with lootings and warrior culture, but the Vikings also practised trade and colonization, and not only did they travel to the west, they also went east, down the Russian rivers as merchants and Varangians. But the vast majority of the population lived a local life as farmers. A contributing reason for the vast expansion was probably the development of ship building technique, it made it possible for the Vikings to travel longer distance (Brink & Price 2008:4; Holmquist 2002:153)

The ships had a great impact on Viking-age society (and also on the periods before the Viking-age) in many ways. Not only in economical, martial, and social perspectives but also in a spiritual way, by burying the dead in boats (Montelius 1886:149; Stjerna 1905:110; Crumlin-Pedersen & Munch Thye 1995:94; Nylén & Schönbäck 1994:128; Arrhenius 1997:175; Brink & Price 2008:170). This type of burial is called boat grave. Boat graves have long been of interest among archaeologists because of their rarity, often richness, and occurrence during a time (Iron Age) when cremation was the most common way of burial traditions (Schönbäck 1983:123).

Many studies have been conducted on the material from boat graves (Montelius 1886:149; Stjerna 1905:110; Crumlin-Pedersen & Munch Thye 1995:94; Nylén & Schönbäck 1994:128; Arrhenius 1997:175; Arwidsson 1983:75; Arbman 1980:22; Lindqvist 1921:83) etc., trying to answer questions like who were the individuals in the boat graves? What part did they play in society? And what were the criteria to be selected for burial in a boat grave? Maybe the individuals went abroad during their lifetime and were placed in a boat as a last resting place and to symbolise their travels? May it even be so that the individuals that were placed in a boat grave came from other places and therefore was given a boat to go back to where they came from? Or the boat might simply represent crossing over to the next life. Those are some of the questions that have made the burial tradition a bit enigmatic and mysterious. There is definitely a social, economic and spiritual aspect to the boat grave tradition that has to be considered when discussing this specific burial ritual.

How do we then approach the individuals that were given this unique final rest? One way is by conducting molecular studies on the individual, more specifically by preforming genetic analyses on the individuals buried in the boat graves and comparing the genetic results of the ancient individuals to modern known genetic populations (modern populations are the best proxy for ancient populations until there is enough ancient data for comparative analyses). In this thesis a total of 11 individuals, from three different boat grave cemeteries were genetically analysed. Four individuals from Tuna in Alsike Uppland, four individuals (RAÄ 40:2) from Sala hytta Västmanland and three individuals from Årby Turinge Södermanland (RAÄ 165:1) (se table 1). With this in mind I would like to point out that this study should be considered as an initial insight into the genetics in boat-cemetery context, and providing some additions to the understanding of the boat grave burial tradition (as it represents the first genomic data collected for a larger project) that is now being undertaken.

Table 1. Table listed graves and the sites that were used for genetic analyse in this thesis.

Boat grave cemeteries sites	Graves
Tuna i Alsike Uppland	Grave XIV, Grave 2, Grave XIII, ANL XI
Sala hytta Västmanland	A1, A2, A3, A4
Årby Turinge Södermanland	A8 II, A18 F3, A71 F66

2. Aims and research questions

The aim of this study is to get a further understanding of the boat grave custom. More specifically, this study focuses on the genetic origin of a specific individual and provides a possibility to highlight the occurrence or non-occurrence of migration in this specific case. Connected to this is the aim to relate the complete genome to the closest existing population. No general discussion of genetic admixture and migration during the Viking-age in northern Europe will be drawn upon the results, duo to the low representatives of the material analysed.

- Where did the individual (that was sampled and successfully analysed), genetically originate from when compared to modern populations?
- Can the genetic results be explained by migration, and if so where did the individual buried in grave A2 (the grave in Sala hytta that provided enough genomic material to analyse) migrate from?

3. Limitation

Only one out of 11 samples that was analysed, produced results. Therefore this study is limited to only this one individual. The sample that worked and could be used for further statistical analyses was from grave A2 in Sala hytta Västmanland. The sample from grave A2 is renamed to sal002 in this study, this is to be congruent with the ATLAS-database, where all the genetic data from this sample is stored under that label (sal002).

As Grave A2 have been dated to the Viking-age (see chapter Grave A2), most focus will be on that specific time period. But also briefly mentioned are the boat graves from Vendel (see chapter Boat graves in Sweden). This study will be geographically limited to the Västmanland County in Sweden, more specifically to the town of Sala, where the burial was excavated. A brief general introduction and problematisation to the phenomenon surrounding the boat graves will be given in the previous research part. Also, the excavation and the site will be presented (see Boat grave cemetery in Sagån Sala hytta Västmanland, but most focus will be on the specific grave where sample sal002 was taken from (grave A2).

As this study is limited to one prehistoric sample (sal002), no general conclusions will be drawn upon inhumation boat graves. The genetic result will be compared to recent modern human populations (see Population genetic analysis). This study will hopefully provide insight into which subject the discussion should be in, whether it is about migration, social structures or burial rituals etc.

4. Previous research

As the boat graves have fascinated archaeologist for more than a century, there are quit a number of studies on them. The first once utilizing ancient DNA were produced within the SIV framework. SIV, Svealand i Vendel- och Vikingatid was a program sponsored by Riksbankens Jubileumsfond, and executed at the Archaeological Research Laboratory and the department of Archaeology at Uppsala University during the 90's. Some of the genetic undertakings of the projects are published in SIV, Sevaland i Vendel –och Vikingatid. Studier från delprojekten vid Stockholms Universitet (1997). Helena Malmström (1996) preformed gender-related studies on individuals from boat graves in Badelunda and Alsike among them molecular sex identifications (Malmström 1996). And there were also genetic work on the boat cemeteries described in Anders Götherströms thesis (*Acquired or inherited prestige? :*

molecular studies of family structures and local horses in Central Svealand during the Early Medieval period, 2001). However, as all of this work was based on PCR targeting, and on single genes, the data is not useful in my analyses but to note that the boat cemeteries have been of archaeogenetic interest for over two decades.

4.1 Boat graves in general

The boat as an element in burial customs can be traced back all the way to the Stone Age in Scandinavia, in the form of single burials in logboats. And during the Bronze-age the boat can be observed in the form of boat-shaped stone setting. During the Iron Age the boat appears as an element in burial customs in the form of boat graves (Schönbäck 1983:123).

Boat graves are a type of burials in which the deceased have been placed in a boat. Often in a rich context, with burial gifts and sacrificed animals that have been placed within the grave. There are both cremations and inhumations. Individuals that are buried in boat graves have to some degree been interpreted by archaeologists as having had a prominent role in society (Schönbäck 1980:108) Both female and male adult individuals were buried in boat graves and there is also a regional and chronological difference that can be studied. And while the phenomenon is often viewed as a rich context, some boat graves are best described as "ordinary" or even "poor". The most expressing part for the complete complex is naturally the boat itself (Crumlin-Pedersen & Munch Thye 1995:92). Ole Crumlin-Pedersen and Munch Thye states in their paper:

"*Thus there is ample indication that boats have been used as an element in Scandinavian graves more or less continuously from the 1st to the 11th centuries, although not everywhere, not by everyone and not always in the same form"(Crumlin-Pedersen & Munch Thye 1995:92).*

The oldest boat grave, ever to be excavated in a northern European context, is dated to the Early Roman Iron Age (1st century) and is located on the Danish island of Bornholm, Slusegård. The Slusegård boat graves extended the chronological range of the burial ritual and ever since, several new sites have been discovered, and also some old discoveries have been re-interpreted as belonging to the boat grave burial custom (Crumlin-Pedersen & Munch Thye 1995:101). Müller-Wille (1974) divided burial rituals into two major groups, inhumation burials and cremations. Inhumation boat graves are found in Germany (Hedeby), Denmark,

Sweden, Norway, The Baltic countries, Great Britain, Scotland, Ireland and Iceland. Cremated boat graves have a more restricted range and are found along the Russian rivers, the coast of the Baltic countries, Finland, Sweden and Norway (Müller-Wille 1974; Hilberg, V & Kalmring 2014:223).There are almost 300 sites that include more than 420 boats graves, both inhumation and cremations (figure 1) (Müller-Wille 1974:187). As my main source-material is ancient DNA, and the DNA is destroyed in the cremation process, the cremation boat burials will not be addressed in this study.

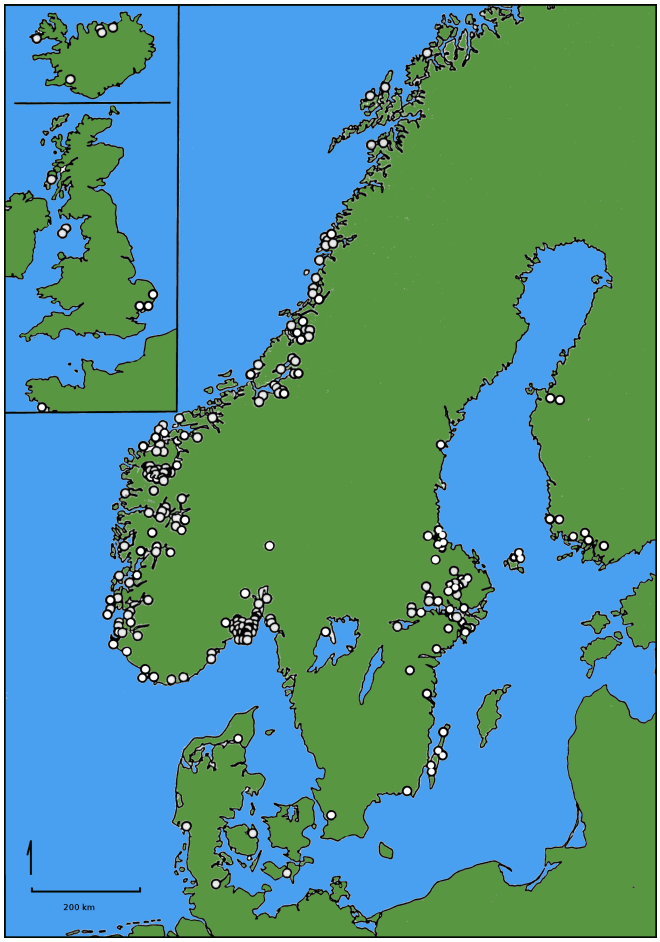

Figure 1. A map that shows the distribution of boat graves (both cremated and inhumation) in northern Europa. Modified from (Crumlin-Pedersen & Munch Thye 1995:100)**.** Note that the boat grave cemetery in Salme is not on the map since it was published after the map was produced.

4.2 Boat Graves in Sweden

In Sweden there are eleven known boat grave cemeteries (were the boat cemeteries at Norra Berga i Mjölby and Malm i Styrstad, Östergötland are visible but yet not excavated, therefore it is not possible to determine if the grave cemeteries are inhumation or cremated) with at

least two graves (see table 2). From the data in figure 1 and table 2, it appears as if the burial ritual had great impact on the Lake Mälardalen region (Hyenstrand 1996:103). But even if there is a strong cultural focus on the Lake Mälaren region, the phenomenon is also present in areas such as the counties of Blekinge, Öland, Småland and Södermanland in Sweden, mostly in the shape of single burials.

Maybe the most famous boat grave cemetery is the one in Vendel north of Uppsala, and it has also given the period its name (Vendeltid). When fist discovered they were thought of as burials of kings of Svealand (Nerman 1914:29), but since then the interpretation has matured due to continuous research, and the burial site in Vendel do not carry that exclusive label anymore (Arrhenius 1995:331). Åke Hyenstrand (1996) suggested that the boat grave phenomenon dates to the Migration and Vendel periods in Vendel and Valsgärde and should be connected to warrior graves due to the weaponry and armour (along the reasoning of Lundberg 1938:38 and Engström 1994:23),and that the burial ritual can be associated with the Frankish empire (Hyenstrand 1996:102). The fifteen known inhumations in boats at Vendel has been dated between the year's 550 CE to 1050 CE (Hyenstrand 1996:92) and the long use of the site is one of the reasons why archaeologist has suggested that power was passed down through kinship in many generations during the Vendel and Viking-age. It should also be mentioned that there are boat graves on both locations that have a contemporary dating. The boat graves during the Vendel period are often described as exclusive, but during the Viking-age they become more ordinary and simple (Schönbäck 1980:108).

The burials in Vendel were looted long before they were excavated. Thus, the find is only a fraction of what originally were buried at the site. Out of 14 boat graves, at least 9 hade been robbed and re-opened during ancient times. Alison Klevnäs (2015) suggest that the reopening of the boat graves in Vendel could be associated to the Christianization and that the skeletal remains could have been reburied in a Christen burial context (Klevnäs 2015:1). Whether this is the case or not, the material is poorer preserved than what is ideal for analyses. And this is actually true for several of the boat graves. Nevertheless, four individuals from Alsike (Grave XIV, Grave 2, Grave XIII, ANL XI) and three from Åby, Turinge (graves; A8 II, A18 F3, A71 F66) were originally a part of the material I extracted. But as they all yielded poor results unsuitable for genomic analyses, I focus on the material from Sala hytta from her on.

Table 2. Inhumation boat grave cemeteries in Sweden, their location, number of boat graves and also their current excavation status. The boat grave cemeteries in Norsa in Köping and Skamby in Kuddby församling could still also contain cremated boat graves, hence their current status.

County	Parish	Number of boat graves	Status
Uppland	Vendels kyrkbacke	14	All have been excavated
	Valsgärde i Gamla Uppsala	15	All have been excavated
	Gamla Uppsala prästgård	4	All have been excavated
	Ultuna i Bondkyrko	2	All have been excavated
	Tuna i Alsike	10-12	All have been excavated
Västmanland	Tuna gravfält i Badelunda	8	All have been excavated
	Norsa i Köping	15	Only one have been excavated
	Sala Stad, Sagoån	4	All have been excavated
Östergötland	Skamby i Kuddby församling	10	Only one has been excavated.

4.3 Problematisation

Much of the work on the boat graves have either related to who were buried in them or why they were buried on boats. Already at an early stage (Montelius 1886:149; Stjerna 1905:110, and later Arbman 1980:19; Arwidsson 1980:45). But also the first attempts on archaeogenetics on these burials related to who they were (Malmström 1996; Götherström 2001). Oscar Montelius (1886) and Knut Stjerna (1905) argue that the boat burial custom practiced by a group that shard the same religious belief and base there idea on tales about the Nordic pre-Christian gods, the description written by Ibn Fadlan, ethnographic data and specific grave features (Montelius 1886:149; Götherström 2001:1; Stjerna 1905:110)

Ole Crumlin-Pedersen (1995) considers the individuals buried in boat graves to be a part of a religious fertility cult and that these individuals had held a special position: priests and priestesses (Crumlin-Pedersen & Munch Thye 1995:94). A similar idea was applied to the boat grave cemeteries in the Lake Mälaren Valley by Bengt Schönbäck (1994). He argues that the division of male and female boat grave cemeteries is an expression of the fertility cultural tradition. He also suggests that the boat graves had been reserved for individuals with a special position in the adult world, as no children have been buried in this way (Nylén & Schönbäck 1994:128). Birgita Arrhenius (1997) also suggests a religious explanation in which the boat graves expresses the presents of a Freja cult and can be traced back to the Egyptian Isis cult (Arrhenius 1997:175).

There is also the interpretation that the boat graves represent the burials of higher strata in the society and that the boats were of a secondary meaning. Holger Arbman (1980) suggests that the boat grave cemeteries from Vendel, Ultuna, Tuna in Alsike and Valsgärde was created by dominant farmers, or more like petty kings and the burial tradition was past down from generation to generation (Arbman 1980:21). Greta Arwidsson (1980) is of similar opinion as the boat graves in Valsgärde expresses wealth and the long continuity use of the tradition which demonstrates stability in social organisation during that time (Arwidsson 1980:45).

Oskar Lundberg (1938) interprets the burial custom as not having any greater symbolic meaning, instead he argues that the burial is an expression in what roll the individual had when he/she was alive. He further suggests that the individuals buried in boat graves was part of the "Ledung" military naval organisation (Lundberg 1938:36). Johan Engström (1994) supports this idea to some degree, at least the military aspect of it. He thinks that buried individuals were apart of a military aristocracy, more specifically assigned to the offensive cavalry. But he fails to explain the element of the boat. His interpretations are based on the weapons and armoury placed within the graves, and naturally the occurrence of horses in them (Engström 1994:23).

Björn Ambrosiani (1983) argues that the buried individuals neither held any political position, nor were they part of any military organisation. He claims the wealth in the Vendel boat graves is best explained by controlling of trade routs to the north (Ambrosiani 1983:18). This idea was criticised as the geographical location of Vendel is not suitable for controlling the trade to the north (Isaksson, S. & Seiler 1997:69). The individuals in the boat graves have also been interpreted as "princes or left-over sons" returning home from travelling abroad (Steuer 1989:100; Hyenstrand 1996:92).

When reading the previous chapter one can get the impression that the boat grave custom is "homogenous" and vast generalisations can be drawn upon the burial tradition. Kerstin Lidén, Sven Isaksson & Anders Götherström looked for dissimilarities and regional traits by studying the boats, kitchen utensils, the individuals in the boat; sex, relations to one another and how they had been deposited. They concluded that these cemeteries had been grouped together based dominantly on the boat used in the burials and that it was probable the only common denominator the cemeteries shared (Lidén, K. Isaksson, S. & Götherström 2001:27).

There is great difference between the boat grave cemeteries. One obvious is the representation of the biological sexes in the cemeteries. In Vendel and Valsgärde there were, as far as we know, only males buried in boat graves, in contrast to the boat grave cemetery in Tuna in Badelunda where only females were buried in boat graves. And in Tuna in Alsike both females and males had been deposited in boat graves (Lidén, K. Isaksson, S. & Götherström 2001:27). In that aspect the boat grave cemetery Sala hytta in Västmanland is (were sample sal002 was extracted from) more similar to the boat grave cemetery in Tuna in Alsike, with both male and female boat grave burials present (and interestingly, Tuna in Alsike may have several contemporary Viking-age boat burials, just like Sala hytta).

Note that in addition to the studies that is presented in this chapter, there are several other relevant studies concerning the boat burial tradition; Arne 1934, Bischoff & Sørensen 2001and Seiler 2001 among others. The latter of these illustrates the important work performed within the SIV project where much of the focus was on boat burials.

4.4 The boat grave field in Sagån, Sala hytta, Västmanland (figure 2)

As mentioned earlier the boat burial cemetery in Sala hytta is more similar to the boat cemetery in Tuna in Alsike in the aspect of both males and females being buried on the same burial grounds. However, at the boat burial cemetery in Sala hytta logboat-based burials had been used. Interestingly, logboats had also been used at the boat grave cemetery at Tuna in Badelunda, where only women have been buried in boats (Nylén & Schönbäck 1994; Larsson 2007).

During the summer of 1901, when the dam from the 16th century was broken and had to be removed, what must be described as a remarkable find was exposed from the mud. A total of 4 graves, buried on the island of Brytilsholmen in the middle of the Sagån River (figure 3), emerged. The first archaeologist on site (Oscar Almgren) concluded that the find was from the Viking-age period. At least two of the individuals had been buried in logboats (hollowed logs of oak)(Almgren 1907:1; Larsson 2007:52).

Unlike the boat grave cemetery in Vendel and Valsgärde, the boat graves in Sagån, Sala hytta in Västmanland have all been interpreted to have had a more contemporary dating to one another, where grave A2 has been dated one hundred year earlier than the rest of the graves. Oscar Almgren dates the graves to the first half of the Viking-age, i.e. to the early 9th century.

The dating of the graves is based on the oval brooches found in the graves (Almgren 1907:17). The topography of *Sagån* and the island of *Brytilsholm* have probably changed considerably since when last used as burial grounds. The graves were likely dug on dry land and were not initially immersed in water. Since then topographical changes, both natural and influenced by humans have likely affected the burial site. Thus the well preserved wooden finds can only be explained in the light of the wet conditions provided by Sagån (Almgren 1907:18).

When excavation of grave A1 was completed, it was confirmed that the individual had been placed in a logboat. The south end of the boat had been damaged, but from the description of the construction workers, that end would have ended one meter from the undisturbed part of the boat. That would have made the length over 4 meters. It was about 0.65 meter wide. The individual had been placed with the head facing the south and the skull was crushed. Within the grave there were a couple of oval brooches, equal-armed brooch, a bronze chain and four pendant ornaments of bronze were found. A total of 57 beads and a pearl of crystalline substance were excavated. East of the southern part of the boat remains from a dog, a sheep and chicken were found (Almgren 1907:3; Larsson 2007:52).

A couple of meter east of grave A1 a third grave was excavated. In the grave there were a pair of oval brooches, of the same type as that excavated in grave A1. And also a equal-armed brooch much similar to the one found in grave A1, but with some differences. Two pendants of tin and four glass beads were also encountered. Similarities to grave A1 are further emphasised due to the finding of dog remains at the feet of the buried individual. But there are differences to grave A1 too as the buried individual in grave A3 had been placed in a wooden coffin instead of a boat (Almgren 1907:7).

In grave A4, to the left of the skeleton, the remains of an iron knife, fragments of arrowheads and a well preserved arrow shaft, a bone comb and a wooden pallet were found. Under the pallet skeletal remains of a sheep were found. There are is still some uncertainties regarding the grave construction. The wooden remains excavated in grave A4 have both been interpreted as the remains of a coffin as well as a stretcher. Oscar Almgren believed that the buried individual in grave A4 was a male and that the grave did not contain any dateable items (Almgren 1907:10).

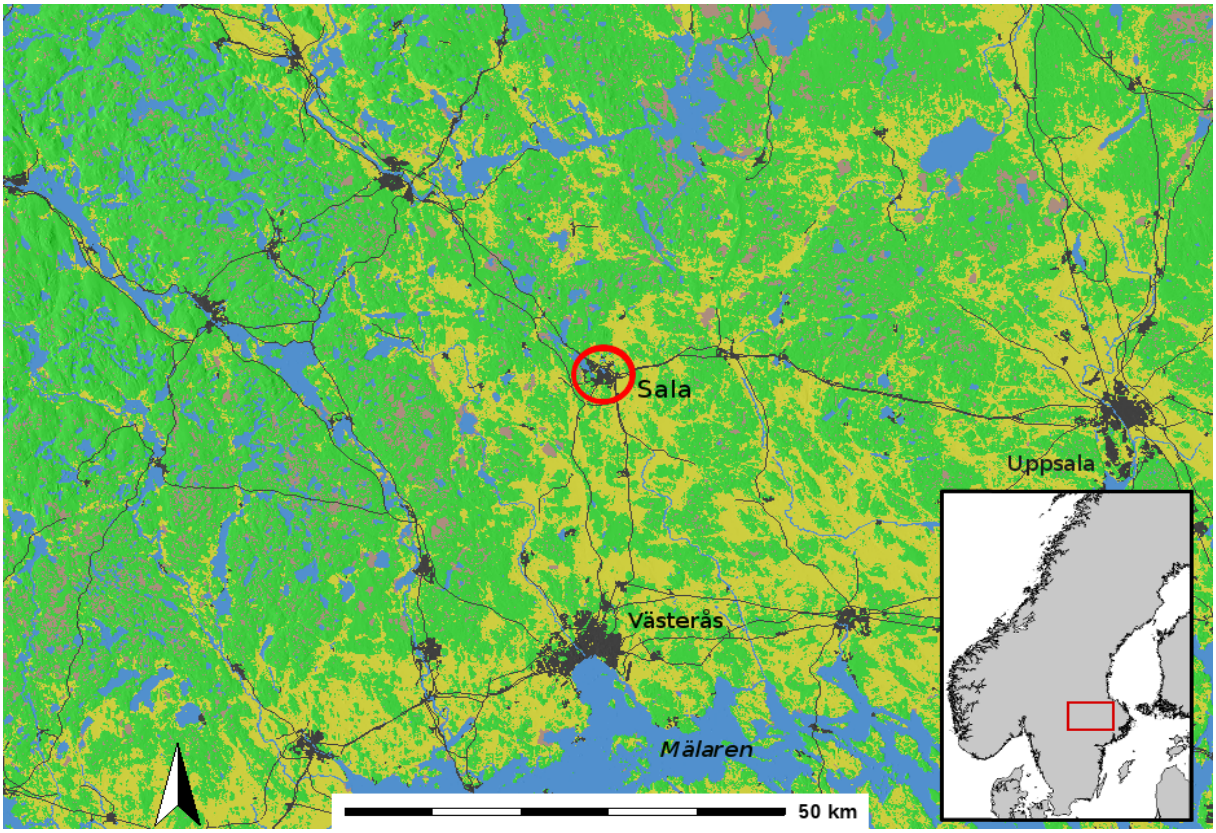

Figure 2. A zoomed in map of the town Sala in Västmarland. Created from data: GSD-Road Map, vector © Lantmäteriet (2017) and GSD-Elevation data, GRID 50+ © Lantmäteriet (2015).

4.5 Grave A2, Sala, Västmanland (sal002)

Mentioned earlier the sample taken from grave A2 was named to sal002 to remain compatible with the classification used in the Atlas project. Figure 4 illustrates the boat grave cemetery on *Brytilsholmen* with grave A2 marked in red. The individual in grave A2 was placed in a stretched out position on the back with the head pointing to the west. The logboat was about 4.5 meter long and 40 centimetres wide. Two oval brooches and an equal-armed brooch hade been placed on the individuals chest (Almgren 1907:5). The two oval brooches have been assigned to the *P51* type, though not identical, but rather two different variants of the same type (figure 4). The *P51* is the most common oval brooch from the Viking-age. The type has a wide distribution in northern Europe, all the way from Ireland and Iceland in the west to Dnieper and Volga rivers in the east. Due to the wide spread and the different variants it is hard to identify where the manufacturing of *P51* have occurred. It is likely that *P51* brooches have been manufactured in different late iron-age settlements in the northern part of Europe. The ones found in grave A2 Sala hytta Västmanland have been dated to the first half of the Viking-age (Almgren 1907:18). The equal-armed brooch belongs to type *P71* (figure 5) witch

had flat ends and has a similar dating as the oval brooches (Almgren 1907:18; Jansson 1985:67, 208). At one of the ends of the boat a wooden box was found. It had a lid on but was found empty (Almgren 1907:6).

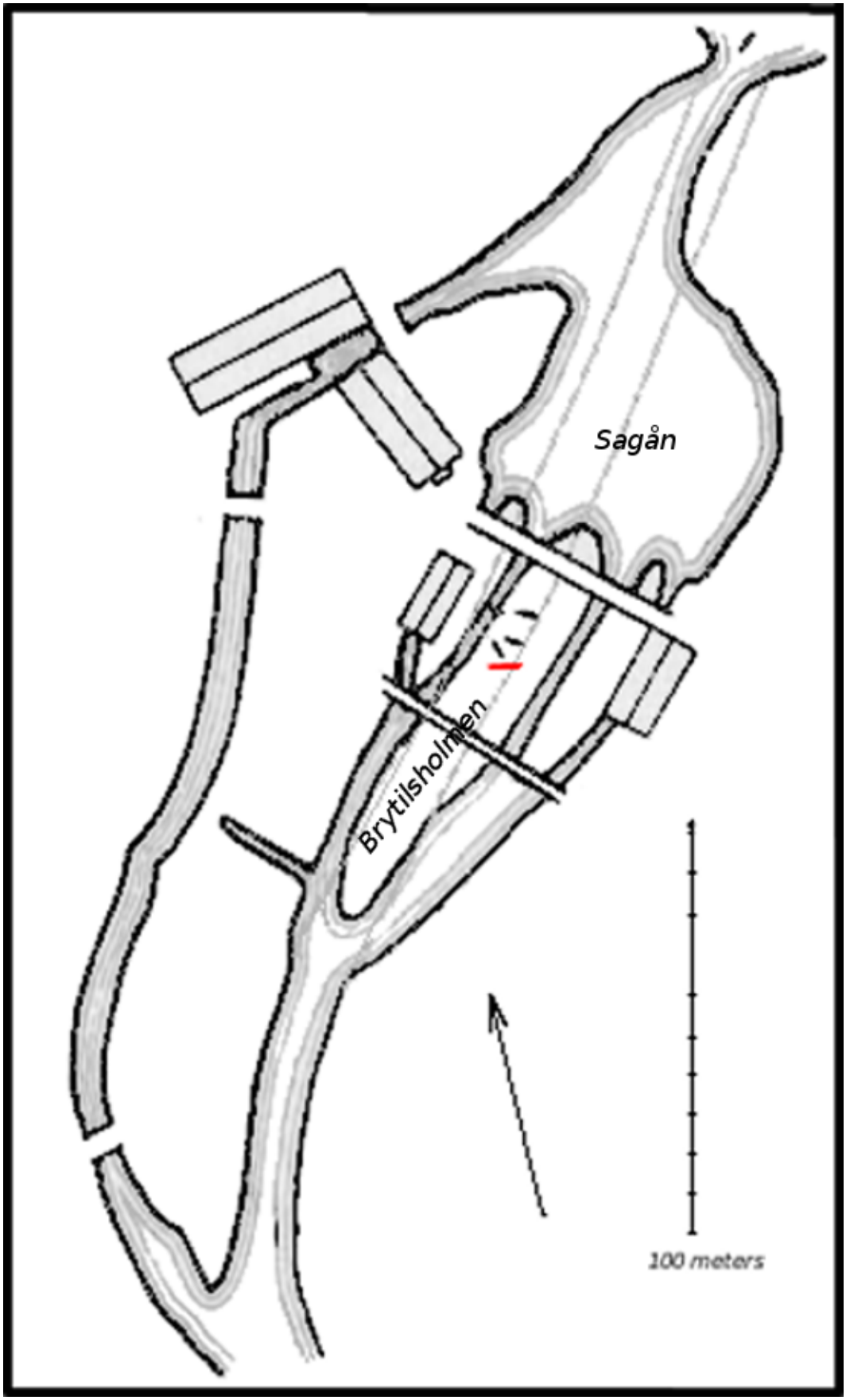

Figure 3. A modified overview of the burial site by Oscar Almgren 1907. The red marking illustrates the location of grave A2.

Figure 4. Oval brooches found in grave A2, Sala hytta, Västmanland.

(http://mis.historiska.se/mis/sok/fid.asp?fid=617647&page=2&in=1)

Figure 5. The *P71* equal-armed brooch found in grave A2 Sala hytta, Västmanland.

(http://mis.historiska.se/mis/sok/fid.asp?fid=372572)

5. Ancient DNA

The field of Ancient DNA (aDNA) studies can broadly be defined as retrieving DNA from biological material post-mortem, often the level of DNA fragmentation is more defining than the actual age. Obtaining DNA from ancient samples is challenging, due to the low amount preserved and the high degradation of the molecule (Pääbo et al. 2004:647). Degradation of the DNA molecule begins when the cell dies and when the enzymatic repair system no longer functions (Lindahl 1993:709). There are several different biomaterials that can be used to extract aDNA, including bone (Hagelberg et al. 1989:485), teeth (Drancourt et al. 1998:12637; Meyer et al. 2000:87; O'Rourke et al. 1996:557), hair and nails (Gilbert et al. 2004:463), intestinal contents (Rollo et al. 2002:12594), coprolites (Poinar et al. 1998:402), soft tissue (Ermini et al. 2008:1687; Rollo et al. 2006:557), plant seed (Rollo et al. 1987:501; Rollo et al. 1991:193) and sediments (Willerslev et al. 2003:791).

Ancient DNA analysis is one of the most direct methods for investigating genetic events from the past. The information obtained from ancient DNA enables the possibility to study past demographic events, like population origins, changes in population size, population variability, migration, expansion and to detect natural selection (Der Sarkissian et al. 2015:370; Ho & Gilbert 2010:1; Knapp et al. 2015:4; Orlando et al. 2015:395; Shapiro & Hofreiter 2014:343; Velasco 2015:3)

6. A Historical overview of the field of Ancient DNA

The history of the field (ancient DNA) began in November 1984 (at least in Europe and the Americas, there were earlier Asian claims). The researchers had managed to extract and sequence a 229 bp mitochondrial DNA fragment from a museum sample, using dried muscle tissue. The sample was of an extinct species, quagga (a form of zebra)(Higuchi et al. 1984:282). The study used the Sanger sequencing method based on chain-terminating inhibitors and this was the beginning of a new interdisciplinary research field (Sanger et al. 1977:5463), bringing together archaeologists, biologists, historians, geneticists, bioinformaticians, anthropologists and palaeontologists all collaborating together under the same scientific umbrella, seeking a common and deeper understanding of the past (Sandoval Velasco 2015:4). The following year Svante Pääbo (1985) managed to clone DNA fragments extracted from a 2400-year-old child mummy (Pääbo 1985:644). Also the same year researchers managed to extract DNA from ancient plant tissue (Rogers & Bendich 1985:69). Mammoth tissue was also extracted and compared with modern elephants (Johnson et al.

1985:1045). All of these studies contributed to the development of the field of ancient DNA and was thought of as ground-breaking work. However, the actual breakthrough, or as some researchers would call it "*the first great revolution in molecular genetics"* came the same year with the introduction of the polymerase chain reaction (PCR). It was now possible to amplify small amounts of DNA and generate thousand to million copies of that specific DNA sequence (Saiki et al. 1985; Mullis et al. 1986). Consequently, the PCR techniques caused the field of ancient DNA to grow rapidly and more studies of extinct species appeared (Cooper et al. 1992:8741; Hagelberg & Clegg 1991:45; Hoss et al. 1994:333; Höss et al. 1996:181; Thomas et al. 1989:465)

During the years 1994 and 1995 the field of ancient DNA suffered setbacks. Some researchers claimed to have successfully extracted DNA from a bone fragment dating to the cretaceous period (80-million-years-old) (Woodward et al. 1994:4541). The DNA extracted from that particular study was later proven to be human contamination (Zischler et al. 1995:1192). The following year Raul Cano & Michael Borucki (1995) and a team of researchers claimed to have had successfully extracted bacterial DNA from an insect trapped in an amber, sounding a lot like the movie Jurassic Park (Cano & Borucki 1995:1060). The results from the Cano et al. 1995 study could not be reproduced and the study's validity was challenged (Austin et al. 1997:303).

Despite the setbacks the field continued to grow and in the year 2001 two independent studies managed to sequence the complete mitochondrial genome of an extinct moa species (Cooper et al. 2001:704; Haddrath & Baker 2001:939). Due to the risk of contamination with modern human DNA, the major focus in the field of ancient DNA was on non-human mammals. When contamination could finally be excluded the field of ancient DNA took ground-breaking steps and made it possible to study human populations, migration and evolution (Velasco 2015:6).

During the early days researchers focused their studies on short fragments of mitochondrial DNA. Mitochondrial DNA is only inherited on the maternal side and does not recombine. It is often used to compare genetic relatedness among species and populations and has a high rate of evolutionary change. And when the DNA quality is such that only mtDNA is retrievable (as it is abundant in higher proportions than nuclear genes), it can be used to seek relatedness among individuals (Bunce et al. 2005:9;Velasco 2015:4; Hofreiter et al. 2004:40).

It is within this experimental context that the early archaeogenetic attempts on boat graves were made. Those were mainly aimed at single nuclear genetic markers (Malmström 1996; Götherström 2001). And although several of them are likely reliable as authentic ancient results, the statistical power is such in genomic single markers that they are not useful for the analyses that has been applied to sal002.

In the year 2005 came "*the next revolution"* in the field of ancient DNA with the introduction of the High Throughput Sequencing (HTS). This new technology offered less time consuming and more cost efficient sequencing strategies, but more importantly the massive amount of data generated by HTS technology made it possible to approach issues such as authenticity and nuclear genomics, that had virtually been impossible to work with previously (Margulies et al. 2005:376). The high throughput sequencing technology allowed sequencing across entire genomes, in contrast to the PCR method where only specific sites on the DNA molecule were targeted by adding artificial primers. Before HTS sequencing, the DNA is converted into library. During the process artificial adapters (synthetic DNA molecules with known base-composition) are added on to all DNA fragments in the sample (see chapter Library Building). The preparation protocol of sequencing libraries is almost the same for ancient samples as it is for modern DNA, except from the shearing step that is not needed when working on ancient samples because the majority of the DNA is already fragmented (Poinar et al. 2006:392; Stoneking & Krause 2011:603; Skoglund 2013:31)

This historical overview is only a brief summary. There are several more studies that contributed to development to the field of ancient DNA; Hagelberg et al. 1989, Gill et al. 1994 and Krings 1997 among others. Methodological and technological improvement has always driven the field of ancient DNA to development. No innovations had greater impact on studying aDNA than the PCR and HTS (but note that these were not exclusively ancient DNA techniques, but relates to the whole field of molecular genetics). It changed the field in a revolutionized way, yet still there are improvements being done, like DNA extraction techniques, library building methods, DNA enrichment and bioinformatics challenges etc. that continue to develop the field of ancient DNA.

7. Authentication & Damage pattern

When working with ancient samples a set of authentication criteria are used to minimize the risk for contamination (Cooper et al. 2000:1139; Gilbert et al. 2005:541). Physically isolated

working areas, negative controls and results that can be reproduced are some of the criteria that were used in this study, but this does not rule out contamination nor does it guarantee authentic results. How then do we know that the sequenced data yield from an ancient sample?

To distinguish between endogenous DNA and contamination; fragmentation patterns and nucleotide misincorporations are used for verification of authenticity. These natural damage patterns can be divided into to two different chemical processes; depurination and deamination (Briggs et al. 2007:1461).

Depurination occurs when a N-glycosyl bond between a sugar and a purine base (adenine or guanine) is lost, resulting in the breakage of the DNA strand. Mapping the aDNA to a reference genome and aligning both sequences to see if the cleaving/cleavage of the ancient sample has occurred at a purine base will detect this damage pattern. This pattern is unique for ancient DNA, and if compared with modern fragmented DNA, the patterned would not be the same. Depurination will change the structure of the DNA molecule resulting in loss of information, and can be used as one of two markers to determine if the sequenced DNA is of ancient origin (Krause et al. 2010:231).

The second damage pattern used to determine if the sequenced DNA is of ancient origin is deamination. Deamination occurs when a methyl group in cytosine is lost resulting in a nucleotide misincorporation. Cytosine is then misread as a thymine in the 5´-ends, and a guanine as a adenine in the 3´-ends of the DNA strand (C-to-T and G-to-A) (Hofreiter et al. 2001:4793). Although deamination can be observed throughout the entire DNA strand it occurs 20 times more often at the end of the sequenced DNA fragments, provided that the sample is of ancient origin (Briggs et al. 2007:1461). The unique characteristics of deamination are thus used to determine authenticity of ancient samples, and especially to distinguish between ancient human DNA and modern human DNA contamination. This is possible because deamination is nearly absent in modern human DNA (Krause et al. 2010a:231; Krause 2010b:11)

Chemical modifications of the DNA molecule, such as depurination and deamination are used to distinguish ancient endogenous DNA from modern exogenous DNA. Most recently published studies on ancient DNA uses these damage patterns to stress that their results are

authentic and of ancient origin. What once limited the field of ancient DNA (contamination, DNA damage and fragmentation) is now possible to either overcome or used to its advantage.

8. Contamination

Contamination can occur when an organism has died and the DNA repair mechanism disappears with time and eventually becomes non-functional (Lindahl 1993:709). Environmental, microbial and several other different sources of contamination are high risk of contaminants for ancient samples.

Other species DNA and also microorganisms that inhabit the tissue after death can contaminate the sample. This type of contamination have been shown to come from the soil where the sample ones came from (Yang & Watt 2005:332). The consequences of sequencing microbial DNA contamination are that it can reduce the proportion of human DNA in the sequenced sample and thus increase sequencing cost (Velasco 2015:22).

Contamination from modern DNA is a major risk when working on ancient human samples and hominines. Much precaution must be taken when the risk of contaminating the ancient sample with a modern counterpart (Velasco 2015:22). Contamination from modern DNA can happen at any point during excavation, storage and when the material is being processed (Götherström & Lidén 199856; Hummel 2003:131; Yang & Watt 2005:332; Linderholm et al. 2008:5; Velasco 2015:22). These days researchers use software to detect sequences that have unique damage patterns characteristic for ancient DNA (see chapter Authentication and Damage pattern) and that are nearly absent in modern DNA (Skoglund et al. 2014:2229). There is also the risk for *cross*-contamination when working on multiple samples at the same time. Contamination can also originate from manufacturers of lab equipment, chemicals and previous samples that were run on the same equipment (Götherström & Liden 1998:56). There is also the risk of ancient cross contamination, between ancient individuals (even though it hasn't been observed on ancient samples before). Ancient cross contamination can occur either before, during or after the individual has been buried. Burial rites and movement of DNA within the burial environment provides some of ancient cross contamination (Brown & Brown 2011:138). One can imagine that the risk of exchanging genetic material between ancient individual is higher in a grave with multiple individuals. However, ancient cross contamination can be detected be estimating the contamination level of the mitochondrial

DNA (Green et al. 2008:416). Overall, contamination is more of an economical problem than a scientific, as we are able to discriminate contaminants with damage patterns.

In this study separated dedicated ancient DNA laboratory facilities with positive air pressure, protective clothes and facemasks, negative blanks and also UV-light irradiation etc. were some of the criteria used to avoid and detect possible contaminants

9. Preparation before extraction

All samples were documented with photographs and placed in new plastic bags. All samples were renamed with three letters and three digits. Before drilling all samples were UV irradiated with 0.5 J/cm^2 at 254 nm on each side. Approximately 1 mm was removed from each sample surface by grinding with the drill. This was done to minimise the risk of dirt and contamination and also contamination from people who had handled the bone samples earlier. All drilling was conducted under a flow hood in the drilling room at the dedicated ancient DNA laboratory at the Department of Archaeology and Classical Studies, Stockholm University, Sweden. Protective clothes were worn all the time and the hood was cleaned using 1% solution of bleach or DNAway (Thermo Scientific), dH_2O (H_2O purified to the level of distillation, here to the level of double distillation) and cleaned with 70% ethanol. The drills were changed between every sample (full protocol in Appendix 1). After drilling, the powder was placed in a 1,5 ml eppendorf tube and weighed to check that no less than 100 mg of bone powder had been collected from every sample.

From sample sal002 DNA was extracted from the mandibule (figure 6). There still are bone materials from all the individuals used in this study (see table 1) left for further analyses.

Figure 6. The bone material (excavated from grave A2 Sala, parish, Västmanland) were sample sal002 were extracted from.

10. Extraction

When extracting DNA from ancient samples it is necessary to select an extraction method that will recover a high amount of DNA as possible and at the same time remove PCR inhibitors, contamination and impurities.

There are several different approaches when extracting DNA from ancient samples. A comparative study of different extraction methods was performed by Rohland & Hofreiter (2007a) and it clearly shows that using a buffer consisting of EDTA (ethylenediaminetetraacetic acid) and *Proteinase K* for bone digestion and binding the DNA to SiO_2 (silicon dioxide), out performs all other methods in terms of yielding as much DNA as possible, and with a high purity (Rohland & Hofreiter 2007:1757). However, the method calls for a pH-adjustment step using HCl (hydrochloric acid). This step is by many researchers viewed as possible source of contamination. Therefore, a silica-column based method was used instead to extract DNA from the samples (Yang et al. 1998:331) and the protocol was modified by adding Urea to the extraction buffer (Svensson et al. 2007:378).

Thereafter purification of DNA was preformed using MinElute PCR Purification Kit (Qiagen) that allows direct purification of double-stranded PCR products. The kit is provided with a MineElute Spin Column that is uniquely designed with a silica membrane (figure 7) and a binding buffer that provides the correct salt concentration and pH so that the silica membrane can absorb the DNA and bind it. The column has a maximum binding capacity of 5 μg DNA and can recover 80 % of DNA from fragments as small as 70 base pairs up to fragments as long as 4000 base pairs. This means that DNA fragments that are shorter than 70 base pairs will not be absorbed to the silica membrane and will be lost during the purification step. This can be a serious disadvantage during extraction of DNA from ancient samples. The extraction took a total of two days' work and can be divided into 4 main steps; release, absorption, washing and elution.

Figure 7. MinElute Spin Column (http://www.gatcat.com/pcr-clean-up/qiaquick-pcr-purification-kit)

10.1 Release

The Yang-Urea buffer contains both 0.5M EDTA pH 8 and 1M Urea, where the EDTA inhibits nuclease activity and decalcifies the bone-material and the Urea is used to enhance the Proteinase K activity (although it is also a chaotropic agent and thus a protein denaturant). Also included in this digestion buffer is Proteinase K that is a broad-spectrum serine protease. Proteinase K can digest keratin and has an optimum at pH 8 and temperature optimum at 55 °C.

1 ml of the Yang-Urea buffer was added to the bone powder and 10 μl Proteinase K (10mg/ml). It was then vortexed and incubated at 38-55 °C in a hybridisation oven overnight.

Additional 10 µl Proteinase K (10mg/ml) was added on to the sample the day after and incubated at 55 °C for two more hours.

10.2 Adsorption

Adsorption step depends on the ability of the DNA to bind to the silicon dioxide, SiO_2 membrane. Nucleic acids can only be adsorbed to the silica surface in presence of a high concentration of chaotropic salts. This is done by adding a binding buffer (Buffer PB). Buffer PB contains a high concentration of chaotropic agent guanidine hydrochloride isopropanol (GuHCl). The PB buffer will provide the correct salt concentration and pH so that the DNA can be adsorbed to the silica membrane in the MinElute Spin Column (but as GuHCl is a chaotropic agent, it also helps to degrade the bone).

10.3 Washing

Buffer PE, which is mainly ethanol, is used to remove unwanted primers and impurities. The MinElute Spin Column is washed with 710 µl of PE Buffer and spun down at 14000 rpm for 1 minute. This is done twice. Any remaining Buffer PE is removed by an extra centrifuge step.

10.4 Elution

When eluting DNA from the silica membrane an Elution Buffer (EB) is used. The Buffer EB contains 10 mM Tris·HCl to set the pH to 8.5 (which is suitable for the other reactives). Elution is most efficient under basic conditions and low salt concentrations. 55 µl of EB Buffer was added on to the MineElute Spin Column, incubated for 10 minutes at 37°C and spun down at 14000 rpm. This was done twice. 110 µl of DNA extract was then collected and placed in new eppendorf tubes for storage in a freezer at -20°C (Schatz & Officer 2008).

Full protocol available in Appendix 2.

11. Building Libraries

Ancient samples are often degraded; containing damaged and fragmented DNA molecules or contamination. This have in some way inhibited the development of ancient DNA studies (Knapp & Hofreiter 2010:227). Therefore methodological improvement has been necessary.

The field of ancient DNA changed dramatically and reached a turning point when next generation sequencing (HTS) was introduced to the field. This made it possible to study whole genome data by DNA library preparation from genomic DNA.

The main purpose for library building is to attach adapters to the DNA molecules so that they can be sequenced on Illumina Genome Analyser platform for highly multiplexed target capture and sequencing (Meyer & Kircher 2010). This will in turn extend and increase the amplification availability of the DNA molecule. Sample specific barcoding (indexes) is placed within the amplification primers instead of being attached at the ends of the templates molecules. Libraries were built according to the protocol described in Meyer & Kricher (2010) with some modifications and was conducted in the dedicated ancient DNA laboratory at the Department of Archaeology and Classical Studies, Stockholm University, Sweden. For this study the MEYER oligos and NEBNext E6070L protocol was used. This protocol can be divided into 4 main steps; Blunt-End Repair, Adaptor ligation, Adaptor fill-in and Library PCR1.

11.1 Blunt-End Repair

This step repairs the ends of fragmented DNA and prepare the ends of the DNA strand, so that the adaptors could be attached. Marcela Sandoval-Velasco (2015) describes this step as polishing the 5' and 3' ends of the DNA fragments using DNA polymerase and a polynucleotide kinase (Velasco 2015:30), by adding a Blunt-End Repair master mix to the DNA (Figure 8). Then the samples was mixed and incubated in a thermal cycler for 15 minutes at 25°C followed by 5 minutes at 12°C and then purified the samples using MinElute (Qiagen).

11.2 Adaptor ligation

In this step the adaptors are attached to the DNA. However, not the whole length of the adaptors is attached to the DNA in this step. Instead, attaching the adaptors to the DNA is divided into two steps (Adaptor ligation and Adaptor Fill-in). This is done to prevent adaptors attaching in the wrong direction of the DNA molecule (Figure 8) (Meyer & Kircher 2010). A master mix were prepared that was mixed with the DNA sample. It was then incubated for 30 minutes at 22°C and purified using MinElute Spin Column.

11.3 Adaptor Fill-in

In the Adaptor Fill-in step the adaptors will be filled up to their full length. A master mix was prepared and mixed with the DNA and incubated for 20 minutes at 37°C then incubated for 20 minutes at 80°C.

11.4 Library PCR1

Indexes were added on to the one of the adaptors for each sample. Samples are then pooled together and sequenced on one single lane. Using a full single lane for one sample is not cost efficient and will produce excessive amount of data that in most cases is not needed (Velasco 2015:31). Therefore it is important to be able to sequence multiple samples on one single lane. As mentioned earlier the indexes are sample specific, therefore it will be possible to identify the correct sequence to the right sample. Indexes are available from 1-228 and less than 4 indexes per sequencing lane should not be used (Meyer & Kircher 2010). A master mix was prepared and added on to the DNA samples. Indexes were added on to each and every sample separately. The samples were then placed on a PCR machine with the following temperature profile:

Initial denaturation	94°C	12 min	
Denaturation/cycle	94°C	30 sec	} 12 cycles
Annealing/cycle	60°C	30 sec	
Elongation/cycle	72°C	45 sec	
Final extension	72°C	10 min	

Full protocol in Appendix 3.

4 µl of DNA from each sample were then loaded on an agarose gel and the DNA was visualised under florescence to reveal which wells contained DNA and to see if the blanks were not contaminated. This procedure will not say anything about the origin of the sample or if it is ancient or not. This step is carried out so that one knows if to proceed to the next steps or not (for more detailed description on how to make an agarose gel see Appendix 4).

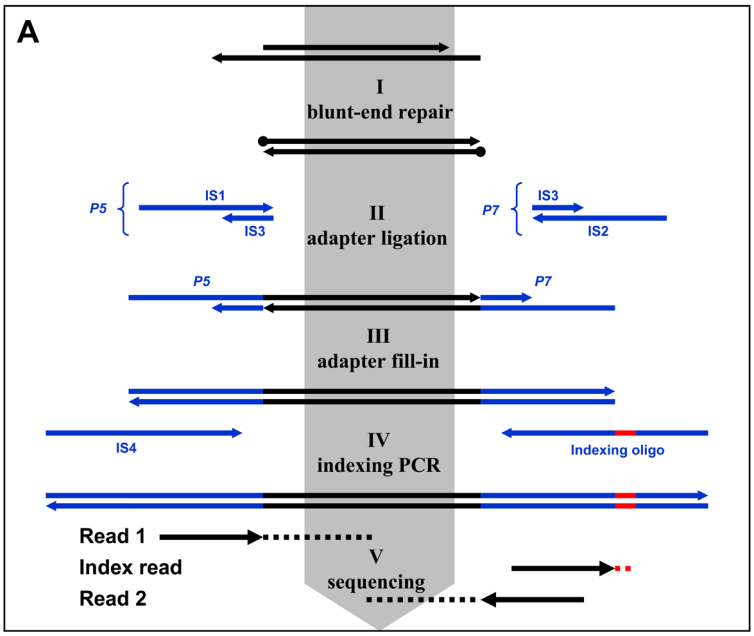

Figure 8. A schematic overview of a double stranded library protocol (modified from Meyer & Kricher 2010)

12. AMPure Beads

Agencourt® AMPure® Beads XP (Beckman Coulter) were used for purification of PCR amplicons. AMPure Beads bind DNA fragments of 100 bp and larger to paramagnetic beads. The outcome of using AMPure beads will result in a purified PCR product by removing unwanted primers, salts, nucleotides and enzymes. The beads are paramagnetic which means that they will only be magnetic in a magnetic field. Each bead has a layer of magnetite that is coated with a carboxyl molecule (Deangelis et al. 1995:4742). The negatively charged DNA will bind to the carboxyl groups on the beads surface when placed on a magnetic rack. Ethanol (70 %) was then used to wash away contaminants. A TET buffer (according to the supplier) was used to elute the clean DNA from the AMPure beads XP (Ronaghi et al. 2011:1pp). Using AMPure Beads XP for purifying PCR products can be divided into 6 main steps; adding AMPure beads, binding, separation, ethanol wash, elution buffer, transfer (figure 9).

Protocol available in Appendix 5.

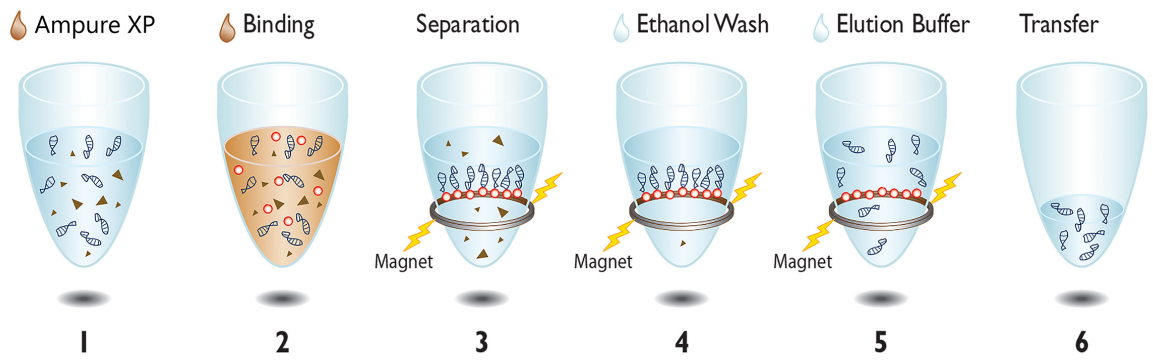

Figure 9. An illustration of purifying PCR amplicons using AMPure Beads XP (modified from Beckman & Coulter) (Ronaghi et al. 2011:1pp).

13. Bioanalyzer

The bioanalyzer is an automated analysing tool for quality control, sizing and quantitation of RNA, DNA and proteins. The results will be provided in high quality digital data. The bioanalyzer is provided with micro-fabricated chips with up to 12-wells. The lab-on-a- chip technology is both cost efficient and less time consuming when compared with regular gel electrophoresis. Analysing samples on the bioanalyzer can be divided in to three easy and quick steps; load the samples on a chip, run analysis and view the data. There are several different types of micro-fabricated chips and for ancient samples High Sensitivity DNA Chips from Agilent Technologies were used. Analyses are completed in 45 minutes and 11 samples can be loaded on a chip simultaneously. The sizing range is from 50-7000 bp. The Bioanalyzer was used for quality control of adapter-ligated library and quantification after PCR (Sciences & Discovery n.d.). The results from the bioanalyzer (sample sal002) is digitally visualised in figure 10.

Full protocol available in Appendix 6.

sal002

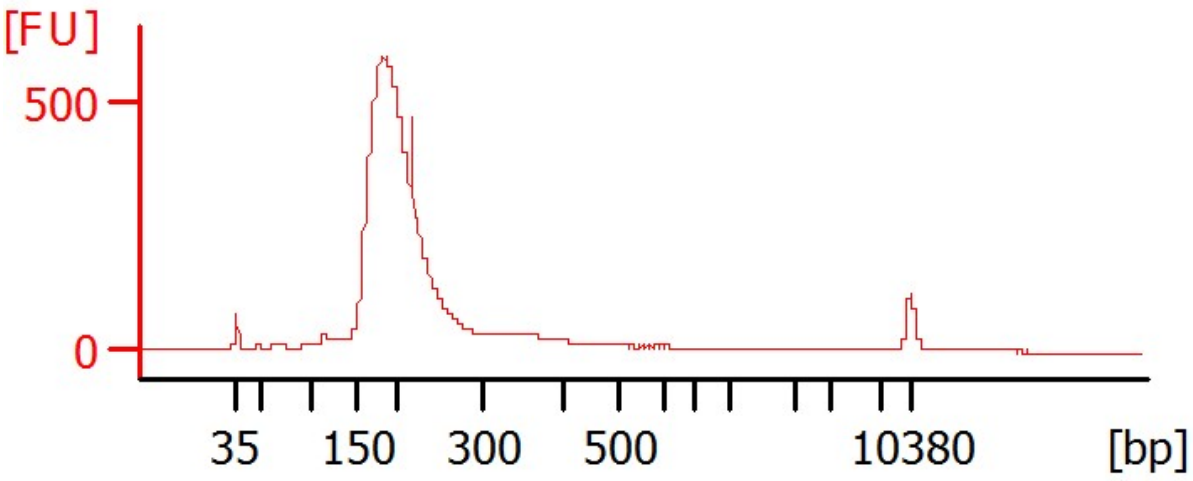

Figure 10. On the *y-axis* the Fluorescence signal strength and on the *x-axis* the length of the DNA fragment is shown in base pairs. From the left the first peak is the lower DNA marker and from the right the upper DNA marker. The sample falls in-between the two markers with an average fragment length of approximately 280bp.

14. Shotgun sequencing

There are several different sequencing strategies to choose from depending on the aim of the study and the condition of the sample material. Shotgun sequencing is in general means sequencing all DNA without targeting, directly and without prior treatment (Velasco 2015:33). Shotgun sequencing was originally developed to handle modern DNA samples. With modern samples the molecules are broken up to smaller DNA fragments and then the fragments are sequenced to determine the order of the DNA bases A, C, G and T. Sophisticated computer programmes then assemble the genome. When using shotgun sequencing on ancient samples the DNA molecules are most likely already heavily fragmented due to post-mortem processes. Shotgun-sequencing will provide an average of all DNA molecules present in the sample, and the resulting data could be used to determine quality and proportion of various origins (Velasco 2015:34).

15. Bioinformatic

15.1 Sequencing

All libraries were sequenced on a Illumina HiSeq2000 platform at the SNP&SEQ Technology Platform SciLife Sequencing Center in Stockholm Libraries that contained less than 1% of

total human DNA were excluded. As a result, only one individual out of eleven was used for second round of sequencing and downstream analysis.

15.2 Processing Data

The Paired-end reads were merged and the adapters were removed simultaneously using *MergeReadsFastQ_cc.py*. PCR duplicate reads with the same start and end coordinates were filtered using *FilterUniqueSAMCons.py* (Kircher 2012:197; Günther et al. 2015:1191). The sequence was then mapped to the reference genome build 36 and 37 using *BWA* (Li et al. 2009:1754). This was done using a script written by Torsten Günther.

15.3 Mitochondrial DNA Haplotypes

The haplotypes were found using a script that is available in the *SAMtools* package. More specifically the sequences were called using *vcfutils.pl (vcf2fq)* and *mpileup* to detect variants (Li et al. 2009:2078). The programme (*SAMtools*) then creates a FASTA (FAST-All, an text based file format for peptides) file with those specific variants and the file is uploaded to *Haplofind* and compared in PhyloTree Build 16 so that the sequence can be assigned a haplogroup and possibly compared with previously known haplogroups (Vianello et al. 2013:11889; van Oven & Kayser 2009:386).

15.4 Mitochondrial DNA authentication

Mitochondrial DNA contamination was estimated by identifying private or near privet alleles for ancient sample by comparing it with 311 worldwide modern human mitochondrial sequences (Green et al. 2008:418). The reads had to have a minimum of 30 mapping score and the coverage hade to be at least 10 and the base quality was 30 (Li & Durbin 2009:1754). Positions that could be due to cytosine deamination were ignored (Günther et al. 2015:8 Supplementary).

15.5 Determining Biological sex

The biological sex was determined by considering the ratio of the sequences aligning to the X and Y-chromosomes. Those that contain less than 1.6% Y chromosomal DNA are considered to be females. Samples that have a mapping quality less than 30 (according to an agreed standard among the users) were then discarded (Skoglund et al. 2013:4477).

16. Population genetic analysis

16.1 Principal Component analysis (PCA)

The method basically identifies the distribution of genetic variation of population structure (McVean et al. 2009:1). PCA is one of the earliest and also most used ways to illustrate genetic variation in multiple-marker datasets (Menozzi et al. 1978:786). It is a statistical analysis that converts highly multidimensional individual data into major components of variation and here relatedness between individuals is based on identifying axes of variation (Engelhardt et al. 2010:1; Novembre et al. 2008:98; Patterson et al. 2006:2074; Skoglund et al. 2012468; Günther et al. 2015:16; Skoglund 2013:21). As multivariate data (and especially the kind of massive data with variable origin that composes a genome) organizes itself in several vectors, and as it is easy to illustrate only two first of these vectors (that is, the two that explains most of the variation) in a common XY-diagram, a PC plot. The two principle component axis usually only include a limited amount of the variation within a genome. Sal002 was merged with a modern reference panel and a PCA was preformed using the SNP's (over 365 thousand SNP's) overlapping between the ancient individual (sal002) and individuals from twenty-one modern populations.

16.2 D-statistics

With *D-statistics* a model is created for historical population relationships and admixture that then can be used for genetic data. Essentially, the overlapping derived mutations in three different taxa (here taxa can be individuals or populations) are compared to an out-group, and are used to determine which two of the three taxa that share most genetic drift (that is, has most derived mutations in common) (Patterson et al. 2012:1065; Günther et al. 2015:17 Supplementary). For the *D-statistics* in this thesis a total of 15 populations (Belarusian, Czech, English Cornwall GB, English Kent GBR, Estonian, Finnish, French, Icelandic, Lithuanian, Norwegian, Orcadian, Russian, Saami, Scottish, Yoruban) from Northern Europa and Africa (Yoruban) were used to compere sample sal002 with.

16.3 f_3-statistics

f_3-statistics is an analytic tool that measures allele frequency correlations across populations, and is based on derived mutations. Thus, the method is not independent from the *D-statistics* test in all aspects. This method tests for historical population mixture and provides models

that fit genetic data. The test provides clear evidence for admixture and gene flow between populations, even though they could have occurred in prehistoric times. The f_3-statistics have two major purposes, it can test if a specific population is more admixed with one of two source populations. And also it can be used to test for shared drift between two populations, using an out-group (Patterson et al. 2012:1065). In this thesis, the second mentioned purpose was used. The results from the f_3-statistics are presented in a heat map (see chapter 17 Results).

17. Results

The genetic sequenced data from sample sal002 reached a genome coverage a total of 3.9x This means that I retrieved the same amount of DNA as it takes to make up 3.9 genomes, but as the DNA was randomly (shotgun) sequenced I may have duplicates and triplicates of parts of the complete genome, and lack other parts. 3.9x coverage must still be considered a well sequenced for an ancient individual given that the vast majority of published ancient data is well below 1x coverage. The cytosine deamination pattern (a damage pattern typical for aDNA based on C to T transitions) exceeded 15% at the 5 ´-end in the sample. This is considered indicative for ancient DNA. The estimated contamination level ranged between 1.18-2.50% (based on variation in mitochondrial SNPs). Thus, at least 97.5% of the DNA is estimated to be of authentic ancient origin. The individual in grave A2 Sala hytta (sample sal002) was assigned the biological sex of a female and was a carrier of mitochondrial haplogroup J1c3b. In table 3 the entire basic statistic for sample sal002 is listed.

Haplogroup J has been dated to ~58 thousand years ago and originate from the Near East, its presence in Europe has been associated with the spread of agriculture during the Neolithic process. The haplogroup is now present in 9 % of all European populations and 13% in Near East populations. Haplogroup J1 (a branch of hapolgroup J) makes up a total of ~80% of the J linage. The branch has been dated to ~33 thousand years ago. J1c is primarily found in central Europe and encompasses almost 80% of the J1 branch. J1c has been dated to ~16 thousand years ago. J1c has also been found in Neolithic samples from Spain, France, Germany and Sweden. J1c has also been detected in a Mesolithic sample from Germany, although the haplogroup is generally assumed to have a later date than the Mesolithic, 4.5–5.5 thousand years (leaving the two possibilities that the molecular clocking is not exact, or an erroneous typing in the German material). J1c3 can be found throughout Europe and have been dated to

~11 thousand years ago. J1c3b is a haplogroup that is most common in the north-western parts of Europe (Pala et al. 2012:919p).

Table 3. Summary statistic of the sequenced data from sal002

	sal002
Sampled material	Mand dx
Biological sex	XX
Proportion human	0.69
Clonality (%)	18.69%
Genome coverage	3.902x
mtDNA coverage	59.02
mtDNA haplogroup	J1c3b
Contamination estimation	1.18-2.50%

The PCA-plot (figure 11) shows a total of twenty-one modern populations (Lithuanian, Finnish, Icelandic, Norwegian, Orcadian (Orkney islanders), Russian, Ukrainian, Hungarian, Croatian, Bulgarian, Italian, Spanish, Basque Spanish, French, Sardinian, Maltese, Cypriot, Libyan Jew, Adygei, Druze). The red square illustrates where's sample sal002 has been plotted in comparison with the modern populations.

D-statistics was conducted to investigate whether or not sample sal002 shared drift with modern populations as described earlier (see chapter *D-statistics)*. The modern populations that were used in the *D*-statistics test were Belarusian, Czech, English Cornwall, English Kent, Estonian, Finnish, French, Icelandic, Lithuanian, Norwegian, Orcadian, Russian, Saami, Scottish and Yoruba that was also used as an out-group in the test. The results provided several non-significant values, but there were some significant Z score values (-2>Z>2, for significance, Z should be outside these values) (Patterson et al. 2012:1065)). The two reference populations that showed highest deviating Z score values were Norwegian and Lithuanian. Apparently individual in grave A2 (sal002) shared most drift with northern European populations, but from which side of the Baltic Sea she may have originated, or even if she had decent from both sides, remains a question. The results from the *D*-statistics, when testing shared drift between sample sal002, Norwegian modern populations and European modern populations are presented in Table 4A. And likewise is shown in Table 4B, when testing shared drift between Lithuanian population, sample sal002 and the reference populations.

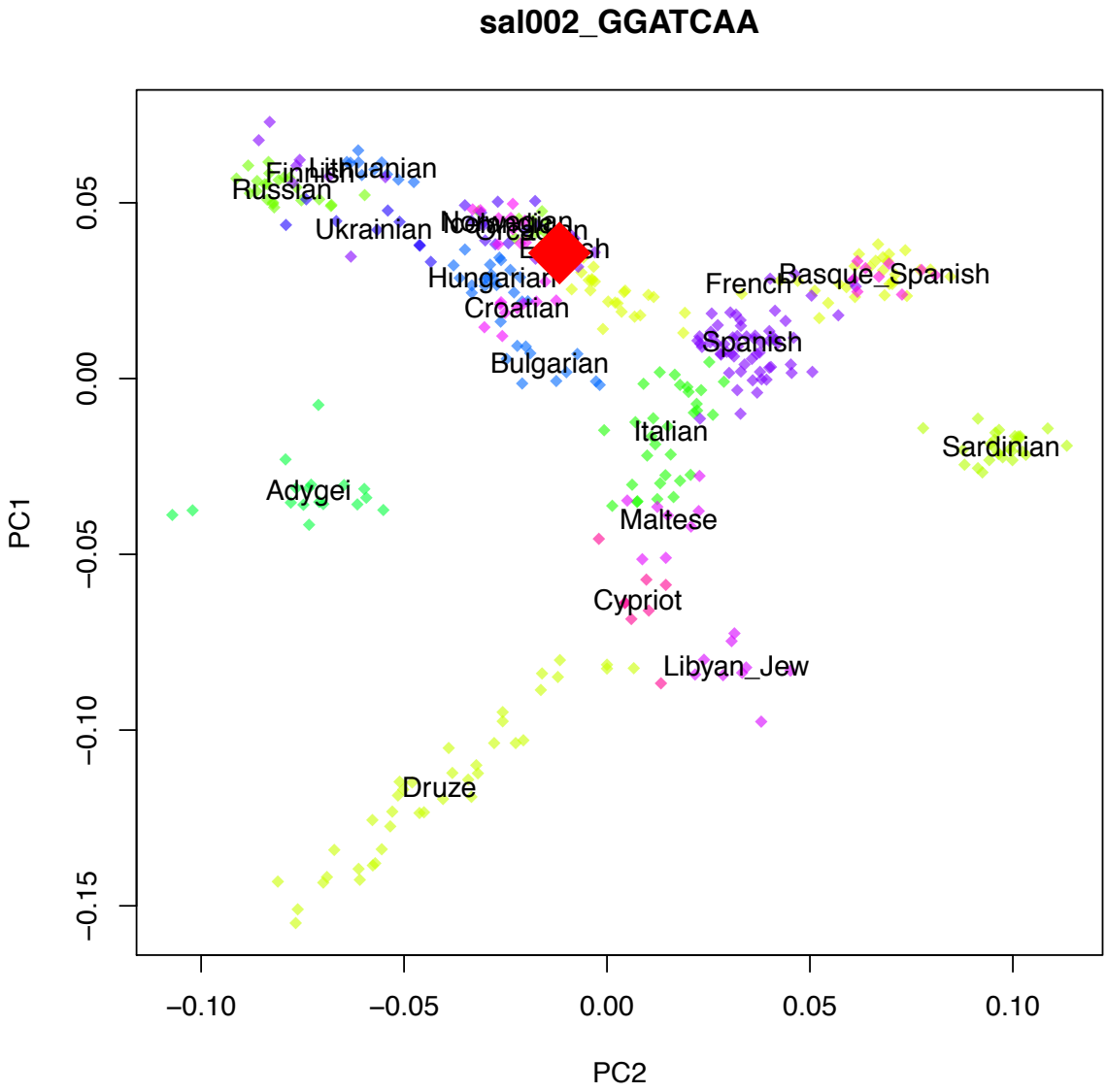

Figure 11. PCA plot where's the modern populations are illustrated with small squares of different colours, and also the name of the populations. The lager red square is illustrates sample sal002.

Table 4A. A selection of the *D*-statistic test results where shared drift is tested between sample sal002, Norwegians and reference populations. The reference population is a comparative population and those used were taken from the Human Origins dataset. The * denotes significant values from the test. Negative value (-) of the *D*-statistic indicates significance for shared drift with the Norwegian population compared to the other population.

	sal002	
Reference population	*D*-statistic	*Z* score
Belarusian	-0.0050	-1.791
Czech	-0.0035	-1.222
English Cornwall GB	0.0019	0.545
English Kent GBR	0.0001	0.031
Estonian	-0.0019	-0.652
Finnish	-0.0053	-1.717
French	-0.0037	-1.577
Icelandic	0.0036	1.329
Lithuanian	0.0018	0.639
Orcadian	-0.0005	-0.195
Russian	-0.0088	**-3.532***
Saami	-0.0199	**-2.867***
Scottish	0.0021	0.562

Table 4B. *D*-statistic test results with Lithuanian population compared to the sample (sal002) and reference population to test for shared drift. The comparative populations were drawn from the Human Origins dataset, as in table 4A. * Significant values. Negative value of the *D*-statistic (-) indicates significance for shared drift with the Lithuanian population compared to the other population.

	sal002	
Reference population	*D*-statistic	*Z* score
Belarusian	-0.0069	**-2.366***
Czech	-0.0053	-1.825
English Cornwall GB	0.0001	0.027
English Kent GBR	-0.0017	-0.479
Estonian	-0.0037	-1.236
Finnish	-0.0072	**-2.217***
French	-0.0055	**-2.217***
Icelandic	0.0018	0.659
Norwegian	-0.0018	-0.639
Orcadian	-0.0024	-0.842
Russian	-0.0106	**-4.276***
Saami	-0.0218	**-3.129***
Scottish	0.0003	0.075

The f_3-statistics that was preformed using a reference panel of 59 populations from the Human Origin reference panel (note that one of them were modern people from Iceland, which was not colonized at the time of the burial, but rather represent descendants from Norwegian colonizers and later additions, note also that there is no Swedish population in this panel, but Norwegians is usually considered a good proxy) with Yoruban population as an out-group. The number of SNPs that was used for the f_3-statistics was 107035, while the standard errors were obtained through a block jackknife, with a total of 703 blocks. The f_3-statistics also indicates that the individual in grave A2 had genetic composition in line with shared drift with the northern part of Europe. From the heat-map (figure 12) it is not possible to segregate whether or not she (sal002) shared more drift with the western part of north Europe from where she was excavated (Sala), or the eastern parts.

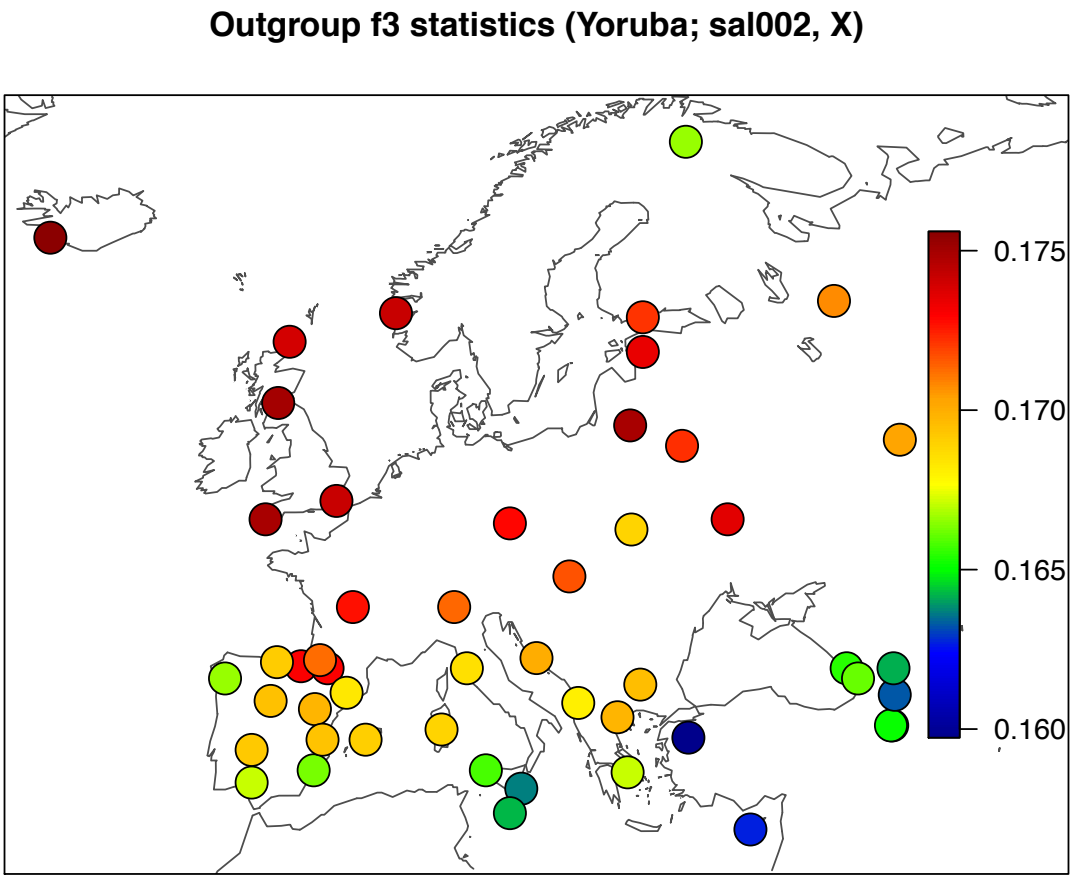

Figure 12. A Heat-map where the red colour illustrates more shared drift and the blue colour illustrates less shared drift.

18. Discussion

18. 1 The Origin of Sample sal002

Genetic data can be studied in a number of ways, my study relies much on two concepts (supported by mtDNA). One is the multivariate-approach used in a PCA, where all data is pooled together, weighted, to produce a detectable pattern. The other is genetic drift, where acquired mutations are used to detect genetic distances, and eventually shared origin in part (when populations hybridize) or in total (when they don't). I could have chosen other

analytical methods and the material most likely will be exposed to such (se chapter Further Studies), but these two concepts are usually the starting-point of a deeper analyses due to their robustness and easiness to use.

The individual (grave A2 Sala hytta Västmanland) had a genetic composition most similar to Northern Europeans, Scandinavians, or populations from the Baltic East Cost. This is supported by the *D*-statistic Z score values. The reason why the sample was not compared to a Swedish modern population is simply because there is no genetic database available on a Swedish modern population with overlapping SNPs compared to the once available. But the population most genetically similar to the Swedish population is the Norwegian population, which can be used as a proxy for modern Swedes. The significant Z score values, when compared with the Lithuanian population are the reason why I chose to use the word regional instead of local (it is still an identifiable part of the world, even if it is not on a scale that can be considered local). And thus the results show that individual in grave A2 is of regional origin. With regional I mean the northern parts of Europe, including both southern and central Scandinavia and also the Eastern Baltic Sea coast. The claim is also supported by the f_3-statistics (Heat map) and the assigned mitochondrial haplogroup (J1c3b).

18.2 Migration

A good start is to define migration and what is not considered as migration. How far do you have to move, in order for it to be defined as migration? This specific individual could have moved from different part of northern Europe and still it wouldn't be detectible in her DNA with the methods I have used here. The Z score values indicate that there could have been some genetic influences from across the Baltic Sea, but that don't necessarily mean that this specific female migrated to Sala hytta from the Baltic areas. Instead it leaves an open possibility that someone from this specific individuals linage could have cross the Baltic Sea and reproduced and vice versa. Actually, much mobility in northern Europe could well have created this kind of pattern. The genes are older than the individual, and one individual contains several genes with different origins. And it is the genes I have been analysing. There are unique cases where individuals are culturally affiliated with a group, but do not have the same genetic composition as the rest of the group (Gamba et al. 2014:1), it does not seem to be the case here. On a regional level it does not seem as if the sampled individual (sal002) have migrated, at least not from a genetically deviating area. And also note that she (sal002) is

compered to modern populations, and that the genetic composition of present populations does not look the same as it did in the past, although what has been published from Swedish Iron age genetics do not indicate any deviation over the latest millennia (Allentoft et al. 2015:167). To get a clearer picture if migration occurred, more statistical analyses and also other types of method should be used on the material (see chapter Further Studies).

18.3 Viking-age society and Burial rituals

The find material is in consensus with the genetic results and the genetics has not provided any surprises here so far. The oval brooches are typical Viking-age women's clothing accessories and biological sex was here determined to a female (XX). All the genetic data generated from this individual point to that she is of regional origin, and if she somehow represents the boat grave burial custom, then we can assume that the tradition of boat grave burials express at least some kind of regionality. As mentioned earlier the burial tradition is heterogeneous and there are notable differences between the many boat burial cemeteries. It is therefore likely that the individual in grave A2 is not representing to the boat graves more specifically than the society in general. But again, if there actually is a common genetic background to a larger part of the boat graves, more than one individual would be needed to identify it. Not acknowledging this would be to allow for the risk of drawing generalising conclusions and suggest that the genetic results of that one individual can represent all the other individuals buried in the same way. One must always keep in mind what the analysed individual actually represent. But for now this is the only individual buried in a boat grave that we have analysed genomic data from and thus the best genetic data we have from the boat burial context. The word "*Regional*" fits better in to the custom per say, due to the wide spread of the tradition (see chapter Previous Research and also figure 1). The practice may have some geographic focus, but as it is well spread, the word "*local*" does not have enough dimensions to explain the custom. As earlier mentioned this thesis is the first part of a more ambitious study in which more boat graves are being included (genetic data from altogether 15 remains are presently being processed) and hopefully a clearer picture will be provided.

18.4 Further Studies

This individual is very well preserved (see chapter Results). Thus there is no need for enrichment strategies (such as targeting specific areas of the genome and projecting all force

on those). We already have enough sequenced data on this specific individual for further and more advanced analyses such as runs of homozygosity (RoH, which will use the variation in the chromosome-pairs within this individual to investigate population-size and population history), treemix (which is a model-based test that investigate genetic distance and possible admixture), ADMIXTURE (which is a technique to investigate from how many major groups this genome origins), and also for looking at expressed genotypes. To mention a few. Simply, there is enough DNA from this individual to preform most of the analyses that can be performed on modern material. The sequenced results from sample sal002 will eventually be exposed to those analyses together with the boat grave material that is now being processed. Phenotypic traits, that are often mentioned are lactase persistence, the CCR5 gene mutation (immunity for the HIV virus), pigmentation, disease resistance, starch digestion etc. But this is just scratching the surface. Basically, there are great opportunities for further genetic studies on this Viking-age woman, that was given a boat grave burial when she died.

The possibility of using other laboratory methods to further understand the individual in the grave is naturally also an opportunity. ^{14}C-dating is sometimes considered as more precise then basing your dating on an artefact (but it depends on the chronology, sometimes artefacts give higher precision, as is the case for the Viking period). Dietary studies using stable isotopes and especially the stable isotope of strontium for studying migration patterns could be an interesting addition. Strontium has two particulars compared with DNA, it is more geographically precise, and it is not cross-generational. Actually, combining strontium with DNA would perhaps provide the best means for studying migration.

19. Conclusion

The aim of this study was to gain new knowledge on the boat grave burial custom, and more specifically the individuals that were buried in boat graves. Only one individual (grave A2) produced enough DNA for statistical analyses. Damage patterns and short fragment length (characteristic for aDNA) could be detected in the sample, which provides arguments for authenticity of the ancient DNA. The sample was also assigned a mitochondrial haplogroup and the biological sex of a female could be determent. Obtained genome coverage of 3.902x was more than enough for statistical tests.

Interestingly, the preservation of DNA in the remains I analysed varied. This is a result of some methodological interest. Material from similar contexts and chronologies provided DNA preservation from extremely poor to extremely good.

The mitochondrial haplogroup is quite common in Europe, and specifically northern and north western Europe. The PCA plot clearly indicates that the individual in grave A2 Sala hytta had a genetic composition most similar to modern northern Europeans, but also looks local. The *D*-statistics in some way indicates the same results as the PCA plot with a significant Z score value against some populations compared to the Norwegian population, but also gives a significant Z score value when the sample is compered between Lithuanian and other populations. The results from the f_3-statsitics are illustrated in a Heat map (figure 12) and display genetic similarity all the way from Iceland in the west to the Baltic regions in the east, basically the distribution of the Viking-world.

With this in mind, a clear conclusion can be drawn upon these results. That the individual in grave A2 had a genetic composition that is similar to modern northern populations. Conclusions whether or not she was local to the western side of the Baltic Sea, or had a genetic origin on the eastern side of the Baltic Sea cannot be drawn at this point. Furthermore it is not possible to draw any general conclusions about the boat burial custom based on this result. One individual is not representative enough. That is, however, a scope for the larger study of boat burials which sample sal002 has initiated.

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21. Appendices

21.1 Appendix 1. Guidelines for the use of Ancient DNA Facilities at AFL, SU

Materials

Reagents

- NaC1O (1 % Bleach)
- dH_2O
- 70 % EtOH

Protocol

Before and after each use wipe all surfaces with 1% solution of bleach (NaClO), twice with dH_2O and dry with 70%EtOH; repeat when leaving the lab for the day.

In the morning rotate the contents of bleach and water baths.

Clean sterile benches before and after each use; wipe with DNA away, then once with dH_2O and dry with 70% EtOH.

For cleaning of precious equipment (sterile benches, crosslinkers, ovens, etc.) Use DNA away NOT bleach.

Consumables which were in contact with DNA (tubes, tips, etc.) should be placed in plastic bags, sealed, disposed of in “farligt avfall” container located in the airlock.

All dustbins should be emptied on daily basis.

Gloves that were in direct contact with skin should be disposed of in the airlock.

Everything that goes in must be thoroughly wiped with 1% bleach.

Reusable plastic- and glass ware (racks, beakers, etc.): soak overnight (minimum 4 hours) in 1% bleach bath and min 2 hours in each of the two water baths.

Do not use the thermocycler in the aDNA lab for PCR reactions.

Turn on the UV lights in the ceiling and the hoods after finishing work (hoods – 3 hrs, lab – overnight).

Cleaning the drill/drill bits

The drill engine (plastic outer layer) can be cleaned with bleach, water and ethanol.

All metal elements should be cleaned with DNA away instead of bleach, and treated with mineral oil after use to reduce/prevent corrosion.

Drill bits: place dirty drill bits in a falcon tube and shake around in 14% bleach for 5 min (repeat if very dirty). Transfer the bits to a fresh tube, wash twice in dH_2O and once with EtOH and EDTA. Spread the bits on paper to dry and transfer onto a piece of aluminium foil. Place inside the crosslinker and radiate on both sides; put the clean and dry drill bits in fresh falcon tube and mark clearly.

21.2 Appendix 2. DNA extraction from human materials

Materials

Regents

- MinElute PCR Purification Kit (For purification of PCR Products (70 bp to 4kb) in low elution volumes
- MinElute Spin Columns
- Buffer PB
- Buffer PE (concentrate, add ethanol)
- Ethanol (96-100%)
- Buffer EB
- Buffer Yang-Urea (6.3 ml of 8M Urea and 43.7 ml of 0.5M EDTA)
- Proteinase K

Equipment

- Rotator
- Hybridization oven
- Centrifuge
- Microcentrifuge
- Amicon® Ultra 4 ml filter
- 1.5 Eppendorf tubes
- Collection tubes (2 ml)

Buffer preparation

Yang-Urea DNA extraction

C (mol/L)=n (mol)/v (L) and $C_{slut} \times V_{slut} = C_{start} \times V_{start}$

Extraction buffer 0.5M EDTA pH 8, 1M Urea, 100 µg/mL Proteinase K (added separately)
Prepare 6.3 mL 8M Urea and 43.7 mL 0.5M EDTA in Falcon tube and UV irradiate.
Also UV the amount of PE, EB and LB needed for extraction.

Protocol

Add 1 ml Yang-Urea buffer to bone powder and at least 10 µl Proteinase K (10mg/ml).
Vortex and incubate at 38-55^{o}C in hybridization oven over night. If powder is fully dissolved,

continue extraction. If not, add 10 µl Proteinase K and incubate for at least a few more hours at 55 ^{o}C.

Spin down at 2000 rpm for 5 min.

Transfer supernatant to Amicon filters and spin down at 4000 rcf (=g) for 10 min to 100 µl. If consecutive extractions have been done, pool them in this step.

Transfer Amicon supernatants to Eppendorf tubes with 5X PB buffer (500 µl) and vortex gently

Transfer the PB-mix to MinElute filter, incubate 10 min, and spin down at 14000 rpm for 1 min.

Discard waste and wipe the opening of the collection tubes with bleached paper.

Add 710 µl PE buffer to MinElute filter.

Spin down at 14000 rpm for 1 min (2washes in total).

Discard waste and wipe the opening of the collection tubes with bleached paper.

Spin down once more at 14000 rpm for 1 min and discard the collection tubes.

Change to new collection tubes (2 mL tubes with removed lids).

Add 55 µl EB buffer. Incubate **10 min at 37^{o}C** and spin down at 14000 rpm for 1 min.

Add additionally 55 µl EB buffer and incubate and spin down as above.

Transfer the 110 µl DNA extract to new eppendorf tubes and store in freeze.

21.3 Appendix 3. Blunt end Illumina libraries (Meyer oligos and NEBNext E6070L kit)

Materials

Reagents

- T4 DNA Polymerase
- T4 Ligase
- BSTPolymerase

Equpment

- Thermal Cycler
- 1.5 ml Eppendorf tubes
- 0.2 ml PCR tubes

Preparation of oligo hybridisation buffer (200 rxns)

500 mM NaCl

10 mM Tris-Cl pH 8 (Trizma from Sigma)

1 mM EDTA pH 8

Preparation of adapter mix (100 µl for 200 rxns)

Make hybridization mix for adapter P5 and adapter P7 in PCR tubes.

Hybridization mix for adapter P5 (200 µM)

40 µl of 500 µM IS1_adapter_P5.F

40 µl of 500 µM IS3_adapter_P5+P7.R

10 µl of 10X Oligo hybridization buffer

10 µl ddH_2O

Hybridization mix adapter P7 (200 µM)

40 µl of 500 µM IS2_adapter_P7.F

40 µl of 500 µM IS3_adapter_P5+P7.R

10 µl of 10X Oligo hybridization buffer

10 µl ddH_2O

Mix and incubate the reactions for 10 sec at 95^{o}C followed by a ramp from 95^{o}C to 12^{o}C at a rate of 0.1^{o}C/sec.

Combine both reactions to get a ready-to-use mix (with 100 µM of each adapter).

Protocol

Blunt End Repair (40 µl final volume/Rx)

Tango Buffer		4 µl
dNTPs	(25mM)	0.16 µl
ATP		0.4 µl
T4 PNK		2 µl
T4 DNA Pol		0.8 µl
H_20		12.64 µl
DNA		20 µl

Mix and incubate in a thermal cycler for 15 min at 25°C followed by 5 min at 12°C

Purify with MinElute

Apply 200ul of PBI Buffer to 40 µl of the repaired DNA to column (mix first). Spin 13000 rpm for 1 min.

Discard waste and wipe with bleach cloth.

Add 700 µl PE Buffer to column, spin 13000 rpm for 1 min and repeat this PE wash one more time (2 washes in total)

Discard waste and wipe with bleach cloth.

Spin 13000 rpm for 1 min.

Change to new 1.5 ml tube.

Add 22 µl EB Buffer to column and incubate at 37^{o}C for 5 min.

Elute DNA by spinning it down for 1 min at 13000 rpm.

Adapter Ligation (40ul final volume/reaction)

Prepare a master mix for the required number of ligation reactions as shown below. If white precipitate is present in the 10X DNA ligase buffer after thawing, warm the buffer to 37°C and vortex until the precipitate has dissolved. Since PEG is highly viscous, vortex the master mix before adding T4 DNA ligase and mix gently thereafter.

H20	10 µl
T4 DNA ligase Buffer (10x)	4 µl
PEG-4000 (50%)	4 µl
Adapter mix	1 µl (1µl of a 1:10 dilution in TE of (the stock = 10pmol in final volume)
T4 DNA ligase (5 U/µl)	1 µl
DNA	20 µl

Incubate for 30 min at 22°C

Purify with MinElute

Apply 200 µl of PBI Buffer to 40 µl of the repaired DNA to column (mix first). Spin 13000 rpm for 1 min.

Discard waste and wipe with bleach cloth.

Add 700 µl PE Buffer to column, spin 13000 rpm for 1 min and repeat this PE wash one more time (2 washes in total).

Discard waste and wipe with bleach cloth.

Spin 13000 rpm for 1 min.

Change to new 1.5 ml tube.

Add 22 µl EB Buffer to column and incubate at 37^{o}C for 5 min.

Elute DNA by spinning it down for 1 min at 13000 rpm.

Adapter Fill in

H20	14.1 µl
Thermopol buffer 10x	4.0 µl
dNTPs (25µM each)	0.4 µl
Bst polymerase, LF (8 U/µL)	1.5 µl
DNA	20 µl

Incubate for 20 min at 37°C and heatkill for 20 min at 80

Library PCR 1

Set up 6 reactions per DNA library in 25 µl reactions containing:

ddH_2O	15.25 µl
10X TaqGold Buffer	2.5 µl
25mM $MgCl_2$	2.5 µl
25mM dNTPs	0.25 µl
IS4_short_amp 10µM	0.5 µl
Index primer (1-22) 10 uM	0.5 µl
AmpliTaq Gold	0.5 µl
Library DNA	3 µl

Mix and spin down and use the following thermal cycler program

Initial denaturation	94°C	12 min		
Denaturation	94°C	}	30 sec	
Annealing	60°C	}	30 sec	12 cycles
Elongation	72°C	}	45 sec	
Final extension	72°C		10 min	
Hold	4	forever		

AmPure bead purification and elution in 20 µl EB Buffer + 0.05% Tween 20.

21.4 Appendix 4. Casting an Agarose gel and Loading DNA samples.

Material

Reagents

- Agarose
- TBE
- GelRed
- Loadin Dye
- DNA-ladder

Equipment

- Gel electrophoresis
- Scale
- Tape
- Glass flask
- Microwave oven
- Pipette and pipette tips

Protocol

Casting an Agarose gel

Begin by taping the sides on the plastic gel tray and attach the plastic gel combs to the gel tray.

Weigh in 0.5 g off agarose and pour it in to a glass flask.

Pour 50 ml off 0.5x TBE to the glass flask mixing it with the agarose.

Place the glass flask in the microwave oven for 40 seconds and set the power to 50%. Take the glass flask out and stir. Place the glass flask back in the microwave oven, but now only for 10 seconds, take it out end gently stir it. Repeat until liquid is clear.

Add 3 µl of GelRed to the glass flask (3 µl/ 50 ml gel) and pour the mixture into the gel tray that you taped the sides on.

Wait for 20 minutes until the gel has solidified.
Now pipette 2 µl loading dye (which is a mixture of 1 µl of 6x Geldye and 1 µl of water) to all the columns that you are going to use.

Now pipette 4 µl DNA of each sample to a sample specific column mixing it with the loadingdye using the pipette.

Now gently remove the plastic gel combs and the tape from the gel.

Place your gel in the gel electrophoresis tank and if needed fill up the container with 1x TBE so that the gel is fully submerged.

Pipette 2 µl of DNA-ladder in the first and last column of every row (This is done so that you can compere your samples). Then pipette 6 µl of DNA of every sample mixed with loading dye to specific wells.

Now place your loaded gel in the electrophoresis apparatus and put the lid on. Set the electrophoresis on 80 V for 40 minutes and 400 Amp.

Wait 40 minutes for results.

21.5 Appendix 5. AMPure beads for purification of high-throughput PCR amplicons.

Material

Reagents

- AMPure Beads XP
- Fresh 70% ethanol
- TET buffer

Equipment

- Pipette and pipette tips
- Vortex
- Rotator
- Magnetic Rack
- LoBind tubes

NB! Let the beads equilibrate at room temp for 30 min

Protocol

Prepare fresh 70% ethanol.

Pool PCR products.

Add 0.5x volume of beads, vortex and spin down quickly and incubate in RT for 5 min.

Place tubes on magnetic rack for 1 min.

Remove supernatant and place it in a fresh LoBind tube.

Add 1.8x volume of beads, vortex and spin down quickly and incubate in RT for 10 min.

Place tubes on magnetic rack for 3 min and discard the supernatant.

Wash the beads 3x with 200ul 70% ethanol; incubate 30s at each step (20s in the last wash).

Elute the beads with 36ul of TET buffer; vortex for 20s, spin down and incubate at RT for 10 min.

Place on magnetic rack for 5 min

Transfer cleaned product to a new LoBind tube.

21.6 Appendix 6. Bioanalyzer protocol. Aglient High Sensitivity DNA Kit.

Material

Reagents

- High Sensitivity DNA ladder
- High Sensitivity DNA Markers 35/10380 bp (4 vails)
- High Sensitivity DNA Dye concentrate [1](1 vial)
- High Sensitivity DNA Gel Matrix (2 vials)

Equipment

- High Sensitivity DNA Chip
- Chip Priming station
- IKA vortex mixer
- Pipetts with compatible tips
- 0.5 ml low-binding microcentrifuge tubes
- Microcentrifuge

Protocol

Setting up the Chip Priming Station

Replace the Syringe with each new reagent kit.

Unscrew the old syringe from the lid of the chip priming station.

Realise the old syringe from the chip. Discard the old syringe.

Remove the plastic cap of the new syringe and insert it into the chip.

Slide it into the hole of the lure lock adapter and screw it tightly to the chip priming station.

Preparing the Gel-Dye mix

Allow the blue-capped High Sensitivity DNA dye concentrate and the red-capped High Sensitivity DNA gel matrix to equilibrate to room temperature for 30 minutes.

Vortex the blue–capped vial with High Sensitivity DNA dye concentrate for 10 seconds and spin down. Make sure the DMSO is completely thawed.

Pipette 15 µl of blue-capped dye concentrate into a red-capped High Sensitivity DNA gel matrix vial. Store the dye concentrate at 4 °C in the dark again.

Cap the tube, vortex for 10 seconds. Visually inspect proper mixing of the gel and dye.

Transfer the complete gel-dye mix to the top receptacle of spin filter.

Place the spin filter in a microcentrifuge and spin for 10 minutes at room temperature at 2240 g ± 20 % (for Eppendorf microcentrifuge, this corresponds to 6000 rpm).

Transfer the complete gel-dye mix to the top receptacle of a spin filter.

Discard the filter according to good laboratory practices. Label the tubes and include the date of preparation.

Loading the Gel-Dye Mix

Allow the gel-dye mix to equilibrate to room temperature for 30 minutes before use. Protect the gel-dye mix from light during this time.

Take a new High Sensitivity DNA Chip out of its sealed bag and place the chip on the chip priming station.

Pipette 9.0 µl of the gel-dye mix at the bottom of the well marked with a "G" and dispense the gel-dye mix.

Set the timer to 60 seconds, make sure that the plunger is positioned at 1ml and then close the chip priming station. The lock of the latch will click when the Priming station is closed correctly.

Press the plunger of the syringe down until it is held by the clip.

Wait for exactly 60 seconds and then release the plunger with the clip release mechanism.

Visually inspect that the plunger moves back at least to the 0.3 ml mark.

Wait for 5 seconds, then slowly pull back the plunger to the 1 ml position.

Pipette 9.0 μl of gel-dye mix in each of the wells marked “G”.

Loading the Marker

Pipette 5 μl of green-capped high sensitivity DNA marker into the well marked with the ladder symbol and into each of the 11 sample wells.

Loading the Ladder and the Samples

Pipette 1 μl of yellow-capped High Sensitivity DNA ladder vial in the well marked with the ladder symbol.

In each of the 11 sample wells pipette 1 μl of sample (used wells) or 1 μl of marker (unused wells).

Place the chip horizontally in the adapter of the IKA vortex mixer and make sure not to damage the bulge that fixes the chip during vortexing.

Vortex for 60 seconds at 2400 rpm.

Insert the loaded chip on to the Bioanalyzer and start up the chip run.


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