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Validating multiplexed STR markers in a Kamchatka brown bear population; system performance and genetic variation

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Abstract

Markers included in a DNA forensic profiling system for wildlife species should be validated according to guidelines developed by the International Society for Forensic Genetics (ISFG). In a former study, a set of twelve dinucleotide STR (short tandem repeats) loci (Mu09, Mu10, Mu23, Mu59, Mu05, G10L, Mu50, Mu51, G1A, G1D, Mu15, G10B) specific for bear were validated in singleplex PCR amplification for eight populations of brown bear (*Ursus arctos).* Studies of the Western European bear populations as well as forensic casework have been conducted applying these twelve validated STRs on non-invasive samples (e.g. hair, faeces). The large brown bear population at Kamchatka, threatened by poaching, has not been studied by the use of autosomal STRs before. As the markers are now multiplexed, a new validation is needed.

The aim of this study was to perform validation of 16 multiplexed dinucleotide STR markers (twelve previously validated, four new ones; G10C, G10J, G10O, G10X) specific to brown bear, and obtain allele frequency estimates as well as relevant forensic and population genetic parameters from a Kamchatka brown bear population by the use of these validated STR markers. Finally a first comparison to the Western European brown bear populations was conducted.

The validation tests (sensitivity, precision, heterozygote balance and stutter) were performed by the use of four brown bear tissue samples with known genotypes in 16 STR loci. Following the validation, DNA from 434 hair samples originating from Kamchatka brown bears were analysed with the same STR markers.

There was satisfactory performance in 15 of the 16 STR loci when template input was ≥ 0.2 ng, while in G10X there was suboptimal amplification of one allele. Most, if not all, Low Copy Number samples could be detected using the current STR guidelines. The tests suggested a homozygote peak height threshold of 1800 relative fluorescence units (RFU) for G10J and G10B, and 800 RFU for the rest of the markers. All markers met the Hardy-Weinberg and linkage equilibrium expectations after Bonferroni corrections of significance levels. Test for genotyping errors or null alleles (Micro-Checker) indicated null alleles at Mu23 and G10O. While G10O had the lowest power of discrimination (PD = 0.667), the PD ranged from 0.794 to 0.962 in the other markers. The total average probability of identity for the 15 markers when accounting for population substructure (F_{ST} = 0.11), was 4.1 x 10^{-13}, and the total average of sibling identity was 6.3 x 10^{-6}. The genetic diversity in the Kamchatka population was high (mean expected heterozygosity 0.79, n = 115). The population pairwise comparisons (pairwise F_{ST}`s) to the Western European populations showed moderate genetic differentiation that, in general, mirrored the geographic distances.

In conclusion, 15 multiplexed dinucleotide STR markers were validated according to ISFG recommendations and applied for the first time in analysis of the Kamchatka brown bear. The discriminating power of the DNA profiles is at a magnitude where they would provide individual specific forensic evidence and they may also be applied in monitoring and population genetic studies.

Sammendrag

Markører inkludert i et rettsgenetisk profilsystem for ville dyr bør valideres etter «the International Society of Forensic Genetics» (ISFG) sine retningslinjer. I en tidligere studie ble tolv bjørnespesifikke dinukleotide STR-loci (Mu09, Mu10, Mu23, Mu59, Mu05, G10L, Mu50, Mu51, G1A, G1D, Mu15, G10B) validert i singleplex PCR-amplifikasjon for åtte populasjoner av brunbjørn *(Ursus arctos*). Studier av bjørnepopulasjoner i Vest-Europa samt rettsgenetiske undersøkelser har blitt utført ved analyser av disse tolv validerte markørene på ikke-invasive prøver (f.eks. hår og fæces). Den store brunbjørnpopulasjonen på Kamchatka, som er truet av ulovlig jakt, har ikke blitt studert ved bruk av autosomale STR-markører tidligere. Da analysene nå utføres i multiplex PCR-oppsett er det behov for en ny validering. Målet med denne studien var å utføre validering av 16 bjørnespesifikke dinukleotide STR-markører (tolv som er validert tidligere, fire nye G10C, G10J, G10O, G10X) i multiplex PCR-analyser og fremskaffe populasjons-spesifikke allelfrekvenser samt retts- og populasjonsgenetiske parametere fra en brunbjørnpopulasjon på Kamchatka ved hjelp av disse validerte markørene. Til sist vil resultatene sammenlignes med data fra bjørnepopulasjoner i Vest-Europa.

Valideringstester (sensitivitet, presisjon, heterozygot balanse og stutter) ble utført på fire ulike vevsprøver fra bjørn med kjent DNA-profil i 16 STR-loci. Etterfulgt av valideringen ble DNA fra 434 hårprøver som stammet fra brunbjørn på Kamchatka analysert med de samme STR-markørene. Valideringen gav tilfredsstillende resultater i 15 av de 16 STR-markørene når templat-tilførselen var $\geq$ 0,2 ng, mens G10X viste suboptimal amplifisering av ett allel. De fleste, om ikke alle, prøvene med lavt kopinummer av DNA (såkalte LCN-prøver) kunne detekteres ved hjelp av nåværende typekriterier. For homozygote genotyper foreslo valideringstestene en grense for allelhøyde på 1800 relativ fluorescensenheter (RFU) for G10J og G10B, og 800 RFU for de andre markørene. Etter Bonferroni-korrigering av signifikansnivået, møtte alle markørene Hardy-Weinberg forventninger og viste uavhengig nedarving. En test for genotype-feil og null-allel (Micro-Checker) indikerte en mulig forekomst av null-allel i Mu23 og G10O. Mens G10O hadde den laveste diskrimineringsevnen (power of discrimination, PD: 0,667), var PD mellom 0,794 og 0,962 for de andre markørene. Det totale estimatet for «sannsynlighet for identitet» (probability of identity) når grad av populasjonsstruktur (F_{ST} 0,11) ble tatt hensyn til var 4,1 x 10^{-13}, og tilsvarende mellom søsken var verdien 6,3 x 10^{-6}. Den genetiske diversiteten i populasjonen på Kamchatka var høy (gjennomsnittlig forventet heterozygositet; H_E 0.79, n =115). Den genetiske distansen mellom Kamchatka-populasjonen og de vesteuropeiske populasjonene estimert ved hjelp av parvise F_{ST}, viste moderat genetisk differensiering som generelt gjenspeiler de geografiske distansene. For å oppsummere; 15 dinukleotide bjørnespesifikke STR-markører i multiplex PCR-oppsett ble validert i henhold til anbefalinger fra ISFG og disse markørene ble videre benyttet for første gang i analyser av brunbjørn fra Kamchatka. Den diskriminerende evnen til disse DNA-profilene er av et slikt omfang at de kan gi individspesifikke rettsgenetiske bevis samt benyttes i monitorering og populasjonsgenetiske studier.

Abbreviations

°C	degrees Celsius
°E	degrees east
°N	degrees north
µl	microliter
µM	micromolar
-1R	minus one repeat
ABI	applied biosystems
APHT	analytical peak height threshold
appr.	approximately
bp	basepairs
BSA	bovine serum albumin
CE	capillary electrophoresis
CI	Confidence interval
CMR	Capture-mark-recapture
ddH_2O	distilled water
DNA	deoxyribonucleic acid
dNTPs	deoxyribose nucleoside triphosphate
F	forward
F_{IS}	inbreeding index (F-statistics)
F_{ST}	index for population substructure (F-statistics)
GDA	genetic data analysis
h	observed homozygosity
H_E	expected heterozygosity
H_O	observed heterozygosity
HPHT	heterozygote peak height threshold
HWE	Hardy-Weinberg equilibrium
ID	identity
IEC	International Electrotechnical Commission
ISFG	international Society for Forensic Genetics
ISO	International Organization for Standardization
KamO	Kamchatka Oblast
km	kilometre
LD	linkage disequilibrium
LCN	low copy number
$MgCl_2$	magnesium chloride
min	minutes
ml	millilitre
mM	millimolar
MP	multiplex assay
n	number
N	number
ND	no dropout
NEB	New England Biolabs

ng	nanogram
NGS	non-invasive genetic sampling
NIBIO	Norwegian Institute of Bioeconomic Research
nt	nucleotides
p	p-value (significance level)
P1-P8	population 1 to population 8
PCoA	principal coordinates
PCR	polymerase chain reaction
PD	probability of discrimination
PE	power of exclusion
PI	probability of identity
PI_{sib}	Probability of identity for siblings
PI_{typ}	typical paternity index
PM	matching probability
POP7	performance optimized polymer - 7
R	reverse
RFU	relative fluorescent unit
SD	standard deviation
S1-S16	station one to station 16
SNP	single-nucleotide polymorphism
S_R	stutter ratio
SSM	slipped strand mispairing
STR	short tandem repeats
T_a	annealing temperature
U	units
Φ_A	height of the allelic peak
Φ_S	height of the stutter peak

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1. Introduction

1.1. General background

Genetic markers applied for forensic purposes, should be validated according to recommended criteria. In human forensics, the markers included in a DNA profiling system have undergone extensive experimental validation. This is often not the case in wildlife forensic science (1). Illegal hunting and trade of wildlife species are growing international problems (2). The need to validate genetic marker systems for wildlife species is both costly and time consuming. Nevertheless, such validation is important for the results from DNA analysis to hold as evidence in court (1). The International Society for Forensic Genetics (ISFG) has therefore developed guidelines for the validation of genetic markers applied to wildlife genetic forensics (3).

In conservation genetics, DNA markers, such as microsatellites, are commonly used for many species as a means of providing not only species identification, but individual specific DNA profiles, often from non-invasively sampled material (e.g. hair, faeces) (4, 5). Few genetic marker sets applied to study wildlife species have been validated according to ISFG recommendations (1). The European brown bear (*Ursus arctos*) has undergone extensive genetic research during the past decades and has played an important role as a model species in the development of methods and analysis in conservation genetics (6). It was also the first wildlife species for which the markers applied in a DNA profiling system were validated according to the ISFG recommendations. This first validation of a set of dinucleotide microsatellites was carried out in 2012 and simultaneously provided allele frequency estimates for eight populations of the Scandinavian brown bear (7).

1.1.1. Brown bear in Europe and Russia

The brown bear populations of Europe vary greatly in size (8, 9). In the 19th and 20th century, they were almost eradicated in several European countries. This was partly a result of habitat loss and fragmentation, but state-financed extermination campaigns to avoid livestock predation also played a crucial role (10, 11). In recent years, bear management policies and genetic monitoring in e.g. Norway and Sweden have contributed to the recovery of brown bears in Scandinavia (10). Now, relatively large populations (Norway appr. 120, Sweden 3.300 and Finland 1600-1800 individuals) exist in the Nordic countries (12-14). From early 1990s, projects involving non-invasive genetic sampling and genetic analysis have been conducted to evaluate the genetic status of small, fragmented and isolated brown bear populations and to assist in conservation strategies in several European countries e.g. Austria and Italy (15, 16).

The largest populations of brown bear are found in Russia (8, 9). The Kamchatka brown bear population is the most eastern population in Russia and has been censused by observations in the field throughout the years (17). As far as we know, the Kamchatka brown bear population has not been

studied by the use of autosomal STRs and non-invasive sampling for genetic purposes has not been conducted in this geographic distant population thus far. Though the brown bears in Kamchatka are abundant, estimates describe a downward trend in the numbers from the 1960s (18000-22000) to 1986-94 (8000-10000). The main threat to brown bears in Kamchatka is poaching (17). To use genetic evidence in court one would need a validated marker set proven to provide individual specific DNA profiles in this population (1).

1.1.2. Non-invasive genetic sampling in bear conservation

Bears are secretive, solitary and often occur at low densities. This makes it difficult to monitor them (6). However, the development of non-invasive genetic sampling (NGS) techniques (collection of hair, faeces etc.) has enabled genetic monitoring (including genetic capture-mark-recapture (CMR) analysis) of wild brown bear populations. This allows biologists to study parameters as abundance and migration, without having to catch or disturb the animal (5, 18, 19). NGS is especially relevant for the monitoring of small, endangered populations and over large areas as opposed to conventional methods (e.g. observation in the field or invasive sampling e.g. from hunting) (6). This method has advantages even in larger, healthy bear populations as a much more extensive portion of the population can be surveyed at a lower cost (20, 21). The success rates when analysing non-invasively collected sample material have been reported to be approximately 55-70 % in the Norwegian monitoring of the brown bear (12, 22, 23). Other studies have reported similar results (24). Hair traps, typically made of barbed wire stretched around some trees with a scent-lure in the centre, are one of the non-invasive sampling techniques that has been utilized in many studies of the black and brown bear (23, 25). Since 2007, this method has been applied in genetic monitoring of the transborder brown bear population of Norway, Finland and Russia (26, 27).

There are several challenges with sample materials like hair and faeces collected in the field when applied to genetic analysis. First, the extracts from these sample materials often have a low quantity yield of DNA that makes PCR amplification challenging. In addition, DNA degradation may decrease effective template concentration. Especially faecal samples may contain PCR inhibitors that lead to negative amplification results despite the presence of sufficient template DNA. Also, a sample may consist of materials (e.g. hair) from more than one individual, providing a mixture of two genotypes rather than an individual genotype (5, 19).

1.2. Microsatellites

Microsatellites, also known as short tandem repeats or STRs, are repetitive DNA sequences (tandem repeats) of 1-6 nucleotides. Typically, the number of repeats in such polymorphic loci vary between 5 and 40 repeats (28). Slipped strand mispairing (SSM) is thought to be the main mechanism for STR instability in vivo. Mutations caused by SSM have a different number of repeats compared to the progenitor allele and SSM is, thus, the main mechanism creating allelic diversity at a microsatellite locus (29). Compared to other genetic markers like SNPs (single-nucleotide polymorphism), the co-dominant STR markers are usually multi-allelic (in contrast to bi-allelic SNPs or indels). Due to this they often have much higher heterozygosity frequencies (30).

In conservation genetics, microsatellites have been used extensively to study e.g. population diversity, impact of genetic drift, and level of inbreeding in a variety of species (4). While tetranucleotide STRs are recommended in human forensics, dinucleotide STRs have been the preferred DNA markers in wildlife genetic studies of many species including the European brown bear (*Ursus arctos*) (3, 31-34). In the early 1990‘s, Pierre Taberlet in France and David Paetkau in Canada were the first to clone and sequence dinucleotide microsatellite loci from the European brown bear (first annotated UarMu loci, now annotated as Mu loci) and the American black bear (annotated as G loci), respectively (32, 35). Although the G loci were identified in American black bear, they were put to use as STR markers in brown bear as well (32). Much of later research by these and other scientists built on analysis of different bear populations applying these markers. The use of the same dinucleotide STR loci in many studies on the brown bear resulted in reference databases in which to match DNA profiles from new samples against in CMR studies (22, 26, 36). Although the ISFG recommends the application of tetranucleotide STRs in wildlife forensic casework, dinucleotide markers that are already widely used in genetic studies of wildlife species, are accepted as preferred markers also for forensic purposes, when the other validation criteria are met (3).

Twelve bear specific dinucleotide STRs have been validated according to ISFG recommendations for non-human forensic DNA typing criteria (7). This DNA profiling system (the combination of the twelve markers) was the first set of STRs applied in wildlife forensic and conservation genetics that provided guidelines for interpretation of genotyping results from dinucleotide STRs (based on experimental validation). Applying these markers, it was shown that the combined power provided individual-specific bear DNA profiles (7, 37).

1.3. Validation of STR markers used in a DNA-profiling system for brown bears; parameters to consider

In the study of Andreassen *et al.* (2012), a set of twelve widely used dinucleotide STR loci were validated with respect to their sensitivity, species specificity and performance (precision, heterozygote balance and stutter ratios). Other genetic parameters (allele frequencies, Hardy-Weinberg equilibrium, linkage equilibrium, heterozygosity frequencies etc.) that are relevant for their use in forensics and conservation genetics were also reported for eight populations of Northern European brown bear (*Ursus arctos)* (7). This DNA profiling system has been applied to establish a reference database for the Scandinavian brown bear that is used in population management and in monitoring of the respective populations (12, 22). The DNA profiling system and the reference database has also been applied in forensic casework (37). The combined results from these STR markers are individual-specific bear DNA profiles that have the power to distinguish even closely related individuals from each other. These DNA profiles may therefore also be applied to study e.g. family relationships (38, 39). They have also been used in numerous studies of geographically defined brown bear populations (especially in Scandinavia and Western Russia) to assess e.g. genetic diversity, level of inbreeding and genetic distance among the respective populations (31, 40-42).

The validation study of Andreassen *et al.* (2012), was carried out on singleplex amplified loci. Later eight of the STRs have been combined into three multiplex assays (MP1, MP2, MP3) routinely analysed in the Norwegian monitoring of the brown bear. The remaining four STRs have been combined with four new STRs (G10C, G10J, G10O, G10X) into two multiplex assays (MP8, MP14). See Table 1 for an overview of the different STRs and their combinations. When STRs are combined into multiplex assays, their performance may change (sensitivity, heterozygote balance etc.). The new multiplex assays should, however, not perform much less than the validated singleplex methods if to replace the validated singleplex-based DNA profiling system. Whether there is a satisfactory performance of the markers when multiplexed needs to be confirmed in new validations of marker performances. Furthermore, the four new STRs need to be validated in a similar manner for the first time. Since the previous validation there is a new capillary electrophoresis used. This may also affect the performance of the markers. Applying the same validation approaches as in Andreassen *et al.* (2012) the performance levels of the new multiplex combinations and the new STR loci may be recorded. A comparison to the performance and the recommended thresholds (current interpretation guidelines) from the older study would then reveal whether the current DNA profiling system has the desired reliability.

Table 1. *The 16 dinucleotide STRs combined into five different multiplex assays.*

Multiplex assay (MP)	STR locus[1]	New STR locus[2]
MP1	Mu09	
	Mu10	
	Mu23	
	Mu59	
MP2	Mu05	
	G10L	
	Mu51	
MP3	XY[3]	
	Mu50	
MP8	G10B	
		G10C
		G10O
		G10X
MP14	G1D	
	G1A	
		G10J
	Mu15	

[1]The singleplex validated dinucleotide STR loci shown as they are combined into multiplex assays.
[2]The new STRs as they are combined with the singleplex validated markers into the multiplex assays MP8 and MP14.
[3]Mu50 is multiplexed with a marker for the sex-specific DNA-sequence of the amelogenin gene denoted XY in MP3.

1.3.1 Sensitivity and species specificity

Results from the sensitivity test of the twelve STRs in Andreassen *et al.* (2012) indicated a successful amplification with template input in the range of 30-0.6 ng for all markers, while dropout of alleles was observed in several markers with a template input of 0.2 ng. The average peak heights (measured in RFUs – relative fluorescence units) of alleles in these samples with known concentrations were also recorded. The peak heights may reflect the template concentrations and may therefore aid in the judgements of template input in samples were effective template concentration is unknown (43). DNA concentration is not measured as these measurements will not provide the amount of bear specific DNA in non-invasive samples (e.g. faeces) (5). Thus, the effective template concentration is unknown in most cases. This test will also reveal if the sensitivity of the markers has changed and if the current guidelines (see section 1.3.4.) are sufficient to discover samples with too low copy numbers of template so that erroneous genotyping due to stochastic effects are avoided.

The twelve STRs were also validated regarding species specificity (7). These results showed that using template from animals other than bear, would give a negative result in the STR analysis. It is unlikely that a change to multiplex PCR amplification would alter these results. Given that twelve STRs were already validated as specific to bear, further analysis of species specificity was not included in our study as conclusions whether a sample consists of bear DNA is not based on the four new ones.

1.3.2. Precision

In the automated fragment analysis, alleles are designated by placing a sized DNA fragment in a size bin. A bin range equal to three standard deviations with no overlapping bin ranges results in 99.7 % of identical alleles being sized within the same bin (44). Single base separation is the aim in fragment analysis of STR markers to precisely score any length variation among alleles. To separate fragments that differ by 1 basepairs (bp) the 3 x SD bin range needs to be less than 0.50 bp (7).

The measurement of precision in Andreassen *et al.* (2012) revealed an ability to discriminate alleles of 2 bp size differences with a confidence of more than 99.7 % in all markers (SD $\leq$ 0.30 bp). Within-run measurement of the least performing locus resulted in considerably improvement of the precision when the between-run factors were eliminated (from 0.30 bp to 0.16 bp). The study concluded that alleles with 1 bp size differences would be detected with the sizing procedure applied. Such small length variation (microvariation) was only observed at locus Mu23 in the previous study. The population studied in this master thesis is from Far East Russia. Although microvariation among alleles has not been observed to a large degree in Western European brown bear populations, there may very well be a larger amount of such variation in the geographically very distant population at Kamchatka.

1.3.3. Stutter ratio and heterozygote balance

1.3.3.1. Stutter ratio

Stutter are artificial alleles generated as by-products in the PCR reaction. They differ in length to the true alleles with one or more repeats (45). An examination of electropherograms from amplification of STRs usually shows stutter peaks with sizes that are from one to several repeats less than the true allele (-1R, -2R, -3R etc.) and in rare cases one repeat larger than the true allele (+1R). The most common and most elevated stutter peak is in position -1R compared to the true alleles. The level of stutter decreases when moving away from the actual allele (7, 45). Figure 1 shows a typical result (electropherogram) from amplification of a heterozygous genotype in a dinucleotide STR locus with typical stutters at positions -1R, -2R and a very small stutter at +1R.

The height of stutter peaks varies among different kinds of microsatellites e.g. dinucleotide STRs have much more pronounced stutter formation than tetranucleotide STRs (45, 46). Comparing same type of microsatellites (e.g. dinucleotide STRs), each marker typically shows individual amounts of stutter (7, 45). In general, there is also a small variation within a locus with increasing amount of stutter with increasing number of repeats (45, 47). Each locus needs to be characterized experimentally to reveal expected stutter levels. Such knowledge is needed to set appropriate interpretation guidelines to assure that stutter is not misinterpreted as an allele (see also section 1.3.4.).

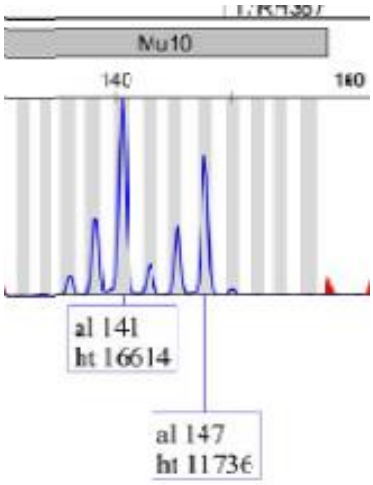


Figure 1. *An electropherogram showing results from a heterozygous sample typed in Mu10 with typical heterozygote balance and stutter products. The alleles (two higher peak heights) are marked as "al 141" and "al 147" designated in basepairs while the peaks to the left of the alleles are stutters (-1R and -2R repeats) generated in the PCR amplification of each allele. There is also a very small stutter at +1R (the small peak to the right of "al 147"). The figure demonstrates a typical heterozygote balance where the shorter allele has the higher peak height (16614 RFU vs. 11736 RFU). (figure from NIBIO Svanhovd).*

1.3.3.2. Heterozygote balance

Like all mammals, brown bears are diploid. The STR genotype of a particular locus is therefore either homozygous (two identical alleles) or heterozygous (two differently sized alleles). In the PCR amplification, the two alleles present in a heterozygous individual rarely amplify in equal proportions. Often the smaller-size allele amplifies slightly better than the larger-size, resulting in a higher peak height of the shorter allele (Figure 1). The imbalance is usually more pronounced when the differences in fragment lengths between the two alleles increases. If the heterozygote imbalance is large, the peak height of the less amplified allele may be below the lower peak height threshold (analytical peak height threshold, APHT) and not recorded as an allele (allele dropout) (43).

Like the stutter ratios, each STR locus therefore needs to be experimentally validated by characterization of heterozygote balance (the ratio between the allele peak heights in a heterozygous genotype). Based on calculated heterozygote balance ratios and APHT, a homozygote peak height threshold (HPHT) can be set. The purpose of having a HPHT is to avoid mistyping a true heterozygous as a homozygous due to a dropout of the larger allele caused by a large heterozygote imbalance. The HPHT is used as a lower peak height threshold (RFU) to accept homozygous STR genotypes (43).

1.3.3.3. Genotyping errors associated with heterozygote balance and stutter

There are two common genotyping errors associated with heterozygote balance and stutter. Applying dinucleotide STRs one need guidelines based on validation tests and trained personal to carry out manual genotyping. As proportions of stutter are much more pronounced and there are larger imbalances in amplification efficiency of two alleles in a heterozygote than what is observed in the tetranucleotide markers used in human forensics, manual genotyping with guidelines based on reliable validations tests is crucial to avoid genotyping errors (7, 45). First, not using validated HPHT based on known heterozygote balance ratios, could result in a failure to detect the less amplified (often the larger) allele. Failing to detect a dropout of an allele would result in mistyping a heterozygous as a homozygous genotype (43). The second common genotyping error is related to stutter. If marker performance were so that the stutter peak is about the same size as the true allele, a homozygous with a high stutter peak would be hard to distinguish from a heterozygous with alleles that are separated by one repeat. Failure in recognizing stutter as an artefact is an error that would result in mistyping a homozygous as a heterozygous genotype (7). Detailed knowledge of the expected heterozygote balance and stutter ratios in the STRs used, may be utilized to provide interpretation guidelines and set a lower threshold for peak heights to avoid the two genotyping errors described above (43, 45).

1.3.3.4. Low Copy Number samples; stutter and heterozygote balance

Typically, stutter proportion relative to the true alleles may increase when amplifying low levels of DNA template with elevated number of PCR cycles. This is due to stochastic effects and in such cases stutter could deviate from expected proportions (45). In heterozygous individuals, it is expected that there are predictable proportions of heterozygote balance when template input is above a certain concentration. However, the level of heterozygote balance is, like stutter ratio, less predictable when there are low amounts of efficient template DNA (Low Copy Number (LCN) samples). As the template concentration decreases there may be stochastic biases leading to an uneven input of template molecules from the two alleles in the PCR reaction. This may lead to selective amplification of one allele over the other and in worst cases dropout of one allele (43).

LCN samples have been referred to as samples that contain less than e.g. 0.2 ng of DNA template (48), but the term is now used to describe partial profiles (the quality of the result), independent of the analysis method used (49, 50). Extracting DNA from non-invasively collected sample material often yield low amounts of DNA (LCN samples) (5). As PCR amplification of low template DNA results in unpredictable proportions of stutter ratio and heterozygote balance, special typing criteria are set to assure that LCN samples which could, when analysed, result in erroneous genotypes are not used. Numerous studies in human forensics have investigated the effect of low template DNA samples on STR analysis and offered interpretation guidelines in order to assure correct genotyping (51-53).

1.3.4. Current interpretation guidelines for genotyping

The current interpretation guidelines for genotyping used at Svanhovd bear laboratory are based on the validation study from 2012 (7) on twelve STRs using singleplex PCR methods. HPHT is 600 RFU for all STRs. In a heterozygous with the shorter allele above 600 RFU, the other larger-size allele is accepted with an APHT of 300 RFU. Manual inspection is needed to assess the quality of the alleles. Any samples where both alleles in a heterozygous or the single allele in a homozygous are less than 600 RFU, are considered unreliable and not used. For comparison, a commonly used APHT in human forensic genetics is 50 RFU, while the HPHT is often set as 150 - 200 RFU (43, 52). The required sample quality (measured as lower peak height thresholds accepted) are thus considerably more stringent than those used in human forensic genetics.

The samples are always analysed in two multiplex assays (MP1 and MP3; five markers and a gender test) as a first test of sample quality. Based on these results, the samples lacking results at any locus are discarded. All positive samples are further analysed twice in MP1, MP2 and MP3 (eight markers). All heterozygous genotypes require two identical, independent results (shorter allele above 600 RFU, longer allele above 300 RFU) to be accepted, while all samples with homozygous genotypes are confirmed with three identical results (homozygous peak above 600 RFU in all cases). If the replicate genotype results at a locus are not identical to each other, a separate singleplex analysis for the locus in question is performed. Whenever a genotype cannot be confirmed at a single locus, the genotype for this locus is discarded. However, when such divergent genotype results or no results are obtained at two or more loci, the whole sample is discarded as an LCN sample. It is recognized as originating from bear (no other species), but not given an individual specific profile.

Probability of allele dropout is increased, and stutter proportions and heterozygote balance are less predictable in LCN samples due to stochastic effects (43, 51, 54). This unpredictability is also experienced at Svanhovd bear laboratory. The purpose of the procedure with repeated analysis of a sample is to identify and discard such samples to avoid erroneous genotyping. This approach has also been recommended in human forensics (55). Experience at Svanhovd laboratory has shown that the stochastic effects of low template input will result in divergent results. Analysis of the same sample at all eight loci and in at least two independent PCR amplifications is therefore part of the protocol used. The duplicate and triplicate runs of heterozygous and homozygous genotypes are important as they are the main mean to identify LCN samples with too low or degraded template DNA. Also, being that the average peak heights of an LCN sample tend to be low, samples with allele peak heights below 1600 RFU are watched carefully as part of the routine at the Svanhovd laboratory (43). The sensitivity test performed in this thesis may reveal if the current interpretation guidelines are sufficient to discover such LCN samples.

Eight of the twelve validated STR markers (Mu09, Mu10, Mu23, Mu59, Mu05, G10L, Mu50, Mu51) and a sex specific marker are used in the yearly monitoring of the Norwegian brown bear (all non-invasive samples collected as well as those from legally shot bears). All samples are typed in these eight markers while the others are applied on selected samples for research purposes. New DNA profiles if reliably typed in all eight STRs as well as the sex specific marker, are registered in the bear reference database.

Since these guidelines and monitoring regimes were implemented, the analysis method has changed from single- to multiplex PCR assays, there has been a switch in the technical equipment and four new STRs have been added as genetic markers to be used in genetic studies of brown bear.

1.3.5. Identification of mixed samples

Samples that consist of DNA from more than one individual are usually termed mixtures. In human forensics where tetranucleotide STRs are analysed, it may be possible to calculate the number of contributors in a mixture and distinguish the different DNA profiles from one another. This is done by the aid of interpretation guidelines based on knowledge of the performance of the STRs used (56). In non-human analysis where dinucleotide STRs are applied, distinguishing individual profiles from mixtures is difficult due to elevated stutter and more skewed heterozygote balance (7, 45). The validation study of the bear DNA profiling system in Andreassen *et al.* (2012) concluded that individual genotypes from a mixed sample cannot be distinguished using these STRs. However, it will be possible, in most cases, to reliably identify a mixed sample as the identification of a sample with DNA from several individuals only requires that there are three alleles in at least one locus (heterozygosity frequencies above 0,70 in most of the twelve STRs) (7). As all samples in this project are from hair materials, extracting DNA from one hair root only may also be a solution if mixed samples are suspected.

1.3.6. Null alleles and microvariation

Null alleles are alleles not being PCR amplified in samples with good DNA quality. This can be due to sequence variation (usually single nucleotide polymorphisms, SNPs) in the part where the primers anneal, or because the complete locus is absent as a result of larger deletions of the complete STR locus (28, 43). The latter was e.g. the likely explanation for null alleles in the STR locus Mu26 (7). The use of PCR primers that hybridize to different parts of the flanking region may solve this problem if null alleles are caused by SNPs (43).

Microvariation, e.g. small deletions or insertions of a number of bases different from two (in dinucleotide STRs), will result in alleles with sizes that are differing from others by less than one repeat. The power to detect such variation depends on the precision of the allele measurements (44,

45). If detected, the cause for the microvariation may be revealed by sequencing the alleles showing such variations.

As the population studied in this master project is from Far East Russia, it would not be unexpected if there exists both null alleles (due to primer site polymorphisms) and microvariation not described in the Scandinavian and Northwest Russian populations studied so far.

1.3.7. Hardy-Weinberg principles and linkage disequilibrium

The Hardy-Weinberg law is one of the fundamental principles of population genetics. According to this law allele frequencies are related to the genotype frequencies and remain constant (equilibrium) from generation to generation in a large population where mating is random and there is no inbreeding, mutation, migration, genetic drift or selection (57). The expectations for Hardy-Weinberg equilibrium (HWE) are rarely met in wildlife species like the brown bear. Many conclusions in population genetic studies are, in fact, based on such observed deviations. If the other factors leading to deviation from the Hardy-Weinberg equilibrium are neglectable, one may use the HWE (or the deviation from it) to argue that one particular evolutionary force is the reason for the observed deviation. Following this logic, deviations from HWE genotype frequencies may be used to indicate inbreeding, population fragmentation, migration etc. (58). Deviations from HWE may be estimated based on the observed genotype frequencies compared to the expected genotype frequencies (estimated from the observed allele frequencies) at any given locus. The chi-squared test, or better Fisher exact test, is commonly applied for this comparison to test whether the observed frequencies are significantly different from the expected ones (59).

Deviations from HWE due to an excess of homozygosity at a single STR loci in a population, may, however, be used to identify typing errors (57, 60). One example from bear STR typing was the large homozygote excess that led to the identification of null alleles in Mu26 (7). Also, the forensic estimations of e.g. how rare a genotype is in a given population assume random combination of alleles (HWE) (61). To test for departures from HWE is therefore an important part of any validation work as it may reveal method-related reasons leading to erroneous genotyping. Absence of large deviations from HWE is needed to demonstrate that the forensic estimations are valid (57).

Genotypes from loci in linkage equilibrium are inherited independently of each other. Conversely, if genotypes from two loci (e.g. two STR loci) are not randomly inherited, but tend to appear together, they are said to be in linkage disequilibrium (LD) (57). The presence of such linked STR loci in a DNA profiling system will invalidate the estimation of forensic parameters like the probability of identity (see section 1.5.) (61). Statistical testing for deviations from linkage equilibrium is the most common procedure applied to identify linked loci (LD) (34, 57).

1.4. Population genetic parameters

1.4.1. Genetic diversity; heterozygosity and allelic diversity

As genetic diversity is vital for the long-term survival of populations, it is an important parameter to estimate. Both allelic diversity and observed *vs* expected heterozygosity are parameters used to describe the populations` genetic diversity (58). The observed heterozygosity at a locus is calculated by dividing the number of heterozygous genotypes with the total number of genotypes (57). If the marker does not deviate from HWE, expected heterozygosity may be estimated based on the observed allele frequencies. Expected heterozygosity is a better estimate for genetic diversity, as it is not dependent on sample size (58). If number of alleles at a locus are many and there is an equal distribution of allele frequencies, this will lead to high expected heterozygosity (57). To characterize genetic diversity in populations, the average expected heterozygosity over all loci (e.g. the twelve STRs) is used (7, 58). Allelic diversity at each locus and across all loci (calculated by dividing the total number of alleles over all loci by the total number of loci) is also used to characterize genetic diversity (62).

A higher heterozygosity and allelic diversity is usually expected and observed in the larger populations compared to the smaller ones (6). Allelic diversity and heterozygosity levels in the Kamchatka brown bear population, studied in this thesis, may be compared to the eight populations previously studied as they are now analysed with the same validated markers. The large differences in population size and how they have been affected by population genetic forces e.g. the Scandinavian populations have been through relatively recent population bottlenecks (10), it is reasonable to believe there are differences in genetic diversity.

1.4.2. Genetic differentiation (population structure) and genetic distance

Genetic differentiation within and between populations can be estimated based on F-statistics. F_{IS}, the inbreeding coefficient, reflects the probability that one individual has two alleles which are identical because they have been inherited from a shared relative (identical by descent). F_{ST}, the fixation index, is an estimate of the genetic differentiation between subpopulations e.g. how likely it is that two members of a subpopulation share identical alleles because they are inherited from a common ancestor (Identical by descent). When reported as a single statistics over loci and populations, F_{ST} renders an estimate of average differentiation (57, 63). F_{ST} is the same as theta (co-ancestry coefficient) when mating within subpopulations is random (57, 64). For the estimation of population substructure, it is recommended to estimate several different estimators and execute caution in their interpretation to avoid erroneous conclusions (65-67). Even so, F_{ST} is widely used and should always be included in a study on genetic differentiation (66). This was also the estimator included in Andreassen *et al.* (2012).

The estimation of pairwise F_{ST} between populations is one way to investigate the genetic distance between populations (68). This has been one of several methods applied to estimate differentiation between brown bear populations (36, 40, 41). The genetic distance between the Kamchatka brown bear population and the eight (Scandinavian and Western Russian) populations studied in Andreassen *et al.* (2012) may be assessed by the analysis of pairwise F_{ST}. Given the large geographic distance between Kamchatka and the other populations, one would assume that isolation by distance (IBD) is reflected in the genetic distance measurements (68).

1.5. Forensic efficiency parameters

The forensic value of each STR marker in a DNA profiling system may be evaluated by a set of forensic efficiency parameters (57). The matching probability (PM) is equal to the probability of identity (PI). It is calculated by summing the square of the genotype frequencies (57). The total average PI is the probability that two different individuals by chance have the same DNA profile in the STR markers applied. Assuming that the loci included in a DNA profiling system are not linked, the total average PI is the product of the average PI for each locus. The estimated average PI for each marker may aid in determining the number of loci required in a DNA profiling system to provide effective identification of individuals (61).

To give a correct PI estimate, one need estimates of allele frequencies and the degree of relatedness must be accounted for, as relatedness leads to small deviations from HWE (61, 69). F_{ST}, or better the estimate of theta, is used in estimations of PI to adjust for the historic relatedness between individuals leading to HWE deviations in allele frequencies (61). It is usually higher in wildlife species than in humans, and therefore an important parameter to include in the estimation of PI (34). F_{IS} is an estimate of the inbreeding in the population. Depending on the mating system in a wildlife species (e.g. if one alpha male parents all offspring in an area), one may observe a significant F_{IS} value. The probability of a random match at homozygous loci will e.g. increase when F_{IS} is high (70). Probability of identity for siblings, PI_{sib}, (and the combined total average PI_{sib}) is a measurement of how likely it is that two individuals are identical by chance when the effect of relatedness (theta) is maximized. It is estimated by using an adjustment of a theta value that assumes all individuals are siblings. A conservative measurement of whether two individuals could show identical genotypes in markers used, would therefore be to estimate the total average PI_{sib}. If the chance of retrieving identical genotypes is still low (total average PI_{sib} is low), the chance of retrieving identical genotypes from two random individuals in a population would be equally low (7, 61, 69).

For a DNA profiling system to be useful in wildlife forensic cases it has been suggested that it should have the power equal to a total average $PI < 0.001$ or $PI < 0.0001$ (depending of the size of the source population) (69). The largest total average PI from the twelve markers in the Western European populations was 1.1×10^{-9} while the largest total average PI_{sib} was 1.3×10^{-4} (7). The total average PI

in the Kamchatka populations should be equally low if arguing that these bear DNA profiles are individual-specific.

The probability of discrimination (PD) is equal to 1 - PI, and if this value is close to one it indicates that the marker has a high discriminating power. The paternity index PI_{typ} is the likelihood that the tested male is the biological father, rather than a randomly selected unrelated male. It is determined by $PI_{typ}= \frac{1}{2h}$ where h=observed homozygosity (frequency). The power of exclusion (PE) or probability of exclusion, on the other hand, is the power that non-related individuals are excluded in a testing of family relationship. It is calculated as $PE=H_O^2 (1- 2H_O (1 - H_O)^2)$ (at any given locus) where H_O=observed heterozygosity (frequency) (57).

1.6. The Kamchatka bear population in this study

The Kamchatka bear population has, to our knowledge, not been studied using autosomal STR markers thus far. The geographic distance (Figure 2) from the Western European populations, in which large amounts of population specific genetic data exist (6, 40-42), is at its maximum. The population material was collected (July-August 2015) in hair traps set up at 16 different locations, covering a very limited area of Kamchatka (Figure 3). See Table 2 for an overview of the hair trap stations, their coordinates and by which river they were located. Although the densities of brown bear on the Kamchatka peninsula are high and among the highest in Russia (17, 71), the population studied here are all individuals sampled in an area less than 200 square km. One would therefore not expect to reveal the total genetic variation of the Kamchatka bear population in this material, only the genetic variation in a smaller geographically limited subpopulation.

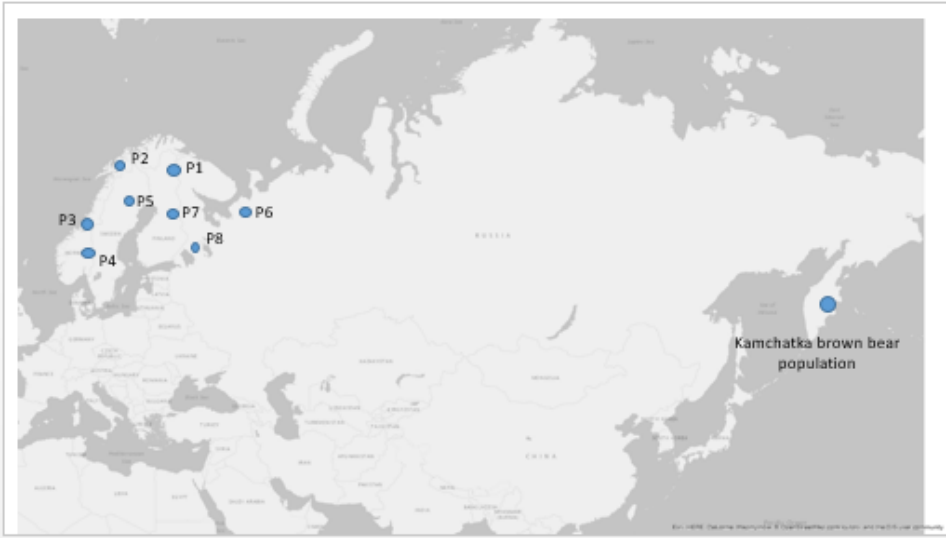


Figure 2: *The figure shows a map of Europe (including Norway, Sweden and Finland) and Russia. The sample locations of each of the eight bear populations, P1-P8, included in Andreassen et al. (2012) and of the Kamchatka brown bear population studied in this thesis, are indicated on the map. P1-P4 are populations in Norway, P5 is a population in Sweden, P6 and P8 are populations in Russia and P7 is a population in Finland.*

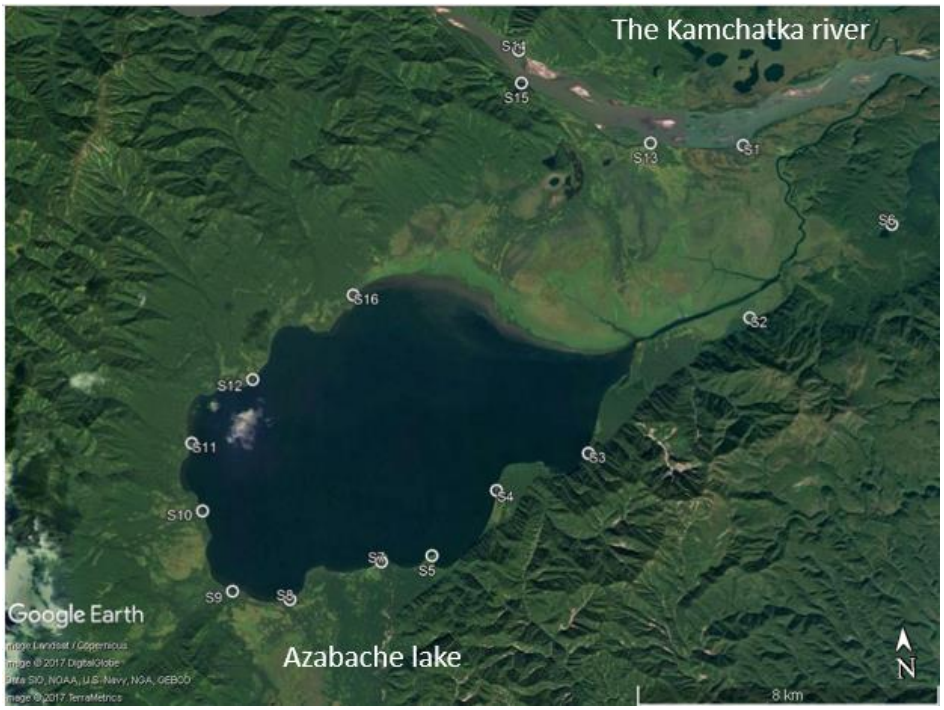


Figure 3. *The figure shows a map of the geographic area (approximately 200 square km) on the Kamchatka peninsula in which 16 hair traps were set up. The different hair traps are marked with a white circle and denoted S1 to S16.*

Table 2. *The sixteen different stations in which the hair traps were set up (S1-S16), their coordinates and by which river they were located.*

Station	Coordinates	River
S1	N56 12.687 E161 57.618	Kamchatka
S2	N56 10.213 E161 57.547	Olezkina
S3	N56 08.395 E161 53.224	Geshkin
S4	N56 07.930 E161 50.822	Lotnaya
S5	N56 07.044 E161 49.083	Vydrovaya
S6	N56 11.443 E162 01.330	Kursinka
S7	N56 06.990 E161 47.780	Bushujka
S8	N56 06.516 E161 45.387	Topolinaya
S9	N56 06.674 E161 43.927	Lamutka
S10	N56 07.842 E161 43.254	Kultuchnaya
S11	N56 08.818 E161 43.057	Arishkin
S12	N56 09.686 E161 44.700	Snovidovskiy
S13	N56 12.787 E161 55.234	Kamchatka
S14	N56 14.209 E161 51.963	Kamchatka
S15	N56 13.737 E161 51.988	Kamchatka
S16	N56 10.824 E161 47.388	Ponomarka

2. Aims of the study

The aims of this project were twofold:

1. One aim of this study was to perform validations of all 16 STR markers currently in use or on trial for brown bear genotyping at the Svanhovd bear laboratory. Twelve of these markers were combined in multiplex assays, while four markers have not been validated at all. Based on the results from the validation the current guidelines could be confirmed as A) reasonable, B) they could be changed according to markers performance or C) if not at all meeting validation criteria, markers could be deemed as unsuitable for use under current conditions. STR markers are validated by conducting a number of tests, in the case of wildlife genetics, the validation tests recommended from ISFG is the "gold standard". Implementing results from this validation, would assure a continued reliable typing of bear samples from all populations routinely typed at Svanhovd bear laboratory.

2. The other aim was to apply the validated STR markers to study a Kamchatka brown bear population. As this is the first such study of brown bear in Far East Russia, it will reveal whether the same markers can be used in this population. The analysis would also provide genotype- and allele frequency estimates allowing for calculations of all relevant forensic genetic parameters needed for their application in such a context. Furthermore, the results will be used to estimate population genetic parameters like diversity (average heterozygosity, allelic diversity) and inbreeding. Finally, they will be used for first comparisons to Western European bear populations and genetic distance will be calculated.

3. Materials and methods

The analyses were performed in February and March 2017 (DNA extraction) and from September 2017 to January 2018 (PCR amplification and fragment analysis) in the laboratory at NIBIO, Svanhovd. See Appendix 1 for an overview of the reagents and instruments used.

3.1. Population material from Kamchatka brown bear

Russian scientists from Lomonosov State University in Moscow provided hair samples from bears in the Kamchatka region (n=434) collected in hair traps (23). The area in which the hair traps were located was at the Kamchatka peninsula (see section 1.6.) encompassing approximately 200 square km (Figure 3). The samples have been stored dry, dark (in paper envelopes) and in room temperature until DNA extraction.

3.2. Materials used in validation tests and as positive controls

Samples from four different individuals of brown bear (*Ursus arctos)* with known genotypes in all twelve STR singleplex validated markers, were included as positive controls in the STR analysis pipeline. These samples consist of DNA which has been extracted (at NIBIO Svanhovd) from bear tissue using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturers protocol for tissue samples. They were from legally shot bears in Norway (T8 (male, 2007), T11 (female, 2009)) and Karelian, Russia (T9 and T10, both male, harvested in 2005). These samples have been used for several years, as positive controls in the accredited analysis (ISO/IEC 17025) carried out in the yearly monitoring of the Norwegian brown bear population.

Two of the control samples (T10 and T11) were analysed with three different template inputs: 0.6 ng, 0.2 ng and 0.05 ng as part of the validation tests (see section 3.2.1.). The other two controls (T8 and T9) were analysed with template input of approximately 0.3 ng. See Appendix 2 for an overview of the initial DNA concentrations in the control samples prior to dilution. Negative controls (ddH_2O, Qiagen) were included for every seventh sample run in the analysis pipeline. The positive controls (T8 and T11) were also used for measurements of stutter and heterozygous balance as part of the validation of the multiplexed 16 STRs.

3.2.1. Sensitivity tests: sample preparation and measurements

Samples from two of the control samples (T10 and T11) were included to test the sensitivity of all 16 markers when analysed in multiplex assays. A NanoDrop™ 1000 spectrophotometer (Thermo Scientific) was used to measure the initial DNA concentrations (ng/µl, in duplicate) of the samples prior to dilution (results in Appendix 2). The two control samples were then diluted in ddH_2O (Qiagen) to obtain the concentrations wanted for this study. The sensitivity was first tested in template inputs of 1.0 ng, 0.6 ng, 0.4 ng, 0.3 ng, 0.2 ng, 0.1 ng and 0.05 ng for MP1, MP2 and MP3. See section 3.4. and 3.5. for PCR amplification and fragment analysis protocol. These concentrations were chosen to span the template input in which dropout occurred in the sensitivity test of Andreassen *et al.* (2012).

To further explore the approximate lower concentration levels where dropout of alleles could be expected, another dilution series with template input of 0.4 ng, 0.3 ng, 0.2 ng, 0.1 ng, 0.05 ng, 0.03 ng and 0.02 ng were also analysed with the same three assays. In Appendix 2 results from this preliminary sensitivity test are summarized for all concentrations. Based on these results and the results of the sensitivity test in Andreassen *et al.* (2012), two control samples with DNA concentrations of 0.6 ng/µl, 0.2 ng/µl and 0.05 ng/µl were included in each set-up (PCR followed by capillary electrophoresis) that was carried out when analysing the 434 samples from Kamchatka. Thus, each of these concentrations were tested 16-20 times in all 16 STR markers. The main purpose of these validation tests was to find the approximate lower limit of template input in which successful amplification of all the markers may be achieved. The peak heights (RFU) of samples where dropout occurred were also registered to find approximate peak height levels where dropout could be expected.

3.3. DNA extraction

Typically, each sample consisted of from 1-10 hairs. The root tip of hairs from each of the 434 samples were cut and DNA was extracted from the roots using the DNeasy Blood & Tissue kit (Qiagen) following the manufacturers protocol (extraction of DNA from tissue). Depending on the number of hairs in each sample, the DNA extract was eluted in 50 µl buffer when 4-10 hairs from a sample were pooled, and in 30 µl when 1-3 hairs from a sample were pooled. With the latter a second elution in 30 µl buffer was performed. The DNA samples were stored frozen at -20 ºC.

3.4. PCR amplification

All the DNA samples from Kamchatka, as well as control samples used in validation tests, were analysed using 16 STRs (of which twelve have been previously validated in singleplex assays (7)) They were also analysed in a sex identification marker (X- and Y- specific DNA sequences of the amelogenin gene) (72). The STRs applied were (new STRs marked in bold): Mu09, Mu10, Mu23, Mu59, G10L, Mu05, Mu50, Mu51, G10B, **G10C**, **G10O**, **G10X**, G1D, G1A, **G10J** and Mu15 (32, 35). The modified forward and reverse primer sequences used in this study, the PCR conditions and the reference numbers to each of the corresponding loci from GenBank are given in Table 3. The table also shows which multiplex assays the different markers are combined into (MP1, MP2, MP3, MP8 and MP14). A short 5´ tail has been added to the reverse primer in eleven of the 16 primer pairs to enhance their amplification success (73).

Multiplex PCRs were performed in 10 µl reaction volumes containing 1 x Multiplex PCR Master Mix (Qiagen), 1 µl primer mix (ABI), 0,5 x BSA (NEB), 2,95 µl ddH_2O (Qiagen) and 1 µl template DNA. The addition of template DNA was mostly performed by a pipetting robot (epMotion 5070, Eppendorf). Due to low volumes of template, some manual pipetting was also executed. PCR conditions were 10 minutes at 95 ºC, 35 cycles of 30 seconds at 94 ºC, 30 s at 58 ºC, and 1 min at 72 ºC, and final extension for 45 min at 72 ºC.

Singleplex PCRs were performed in a few cases on samples where the genotyping of a single STR locus was incomplete (see section 3.5.). These PCRs were performed in 10 µl reaction volumes containing 1,5 mM PCR Gold buffer (ABI), 200 µM dNTP (Thermo Scientific), 1,5 mM $MgCl_2$ (ABI), 0,5 µM of each primer (ABI), 1 U AmplitaqGold DNA polymerase (ABI), 1 x BSA (NEB) and 1 µl template DNA. PCR conditions were as aforementioned for all STRs except the singleplex analysis of G10X that used an annealing temperature (T_a) of 54 ºC.

Table 3: *Primer sequences for 16 STR markers and for the X- and Y- specific DNA sequences of the amelogenin gene. The four new STR loci included in MP8 and MP14 are marked in bold.*

MP[3]	locus	Primer sequences (5'-3') F=forward, R=reverse	Allele size range (bp)	multiplex PCR conditions[4]	GenBank accession no.
MP1	Mu09	F:GCCAGCATGTGGGTATATGTGT	98-128	0,1 µM, FAM	Y09641.1
		R:*GTTTCTT*AGCAGCATATTTTTGGCTTTGAT[1]			
	Mu10	F: TTCAGATTTCATCAGTTTGAC	133-153	0,3 µM, FAM	Y09642.1
		R:TTTGTATCTTGGTTGTCAGC			
	Mu23	F:GCCTGTGTGCTATTTTATCC	164-180	0,05 µM, FAM	Y09645.1
		R:*GTTTCTT*TTGCTTGCCTAGACCACC[1]			
	Mu59	F:GCTGCTTTGGGACATTGTAA	224-256	0,6 µM, FAM	Y09649.1
		R:*GTTTCTT*CAATCAGGCATGGGGAAGAA[1]			
MP2	Mu05	F:ATGTGGATACAGTGGAATAGACC	109-133	0,1 µM, FAM	Y09640.1
		R:*GTTTCTT*GTGACATGAACTGAAACTTGTTA[1]			
	G10L	F:CAGGACAGGATATTGACATTGA	166-196	0,05 µM, FAM	U22088.1
		R:GATACAGAAACCTACCCATGC			
	Mu51	F: GCCAGAATCCTAAGAGACCT	130-152	0,05 µM, VIC	Y09648.1
		R:*GTTTCTT*GAAAGGTTAGATGGAAGAGATG[1]			
MP3	XY[2]	F:CAGCCAAACCTCCCTCTGC	94 and 149	0,2 µM, FAM	
		R: AGGTGGCTGTGGCGGCA			
	Mu50	F:GTCTCTGTCATTTCCCCATC	106-136	0,2 µM, PET	Y09647.1
		R:GAGCAGGAAACATGTAAGATG			
MP8	G10B	F:ATTTTCTTGAGGACTTTTGCATATA	94-122	0,2 µM, FAM	U22084.1
		R:*GTTTCTT*AACCTCCATCCATACAATACAAC[1]			
	G10C	F:CAACAAAAGGTTGAAGGGAG	153-167	0,1 µM, FAM	U22085.1
		R:*GTTTCTT*AAACACCGAGACAGCAGG[1]			
	G10O	F:CTTTGGCTACCTTCAGATGG	195-207	0,1 µM, FAM	U22090.1
		R:*GTTTCTT*TGCCTACTGCACCAACAG[1]			
	G10X	F:TTCCAATTCTCCCAGTAGC	174-196	0,1 µM, VIC	U22093.1
		R:*GTTTCTT*ATCTGTGAAATCAAAACAAACA[1]			
MP14	G1D	F:TCTCTTTTCCTTTAGGGGACT	123-139	0,2 µM, FAM	U22094.1
		R:CTAGCACCCAGCAAGGTATAATA			
	G1A	F:ACCCTGCATACTCTCCTCTGATG	177-195	0,1 µM, FAM	U22095.1
		R:GCACTGTCCTTGCGTAGAAGTGAC			
	G10J	F :GCTTTTGTGTGTGTTTTTGC	99-127	0,15 µM, VIC	U22087.1
		R:*GTTTCTT*TACTGGGAAAATCACTCACC [1]			
	Mu15	F:CATCTGAATTATGCAATTAAACAGC	96-120	0,1 µM, NED	Y09644.1
		R:*GTTTCTT*GTTTTTGTTTAGCAGGTTTGTCTC[1]			

[1] A tail (in italic) has been added to the reverse primer (73).
[2] Primer sequence for amplification of the sex-specific DNA fragment of the amelogenin gene (72).
[3] MP= multiplex assay, the different multiplex assays denoted MP1, MP2, MP3, MP8 and MP14.
[4] The fluorescent label and concentration (in the PCR reaction mix) of each primer.

3.5. Fragment analysis

Capillary electrophoresis (CE) with 4 seconds injection time, was carried out on an ABI 3730, and the PCR fragments were analysed in GeneMapper 4.0 (ABI). Formamide (10 µl, ABI) was added for denaturation of the PCR product (1 µl) prior to CE and allele sizes were measured using Genescan 500LIZ standard (ABI). At least two independent PCR amplifications followed by fragment analysis were performed on each sample. The marker for sex determination was run in three independent replicates for each sample. Samples which were initially typed as a homozygous genotype at any locus were confirmed by a minimum of three replicates (peak height threshold values of 600 RFU) and heterozygous genotypes were confirmed by two replicates (shorter-size allele with peak height above 600 RFU and larger-size allele with peak height above 300 RFU), in accordance with the current genotyping guidelines. If less, it was not accepted as a reliable result. Based on the validation tests carried out in this project, these peak height thresholds were changed. As a consequence, all samples were re-analysed and whether genotypes were accepted as reliable results were based on the new guidelines for peak height thresholds (see section 5.1.3.). The third replicate typing of a particular STR locus was for the most part amplified in a singleplex PCR assay for the specific marker in question.

Fragments were sized by their length in basepairs. The four positive controls (from the tissue samples) were used as allelic ladders to adjust for between-run variation (see section 3.2. and 3.2.1.). Results from these control samples, each run ≥ 20 times, were also used in the measurement of precision, stutter ratio and heterozygote balance (section 3.6.). The final allele designation included manual inspection of electropherograms.

3.5.1. Designation of an individual DNA profile

All samples were initially analysed using a standard set of eight STR loci (MP1, MP2 and MP3). A satisfactory result in all eight loci, as well as a confirmed gender, was required to be accepted as a sample providing a result that could differ between individuals (individual ID profile). The power estimates that were later carried out based on allele frequencies in the Kamchatka population, confirmed that this was a reliable assumption. Any sample with results in these eight markers that were identical to another sample, was assumed to be a recapture of the same individual. Using this strategy, the 434 samples were sorted into DNA profiles originating from different individuals, some recaptured several times (replicates of identical profiles). For analysis of the remaining eight STR loci (MP8 and MP14), only one high quality sample was analysed from each individual. The individual ID profiles obtained from the Kamchatka samples were consecutively named KamOx (KamO = Kamchatka Oblast, x = numbers starting from 1).

3.5.2. Samples denoted as negative, unknown or mixed samples

Samples with no results in any loci were defined as negative for DNA from bear. All samples with a satisfactory genotype result in at least one STR marker were designated as positive for DNA from bear. Of these samples, the ones with genotype results in less than eight markers were designated as a bear without an individual specific DNA profile, denoted as an unknown individual. A sample with three alleles or more in at least one marker, was discarded as a mixed sample (more than one contributor).

3.6. Validation tests of precision, stutter ratio and heterozygote balance

Between-run precision was measured in all candidate STRs by 20 or more independent amplifications and subsequent runs of the two heterozygote positive controls T8 (MP1, MP2, MP3) and T11 (MP8, MP14). Template input was 0.3 ng and 0.6 ng, respectively. Measurements of stutter ratio and heterozygote balance were also acquired from these runs. Stutter ratio was calculated by dividing the peak height (RFU) of the stutter peak in position -1R (one repeat less than the true allele) by the peak height of the true allele.

Stutter ratio: $S_R = \frac{\emptyset S}{\emptyset A}$ were Φ_s = height of the stutter peak and Φ_A= height of the allelic peak (47).

To give information of the direction of the imbalance, heterozygote balance was calculated by dividing the peak height (RFU) of the short allele by the peak height of the longer allele (45). This results in a value over 1 if the shorter allele amplifies better than the smaller allele. It will be 2 if the larger allele is half the height of the smaller allele.

3.7. Analysis of data

The GDA software v.1.1 was used for evaluation of Hardy-Weinberg equilibrium (HWE) and Linkage disequilibrium (LD) by applying Fishers exact test and permutations (3200 shuffles) (74, 75). Each statistical test performed in a set of data is related to a hypothesis and a p-value is estimated for each test. The possibility of revealing a significant p-value ($p < 0.05$) by random chance increases by the number of independent tests performed. There are different methods available to adjust the p-value to avoid such false positives (type I error) when multiple comparisons are made. The Bonferroni correction is a simple method to use in that the chosen p-value is adjusted by dividing it by the number of tests performed (76). The significance levels ($p = 0.05$) were Bonferroni corrected in the testing of LD (120 tests, $p = 0.0004$) and HWE (16 tests, $p = 0.003$).

Estimations of expected heterozygosity (H_E) and population structure (F_{IS} and F_{ST}) were also performed with GDA v.1.1 (75). To estimate F_{ST} (both total and pairwise), data from the eight populations studied in Andreassen *et al.* (2012) were compared to the data obtained from the Kamchatka population, using the F-statistics analysis (95 % bootstrap confidence interval, 1000 permutations) in the GDA software (64). In addition, pairwise F_{ST} was estimated among these populations by the use of GenAlEx v.6.501 (77, 78).

Observed allele frequencies were obtained by the use of the allele count method (PowerStats v.1.2) (79). Observed heterozygosity (H_O) as well as estimations of different forensic efficiency parameters were also obtained by the use of PowerStats v.1.2 (79). The estimation of average probability of identity (PI) for each marker as well as total average PI was performed with the program API-Calc v.1.0 (61). This statistical program allows for estimations of average PI based on allele frequencies when accounting for the effects of population substructure (theta), inbreeding (F_{IS}) and close relatedness (e.g. siblings; PI_{sib}) (61). Micro-checker v.2.2.3 was used to test for possible allelic dropout, presence of null alleles, and scoring errors caused by stutter, by comparing expected and observed genotype frequencies (60). The confidence interval was set to 95 % and the analysis were run 1000 times.

4. Results

4.1. Results from validation tests of 16 STRs amplified in five multiplex PCR reactions

4.1.1. Sensitivity of the STR markers

The sensitivity tests showed that there was a successful amplification, with correct genotypes in all of the multiplexed markers in all cases (N ≥ 16), when template input was 0.6 and 0.2 ng. The only exception was MP14, where a single dropout was observed at locus Mu15 when template concentration was 0.2 ng/µl. All results are shown in Table 4. Dropout of an allele (shorter or longer) was defined as a peak height of the allele below 300 RFU (APHT), and dropout of both alleles as both allele peak heights below 600 RFU. For STR markers Mu05 and G10O, one of the control samples was homozygous at these loci. Thus, for these markers, dropout events of one allele in a heterozygous

Table 4. *Results from the sensitivity test of the 16 STR markers from analysis of two control samples (T10 and T11) with different template input – number of dropouts given at each locus.*

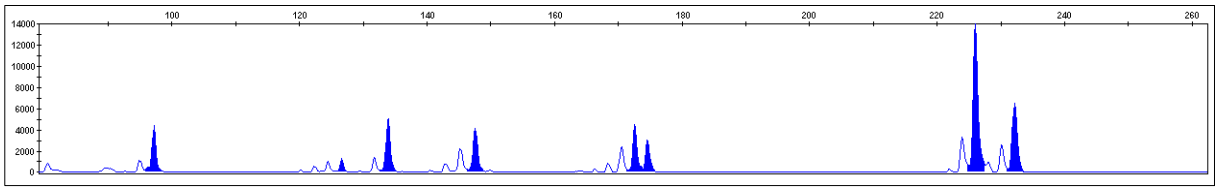
		Dropout[1] (n ≥ 16)[2] Template input (ng/µl)		
Multiplex assay (MP)	Locus	0.6	0.2	0.05
MP1	Mu09	ND	ND	5
	Mu10	ND	ND	3
	Mu23	ND	ND	1
	Mu59	ND	ND	4
MP2	Mu51	ND	ND	2
	Mu05	ND	ND	ND
	G10L	ND	ND	ND
MP3[3]	Mu50	ND	ND	3
MP8	G10B	ND	ND	7
	G10C	ND	ND	6
	G10O	ND	ND	5
	G10X	ND	ND	10
MP14	G1D	ND	ND	6
	G1A	ND	ND	4
	G10J	ND	ND	2
	Mu15	ND	1	14

[1]If no dropout was detected this is denoted ND (no-dropout), numbers indicate number of cases with dropout (either shorter or longer allele (APHT 300 RFU), or both (both alleles < 600 RFU) in the marker with the given template concentration.
[2]For MP8, MP14: N=16 and for MP1, MP2, MP3: N=20.
[3]In MP3 the dinucleotide STR marker, Mu50 was combined with a sex specific marker which is not a microsatellite. Thus for MP3 only results for Mu50 are shown.

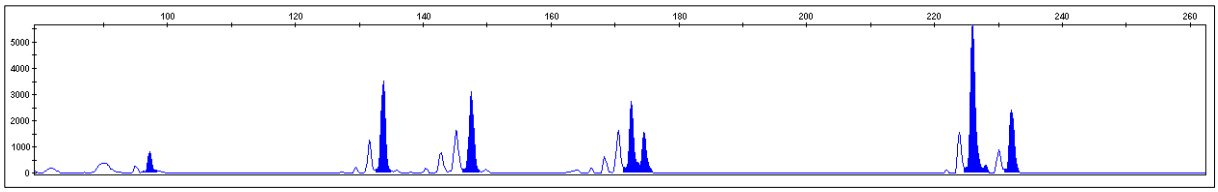
were only tested in ten and eight cases, respectively. When lowering the concentration of the template to 0.05 ng/µl dropout was observed occasionally in six of the eight markers (Mu09, Mu10, Mu23, Mu59, Mu51, Mu50) in MP1, MP2 and MP3, while two markers (G10L, Mu05) were successfully amplified in all cases (N=20). There were considerably more cases of dropout in MP8 and MP14 when template input was 0.05 ng (Table 4). The peak heights of all alleles (with the exception of one case of dropout in Mu15) were above 350 RFU when 0.2 ng/µl template concentration was applied, and above

500 RFU with 0.6 ng template input. Figure 4 shows electropherograms with results from a sample with template input of 0.2 ng and 0.05 ng analysed with MP1 (Mu09, Mu10, Mu23 and Mu59). The figures in 4b and 4d illustrate the allelic dropout of the larger-size allele at marker Mu09 when the template input was decreased to 0.05 ng.

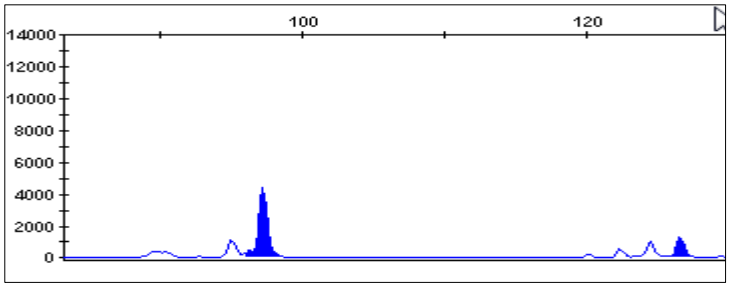
a)



b)



c)



d)

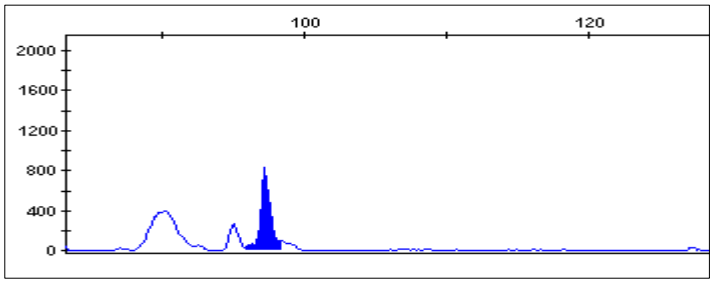


Figure 4. *Electropherograms with genotype results from a control sample after a multiplex (MP1: Mu09, Mu10, MU23 and Mu59) PCR amplification and subsequent CE. a) Results from all four markers when template input was 0.2 ng. Successful amplification is shown for all markers. b) Results from PCR amplification and CE when template input was 0.05 ng. The electropherogram shows dropout of the larger-size allele at locus Mu09. c) and d) Close-ups of Mu09 from the same electropherogram as in a) and b), respectively.*

4.1.1.1. Results from Low Copy Number samples

The 0.05 ng/µl samples represent concentrations where dropout occurred due to stochastic effects (Low Copy Number samples; LCN). The results of repeated runs of these samples were used to assess whether the current guidelines would identify these as divergent results in at least two loci (and discard further use of such a sample). All genotyping of these samples (N=16 for MP8 and MP14, N=20 for MP1, MP2 and MP3) as well as tables listing number of cases were peak heights were below 1600 RFU (for all concentrations) are shown in Appendix 3. Comparing duplicate runs of these samples revealed that, in most cases there were at least two loci with divergent results. For both control samples, one case of duplicate runs at eight markers (MP1, MP2 and MP3), resulted in correct genotypes at all loci (Table A3a run 1a and 1b and Table A3b run 4a and 4b, Appendix 3). Also, as expected from LCN samples, the results showed unexpected heterozygote balance ratios due to stochastic effects. In fact, the heterozygote balance ratios ranged from 0.24 to 8.55 in all the loci and allele combinations in runs of these low template samples. In addition, the peak height of the remaining allele (a false homozygous) in a sample with dropout ranged from 670-7110 RFU. The average peak heights in these LCN samples were also overall lower than the peak heights from samples analysed with higher template input (Tables A3e and A3f in Appendix 3).

The results also showed that more dropout events and spurious genotype results were encountered when amplifying the loci in MP8 and MP14, indicating that the eight markers amplified in MP8 and MP14 are slightly less sensitive than the markers in MP1, MP2 and MP3 (Appendix 3). Also, for MP14 there were far more cases of dropout events in Mu15 (14 cases) than in G10J (2 cases) with template input of 0.05 ng (Table 4).

4.1.2. Measurement of precision, stutter ratio and heterozygote balance

The results from measurements of precision, stutter ratio and heterozygote balance are from more than 20 independent analyses of two heterozygous control samples. For the markers in MP1, MP2 and MP3, results from the control sample T8 was used (0.3 ng template input), and for the markers in MP8 and MP14, results from the control sample T11 (0.6 ng template input) was used for the calculations. This would provide expected variation in precision, stutter ratio, and heterozygote balance ratio when template input is above LCN levels. The results are summarized in Tables 5 and 6. Table 5 shows the multiplexed DNA profiling system markers that were previously validated in Andreassen *et al.* (2012). Table 6 shows the results for the four new markers (G10C, G10O, G10X, G10J). At locus G10X and locus G10J (Table 5) performances were tested in one additional sample (T8, 0.3 ng). This was done to verify some unexpected results when amplifying allele 174 at locus G10X, and to explore further what seemed to be a large heterozygote imbalance between alleles 101 and 115 at G10J.

Measurements of the precision in the twelve previously validated markers showed standard deviations (SD) that were 0.16 bp or less in six of the markers and from 0.16 to 0.22 bp in the six remaining markers (Table 5). The between-run measurement of precision performed on the four new markers (Table 6) revealed SD that were 0.15 bp or less at loci G10C and G10O, while at G10X and G10J the between-run measurement of precision showed SD from 0.17 to 0.24 bp.

Table 5. *Between-run measurements of precision, heterozygote balance and stutter ratio in twelve dinucleotide STRs (previously validated in singleplex PCR assays) from ≥ 20 runs of two heterozygous control samples (tissue) analysed in multiplex PCR assays.*

Multiplex assay	Locus	Alleles genotype[a]	Mean (bp)[b]	SD (bp)[c]	Heterozygote balance[d]	Stutter ratio[e]
	MU09 allele A	110	109.72	0.18		0.46 (0.52)
MP1	MU09 allele B	116	115.97	0.18	1.55 (1.30-1.98)	0.60 (0.63)
	MU10 allele A	149	149.90	0.16		0.48 (0.52)
MP1	MU10 allele B	151	152.11	0.17	1.57 (1.41-1.94)	–
	MU23 allele A	166	166.29	0.19		0.50 (0.65)
MP1	MU23 allele B	173	173.62	0.22	1.18 (0.92-1.58)	0.65 (0.73)
	MU59 allele A	240	240.36	0,15		0.51 (0.60)
MP1	MU59 allele B	256	256.58	0,14	1.69 (1.15-2.18)	0.77 (0.83)
	MU05 allele A	125	125.27	0.15		0.50 (0.56)
MP2	MU05 allele B	127	127.42	0.13	1.61 (1.29-1.82)	–
	MU51 allele A	140	139.63	0.15		0.30 (0.33)
MP2	MU51 allele B	150	150.41	0.14	1.54 (1.05-1.78)	0.54 (0.58)
	G10L allele A	174	173.90	0.22		0.36 (0.39)
MP2	G10L allele B	182	182.25	0.22	1.38 (1.03-1.78)	0.44 (0.52)
	MU50 allele A	124	123.70	0.16		0.51 (0.57)
MP3	MU50 allele B	128	127.88	0.15	1.55 (1.11-2.06)	0.70 (0.78)
	G10B allele A	98	97.13	0.15		0.29 (0.33)
MP8	G10B allele B	110	109.62	0.14	2.85 (1.78-4.92)	0.52 (0.57)
	G1D allele A	129	129.46	0.13		0.40 (0.43)
MP14	G1D allele B	133	133.48	0.12	1.08 (0.80-2.26)	0.44 (0.52)
	G1A allele A	181	180.98	0.17		0.46 (0.52)
MP14	G1A allele B	189	189.31	0.16	1.29 (0.98-2.18)	0.64 (0.66)
	MU15 allele A	110	109.98	0.17		0.49 (0.57)
MP14	MU15 allele B	116	116.28	0.16	1.41 (0.95-1.88)	0.62 (0.67)

[a]Alleles as designated with a size-based nomenclature (basepairs).
[b]Mean value of allele sizes when measured with POP7 on ABI3730.
[c]Standard deviations (SD) from between-run measurements of ≥ 20 runs of a control sample.
[d]Median heterozygote balance ratio with upper and lower 95 percentiles in parenthesis.
[e]Median stutter ratios of alleles with upper 95 percentiles in parenthesis.

Stutter was observed as peaks at positions that were from one to several repeat units less than the true allele (-1R, -2R, -3R etc.). The stutter ratios (peak height of stutter in position -1R divided by peak height of the true allele) were calculated for short allele and large allele at all loci but Mu05 and Mu10

(Table 5), as well as G10O and G10J (alleles 99/101) (Table 6). At these loci, the alleles were separated by only one repeat in the control samples used. As a consequence the stutter ratios could only be recorded for the short allele. The median stutter ratios along with their upper 95 percentiles are given in Tables 5 and 6. The median stutter ratios at the different loci ranged from 0.18 to 0.51 for the shorter alleles and from 0.39 to 0.77 for the larger alleles. The single largest stutter ratio observed in any marker was 0.83 at MU50 (one case) and Mu59 (four cases).

The variation in heterozygote balance was recorded for all loci. To provide values that give information about the direction of the imbalance, heterozygote balance was calculated by dividing the peak height (RFU) of the short allele by the peak height (RFU) of the larger allele. Using this approach a heterozygous with larger-size allele that is half the height of the smaller-size allele, would e.g. have a heterozygote balance ratio of 2. The median heterozygote balance ratios from each locus are given in Tables 5 and 6 with upper and lower 95 percentile in parenthesis. The median heterozygote balance ratios were above 1 in 15 STRs with the exception of G10X (see below). They ranged from 1.18 at Mu23 to 2.85 at G10B for the twelve previously validated loci (Table 5), and from 1.42 at G10O to 3.02 at G10J (alleles 101/115) for three (G10O, G10C, G10J) of the new STR loci (Table 6). However, ratios slightly less than one were in a few cases observed at four of these 15 loci; Mu23, G1D, G1A and Mu15 (Table 5). This resulted in lower 95 percentile values ranging from 0.80

Table 6. *Between-run measurements of precision, heterozygote balance and stutter ratio in four dinucleotide STRs (novel validation) from ≥ 20 runs of two control samples (tissue) analysed in multiplex PCR assays.*

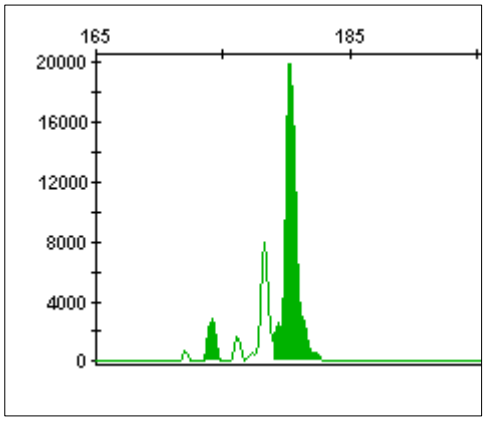
Multiplex assay	Locus	Alleles Genotype[a]	Mean (bp)[b]	SD (bp)[c]	Heterozygote balance[d]	Stutter ratio[e]
	G10C allele A	155	154.80	0.09		0.42 (0.47)
MP8	G10C allele B	163	163.21	0.11	1.58 (1.20-2.21)	0.56 (0.65)
	G10O allele A	201	201.23	0.15		0.28 (0.32)
MP8	G10O allele B	203	203.26	0.12	1.42 (1.04-1.72)	–
	G10X allele A	180	179.87	0.19		0.36 (0.40)
MP8	G10X allele B	188	188.14	0.17	1.17 (0.91-1.85)	0.54 (0.57)
	G10X allele A	174	173.77	0.20		0.27 (0.34)
MP8	G10X allele B	180	179.95	0.18	0.27 (0.14-0.62)	0.39 (0.45)
	G10J allele A	99	98.96	0.24		0.18 (0.20)
MP14	G10J allele B	101	101.04	0.22	1.26 (1.01-2.84)	–
	G10J allele A	101	101.20	0.19		0.24 (0.25)
MP14	G10J allele B	115	115.54	0.17	3.02 (1.75-5.53)	0.52 (0.56)

[a]Alleles as designated with a size-based nomenclature (basepairs).
[b]Mean value of allele sizes when measured with POP7 on ABI3730.
[c]Standard deviations (SD) from between-run measurements of ≥ 20 runs of a control sample.
[d]Median heterozygote balance with upper and lower 95 percentiles in parenthesis.
[e]Median stutter ratios of alleles with upper 95 percentiles in parenthesis.

to 0.98 in these markers. Nonetheless, the upper 95 percentile of stutter ratios and the lower 95 percentile of heterozygote balance ratios did not overlap at any of these loci (Table 5).

Locus G10X (alleles 180/188) (Table 6) showed, in general, heterozygote balance ratios comparable to other loci. However, in samples where the 174 allele was the smaller-sized allele (e.g. 174/180, Table 6), and regardless whether the samples were from Western Europe or Kamchatka, the heterozygote balance ratio was much lower than one. The median heterozygote balance value in this combination was 0.27 (the shorter-size allele is less than 30 % of the peak height of the larger-size allele) and the lower 95 percentile was 0.14. Figure 5a shows an electropherogram with results from genotypes with the allele combination 174/180. The electropherogram illustrates the unexpected heterozygote balance ratio typical for this allele combination. To investigate if the effect was caused by multiplexing several STRs, the control sample was also amplified in singleplex (four cases) with a change in the annealing temperature from 58 °C used in the multiplex to 54 ºC which is the optimal annealing temperature (T_a) for the primer pair at G10X. Amplification using these conditions greatly improved the heterozygote balance (Figure 5b), but the smaller-sized allele (174) still showed a lower peak height (heterozygote balance ratio of 0.89 in Figure 5b) in all measurements.

a)



b)

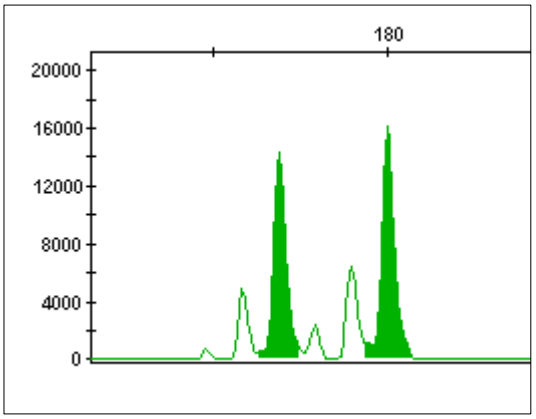


Figure 5. *Electropherograms (same sample, different PCR conditions) with heterozygous genotype results at locus G10X for the control sample with allele combination 174/180 (basepairs). The two alleles are marked in green. (a) Results from a multiplex PCR assay (T_a=58 °C). Allele 174 has a much lower peak height than allele 180. Heterozygote balance ratio: 0.14. (b) Results from a singleplex PCR assay where T_a=54 °C. Heterozygote balance ratio: 0.89.*

The single largest heterozygote balance ratios were 6.08 at G10J (alleles 101/115) and 5.14 at G10B. Figures 6 and 7 show the electropherograms from two control samples that are heterozygous at loci G10J (alleles 101/115) and G10B (alleles 98/110), respectively. The figures illustrate the typical heterozygote balance ratios in these markers at these specific allele combinations. Apart from loci G10X, G10B and G10J, the single largest heterozygote balance ratio was 2.45 (G10C).

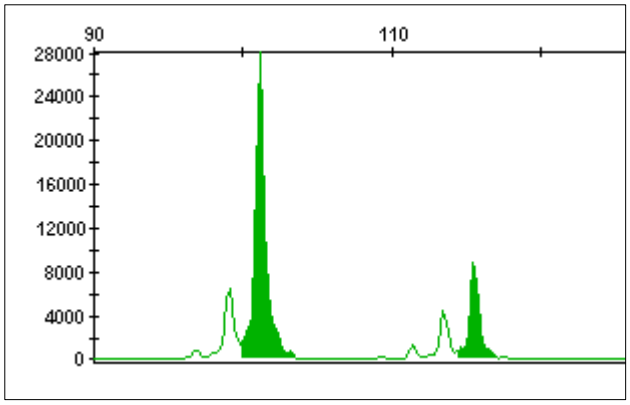


Figure 6. *The figure shows an electropherogram with a heterozygous genotype result from a control sample analysed at locus G10J (alleles 101/115). The two alleles are marked in green. Heterozygote balance ratio: 3.19.*

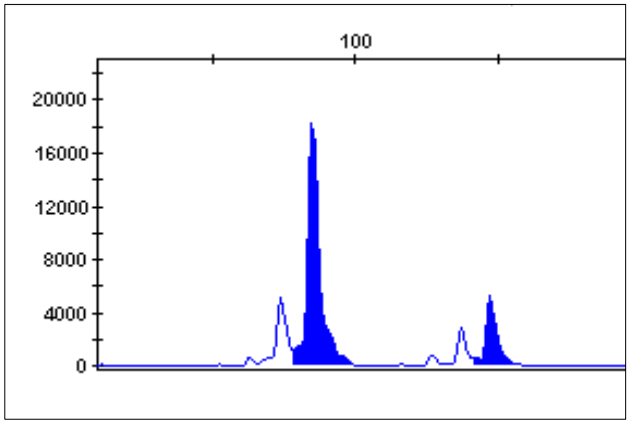


Figure 7. *The figure shows an electropherogram with a heterozygous genotype result from a control sample analysed at locus G10B (alleles 98/110). The two alleles are marked in blue. Heterozygote balance ratio 3.45.*

4.1.3. Success rate; STR typing of hair samples

There were in total 434 hair samples from the Kamchatka population that were analysed in this study. The success rate of genotyping was very much influenced by the number of hair roots in a sample. Figure 8 gives an overview of success rate when samples were differentiated into DNA extracts from 1-3 hair roots (30 µl eluate) *versus* 4-10 hair roots (50 µl eluate). 79.2 % of the 50 µl eluate DNA samples (n = 240) were successfully genotyped in eight or more (for the one sample from each individual that was analysed in MP8 and MP14 as well) STRs. These samples were denoted as samples with individual ID profiles (Figure 8), as the combined power of these markers were expected to be of such a magnitude that it would be possible to differ among individuals. In contrast, among the 30 µl eluate DNA samples (n=194) only 49.5 % provided an individual ID profile.

As a higher percentage of individual ID profiles were retrieved in the samples with DNA from 4-10 hair roots, there were lower percentages of such samples in the group of bear positive samples (from 1-7 STRs successfully typed, denoted as unknown in Figure 8) and the group of negative samples

(denoted as neg in Figure 8). The group of 50 µl DNA extracts contained, however, a higher percentage of mixed samples (a sample with DNA from more than one contributor) than the 30 µl DNA extracts (5 % *versus* 0.5 %, Figure 8).

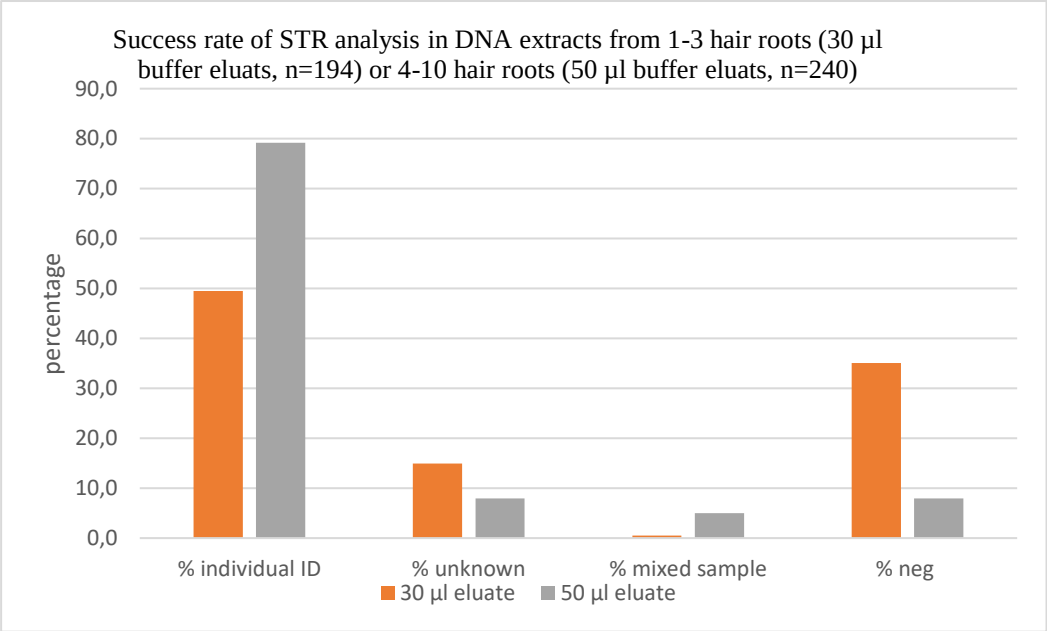


Figure 8. *The figure gives an overview of success rate (percentage) of samples with DNA extracted from 1-3 hair roots (30 µl eluate, orange bars) compared to samples with DNA extracted from 4-10 hair roots (50 µl eluate, grey bars). The results are differentiated into percentage of samples that provided an individual ID and those samples denoted as unknown, mixed or negative (neg). Out of the total number of samples extracted from 4-10 hair roots, 79.2 % provided an individual ID profile.*

Summarizing the results for all 434 samples, 347 (80.0 %) were positive for bear DNA (a genotype result in at least one marker) while 286 samples (65.9 %) resulted in successful typing of eight or more STRs (individual ID profiles). Of the remaining 61 samples, 48 (11.1 %) were denoted as unknown meaning that the samples were of too low quality or quantity to provide sufficient results for an individual ID profile, while 13 samples (3.0 %) were denoted as a mixed sample. In the 286 samples that resulted in individual ID profiles there were 115 individuals (59 males and 56 females), while the remaining 171 samples were recaptures of these individuals. In Appendix 4 results from all samples are presented.

4.2. Results from population data and analysis of a Kamchatka brown bear population

4.2.1. DNA profiles, allele frequencies and forensic efficiency parameters

There were 115 individuals in the Kamchatka bear population sampled (n = 434). Of these, 109 were successfully typed in 16 STR loci (G1A, G1D, G10B, G10C, G10J, G10O, G10L, G10X, Mu05, Mu09, Mu10, Mu15, Mu23, Mu26, Mu50, Mu51, Mu59). The six remaining individuals were successfully typed in 8-15 of the 16 STR loci applied. A list of the 115 (59 male and 56 female) individuals with their unique DNA profiles is given in Appendix 7. Based on the genotypes from these individuals, observed allele frequencies at each locus were calculated. These allele frequencies together with observed and expected heterozygosities (H_O and H_E, respectively) and results from estimations of commonly used forensic efficiency parameters are summarized in Tables 7 and 8. H_O and H_E will be further described in section 4.2.3.

Several new alleles not previously observed in the Western European populations (7) and present in a rather high frequency, were detected in Mu05, Mu15, Mu50, Mu59 and G10L. These allele frequencies, ranging from 0.018 to 0.186 are marked in bold in Tables 7 and 8. In markers Mu05, Mu15 and G10L, the Kamchatka population also revealed alleles that, prior to this study, had only been observed once (a single allele) in individuals from Arkhangelsk and Kami Oblast in Russia (Svanhovd database). These three allele frequencies are marked in bold and cursive in Tables 7 and 8. One of these alleles (96) was among the most frequent ones in Mu15. No microvariation (size differences of 1 bp) was discovered in our sample materials.

The forensic efficiency parameters indicate the usefulness and power of a marker in a forensic context. These parameters are given for each of the STR markers in Tables 7 and 8. The twelve markers studied before in the Western European bear populations, revealed a power of discrimination (PD) ranging from 0.855 (Mu51) to 0.962 (G10L) for eleven of the markers in the Kamchatka brown bear population, while Mu10 revealed a somewhat lower PD of 0.797. Two of the four new markers (G10C and G10O), displayed a somewhat less power than the others. In particular, G10O, with one frequent allele and only four different alleles in total, showed a reduced PD of 0.667.

Table 7. *Allele frequencies, observed and expected heterozygosity (H_O and H_E) and commonly used forensic efficiency parameters for eight dinucleotide STR loci in a Kamchatka brown bear population (n=115).*

Allele	G10B	MU09	MU15[a]	MU50[a]	**Allele**	Mu05[a]	G1D	MU10	Mu51
96	-	-	***0.218***	-	**113**	0.157	-	-	-
98	0.044	-	-	-	**115**	0.217	-	-	-
102	-	0.035	-	-	**117**	0.209	-	-	-
104	-	0.122	-	-	**119**	***0.022***	-	-	-
106	0.259	-	-	-	**121**	-	-	-	-
108	0.044	-	-	-	**123**	0.078	-	-	-
110	-	0.248	0.045	0.126	**125**	0.022	0.023	-	-
112	0.254	0.087	0.105	-	**127**	0.113	0.090	-	-
114	0.110	0.209	0.168	-	**129**	0.078	0.194	-	-
116	0.018	0.004	0.277	-	**131**	0.052	0.248	-	-
118	0.175	0.009	0.155	-	**133**	0.048	0.378	-	-
120	0.083	0.057	0.014	-	**135**	**0.004**	0.045	-	-
122	0.013	0.157	**0.018**	0.135	**137**	-	0.005	-	-
124	-	0.070	-	0.013	**139**	-	0.018	0.013	-
126	-	-	-	0.057	**141**	-	-	0.017	-
128	-	0.004	-	0.187	**142**	-	-	-	0.053
130	-	-	-	0.304	**143**	-	-	0.187	-
132	-	-	-	0.039	**144**	-	-	-	0.132
134	-	-	-	0.126	**145**	-	-	0.235	-
136	-	-	-	0.004	**146**	-	-	-	0.364
138	-	-	-	**0.009**	**147**	-	-	0.530	-
139	-	-	-	-	**148**	-	-	-	0.333
141	-	-	-	-	**149**	-	-	0.013	-
142	-	-	-	-	**150**	-	-	-	0.118
143	-	-	-	-	**151**	-	-	0.004	-
H_O	0.772	0.852	0.736	0.887	**H_O**	0.843	0.793	0.678	0.746
H_E	0.818	0.842	0.814	0.821	**H_E**	0.857	0.747	0.631	0.725
PD	0.941	0.952	0.938	0.933	**PD**	0.956	0.887	0.797	0.855
PE	0.548	0.699	0.487	0.769	**PE**	0.682	0.586	0.395	0.502
PM	0.059	0.048	0.062	0.067	**PM**	0.044	0.113	0.203	0.145
PI_{typ}	2.19	3.38	1.90	4.42	**PI_{typ}**	3.19	2.41	1.55	1.97
N	114	115	109	115	**N**	115	111	115	114

Observed and expected heterozygosity (H_O, H_E), Power of Discrimination (PD), Power of Exclusion (PE), Matching probability (PM), typical paternity index (PI_{typ}). N=number of individuals genotyped at each locus.
[a]Allele frequencies that are marked in bold represents new alleles not previously observed in the Western European brown bear populations (7). Alleles that, prior to this study, had only been observed once (a single allele) in individuals from Arkhangelsk and Kami Oblast in Russia (Svanhovd database), are marked in bold and cursive (the respective allele frequencies).

Table 8. *Allele frequencies, observed and expected heterozygosity (H_O and H_E) and commonly used forensic efficiency parameters for eight dinucleotide STR loci in a Kamchatka brown bear population (n=115).*

Allele	G10L[a]	G1A	Mu59[a]	MU23	**Allele**	G10J	G10C	G10X	G10O
168	-	-	-	0.100	**99**	0.305	-	-	-
170	-	-	-	0.200	**101**	0.009	-	-	-
172	-	-	-	0.017	**111**	0.027	-	-	-
174	0.167	-	-	0.448	**113**	0.004	-	-	-
176	-	-	-	0.183	**115**	0.142	-	-	-
177	-	0.308	-	-	**117**	0.274	-	-	-
178	0.018	-	-	0.052	**119**	0.031	-	-	-
179	-	0.018	-	-	**121**	0.142	-	-	-
180	0.110	-	-	-	**123**	0.053	-	-	-
181	-	0.022	-	-	**125**	-	-	-	-
182	0.022	-	-	-	**127**	0.013	-	-	-
183	-	0.161	-	-	**153**	-	0.004	-	-
184	0.202	-	-	-	**155**	-	0.540	-	-
185	-	0.281	-	-	**157**	-	0.263	-	-
186	0.053	-	-	-	**159**	-	0.058	-	-
187	-	0.129	-	-	**161**	-	0.085	-	-
188	0.057	-	-	-	**163**	-	0.009	-	-
189	-	0.071	-	-	**165**	-	0.018	-	-
190	0.053	-	-	-	**167**	-	0.022	-	-
192	0.044	-	-	-	**174**	-	-	0.104	-
193	-	0.009	-	-	**176**	-	-	0.059	-
194	0.180	-	-	-	**178**	-	-	0.041	-
196	***0.004***	-	-	-	**180**	-	-	0.045	-
198	**0.048**	-	-	-	**182**	-	-	0.532	-
202	**0.044**	-	-	-	**184**	-	-	0.153	-
228	-	-	0.004	-	**186**	-	-	0.005	-
232	-	-	0.053	-	**192**	-	-	0.005	-
234	-	-	0.040	-	**194**	-	-	0.023	-
238	-	-	**0.186**	-	**195**	-	-	-	0.045
242	-	-	0.142	-	**196**	-	-	0.036	-
244	-	-	0.119	-	**201**	-	-	-	0.692
246	-	-	0.066	-	**203**	-	-	-	0.214
248	-	-	0.058	-	**205**	-	-	-	-
250	-	-	0.168	-	**207**	-	-	-	0.049
252	-	-	0.164	-	**209**	-	-	-	-
H_O	0.877	0.759	0.841	0.626	**H_O**	0.796	0.652	0.667	0.402
H_E	0.875	0.781	0.868	0.716	**H_E**	0.790	0.625	0.677	0.473
PD	0.962	0.918	0.960	0.878	**PD**	0.917	0.794	0.871	0.667
PE	0.749	0.525	0.677	0.323	**PE**	0.592	0.358	0.379	0.115
PM	0.038	0.082	0.040	0.122	**PM**	0.083	0.206	0.129	0.333
PI_{typ}	4.07	2.07	3.14	1.34	**PI_{typ}**	2.46	1.44	1.50	0.84
N	114	112	113	115	**N**	113	112	111	112

Observed and expected heterozygosity (H_O, H_E), Power of Discrimination (PD), Power of Exclusion (PE), Matching probability (PM), typical paternity index (PI_{typ}). N=number of individuals genotyped at each locus.
[a]Allele frequencies that are marked in bold represents new alleles not previously observed in the Western European brown bear populations (7). Alleles that, prior to this study, had only been observed once (a single allele) in individuals from Arkhangelsk and Kami Oblast in Russia (Svanhovd database), are marked in bold and cursive (the respective allele frequencies).

4.2.2. Inbreeding coefficient (F_{IS}) and population substructure (F_{ST})

The results from estimations of the inbreeding coefficient (F_{IS}) applying the 16 STR markers included in this study are shown in Table 9. As locus G10X needs further investigation before being fully included as a validated marker (see section 5.1.2.3.), the F_{IS}-value was also estimated with 15 loci included (G10X removed). The inbreeding coefficient F_{IS} was 0.011 (also when G10X was removed), but it was not significant as the 95 % bootstrap confidence interval contained zero.

The results from the eight populations studied in Andreassen *et al*. (2012) were applied in the measurement of population substructure (F_{ST}). The overall F_{ST}-value was 0.11 (95 % bootstrap confidence interval 0.09-0.12) when comparing genotypes from the Kamchatka population to all the other populations (Table 9).

Table 9. *Results from measurement of the inbreeding coefficient (F_{IS}) and estimation of F_{ST}.*

Number of loci included	F_{IS} (bootstrap 95 % CI)[a]	F_{ST} (bootstrap 95 % CI)[b]
15 loci (G10X removed)	0.011 (-0.018-0.043)	
16 loci	0.011 (-0.017-0.042)	
12 loci		0.11 (0.09-0.12)

[a]The F_{IS}-value and 95 % bootstrap confidence interval was estimated for all 16 loci included in this study and with G10X removed (15 loci). (GDA v.1.1)

[b]The overall F_{ST}-value and 95 % bootstrap confidence interval was estimated after comparing DNA profiles from the eight populations studied in Andreassen *et al.* (2012) with the profiles in this project (GDA v.1.1, twelve STRs applied).

4.2.3. Heterozygosity and test for deviation from Hardy-Weinberg equilibrium

The observed heterozygote frequencies (H_O) ranged from 0.626 (Mu23) to 0.887 (Mu50) among the 16 loci, with an exception for locus G10O that displayed a heterozygote frequency of 0.402 (Table 10). The mean H_O and H_E values averaged across all loci were near identical. While the mean H_E when applying all 16 markers was 0.75, it was 0.76 when G10X was removed from the marker system. For comparison to Andreassen *et al.* (2012), the mean H_E when applying the twelve previously validated STR markers was also estimated (0.79).

Deviations from ($p < 0.05$) Hardy-Weinberg equilibrium (HWE) were observed in two (Mu23 and G10O) out of 16 tests (p-values marked in bold in Table 10). These two markers also flagged the presence of null alleles due to differences in observed and expected homozygote genotype frequencies when analysed in Micro-checker v. 2.2.3 (results are shown in Appendix 5). The differences between observed and expected heterozygosity at these two loci were H_E: 0.716 and H_O: 0.626 (Mu23) and H_E: 0.473 and H_O: 0.402 (G10O). After Bonferroni correction (16 tests, $p = 0.003$) of significance levels, there were no deviations from HWE in any of the applied markers.

Table 10. *Population data from a Kamchatka brown bear population (n=115).*

Locus	H_O	H_E	HWE_p	No. alleles	Av.PI (F_{ST})[a]	Av. PI_{sib} (F_{ST}) [a]
G1D	0.793	0.747	0.7922	8	0.17	0.47
G10B	0.772	0.818	0.2956	9	0.12	0.42
Mu05	0.843	0.857	0.1972	11	0.09	0.39
Mu09	0.852	0.842	0.5831	11	0.10	0.40
Mu15	0.736	0.814	0.6722	8	0.12	0.42
G1A	0.759	0.781	0.8528	8	0.15	0.44
G10L	0.877	0.875	0.0681	13	0.08	0.38
Mu10	0.678	0.631	0.5791	7	0.27	0.54
Mu23	0.626	0.716	**0.0084**	6	0.19	0.48
Mu50	0.887	0.821	0.4434	10	0.12	0.41
Mu51	0.746	0.725	0.0769	5	0.19	0.48
Mu59	0.841	0.868	0.3022	10	0.08	0.39
G10J	0.796	0.790	0.6181	10	0.14	0.43
G10C	0.652	0.625	0.1331	8	0.26	0.54
G10X	0.667	0.677	0.2103	10	0.22	0.51
G10O	0.402	0.473	**0.0319**	4	0.41	0.64
$Mean_{16}$[c]	0.75	0.75		8.63		
$Mean_{12}$[c]	0.78	0.79		8.83		
$Mean_{15}$[c]	0.75	0.76		8.53		

Observed (H_O) and expected (H_E) heterozygosities. Significant deviations from Hardy-Weinberg equilibrium (HWE_p) before Bonferroni corrections of significance levels (12 tests, $p < 0.003$) are marked in bold.
[a]Average probability of identity at each of the loci estimated from allele frequencies in the total population: **av. PI** (F_{ST}=0.11), **av. PI_{sib}:** average probability of sibling identity (F_{ST} = 0.11, Sibling probability: 0.999).
[c]The mean H_O, H_E and average number of alleles in the population applying twelve (previously validated, $mean_{12}$), 15 (G10X removed, $mean_{15}$) or all 16 ($mean_{16}$) loci.

4.2.4. Test for deviations from linkage equilibrium

Tests for deviation from linkage equilibrium across loci revealed that 15 out of 120 tests (12.5 %) showed linkage disequilibrium (LD) at a significance level of $p < 0.05$ (Table 11). Ten of these significant LD were in pairwise combinations of loci Mu23 or G10O to another locus. Since these two loci also showed departures from Hardy-Weinberg equilibrium (HWE) before Bonferroni corrections, the HWE effect was controlled in a new LD test ("the preserving genotypes" option in GDA). This reduced the number of tests with LD to eight out of 120 (6.7 %). However, after Bonferroni corrections of significance levels (120 tests, $p < 0.0004$) none of the pairwise combinations tested remained significant. Results are presented in Table 11 (results with deviations) and Appendix 6 (all results, rawdata).

Table 11. *Test for deviation from linkage equilibrium (pairwise comparisons of all 16 loci). Results are presented for those pairwise comparisons that showed deviations before Bonferroni corrections of significance level.*

Loci pairwise comp.[a]	P[b]	Preserv. P[c]
Mu23/Mu59	**0.01750**	0.12188
Mu23/Mu51	**0.00375**	0.05063
Mu23/G10J	**0.00063**	**0.01938**
Mu23/Mu05	**0.02281**	0.20844
Mu23/Mu15	**0.02906**	0.25906
Mu59/G10O	**0.01250**	0.06563
Mu51/G10O	**0.00563**	**0.03969**
G10O/G10X	**0.00125**	**0.00969**
G10O/Mu15	**0.00156**	**0.01750**
Mu10/G10O	**0.03469**	0.14406
Mu59/Mu15	**0.02688**	**0.02469**
Mu05/Mu15	**0.04563**	0.05375
Mu51/G10C	**0.01500**	**0.01469**
Mu51/Mu15	**0.02688**	**0.03125**
G10B/Mu15	**0.01781**	**0.01188**

[a]An overview of the pairwise comparisons that showed linkage disequilibrium (LD) at a significance level of $p < 0.05$ prior to Bonferroni corrections of significance level.
[b]P-values after tests for deviations from LD.
[c]P-values after controlling for the HWE effect on the LD test («the preserving genotypes» option in GDA).
Significant deviations ($p < 0.05$) before Bonferroni corrections of significance levels are marked in bold. After Bonferroni corrections of significance levels (120 tests, $p < 0.0004$), none of these deviations remained significant.

4.2.5. Total average probability of identity (PI)

Average PI was estimated for all loci based on population allele frequencies (Tables 7 and 8) and accounting for population substructure (overall $F_{ST} = 0.11$). For each locus, there is also one estimation when assuming that all samples are from siblings (sib: 0.999). All results are shown in Table 10. An estimate of total F_{IS} ($F_{IS} = 0.011$) was not included as it was not significant being that the 95 % confidence interval contained zero (see Table 9). G10L and Mu59 displayed the highest discriminating power (PI 0.08) while G10O revealed the lowest discriminating power (PI 0.41) among the 16 STR markers (Table 10). PI for siblings (PI_{sib}), for the same markers were 0.38 (G10L), 0.39 (Mu59) and 0.64 (G10O).

The total average PI for this population refers to the probability that two different individuals would receive an identical DNA profile when applying the STRs in this profiling system (eight, twelve, 15 or 16 loci) (61). Table 12 shows all estimates of total average PI, when accounting for population substructure ($F_{ST} = 0.11$). Since individual ID profiles were based on the initial analysis of eight loci (Mu05, Mu09, Mu10, Mu23, Mu50, Mu51, Mu59 and G10L), the total average PI when including only these eight STR markers was estimated as well. Locus G10X needs further investigation before being included as a validated marker (see section 5.1.2.3.), so the total average PI was also estimated with 15 loci (G10X removed). The total average PI accounting for population substructure (F_{ST}) was 9.6×10^{-8} applying eight loci and 4.1×10^{-13} applying 15 loci. The total average PI_{sib} for the same applications were 1.6×10^{-3} and 6.3×10^{-6}, respectively. For comparison to results in Andreassen *et al*.

(2012), the total average PI was also estimated with the same twelve loci included; total average PI was 1.2×10^{-10} and total average PI_{sib} was 4.3×10^{-4}.

Table 12. *Results from estimations of the total average probability of identity.*

	Total average PI[a]	**Total average PI_{sib}[b]**
8 loci	9.6×10^{-8}	1.6×10^{-3}
12 loci	1.2×10^{-10}	4.3×10^{-4}
15 loci	4.1×10^{-13}	6.3×10^{-6}
16 loci	9.1×10^{-14}	3.3×10^{-6}

[a]The total average probability of identity when applying eight (MP1, MP2, MP3), twelve (previously validated), 15 (G10X removed) or all 16 STRs and population specific allele frequencies ($F_{ST} = 0.11$).
[b]The total average probability of sibling identity when applying eight (MP1, MP2, MP3), twelve (previously validated), 15 (G10X removed) or all 16 STRs and population specific allele frequencies ($F_{ST} = 0.11$, sib 0.999).

4.2.6. Genetic distance

All results from estimations of pairwise F_{ST} (GDA v.1.1) are significant ($p < 0.002$) are given in Table 13. The first column in the table also lists the populations from small to larger genetic distance (measured as F_{ST}) to the Kamchatka population. Figure 9 shows the geographic distance between all populations. The genetic distance (pairwise F_{ST}) between the nine populations measured with GenAlEx is also illustrated in a principal coordinates (PCoA) plot in Figure 10. The pairwise F_{ST}`s when comparing Kamchatka population to the other eight populations ranged from 0.0837 (comparison to Karelia) to 0.1673 (comparison to Vesterbotten). Largest distances were to Vesterbotten, Hedmark and Trøndelag. This was also the results from the PCoA plot where these three populations formed a cluster that had the largest genetic distance to Kamchatka. The genetically closest populations were Karelia, Kainuu and Pinega that formed another cluster in the PCoA plot (possibly including Finnmark).

Table 13. *Results from estimations of pairwise F_{ST} between nine brown bear populations.*

	Kamchatka	Karelia	Kainuu	Pinega	Finnmark	Troms	Trøndelag	Hedmark
Karelia (P8)	0.0837							
Kainuu (P7)	0.0892	0.0088						
Pinega (P6)	0.0924	0.0459	0.0556					
Finnmark (P1)	0.1067	0.0477	0.0612	0.0720				
Troms (P2)	0.1417	0.1180	0.1304	0.1311	0.1031			
Trøndelag (P3)	0.1622	0.0977	0.1204	0.1043	0.0852	0.0990		
Hedmark (P4)	0.1670	0.1057	0.1267	0.1189	0.1073	0.1566	0.0516	
Vesterbotten (P5)	0.1673	0.0995	0.1211	0.0982	0.0918	0.1122	0.0131	0.0532

All estimates of pairwise F_{ST} were significant ($p < 0.002$). P1-P5 = Norway: P1, Finnmark (n = 74); P2, Troms (n = 34); P3, Trøndelag (n = 81); P4, Hedmark (n = 101). P5, Vesterbotten - Sweden (n = 84); P6, Pinega–Russia (n = 26); P7, Kainuu–Finland (n = 44); P8, Karelia–Russia (n = 35); Kamchatka (n = 115).

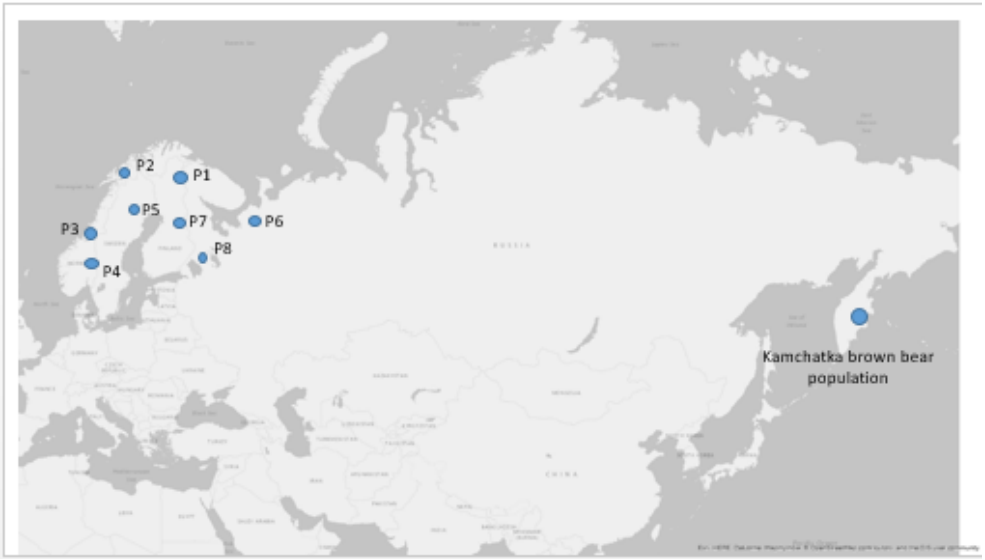


Figure 9. *The figure shows a map of Europe (including Norway, Sweden and Finland) and Russia. The sample locations of each of the eight bear populations, P1-P8 included in Andreassen e. al. (2012) are shown, and the sample location of the Kamchatka (KAM, n = 115) brown bear population studied in this thesis is also indicated on the map. P1= Finnmark (F) n = 74, P2=Troms (TR) n = 34, P3=Trøndelag (NT) n = 81, P4=Hedmark (H) n = 101 (P1-P4, Norway), P5=Vesterbotten (V, Sweden) n = 84, P6=Pinega (P, Russia) n = 27, P7=Kainuu (KAA, Finland) n = 44 and P8=Karelia (KAR, Russia) n = 35.*

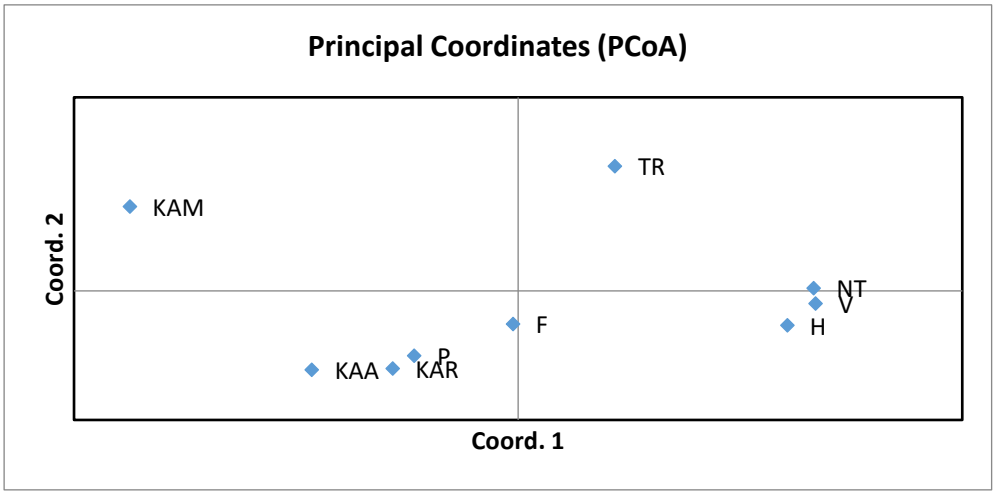


Figure 10. *A principal coordinates plot (PCoA plot) of the genetic distance based on pairwise F_{ST}-values (GenAlEx v. 6.501) between nine brown bear populations: Kamchatka (KAM), Kainuu (KAA, P7), Karelia (KAR, P8), Pinega (P, P6), Finnmark (F, P1), Troms (TR, P2), Trøndelag (NT, P3), Vesterbotten (V, P5) and Hedmark (H, P4).*

5. Discussion

5.1. Validation of 16 STRs amplified in five multiplex PCR reactions

The need for validation of genetic markers in wildlife forensic science is increasing as illegal hunting and trade of wildlife species are growing international problems (1). The main objective of this study was to validate all 16 STR markers currently in use or on trial for brown bear genotyping of non-invasively collected samples at the Svanhovd bear laboratory, following ISFG recommendations (3). Extracting DNA from non-invasive samples often yield low amounts of DNA, therefore, PCR amplification in multiplex assays is a way to obtain as much information as possible from the little material available (5). It is also time and cost efficient. However, these multiplex assays should not perform much less than the previously validated singleplex methods (7).

5.1.1. Sensitivity and Low Copy Number samples – interpretation guidelines

Our findings showed that all markers were successfully amplified with template input of 0.6. Also, the validation indicated that when lowering the template input to 0.2 ng, there would be rare cases of allele dropout (Mu15, one case of dropout, n=16). In general, genotypes from samples with template input ≥ 0.2 ng would be reliably detected using the current interpretation guidelines (APHT 300 RFU) for the multiplexed 16 STRs tested here. Furthermore, the sensitivity test revealed dropout of alleles when the template input was 0.05 ng, which is similar to findings in other studies (51). The repeated individual tests of two samples using 0.05 ng template input could therefore be used to provide information about stochastic variation, average peak heights, and heterozygote balance in LCN samples when analysed with current multiplex protocols.

In human forensic genetics, the concentration of human DNA in a sample may be measured by methods that target only the human DNA, and the measured concentration may then be used to identify LCN samples (80). If the concentration is too low, such samples are discarded after concentration measurements. However, the non-invasive samples used for bear DNA typing consists of large amounts of bacteria (faeces) and the DNA originating from bear cannot be properly measured (5). Direct identification of LCN samples, e.g. those with bear DNA concentrations below 0.2 ng, is not possible and must rely on other means.

Theoretically, when one allele drops out, the low peak height of the remaining allele could be used to identify LCN samples (a minimum peak height threshold). Such thresholds are commonly used in human forensics, as the STRs in the forensic multiplex kits seem to perform in certain predicted ways (51-53). However, the repeated tests of the 0.05 ng LCN samples in the bear multiplexes did show that the stochastic variation was too large to adapt similar guidelines. The heterozygote balance ratios ranged from 0.24 to 8.55 in all loci and allele combinations. The height of the remaining allele in loci with dropout ranged from 670 to 7110 RFU. Although the general profile, with lower than average

allele peak heights and very large heterozygote balance ratios could indicate that this was an LCN sample, a fixed peak height threshold could not be set. Instead, the protocol used at the Svanhovd bear laboratory apply repeated measurements (independent amplification and analysis) to aid in the identification of LCN samples. Any samples showing diverging results in duplicate (heterozygotes) or triplicate (homozygotes) runs at two or more loci would not be trusted but be singled out as LCN samples (and removed). This procedure has also been recommended in human forensics (55).

The results from the repeated analysis of the 0.05 ng samples (Appendix 3) were used to test whether the current protocol did manage to identify such LCN samples. Incorrect genotypes were never registered as identical results twice in independent, subsequent amplifications of the same control sample. In all duplicate runs with incorrect genotypes, there were diverging results. Out of all combinations of results from the ten runs (eight for MP8 and MP14) of each of the samples, there were two cases where an incorrect genotype could have passed based on a duplicate run (if combining 2b and 4a or 4b and 5a in Table A3a, Appendix 3). However, these incorrect genotypes were false homozygotes, and passing homozygous genotypes requires identical results in triplicate. Our findings suggest that the diverging results, together with low average peak heights and large deviations from expected heterozygote balance in LCN samples would lead to these being identified in manual inspection of the electropherograms by a trained technician. In summary, the repeated analysis protocol and the general knowledge about LCN sample profiles, seems to be the best solution to identify and discard LCN samples. The adaptation of these typing guidelines for the multiplex assays therefore seems to be best approach to avoid including erroneous genotypes in the database.

As the sensitivity was slightly better for MP1, MP2 and MP3 (fewer cases of dropout) than MP8 and MP14 with template input of 0.05 ng, one could question if the two latter multiplexes could be improved (e.g. better loci balance). Locus G10J in MP14 had e.g. overall much higher peak heights than Mu15. Exploring if MP8 and MP14 would benefit from a slightly different protocol might be included in further studies.

While Andreassen *et al.* (2012) revealed an equally high sensitivity at all markers with a sample concentration of ≥ 0.6 ng/µl, this study showed a better sensitivity with successful amplification across all 16 markers (one exception: Mu15) when DNA input was ≥ 0.2 ng. Even though the quality of results decreased considerably at template inputs of 0.05 ng, the change from single- to multiplex assays for the 16 STRs included in this study, has not lowered the sensitivity of the markers.

5.1.2. Precision, stutter ratio and heterozygote balance

5.1.2.1. Precision

A bin range equal to three standard deviations with no overlapping bin ranges results in 99.7 % of identical alleles being sized within the same bin (44). In this study, the measurements of precision indicate that alleles with 2 bp size differences can be separated with a confidence of more than 99.7 % in all loci. These results are the same as the ones described in Andreassen *et al.* (2012). In this previous validation study, microvariants at Mu23 required a typing procedure able to detect size differences of 1 bp. Six of the twelve loci showed a between-run precision of 3 x SD ≤ 0.48 bp in the aforementioned study. In comparison, eight of the sixteen loci (six previously validated, two new loci) in this study revealed a between-run precision that allows a similar high discrimination of 1 bp size differenced alleles. Lowering the expected confidence to 95 %, all markers revealed a precision able to separate alleles that differ in length by 1 bp (2 x SD ≤ 0.48 for the least performing locus: G10J, and 2 x SD ≤ 0.44 for all other markers). For the least performing locus, it is reasonable to assume that the precision will improve when eliminating the between-run factors, as was demonstrated at locus G10L and G10B in Andreassen *et al*. (2012). With an analysing protocol like the one applied in this study, where all samples are run at least twice, it is safe to presume that also microvariation (1 bp size variation) will be detected. Such microvariation was not observed at any locus in our materials from the Kamchatka brown bear population. In summary, the precision of the multiplexed markers, including the new STRs, would have the ability to detect alleles with 1 bp length differences using the current analysis protocol.

5.1.2.2. Stutter and heterozygote balance – suggested adjustment of typing guidelines

The stutter ratios observed in the 16 dinucleotide loci were similar to those registered in Andreassen *et al.* (2012), with the shorter allele revealing the lowest ratio within a locus. The single largest stutter ratios observed at any locus was 0.83, i.e. a stutter with peak height well below the true allele. In summary, the proportion of stutter in all loci was well below the true allele. A true homozygous would therefore never be mistaken as a heterozygous if using the current interpretation rule that in a homozygous the true allele is the largest in size with the highest peak height.

Dinucleotide loci are expected to have larger differences in peak heights between alleles than tetranucleotide loci and therefore a higher heterozygote balance ratio (3, 7, 81). Our results are in concordance with the previous validation study at this point (7). At all loci but G10X, the direction of the heterozygote imbalance was such that the shorter allele had the largest peak heights in most cases. The two loci G10J (alleles 101/115) and G10B showed the largest heterozygote imbalances, with a median ratio of 2.85 and 3.02 (large allele approximately 30 % of the peak height of the short allele), respectively. The single largest heterozygote balance ratios were also in these loci, with values of 6.08

and 5.14, respectively. This differs from the results in Andreassen *et al.* (2012) where no heterozygote balance ratios were larger than 3.3. Multiplex assay with a combination of dinucleotide markers may result in less performing markers, as they are optimized towards the best fit combination for all loci (82). Failure to detect the larger-size allele could result in falsely genotyping the sample as a homozygous (7, 43). To avoid such false homozygous genotypes, we suggest that the HPHT should be around 1800 RFU for G10J and G10B. This would be required to reveal if the homozygous genotype in fact was a heterozygous (as the APHT is 300 RFU, 1800/300=6.0). As the single largest heterozygote balance ratio apart from loci G10J and G10B was 2.45 (G10C), the results suggest that the HPHT in the thirteen other markers should be around 800 RFU. This would ensure that a true heterozygous genotype is rarely missed using the 300 RFU threshold for allele detection (800/300=2.66). Manual inspection by expert analysts and a procedure, as ours, where all homozygous genotypes are typed in triplicate, would further limit the chance that random variation of heterozygote balance in a single typing could lead to genotyping errors.

Heterozygote balance ratios slightly less than one were observed in a few cases at Mu23, G1D, G1A and Mu15. Theoretically, a heterozygote balance below 1 could pose a problem if the heterozygous has alleles that are separated by only one repeat. A heterozygous with a lower peak height of the smaller-size allele could then potentially be mistyped as a homozygous with a large stutter (wrongly assuming the smaller-size allele is a large stutter). However, the upper 95 percentile of stutter and the lower 95 percentile of heterozygote balance ratios did not overlap at any of these loci (Table 5). As all heterozygous samples are run twice (and putative homozygous samples three times), the results suggest that these four markers would not be incorrectly genotyped as homozygous, if using a heterozygote balance ratio above the lower 95 percentile as an absolute lower peak height threshold for accepting the smaller-size allele. These cases exemplify why there is a need for individual guidelines for the different markers and manual genotyping by trained staff. This potential problem applies to heterozygous genotypes with alleles separated by only one repeat in loci Mu23, G1A, G1D and Mu15.

5.1.2.3. Suboptimal PCR amplification at the locus G10X

One possible explanation for the observed suboptimal PCR amplification at allele 174 in G10X is that there is a variation at the primer binding site, which makes the amplification of this particular allele less efficient (83). Increasing the temperature to 58 °C, as used in the multiplex, seemed to make the amplification of this allele even less efficient (increased stringency for primer binding) (82). This STR locus should be further investigated regarding mutations in the region for primer binding, possibly change primers (located 5‘and 3‘ of the original forward and reverse primers), before one can fully include G10X as a validated marker . The G loci are cloned from the American black bear (*Ursus americanus*) but have been applied to studies of the black bear, polar bear and grizzly bear as well as

the brown bear (32, 35, 84, 85). Even so, in brown bears there might be alleles at this locus with slightly different DNA sequence in the region for primer binding that has not been investigated thus far.

In our sample materials, the frequencies of homozygous genotypes at G10X were not larger than expected. and the allelic diversity for the marker was high (10 alleles). In addition, no deviations from HWE or linkage equilibrium were observed. The heterozygosity was in the lower range (H_O 0.667), though. This low estimate may indicate genotyping errors caused by the problems we experienced with typing. No such genotyping errors were detected by the software Micro-Checker indicating this was not a large problem in our materials (60). We believe that G10X can be an informative putative genetic marker in future studies if the typing criteria are met, but for now it should not be included as a validated marker. One might also consider if the marker should be in a different multiplex combination with a lower T_a (possibly 54ºC).

5.1.3. Interpretation guidelines – summary

In summary, one could propose that there should be minor changes in the interpretation guidelines in line with the results from this validation. The recommendations for new guidelines are to exclude G10X as a marker while awaiting more investigation regarding primer binding site mutations. For the 15 other markers, HPHT should be set at 1800 RFU for G10J and G10B, while it could be 800 RFU for all other loci (APHT 300 RFU). For all heterozygous genotypes, heterozygote balance ratios should be above the lower 95 percentile for Mu23, G1D, G1A and Mu15 where ratios below 1 were detected. In line with current typing guidelines, all genotypes should be confirmed with independent runs, heterozygous genotypes with duplicate and homozygous genotypes with triplicate runs, to be accepted.

5.1.4. Success rate of the samples

PCR amplification of 16 STR loci in five multiplex assays resulted in an individual ID profile in 286 of 434 samples, yielding a success rate of 65.9 %. This is in line with other studies where the sample material has been hair collected in hair traps (26, 27). These other studies give no information on the quality of the DNA samples, e.g. number of hairs in each sample. Our results indicate that there was, in general, relative high sample quality, and that number of hair roots available for DNA extraction was the most important factor affecting the success rate. There is a considerably higher number of positive results yielding a DNA profile with samples of DNA extracted from more than three hair roots (79.2 % *vs* 49.5 % for DNA samples from 1-3 roots). These results give a strong indication that a high-quality hair sample should contain close to ten hairs. On the other hand, an increased number of hairs added to the sample gives a slightly higher risk of mixed samples. These mixed samples are detected quite easily by observing three or more alleles in at least one locus (heterozygosity levels above 0.70 for most markers). Thus, an increase in mixed samples by 4.5 % when increasing the

number of hair roots up to ten may be a low price to pay to achieve a 30 % increase in number of samples with an individual ID profile. Whenever the fieldworker believe that the sample may be from more than one bear, such information should be noted. This would allow the lab technician to take knowledge-based choices and in such cases extract DNA from one hair root only.

5.2. Genetic variation in the Kamchatka brown bear population.

The second aim of this study was to apply the validated markers to study the Kamchatka brown bear and reveal whether or not the same markers can be used in a geographically very distant population. To facilitate this aim, genotype and allele frequency estimates, forensic efficiency parameters and population genetic parameters were acquired for all markers from this population. Our study confirmed that the previously validated dinucleotide STR markers together with the four new markers work well in the Kamchatka brown bear population. However, as G10X needs more exploration until being fully included in the DNA profiling system (also for the Western European brown bear), it should be temporarily removed.

5.2.1. DNA profiles, allele frequencies and forensic efficiency parameters

Allele frequencies and population data for all 16 markers were obtained from the 115 individuals in the materials from the Kamchatka brown bear. The population of bears in the Kamchatka area is large (>8000, censused in 1986-1994) (17). The sampled population included in this study will only represent a small subpopulation of all brown bears in Kamchatka being the small geographic sampling area (approximately 200 square km). Sampling over a larger area would presumably result in higher heterozygosity, as many of the individuals in a small area are likely to be related due to home range overlap for related females (38, 86), and cubs (1-4 siblings) following their mother for 1.5-3.5 years (87). A recent study using satellite tracking of four brown bears in Kamchatka found significantly overlapping home ranges of two female brown bears (71). A geographically restricted sample of 115 individuals should therefore be a sufficient number to make conservative forensic power estimates representative for the Kamchatka brown bear population. In human forensics, most published population data include 100-200 individuals per population (57). A paper from 1992 stated that100 - 150 individuals per population would be sufficient for forensic power estimates in human forensics (88).

STR markers included in a DNA profiling system should possess a high discriminating power (PD), and a sufficient number of markers should be combined so that the profiling system is able to distinguish individuals from one another (61). A high heterozygosity at a locus results in a high power of discrimination and this is influenced by number of alleles and the distribution of the allele frequencies at a locus (57). With the exception of locus G10O (PD=0.667), the PD for all loci were above 0.794. If removing one marker from the profiling system, G10O would be the obvious one.

Overall, the forensic efficiency parameters estimated showed that all loci work well when combined in a DNA profiling system. Compared to results in Andreassen *et al.* (2012), the power of discrimination was equally high when these markers were applied in the Kamchatka population.

In wildlife forensic science, an estimate of average PI has been used in several studies (7, 33, 89, 90). This is the probability of randomly observing two identical copies of a given genotype in the population (61). The average PI (and other power estimates) assumes no large deviations from HWE and that the loci combined are not in LD (57, 61). This was also the case for the markers (no large deviations) validated in this thesis. A low value of average PI at a locus indicate a high discriminating power. As they are not in LD, the combined power of all markers could be obtained by multiplying the average PI for all loci (61).

The estimates in this thesis are accounting for population substructure (theta), and an estimate assuming close relatedness between individuals (PI_{sib}), was also included. The inclusion of an estimate for theta is particularly important in wildlife species where theta values typically are higher than in humans, due to more pronounced population structure (34). The estimate of theta for the Kamchatka population was obtained by comparing data from the eight populations studied in Andreassen *et al.* (2012) to the Kamchatka population. A total F_{ST} of 0.11 is a moderate level of population substructure (68) and is similar to the findings in Andreassen *et al.* (2012) (0.09) and for the Eurasian badger (0.12) (34). Ideally, the estimation of population substructure should have been obtained by comparing the population in this study to a brown bear population geographically closer to Kamchatka. Such data was not available, but we believe that it is still a valid estimate of the historic relatedness within the population.

The inbreeding coefficient was not significant and thus, was not included in the estimation of total average PI. As significant inbreeding in a population would result in higher match probabilities at homozygous and lower match probabilities at heterozygous loci, an inclusion of an estimate of inbreeding at homozygous loci is common, but in this case negligible (34, 70). The most conservative approach to reveal whether a DNA profiling system has enough power for forensic purposes, would be to assume that all individuals are siblings and estimating a sibling match probability (61, 91). The magnitude of this estimate for twelve or all 15 STRs strongly indicates that applying this DNA profiling system one may discriminate even between close relatives (PI_{sib} for twelve loci 4.3×10^{-4} or all 15 STRs: PI_{sib}: 6.3×10^{-6}). The individual ID profiles in this thesis were based upon eight loci. The power of the combination of these markers are also at a magnitude where it provides individual specific identification of bears (for eight loci: PI 9.6×10^{-8} and PI_{sib} 1.6×10^{-3}). In summary, the power of the combined markers (DNA profiling system) when applied for DNA profiling in Kamchatka is at a magnitude where it would provide individual-specific identification of bears.

5.2.2. Comments to findings in G10O and Mu23

Test for deviation from HWE showed deviations in G10O and Mu23 at a significance level of 0.05. However, after Bonferroni corrections (16 tests, p = 0.003) of significance levels none of these deviations remained significant. These markers did flag with null alleles in Micro-Checker which also is mostly based on the HWE comparisons (60). If there, in fact, exists null alleles at G10O and Mu23, these markers may still be used in monitoring of the Kamchatka brown bear as recapture of such individuals will still result in identical DNA profiles. These markers, if having null alleles, will work less well if applied in parentage analysis as relatedness testing rely on correct genotypes to reveal alleles that are inherited from parents to offspring. If the alleged parent and the offspring seem to be homozygous at different alleles in the same locus, the child may have inherited a null allele from the parent (92). Several studies have addressed this problem and investigated the effect of null alleles on parentage testing (93-95).

5.2.3. Genetic variation and genetic distance measurements

With the exception of G10O, the heterozygosity observed at the 16 loci (Table 10) suggests that all loci are highly polymorphic. The expected heterozygosity averaged across all loci is a parameter commonly used to describe the genetic diversity in brown bear populations and studies have shown higher expected heterozygosity in large populations compared to small populations (6). A paper including 30 brown bear populations from ten different studies has proposed a method to better compare genetic diversity across populations when there are differences in loci and sample size (96). The Kamchatka brown bear population studied in this thesis has been analysed with the same validated markers as for the eight populations in Andreassen *et al.* (2012). Thus, no adjustment for comparison of genetic diversity (with regard to loci differences) in these populations is needed. Our findings showed little difference in the expected and observed heterozygosity averaged over the twelve identical loci (0.79 *vs*. 0.78, respectively) in the Kamchatka population. As expected, given the large population size, the genetic diversity in the Kamchatka brown bear population is high and slightly higher than the diversity (heterozygosity levels: 0.69-0.75) in the Scandinavian (Norway and Sweden, P2 -P5) populations studied in Andreassen *et al.* (2012), one exception being the Finnmark population (P1), with a heterozygosity of 0.80. In the Russian and the Finnish populations (P6-P8), heterozygosity values (0.78-0.83) similar to and slightly higher than the ones in this thesis, were observed. These differences could have been caused by the recent bottleneck events in the Scandinavian populations combined with low connectivity to the larger populations in Finland and Russia (9, 10, 42, 97). A study comparing the average expected heterozygosity at eight microsatellite loci in Scandinavian subpopulations to genetic diversity in brown bear populations from North America, concluded, though, that the Scandinavian brown bear despite recent bottleneck events, revealed a relative high degree of genetic diversity (36).

Some new alleles were detected in the remote Kamchatka population that has never been registered before. This is not surprising given the large geographic distance to the other populations examined earlier (7). The 96 allele was among one of the most frequent alleles at Mu15 and has only been observed once (a single allele, registered in the Svanhovd database) before in a Western Russian population. This indicates it being one of the most common alleles in the Far East Russian bear population. At Mu23, there are microvariant alleles (169, 171, 173 and 177) in the Western European populations (7). However, these microvariants were not observed in the Kamchatka population. All alleles observed at Mu23 in our study were previously seen in the Western European populations. Two other Mu23 alleles (164 and 166) observed in the Western European populations (7), were also lacking in the Kamchatka population. These findings might suggest that the microvariant alleles at Mu23 are a result of relative recent mutations at this locus in the Western European populations, and that the ones with similar sizes in all populations (including Kamchatka) are the ancestral alleles in this marker.

The genetic distance to the eight populations studied in Andreassen *et al.* (2012) was assessed by the use of estimation of pairwise F_{ST}`s (both GDA and GenAlEx) (64, 75, 77, 78). This is a widely used estimator applied to investigate the genetic differentiation between populations (40, 41, 66) enabling comparisons of estimated genetic distances across studies. In general, these genetic distance measurements mirrored the geographic distances of the Western European populations to Kamchatka. Interestingly, the pairwise F_{ST}`s indicated the same genetic distance between Kamchatka population and the population in Finnmark as between the Finnmark population and the geographically much closer populations in Northwest (Troms) and Southern (Hedmark) Norway. Likewise, the genetic differentiation between the populations of Kamchatka and e.g. Trøndelag is twice the size of the differentiation between the Kamchatka population and the populations in Karelia and Kainuu. These differences do not mirror the overall large geographic distance from the different populations to Kamchatka. Studies have implied an eastern-western barrier to gene flow that is not solely explained by geographic distance or physical boundaries (42, 98). Our findings are in concordance with the results from these studies. This is a first estimate of the genetic distance from the Kamchatka population to other populations. Further research must be conducted, and more estimators need to be assessed to fully explore this topic (66, 67).

This is the first time non-invasively collected hair from brown bears at Kamchatka has been analysed with autosomal STR markers, and novel genetic information has been gathered. There is not much prior knowledge about the dispersal of the bears in the area. Earlier research has been performed by actual observation (also of marking activity) of the animals (17, 99, 100). One study applying satellite tracking on four brown bears in Kamchatka offer a first insight into the dispersal of male and female brown bears on the Kamchatka peninsula (71). The study revealed an overlap of home ranges for two female brown bears and different seasonal dispersal patterns where the brown bears aggregate by rivers during salmon spawning. The sampled population in this study will only represent a small

subpopulation of all brown bears in Kamchatka due to the limited geographic sampling area. To obtain more information on this geographically remote population, sampling over a larger area is needed.

5.3. Conclusion and further prospects

The main objective of this study was to validate 16 multiplexed dinucleotide STR markers specific for bear and change interpretation guidelines in concordance with the results from the experimental validation. Also, the objective was to apply the validated markers to study a Kamchatka brown bear population.

Our findings resulted in the validation of 15 of the STR markers in line with ISFG recommendations, while G10X was removed awaiting further investigation regarding possible primer site mutations. The validated markers proved to work well in the Kamchatka brown bear population indicating that these markers may be applied to brown bear populations over a large geographic area. The discriminating power of the combined marker set is at a magnitude where it would provide individual specific identification of even closely related bears. In conclusion, this DNA profiling system may be applied in forensic casework for both Western European and Kamchatka brown bear populations. The expected heterozygosity estimate indicates a high genetic diversity, as would be expected in a large and dense population of bears. First estimates of genetic distance to the Western European populations were also obtained. In general, these measurements mirrored the geographic distance.

In future studies, G10X should be further investigated, as it may work well as a putative forensic STR marker. In addition, the Kamchatka brown bear population needs to be studied over a larger geographic area to assess allele frequencies and diversity that reflects the total population material. Likewise, the genetic distance to the Western European populations may be investigated further.

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Appendix 1. Products and manufacturers

Products	Manufacturers	Country	Catalogue no.
ABI 3730 Buffer with EDTA	ABI	USA	4335613
ABI 3730 DNA Analyzer	ABI	USA	3730S
AmplitaqGold DNA polymerase with Gold Buffer & $MgCl_2$	ABI	USA	4311814
BSA	NEB	USA	B9000S
Centrifuge 5430	Eppendorf	Germany	5427000410
RNase-free water (multiplex PCR kit)	Qiagen	Germany	1012888
Dneasy Blood & Tissue Kit	Qiagen	Germany	69504
dNTP	Thermo Scientific	USA	ABO196
epMotion barrier tips 50 µl reload	Eppendorf	Germany	0030014430
epMotion Dispension Tool TS 50	Eppendorf	Germany	960001010
epMotion® 5075	Eppendorf	Germany	960020006
Eppendorf Safe-Lock Tubes 1.5 ml	Eppendorf	Germany	0030 123.328
Ethanol	Antibac	Norway	600051
Formamide	ABI	USA	4311320
Galaxy 14D (1814) centrifuge	VWR	Germany	37001-296
Genescan 500LIZ	ABI	USA	4322682
MicroAmp Clear Adhesive Film	ABI	USA	4306311
MicroAmp Optical 96-Well Reaction Plate	ABI	USA	4316813
Microtube thermo-shaker block	VWR	Germany	97043-550
MiniSpin plus centrifuge	Eppendorf	Germany	5453000011
Molecular Biology Water, ddH_2O	AccuGENE, Lonze	Belgium	BE51200
NanoDrop™ 1000 Spectrophotometer	Thermo Scientific	USA	Serial no. 9708
Pipette (barrier) tips	Thermo Scientific	USA	variable
Plate septa, 96 well	ABI	USA	4315933
POP-7™ polymer	ABI	USA	4335615
Qiagen Multiplex PCR PlusKit	Qiagen	Germany	206152
SimpliAmp Thermal Cycler (PCR)	ABI	USA	A24811
STR locus specific primers	ABI	USA	variable
Vortex-Genie 1	Scientific Industries	USA	SI-0156
Vortex-Genie 2T	Scientific Industries	USA	SI-G560E

New England Biolabs Inc=NEB
Applied biosystems=ABI

Appendix 2. Sensitivity test. Results from preliminary analysis.

Table A2a. *DNA concentrations of positive controls T10 and T11 prior to dilutions (Nanodrop).*

#	Sample ID	Date	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	Blank	25.09.2017								
2	blank	25.09.2017	0,2	ng/µl	0,004	0,005	0,9	0,59	DNA	50
3	T8stock	25.09.2017	6,0	ng/µl	0,119	0,064	1,86	1,32	DNA	50
4	T8stock	25.09.2017	5,6	ng/µl	0,111	0,058	1,91	1,30	DNA	50
5	T9stock	25.09.2017	9,8	ng/µl	0,191	0,099	1,93	1,41	DNA	50
6	T9stock	25.09.2017	10.1	ng/µl	0,202	0,102	1,98	1,53	DNA	50
7	T10stock	25.09.2017	19,2	ng/µl	0,385	0,200	1,93	1,31	DNA	50
8	T10stock	25.09.2017	18,6	ng/µl	0,373	0,189	1,97	1,55	DNA	50
9	T11stock	25.09.2017	22,4	ng/µl	0,447	0,230	1,94	1,51	DNA	50
10	T11stock	25.09.2017	22,1	ng/µl	0,442	0,228	1,94	1,61	DNA	50
11	Blank	25.09.2017								
12	blank	25.09.2017	-0,1	ng/µl	-0,002	-0,004	0,41	0,07	DNA	50

Table A2b. *Results from the preliminary sensitivity test of controls T10 (male) and T11 (female) after PCR amplification and fragment analysis (same protocol as for the samples). Dilutions were in ng/µl:* ***1.0, 0.6****, 0.4, 0.3, 0.2, 0.1, 0.05,* ***0.03 and 0.02*** *(n=4, for dilutions marked in bold: n=2). Analytical threshold: 300 RFU (allele peak heights below this was considered a dropout). Manual inspection of the electropherograms. The table lists the number of dropout events (one or both alleles (both alleles < 600 RFU)) in each marker with the different template input.*

		Dropout[1] (N=2[2] and N=4[3]) Template input (ng/µl)								
Multiplex assay (MP)	locus	**1.0**	**0.6**	0.4	0.3	0.2	0.1	0.05	**0.03**	**0.02**
MP1	Mu09	ND	ND	ND	ND	1	ND	1	1	1
	Mu10	ND	ND	ND	ND	ND	ND	1	1	1
	Mu23	ND	ND	ND	ND	ND	ND	2	1	1
	Mu59	ND	ND	ND	ND	ND	ND	1	1	1
MP2	Mu05	ND	ND	ND	ND	1	ND	ND	2	1
	Mu51	ND	ND	ND	ND	ND	ND	1	2	2
	G10L	ND	ND	ND	ND	ND	ND	ND	1	2
MP3	MU50[4]	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1]If no dropout was detected this is denoted ND (no-dropout), numbers indicate number of cases with dropout (either shorter or longer allele, or both) in the marker with the given template concentration.

[2]N=2: Analysing two controls with template input of 1.0, 0.6, 0.03 or 0.02 ng (marked in bold) in one PCR assay and subsequent fragment analysis.

[3]N=4: Analysing two controls (0.4, 0.3, 0.2, 0.1 or 0.05 ng template input) in two separate PCR assays and subsequent fragment analysis.

[4]In MP3 the dinucleotide STR marker, Mu50 was combined with a sex specific marker which is not a microsatellite. Thus for MP3 only results for Mu50 are shown.

Appendix 3. Genotype results from Low Copy Number samples

Table A3a. *The table lists genotype results from a control sample (T10) analysed ten times (five duplicate runs) in eight markers (MP1, MP2 and MP3) with a 0.05 ng template input.*

	MP1				MP2			MP3
	Mu09	Mu10	Mu23	Mu59	Mu05	G10L	Mu51	Mu50
T10[a]	116/122	141/143	169/172	224/252	113/227	194/194	138/148	110/124
run[b]								
1a	116/122	141/143	*169/172*	224/252	113/127	194/194	138/148	110/124
1b	116/122	141/143	169/172	224/252	113/127	194/194	138/148	110/124
2a	NR	*141/143*	172/172	224/252	113/127	194/194	138/148	110/124
2b	116/116	141/143	169/172	224/252	113/127	194/194	138/148	110/124
3a	116/122	141/143	172/172	224/252	113/127	194/194	138/148	110/110
3b	116/122	141/143	169/172	224/252	113/127	194/194	148/148	110/124
4a	116/116	141/143	169/172	224/252	113/127	194/194	138/148	110/124
4b	116/122	141/143	169/172	224/224	113/127	194/194	138/148	110/124
5a	116/122	141/143	*169/172*	224/224	113/127	194/194	138/148	110/124
5b	116/122	141/143	169/169	224/252	113/127	194/194	138/148	110/124

[a]The correct genotypes for control sample T10 listed and highlighted in grey.
[b]Letters a and b represents the duplicate runs of one sample.
Erroneous genotype results are highlighted in blue. This is caused by allele dropout (shorter or longer allele, APHT 300 RFU) or heterozygote imbalance resulting in a heterozygous with alleles separated by one repeat, being incorrectly typed as a homozygous. NR – no result (a homozygous with peak height below 600 RFU or a heterozygous with both allele peak heights below 600 RFU), also highlighted in blue.

Table A3b. *The table lists genotype results from a control sample (T11) analysed ten times in eight markers (MP1, MP2 and MP3) with a 0.05 ng template input.*

	MP1				MP2			MP3
	Mu09	Mu10	Mu23	Mu59	Mu05	G10L	Mu51	Mu50
T11[a]	98/126	135/147	172/174	226/232	115/115	182/184	144/146	110/132
run[b]								
1a	98/126	135/147	172/174	232/232	115/115	182/184	144/146	110/110
1b	98/98	135/147	172/174	226/232	115/115	182/184	146/146	110/132
2a	98/126	135/147	172/174	226/232	115/115	182/184	144/146	110/132
2b	98/126	147/147	172/174	226/232	115/115	182/184	NR	132/132
3a	98/126	135/135	172/174	226/232	115/115	182/184	144/146	110/132
3b	98/126	135/147	172/174	226/232	115/115	182/184	144/146	110/132
4a	98/126	135/147	172/174	226/232	115/115	182/184	144/146	110/132
4b	98/126	135/147	172/174	226/232	115/115	182/184	144/146	110/132
5a	98/126	147/147	172/174	226/232	115/115	182/184	146/146	110/132
5b	126/126	135/147	172/174	232/232	115/115	*182/184*	144/146	110/132

[a]The correct genotypes for control sample T11 listed and highlighted in grey.
[b]Letters a and b represents the duplicate runs of one sample.
Erroneous genotype results are highlighted in blue. This is caused by allele dropout (shorter or longer allele, APHT 300 RFU) or heterozygote imbalance resulting in a heterozygous with alleles separated by one repeat, being incorrectly typed as a homozygous. NR – no result (a homozygous with peak height below 600 RFU or a heterozygous with both allele peak heights below 600 RFU), also highlighted in blue.

Table A3c. *The table lists genotype results from a control sample (T10) analysed eight times (four duplicates) in eight markers (MP8 and MP14) with a 0.05 ng template input.*

	MP8				MP14			
	G10B	G10C	G10O	G10X	G1D	G1A	G10J	Mu15
T10[a]	110/112	155/167	201/201	178/182	130/138	179/181	101/109	116/120
run[b]								
1a	110/112	155/167	201/201	178/178	138/138	179/181	101/109	NR
1b	110/112	NR	201/201	178/182	130/138	179/181	101/109	NR
2a	110/112	155/167	201/201	178/182	130/138	179/181	109/109	NR
2b	112/112	NR	201/201	178/178	130/130	179/181	101/109	120/120
3a	110/110	155/167	201/201	178/182	138/138	179/181	101/109	NR
3b	110/112	155/167	201/201	182/182	NR	179/181	109/109	NR
4a	110/112	155/167	201/201	178/178	130/138	179/181	101/109	116/120
4b	110/112	155/167	201/201	178/182	130/138	181/181	101/109	116/120

[a]The correct genotypes for control sample T10 listed and highlighted in grey.
[b]Letters a and b represents the duplicate runs of one sample.
Erroneous genotype results are highlighted in blue. This is caused by allele dropout (shorter or longer allele, APHT 300 RFU) or heterozygote imbalance resulting in a heterozygous with alleles separated by one repeat, being incorrectly typed as a homozygous. NR – no result (a homozygous with peak height below 600 RFU or a heterozygous with both allele peak heights below 600 RFU), also highlighted in blue.

Table A3d. *The table list genotype results from one control sample (T11) with known genotypes analysed eight times (four duplicates) in eight markers (MP8 and MP14) with a 0.05 ng template input.*

	MP8				MP14			
	G10B	G10C	G10O	G10X	G1D	G1A	G10J	Mu15
T11[a]	98/110	155/163	201/203	180/188	130/134	181/189	99/101	110/116
run[b]								
1a	NR	155/163	NR	NR	130/134	181/189	99/101	110/110
1b	NR	155/155	NR	180/188	130/134	181/189	99/101	NR
2a	98/110	NR	201/203	180/188	NR	189/189	99/101	NR
2b	NR	155/155	NR	NR	130/134	181/181	99/101	NR
3a	98/98	163/163	201/203	NR	130/130	189/189	99/101	NR
3b	98/110	155/163	201/201	NR	130/134	181/189	101/101	110/110
4a	NR	155/163	201/201	NR	130/134	NR	99/101	NR
4b	110/110	155/163	203/203	NR	130/134	181/189	101/101	NR

[a]The correct genotypes for control sample T11 listed and highlighted in grey.
[b]Letters a and b represents the duplicate runs of one sample.
Erroneous genotype results are highlighted in blue. This is caused by allele dropout (shorter or longer allele, APHT 300 RFU) or heterozygote imbalance resulting in a heterozygous with alleles separated by one repeat, being incorrectly typed as a homozygous. NR – no result (a homozygous with peak height below 600 RFU or a heterozygous with both allele peak heights below 600 RFU), also highlighted in blue.

Table A3e. The table lists number of cases where alleles had peak heights below 1600 RFU in two control samples analysed at eight STR markers (MP1, MP2 and MP3) with a template input of 0.6 ng (n=20), 0.2 ng (n=20) and 0.05 ng (n=20).

Peak heights below 1600 RFU (n=20)								
Template input (ng)	MP1				MP2			MP3
	Mu09	Mu10	Mu23	Mu59	Mu05	G10L	Mu51	Mu50
0.6	-	-	1	2	-	-	-	3
0.2	5	2	3	3	-	1	-	4
0.05	18	3	12	7	8	9	7	12

Table A3f. The table lists number of cases the peak heights were below 1600 RFU in two control samples analysed at eight STR markers (MP8 and MP14) with a template input of 0.6 ng (n=16), 0.2 ng (n=16) and 0.05 ng (n=16).

Peak heights below 1600 RFU (n=16)								
Template input (ng)	MP8				MP14			
	G10B	G10C	G10O	G10X	G1D	G1A	G10J	Mu15
0.6	1	1	1	1	-	1	-	4
0.2	3	6	5	4	3	2	1	9
0.05	11	14	9	13	14	13	4	16

Appendix 4. An overview of the samples, stations and analysis results.

P/N: Positive/ Negative results. A negative result indicates that there were no bear specific PCR products amplified. A positive result can either be of good quality e.g. give an individual ID (genotypes in ≥8 STRs), be assigned the result "unknown" (from 1-7 STRs successfully typed (see section 3.5.2.)) or be assigned the result "mixture"= a sample from ≥ two contributors.

Gender (F=female, M=male), Individual ID (KamOx= Kamchatka Oblast, x=numbers starting from 1)

Date of collection	St. №	External sample №	River	Svanhovd-sample №	P/N	gender	Individual ID
23.07.2015	S1	1001	Kamchatka	17RH001	N	–	–
23.07.2015	S1	1002	Kamchatka	17RH002	P	F	KamO1
23.07.2015	S1	1003	Kamchatka	17RH003	P	F	KamO1
23.07.2015	S1	1004	Kamchatka	17RH004	P	F	KamO1
23.07.2015	S1	1005	Kamchatka	17RH005	N	–	–
26.07.2015	S1	1006	Kamchatka	17RH006	P	M	unknown
26.07.2015	S1	1007	Kamchatka	17RH007	P	M	KamO2
30.07.2015	S1	1008	Kamchatka	17RH008	P	M	KamO3
30.07.2015	S1	1009	Kamchatka	17RH009	P	M	KamO3
06.08.2015	S1	1010	Kamchatka	17RH010	P	M	KamO111
25.07.2015	S2	1011	Olezkina	17RH011	P	F	KamO4
25.07.2015	S2	1012	Olezkina	17RH012	N	–	–
25.07.2015	S2	1013	Olezkina	17RH013	P	F	KamO5
25.07.2015	S2	1014	Olezkina	17RH014	P	F	KamO5
25.07.2015	S2	1015	Olezkina	17RH015	P	–	mixture
25.07.2015	S2	1016	Olezkina	17RH016	P	F	unknown
25.07.2015	S2	1017	Olezkina	17RH017	P	F	KamO5
25.07.2015	S2	1018	Olezkina	17RH018	P	F	KamO5
25.07.2015	S2	1019	Olezkina	17RH019	P	F	KamO5
25.07.2015	S2	1020	Olezkina	17RH020	P	F	KamO5
25.07.2015	S2	1021	Olezkina	17RH021	P	F	unknown
25.07.2015	S2	1022	Olezkina	17RH022	N	–	–
25.07.2015	S2	1023	Olezkina	17RH023	N	–	–
25.07.2015	S2	1024	Olezkina	17RH024	P	M	KamO106
25.07.2015	S2	1025	Olezkina	17RH025	N	–	–
25.07.2015	S2	1026	Olezkina	17RH026	P	F	unknown
25.07.2015	S2	1027	Olezkina	17RH027	N	–	–
25.07.2015	S2	1028	Olezkina	17RH028	P	F	unknown
31.07.2015	S2	1029	Lotnaya	17RH029	P	F	KamO6
31.07.2015	S2	1030	Lotnaya	17RH030	N	–	–
31.07.2015	S2	1031	Lotnaya	17RH031	N	–	–
31.07.2015	S2	1032	Lotnaya	17RH032	N	–	–
07.08.2015	S2	1033	Olezhkina	17RH033	P	F	KamO5
07.08.2015	S2	1034	Olezhkina	17RH034	N	–	–
07.08.2015	S2	1035	Olezhkina	17RH035	N	–	–
07.08.2015	S2	1036	Olezhkina	17RH036	N	–	–
07.08.2015	S2	1037	Olezhkina	17RH037	P	F	KamO8
07.08.2015	S2	1038	Olezhkina	17RH038	P	M	KamO7
07.08.2015	S2	1039	Olezhkina	17RH039	P	M	KamO7
07.08.2015	S2	1040	Olezhkina	17RH040	P	F	KamO5
07.08.2015	S2	1041	Olezhkina	17RH041	P	F	unknown
14.08.2015	S2	1042	Olezhkina	17RH042	N	–	–
14.08.2015	S2	1043	Olezhkina	17RH043	P	F	KamO8
14.08.2015	S2	1044	Olezhkina	17RH044	P	F	KamO9
14.08.2015	S2	1045	Olezhkina	17RH045	N	–	–
14.08.2015	S2	1046	Olezhkina	17RH046	P	F	unknown
14.08.2015	S2	1047	Olezhkina	17RH047	P	–	mixture
14.08.2015	S2	1048	Olezhkina	17RH048	N	–	–
14.08.2015	S2	1049	Olezhkina	17RH049	P	M	KamO10

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25.07.2015	S3	1050	Geshkin	17RH050	P	F	KamO11
25.07.2015	S3	1051	Geshkin	17RH051	P	F	KamO13
25.07.2015	S3	1052	Geshkin	17RH052	N	–	–
25.07.2015	S3	1053	Geshkin	17RH053	P	F	KamO12
25.07.2015	S3	1054	Geshkin	17RH054	N	–	–
25.07.2015	S3	1055	Geshkin	17RH055	P	F	KamO13
25.07.2015	S3	1056	Geshkin	17RH056	P	F	KamO13
25.07.2015	S3	1057	Geshkin	17RH057	P	F	KamO11
25.07.2015	S3	1058	Geshkin	17RH058	N	–	–
25.07.2015	S3	1059	Geshkin	17RH059	P	F	unknown
25.07.2015	S3	1060	Geshkin	17RH060	P	F	KamO13
25.07.2015	S3	1061	Geshkin	17RH061	P	F	KamO11
25.07.2015	S3	1062	Geshkin	17RH062	P	M	KamO14
25.07.2015	S3	1063	Geshkin	17RH063	P	M	KamO14
25.07.2015	S3	1064	Geshkin	17RH064	P	M	unknown
25.07.2015	S3	1065	Geshkin	17RH065	P	F	KamO11
25.07.2015	S3	1066	Geshkin	17RH066	P	–	unknown
25.07.2015	S3	1067	Geshkin	17RH067	P	F	KamO13
25.07.2015	S3	1068	Geshkin	17RH068	P	F	KamO15
25.07.2015	S3	1069	Geshkin	17RH069	P	F	unknown
25.07.2015	S3	1070	Geshkin	17RH070	P	F	KamO11
31.07.2015	S3	1071	Lotnaya	17RH071	N	–	–
31.07.2015	S3	1072	Lotnaya	17RH072	N	–	–
31.07.2015	S3	1073	Lotnaya	17RH073	P	F	KamO16
31.07.2015	S3	1074	Lotnaya	17RH074	N	–	–
31.07.2015	S3	1075	Lotnaya	17RH075	N	–	–
31.07.2015	S3	1076	Lotnaya	17RH076	P	F	KamO16
31.07.2015	S3	1077	Lotnaya	17RH077	P	F	unknown
07.08.2015	S3	1078	Geshkin	17RH078	P	M	KamO17
07.08.2015	S3	1079	Geshkin	17RH079	P	M	KamO18
07.08.2015	S3	1080	Geshkin	17RH080	N	–	–
25.07.2015	S4	1081	Lotnaya	17RH081	N	–	–
25.07.2015	S4	1082	Lotnaya	17RH082	P	M	KamO19
25.07.2015	S4	1083	Lotnaya	17RH083	P	M	KamO19
25.07.2015	S4	1084	Lotnaya	17RH084	N	–	–
25.07.2015	S4	1085	Lotnaya	17RH085	P	F	KamO11
25.07.2015	S4	1086	Lotnaya	17RH086	P	M	KamO20
25.07.2015	S4	1087	Lotnaya	17RH087	P	F	KamO21
25.07.2015	S4	1088	Lotnaya	17RH088	P	M	KamO22
25.07.2015	S4	1089	Lotnaya	17RH089	P	M	KamO22
25.07.2015	S4	1090	Lotnaya	17RH090	P	–	mixture
25.07.2015	S4	1091	Lotnaya	17RH091	N	–	–
25.07.2015	S4	1092	Lotnaya	17RH092	P	M	KamO22
25.07.2015	S4	1093	Lotnaya	17RH093	N	–	–
25.07.2015	S4	1094	Lotnaya	17RH094	P	–	unknown
25.07.2015	S4	1095	Lotnaya	17RH095	P	M	KamO22
25.07.2015	S4	1096	Lotnaya	17RH096	P	M	KamO22
25.07.2015	S4	1097	Lotnaya	17RH097	N	–	–
25.07.2015	S4	1098	Lotnaya	17RH098	P	M	KamO22
25.07.2015	S4	1099	Lotnaya	17RH099	N	–	–
25.07.2015	S4	1100	Lotnaya	17RH100	N	–	–
29.07.2015	S4	1101	Lotnaya	17RH101	P	F	KamO23
29.07.2015	S4	1102	Lotnaya	17RH102	P	F	KamO23
29.07.2015	S4	1103	Lotnaya	17RH103	P	F	KamO23
29.07.2015	S4	1104	Lotnaya	17RH104	N	–	–
29.07.2015	S4	1105	Lotnaya	17RH105	N	–	–
29.07.2015	S4	1106	Lotnaya	17RH106	P	M	KamO22

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29.07.2015	S4	1107	Lotnaya	17RH107	P	F	KamO24
29.07.2015	S4	1108	Lotnaya	17RH108	P	F	KamO24
29.07.2015	S4	1109	Lotnaya	17RH109	P	F	KamO24
29.07.2015	S4	1110	Lotnaya	17RH110	P	F	KamO24
29.07.2015	S4	1111	Lotnaya	17RH111	N	–	–
29.07.2015	S4	1112	Lotnaya	17RH112	P	F	KamO25
29.07.2015	S4	1113	Lotnaya	17RH113	P	F	KamO26
29.07.2015	S4	1114	Lotnaya	17RH114	P	F	KamO24
29.07.2015	S4	1115	Lotnaya	17RH115	N	–	–
29.07.2015	S4	1116	Lotnaya	17RH116	P	F	KamO26
29.07.2015	S4	1117	Lotnaya	17RH117	N	–	–
29.07.2015	S4	1118	Lotnaya	17RH118	P	–	unknown
29.07.2015	S4	1119	Lotnaya	17RH119	P	F	KamO26
29.07.2015	S4	1120	Lotnaya	17RH120	P	F	KamO26
29.07.2015	S4	1121	Lotnaya	17RH121	P	F	KamO27
05.08.2015	S4	1122	Lotnaya	17RH122	N	–	–
05.08.2015	S4	1123	Lotnaya	17RH123	N	–	–
05.08.2015	S4	1124	Lotnaya	17RH124	N	–	–
05.08.2015	S4	1125	Lotnaya	17RH125	P	M	KamO28
05.08.2015	S4	1126	Lotnaya	17RH126	P	F	KamO29
05.08.2015	S4	1127	Lotnaya	17RH127	P	F	KamO30
05.08.2015	S4	1128	Lotnaya	17RH128	P	F	KamO30
05.08.2015	S4	1129	Lotnaya	17RH129	P	F	KamO31
05.08.2015	S4	1130	Lotnaya	17RH130	P	F	KamO31
13.08.2015	S4	1131	Lotnaya	17RH131	N	–	–
13.08.2015	S4	1132	Lotnaya	17RH132	P	F	KamO11
13.08.2015	S4	1133	Lotnaya	17RH133	P	M	KamO32
13.08.2015	S4	1134	Lotnaya	17RH134	P	M	KamO32
13.08.2015	S4	1135	Lotnaya	17RH135	P	M	KamO32
13.08.2015	S4	1136	Lotnaya	17RH136	P	F	KamO33
29.07.2015	S5	1137	Vydrovaya	17RH137	N	–	–
29.07.2015	S5	1138	Vydrovaya	17RH138	N	–	–
29.07.2015	S5	1139	Vydrovaya	17RH139	P	M	KamO34
29.07.2015	S5	1140	Vydrovaya	17RH140	N	–	–
29.07.2015	S5	1141	Vydrovaya	17RH141	P	M	KamO20
29.07.2015	S5	1142	Vydrovaya	17RH142	P	M	KamO20
29.07.2015	S5	1143	Vydrovaya	17RH143	P	M	unknown
29.07.2015	S5	1144	Vydrovaya	17RH144	N	–	–
29.07.2015	S5	1145	Vydrovaya	17RH145	N	–	–
29.07.2015	S5	1146	Vydrovaya	17RH146	P	F	KamO35
29.07.2015	S5	1147	Vydrovaya	17RH147	P	F	KamO36
29.07.2015	S5	1148	Vydrovaya	17RH148	P	F	KamO35
29.07.2015	S5	1149	Vydrovaya	17RH149	N	–	–
29.07.2015	S5	1150	Vydrovaya	17RH150	N	–	–
29.07.2015	S5	1151	Vydrovaya	17RH151	P	M	KamO37
29.07.2015	S5	1152	Vydrovaya	17RH152	P	F	KamO35
29.07.2015	S5	1153	Vydrovaya	17RH153	P	F	KamO35
29.07.2015	S5	1154	Vydrovaya	17RH154	P	F	KamO35
29.07.2015	S5	1155	Vydrovaya	17RH155	P	F	unknown
29.07.2015	S5	1156	Vydrovaya	17RH156	P	F	KamO35
29.07.2015	S5	1157	Vydrovaya	17RH157	N	–	–
29.07.2015	S5	1158	Vydrovaya	17RH158	P	F	KamO35
29.07.2015	S5	1159	Vydrovaya	17RH159	P	M	KamO38
29.07.2015	S5	1160	Vydrovaya	17RH160	P	–	mixture
05.08.2015	S5	1161	Vydrovaya	17RH161	P	M	unknown
05.08.2015	S5	1162	Vydrovaya	17RH162	P	M	KamO39
05.08.2015	S5	1163	Vydrovaya	17RH163	N	–	–

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05.08.2015	S5	1164	Vydrovaya	17RH164	P	F	KamO40
05.08.2015	S5	1165	Vydrovaya	17RH165	P	–	mixture
05.08.2015	S5	1166	Vydrovaya	17RH166	P	F	KamO40
05.08.2015	S5	1167	Vydrovaya	17RH167	P	F	KamO40
05.08.2015	S5	1168	Vydrovaya	17RH168	P	F	KamO40
05.08.2015	S5	1169	Vydrovaya	17RH169	P	F	KamO40
05.08.2015	S5	1170	Vydrovaya	17RH170	P	F	KamO41
05.08.2015	S5	1171	Vydrovaya	17RH171	P	F	KamO4
13.08.2015	S5	1172	Vydrovaya	17RH172	P	M	KamO42
13.08.2015	S5	1173	Vydrovaya	17RH173	P	M	KamO42
13.08.2015	S5	1174	Vydrovaya	17RH174	P	F	KamO43
13.08.2015	S5	1175	Vydrovaya	17RH175	P	M	KamO42
13.08.2015	S5	1176	Vydrovaya	17RH176	P	F	KamO43
13.08.2015	S5	1177	Vydrovaya	17RH177	P	F	KamO43
13.08.2015	S5	1178	Vydrovaya	17RH178	P	F	KamO43
13.08.2015	S5	1179	Vydrovaya	17RH179	P	F	KamO43
13.08.2015	S5	1180	Vydrovaya	17RH180	P	M	KamO42
13.08.2015	S5	1181	Vydrovaya	17RH181	P	F	KamO107
13.08.2015	S5	1182	Vydrovaya	17RH182	P	M	KamO42
13.08.2015	S5	1183	Vydrovaya	17RH183	P	M	KamO44
13.08.2015	S5	1184	Vydrovaya	17RH184	P	M	KamO44
13.08.2015	S5	1185	Vydrovaya	17RH185	P	–	unknown
13.08.2015	S5	1186	Vydrovaya	17RH186	N	–	–
13.08.2015	S5	1187	Vydrovaya	17RH187	P	M	KamO45
13.08.2015	S5	1188	Vydrovaya	17RH188	N	–	–
13.08.2015	S5	1189	Vydrovaya	17RH189	P	M	KamO46
13.08.2015	S5	1190	Vydrovaya	17RH190	P	M	KamO46
27.07.2015	S6	1191	Kursinka	17RH191	P	–	unknown
27.07.2015	S6	1192	Kursinka	17RH192	P	F	KamO47
10.08.2015	S6	1193	Kursinka	17RH193	P	M	KamO114
10.08.2015	S6	1194	Kursinka	17RH194	P	F	KamO48
29.07.2015	S7	1195	Bushujka	17RH195	P	M	KamO49
29.07.2015	S7	1196	Bushujka	17RH196	P	–	unknown
29.07.2015	S7	1197	Bushujka	17RH197	P	M	KamO50
29.07.2015	S7	1198	Bushujka	17RH198	P	M	KamO50
29.07.2015	S7	1199	Bushujka	17RH199	P	M	KamO49
29.07.2015	S7	1200	Bushujka	17RH200	N	–	–
29.07.2015	S7	1201	Bushujka	17RH201	P	M	KamO49
29.07.2015	S7	1202	Bushujka	17RH202	P	M	KamO50
29.07.2015	S7	1203	Bushujka	17RH203	P	M	KamO50
29.07.2015	S7	1204	Bushujka	17RH204	P	M	KamO32
29.07.2015	S7	1205	Bushujka	17RH205	P	M	KamO32
29.07.2015	S7	1206	Bushujka	17RH206	P	M	KamO32
05.08.2015	S7	1207	Bushujka	17RH207	P	M	KamO51
05.08.2015	S7	1208	Bushujka	17RH208	P	M	KamO32
05.08.2015	S7	1209	Bushujka	17RH209	N	–	–
05.08.2015	S7	1210	Bushujka	17RH210	P	M	KamO51
13.08.2015	S7	1211	Bushujka	17RH211	P	F	KamO31
13.08.2015	S7	1212	Bushujka	17RH212	P	–	mixture
13.08.2015	S7	1213	Bushujka	17RH213	P	F	KamO52
13.08.2015	S7	1214	Bushujka	17RH214	P	M	KamO28
13.08.2015	S7	1215	Bushujka	17RH215	P	F	KamO52
13.08.2015	S7	1216	Bushujka	17RH216	P	F	KamO52
13.08.2015	S7	1217	Bushujka	17RH217	P	F	KamO52
13.08.2015	S7	1218	Bushujka	17RH218	P	F	KamO52
13.08.2015	S7	1219	Bushujka	17RH219	P	F	KamO52
13.08.2015	S7	1220	Bushujka	17RH220	P	F	KamO52

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13.08.2015	S7	1221	Bushujka	17RH221	P	M	KamO53
13.08.2015	S7	1222	Bushujka	17RH222	P	F	KamO54
29.07.2015	S8	1223	Topolinaya	17RH223	P	F	KamO55
29.07.2015	S8	1224	Topolinaya	17RH224	P	F	KamO55
29.07.2015	S8	1225	Topolinaya	17RH225	P	F	KamO55
29.07.2015	S8	1226	Topolinaya	17RH226	P	F	KamO56
29.07.2015	S8	1227	Topolinaya	17RH227	N	–	–
29.07.2015	S8	1228	Topolinaya	17RH228	P	F	KamO55
29.07.2015	S8	1229	Topolinaya	17RH229	P	F	KamO55
05.08.2015	S8	1230	Topolinaya	17RH230	P	F	KamO57
05.08.2015	S8	1231	Topolinaya	17RH231	P	F	unknown
05.08.2015	S8	1232	Topolinaya	17RH232	P	F	KamO55
05.08.2015	S8	1233	Topolinaya	17RH233	P	F	KamO55
05.08.2015	S8	1234	Topolinaya	17RH234	P	F	KamO55
05.08.2015	S8	1235	Topolinaya	17RH235	P	F	KamO55
05.08.2015	S8	1236	Topolinaya	17RH236	P	–	mixture
05.08.2015	S8	1237	Topolinaya	17RH237	P	F	KamO55
13.08.2015	S8	1238	Topolinaya	17RH238	P	M	KamO58
13.08.2015	S8	1239	Topolinaya	17RH239	P	–	unknown
13.08.2015	S8	1240	Topolinaya	17RH240	P	–	unknown
13.08.2015	S8	1241	Topolinaya	17RH241	P	M	KamO115
29.07.2015	S9	1242	Lamutka	17RH242	P	M	KamO59
29.07.2015	S9	1243	Lamutka	17RH243	P	F	KamO60
29.07.2015	S9	1244	Lamutka	17RH244	P	F	KamO60
29.07.2015	S9	1245	Lamutka	17RH245	P	F	KamO60
29.07.2015	S9	1246	Lamutka	17RH246	N	–	–
05.08.2015	S9	1247	Lamutka	17RH247	P	M	KamO61
05.08.2015	S9	1248	Lamutka	17RH248	P	M	KamO61
05.08.2015	S9	1249	Lamutka	17RH249	P	M	KamO61
05.08.2015	S9	1250	Lamutka	17RH250	P	M	KamO61
05.08.2015	S9	1251	Lamutka	17RH251	P	M	KamO61
05.08.2015	S9	1252	Lamutka	17RH252	P	F	KamO62
05.08.2015	S9	1253	Lamutka	17RH253	P	F	KamO62
05.08.2015	S9	1254	Lamutka	17RH254	P	M	KamO61
05.08.2015	S9	1255	Lamutka	17RH255	P	M	KamO61
05.08.2015	S9	1256	Lamutka	17RH256	N	–	–
13.08.2015	S9	1257	Lamutka	17RH257	P	M	KamO63
13.08.2015	S9	1258	Lamutka	17RH258	P	–	mixture
29.07.2015	S10	1259	Kultuchnaya	17RH259	P	M	KamO58
29.07.2015	S10	1260	Kultuchnaya	17RH260	P	M	KamO58
29.07.2015	S10	1261	Kultuchnaya	17RH261	P	M	KamO58
29.07.2015	S10	1262	Kultuchnaya	17RH262	N	–	–
29.07.2015	S10	1263	Kultuchnaya	17RH263	P	F	KamO64
29.07.2015	S10	1264	Kultuchnaya	17RH264	P	M	KamO49
29.07.2015	S10	1265	Kultuchnaya	17RH265	P	–	mixture
29.07.2015	S10	1266	Kultuchnaya	17RH266	P	F	KamO65
29.07.2015	S10	1267	Kultuchnaya	17RH267	P	–	mixture
29.07.2015	S10	1268	Kultuchnaya	17RH268	P	F	KamO66
29.07.2015	S10	1269	Kultuchnaya	17RH269	P	M	KamO58
29.07.2015	S10	1270	Kultuchnaya	17RH270	P	M	KamO58
29.07.2015	S10	1271	Kultuchnaya	17RH271	P	M	KamO58
29.07.2015	S10	1272	Kultuchnaya	17RH272	P	M	KamO58
29.07.2015	S10	1273	Kultuchnaya	17RH273	P	M	KamO58
29.07.2015	S10	1274	Kultuchnaya	17RH274	P	M	KamO58
29.07.2015	S10	1275	Kultuchnaya	17RH275	P	F	KamO57
29.07.2015	S10	1276	Kultuchnaya	17RH276	P	F	KamO66
29.07.2015	S10	1277	Kultuchnaya	17RH277	N	–	–

Date of collection	St. №	External sample №	River	Svanhovd-sample №	P/N	gender	Individual ID
05.08.2015	S10	1278	Kultuchnaya	17RH278	N	–	–
05.08.2015	S10	1279	Kultuchnaya	17RH279	P	M	KamO67
05.08.2015	S10	1280	Kultuchnaya	17RH280	P	M	KamO67
05.08.2015	S10	1281	Kultuchnaya	17RH281	P	F	KamO69
05.08.2015	S10	1282	Kultuchnaya	17RH282	P	M	KamO68
05.08.2015	S10	1283	Kultuchnaya	17RH283	P	F	unknown
05.08.2015	S10	1284	Kultuchnaya	17RH284	P	F	KamO69
05.08.2015	S10	1285	Kultuchnaya	17RH285	P	M	KamO70
05.08.2015	S10	1286	Kultuchnaya	17RH286	P	M	KamO68
13.08.2015	S10	1287	Kultuchnaya	17RH287	P	F	unknown
13.08.2015	S10	1288	Kultuchnaya	17RH288	P	M	KamO108
13.08.2015	S10	1289	Kultuchnaya	17RH289	P	–	unknown
13.08.2015	S10	1290	Kultuchnaya	17RH290	P	M	KamO71
13.08.2015	S10	1291	Kultuchnaya	17RH291	P	–	unknown
13.08.2015	S10	1292	Kultuchnaya	17RH292	P	F	KamO72
13.08.2015	S10	1293	Kultuchnaya	17RH293	N	–	–
13.08.2015	S10	1294	Kultuchnaya	17RH294	P	M	KamO73
13.08.2015	S10	1295	Kultuchnaya	17RH295	P	F	KamO72
13.08.2015	S10	1296	Kultuchnaya	17RH296	P	F	KamO72
13.08.2015	S10	1297	Kultuchnaya	17RH297	P	M	KamO42
29.07.2015	S11	1298	Arishkin	17RH298	P	–	unknown
29.07.2015	S11	1299	Arishkin	17RH299	P	M	KamO74
29.07.2015	S11	1300	Arishkin	17RH300	P	M	unknown
29.07.2015	S11	1301	Arishkin	17RH301	P	–	unknown
29.07.2015	S11	1302	Arishkin	17RH302	N	–	–
29.07.2015	S11	1303	Arishkin	17RH303	P	M	KamO49
29.07.2015	S11	1304	Arishkin	17RH304	N	–	–
29.07.2015	S11	1305	Arishkin	17RH305	P	–	unknown
29.07.2015	S11	1306	Arishkin	17RH306	P	M	unknown
29.07.2015	S11	1307	Arishkin	17RH307	P	M	KamO75
29.07.2015	S11	1308	Arishkin	17RH308	P	M	KamO75
29.07.2015	S11	1309	Arishkin	17RH309	N	–	–
29.07.2015	S11	1310	Arishkin	17RH310	P	–	unknown
29.07.2015	S11	1311	Arishkin	17RH311	N	–	–
29.07.2015	S11	1312	Arishkin	17RH312	P	M	KamO76
29.07.2015	S11	1313	Arishkin	17RH313	P	M	KamO76
29.07.2015	S11	1314	Arishkin	17RH314	N	–	–
05.08.2015	S11	1315	Arishkin	17RH315	P	F	KamO72
05.08.2015	S11	1316	Arishkin	17RH316	P	F	KamO72
05.08.2015	S11	1317	Arishkin	17RH317	P	–	unknown
05.08.2015	S11	1318	Arishkin	17RH318	P	F	KamO72
05.08.2015	S11	1319	Arishkin	17RH319	P	F	KamO72
05.08.2015	S11	1320	Arishkin	17RH320	P	F	KamO57
05.08.2015	S11	1321	Arishkin	17RH321	P	F	KamO57
05.08.2015	S11	1322	Arishkin	17RH322	P	M	KamO77
05.08.2015	S11	1323	Arishkin	17RH323	N	–	–
05.08.2015	S11	1324	Arishkin	17RH324	P	F	KamO72
05.08.2015	S11	1325	Arishkin	17RH325	N	–	–
05.08.2015	S11	1326	Arishkin	17RH326	P	M	KamO75
05.08.2015	S11	1327	Arishkin	17RH327	P	F	KamO72
05.08.2015	S11	1328	Arishkin	17RH328	N	–	–
13.08.2015	S11	1329	Arishkin	17RH329	P	M	KamO78
13.08.2015	S11	1330	Arishkin	17RH330	P	F	KamO79
13.08.2015	S11	1331	Arishkin	17RH331	P	F	KamO79
13.08.2015	S11	1332	Arishkin	17RH332	N	–	–
13.08.2015	S11	1333	Arishkin	17RH333	P	M	KamO80
13.08.2015	S11	1334	Arishkin	17RH334	P	M	KamO80

Date of collection	St. №	External sample №	River	Svanhovd-sample №	P/N	gender	Individual ID
29.07.2015	S12	1335	Snovidovskiy	17RH335	P	F	KamO72
29.07.2015	S12	1336	Snovidovskiy	17RH336	N	–	–
29.07.2015	S12	1337	Snovidovskiy	17RH337	P	–	unknown
29.07.2015	S12	1338	Snovidovskiy	17RH338	P	M	KamO81
29.07.2015	S12	1339	Snovidovskiy	17RH339	N	–	–
29.07.2015	S12	1340	Snovidovskiy	17RH340	P	F	KamO109
29.07.2015	S12	1341	Snovidovskiy	17RH341	N	–	–
29.07.2015	S12	1342	Snovidovskiy	17RH342	P	–	unknown
29.07.2015	S12	1343	Snovidovskiy	17RH343	P	–	mixture
29.07.2015	S12	1344	Snovidovskiy	17RH344	P	F	KamO72
29.07.2015	S12	1345	Snovidovskiy	17RH345	P	–	mixture
29.07.2015	S12	1346	Snovidovskiy	17RH346	P	F	KamO113
29.07.2015	S12	1347	Snovidovskiy	17RH347	P	M	KamO82
29.07.2015	S12	1348	Snovidovskiy	17RH348	N	–	–
29.07.2015	S12	1349	Snovidovskiy	17RH349	P	M	KamO81
29.07.2015	S12	1350	Snovidovskiy	17RH350	N	–	–
29.07.2015	S12	1351	Snovidovskiy	17RH351	P	M	KamO81
29.07.2015	S12	1352	Snovidovskiy	17RH352	P	F	KamO72
29.07.2015	S12	1353	Snovidovskiy	17RH353	P	F	KamO72
29.07.2015	S12	1354	Snovidovskiy	17RH354	P	F	KamO72
29.07.2015	S12	1355	Snovidovskiy	17RH355	P	F	KamO72
29.07.2015	S12	1356	Snovidovskiy	17RH356	P	F	KamO72
29.07.2015	S12	1357	Snovidovskiy	17RH357	P	F	KamO72
05.08.2015	S12	1358	Snovidovskiy	17RH358	P	F	KamO72
05.08.2015	S12	1359	Snovidovskiy	17RH359	P	F	KamO72
05.08.2015	S12	1360	Snovidovskiy	17RH360	P	F	KamO72
05.08.2015	S12	1361	Snovidovskiy	17RH361	P	M	KamO83
05.08.2015	S12	1362	Snovidovskiy	17RH362	P	F	Unknown
05.08.2015	S12	1363	Snovidovskiy	17RH363	P	M	KamO83
05.08.2015	S12	1364	Snovidovskiy	17RH364	N	–	–
05.08.2015	S12	1365	Snovidovskiy	17RH365	P	F	KamO84
05.08.2015	S12	1366	Snovidovskiy	17RH366	P	F	KamO84
05.08.2015	S12	1367	Snovidovskiy	17RH367	N	–	–
05.08.2015	S12	1368	Snovidovskiy	17RH368	P	F	KamO85
05.08.2015	S12	1369	Snovidovskiy	17RH369	P	F	KamO85
05.08.2015	S12	1370	Snovidovskiy	17RH370	N	–	–
05.08.2015	S12	1371	Snovidovskiy	17RH371	P	–	mixture
05.08.2015	S12	1372	Snovidovskiy	17RH372	N	–	–
13.08.2015	S12	1373	Snovidovskiy	17RH373	P	F	KamO86
13.08.2015	S12	1374	Snovidovskiy	17RH374	P	F	KamO87
13.08.2015	S12	1375	Snovidovskiy	17RH375	P	M	KamO74
13.08.2015	S12	1376	Snovidovskiy	17RH376	P	M	unknown
13.08.2015	S12	1377	Snovidovskiy	17RH377	P	M	KamO88
13.08.2015	S12	1378	Snovidovskiy	17RH378	P	M	KamO88
13.08.2015	S12	1379	Snovidovskiy	17RH379	P	M	KamO88
13.08.2015	S12	1380	Snovidovskiy	17RH380	P	F	KamO87
13.08.2015	S12	1381	Snovidovskiy	17RH381	N	–	–
30.07.2015	S13	1382	Kamchatka	17RH382	P	–	unknown
30.07.2015	S13	1383	Kamchatka	17RH383	N	–	–
30.07.2015	S13	1384	Kamchatka	17RH384	N	–	–
12.08.2015	S13	1385	Kamchatka	17RH385	P	M	KamO89
12.08.2015	S13	1386	Kamchatka	17RH386	N	–	–
12.08.2015	S13	1387	Kamchatka	17RH387	P	M	KamO90
30.07.2015	S14	1388	Kamchatka	17RH388	P	F	KamO91
30.07.2015	S14	1389	Kamchatka	17RH389	N	–	–
30.07.2015	S14	1390	Kamchatka	17RH390	P	F	unknown
30.07.2015	S14	1391	Kamchatka	17RH391	P	M	KamO92

Date of collection	St. №	External sample №	River	Svanhovd-sample №	P/N	gender	Individual ID
30.07.2015	S14	1392	Kamchatka	17RH392	P	F	KamO91
30.07.2015	S14	1393	Kamchatka	17RH393	P	F	KamO91
30.07.2015	S14	1394	Kamchatka	17RH394	P	F	unknown
06.08.2015	S14	1395	Kamchatka	17RH395	P	M	KamO93
06.08.2015	S14	1396	Kamchatka	17RH396	P	M	KamO93
06.08.2015	S14	1397	Kamchatka	17RH397	P	F	KamO94
06.08.2015	S14	1398	Kamchatka	17RH398	P	M	KamO93
30.07.2015	S15	1399	Kamchatka	17RH399	P	F	KamO95
06.08.2015	S15	1400	Kamchatka	17RH400	P	F	KamO96
06.08.2015	S15	1401	Kamchatka	17RH401	P	M	KamO97
06.08.2015	S15	1402	Kamchatka	17RH402	P	F	unknown
06.08.2015	S15	1403	Kamchatka	17RH403	P	F	KamO94
06.08.2015	S15	1404	Kamchatka	17RH404	P	F	KamO96
06.08.2015	S15	1405	Kamchatka	17RH405	P	F	KamO110
06.08.2015	S15	1406	Kamchatka	17RH406	P	F	KamO110
06.08.2015	S15	1407	Kamchatka	17RH407	P	F	KamO94
31.07.2015	S16	1408	Ponomarka	17RH408	P	F	KamO104
31.07.2015	S16	1409	Ponomarka	17RH409	P	F	KamO104
31.07.2015	S16	1410	Ponomarka	17RH410	P	M	KamO98
31.07.2015	S16	1411	Ponomarka	17RH411	P	F	KamO99
31.07.2015	S16	1412	Ponomarka	17RH412	P	–	unknown
31.07.2015	S16	1413	Ponomarka	17RH413	P	M	KamO88
31.07.2015	S16	1414	Ponomarka	17RH414	N	–	–
31.07.2015	S16	1415	Ponomarka	17RH415	N	–	–
31.07.2015	S16	1416	Ponomarka	17RH416	P	M	KamO100
31.07.2015	S16	1417	Ponomarka	17RH417	P	–	unknown
31.07.2015	S16	1418	Ponomarka	17RH418	P	–	unknown
31.07.2015	S16	1419	Ponomarka	17RH419	P	F	unknown
31.07.2015	S16	1420	Ponomarka	17RH420	P	F	KamO104
31.07.2015	S16	1421	Ponomarka	17RH421	P	M	KamO101
31.07.2015	S16	1422	Ponomarka	17RH422	P	M	KamO112
31.07.2015	S16	1423	Ponomarka	17RH423	N	–	–
05.08.2015	S16	1424	Ponomarka	17RH424	P	M	KamO88
05.08.2015	S16	1425	Ponomarka	17RH425	P	–	unknown
05.08.2015	S16	1426	Ponomarka	17RH426	P	F	KamO102
05.08.2015	S16	1427	Ponomarka	17RH427	P	F	KamO102
05.08.2015	S16	1428	Ponomarka	17RH428	P	M	KamO103
05.08.2015	S16	1429	Ponomarka	17RH429	P	M	KamO101
13.08.2015	S16	1430	Ponomarka	17RH430	P	M	KamO105
13.08.2015	S16	1431	Ponomarka	17RH431	N	–	–
13.08.2015	S16	1432	Ponomarka	17RH432	P	–	unknown
13.08.2015	S16	1433	Ponomarka	17RH433	P	F	KamO104
13.08.2015	S16	1434	Ponomarka	17RH434	P	M	KamO88

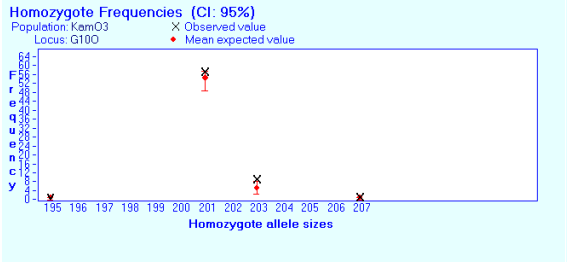
Appendix 5. Results from Microchecker v.2.2.3

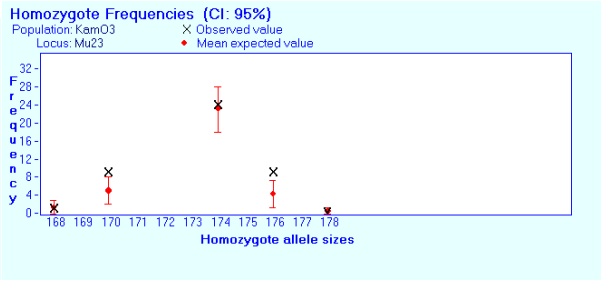
Rawdata: 95 % CI

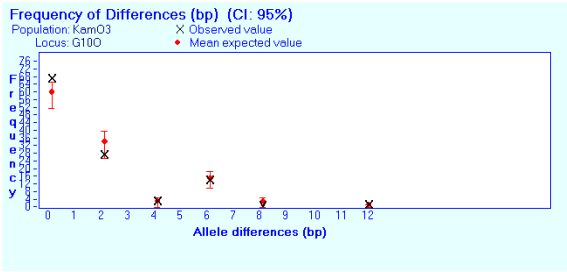
Locus	Null Present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Mu09	no	-0,0045	-0,0028	-0,0025	0
Mu10	no	-0,042	-0,0386	-0,0309	0
Mu23	yes	0,0556	0,065	0,0508	0,0508
Mu59	no	0,0134	0,0136	0,0125	0,0604
Mu05	no	0,0034	0,006	0,0055	0,0055
G10L	no	-0,0026	-0,0033	-0,0031	0,0304
Mu51	no	-0,02	-0,0159	-0,0136	0,0371
Mu50	no	-0,0436	-0,0408	-0,0382	0
G10B	no	0,0279	0,0267	0,0233	0,0538
G10C	no	-0,0292	-0,0191	-0,015	0,0677
G10O	yes	0,0695	0,0792	0,047	0,1411
G10X	no	-0,0076	0,0057	0,0046	0,1113
G1D	no	-0,0378	-0,0298	-0,0262	0,0671
G1A	no	0,0133	0,012	0,0104	0,086
G10J	no	-0,006	-0,0062	-0,0055	0,0568
Mu15	no	0,0468	0,0475	0,0406	0,1358

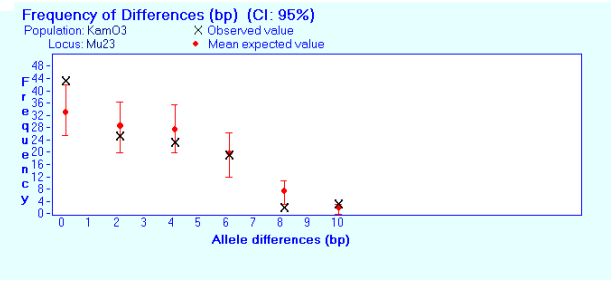
Two loci show evidence for a null allele.

This population is possibly in Hardy Weinberg equilibrium with loci Mu23, G10O, showing signs of a null allele.









Appendix 6. Fishers exact test for linkage equilibrium

Rawdata: (Fishers exact test for linkage and Hardy-Weinberg disequilibrium). GDA v.1.1.

Exact tests for linkage and Hardy-Weinberg disequilibrium:

Subsets of loci will be analyzed

Subsets will be comprised of up to 2 loci

Individuals with missing data will be discarded

Number of runs: 3200

Measure: Fisher

Permute methods string: bbbbbbbbbbbbbbb

Runs	Prob	Locus combination
3200	0.596250	MU09
3200	0.579063	MU10
3200	**0.008438**	MU23
3200	0.302187	MU59
3200	0.185625	MU05
3200	0.068125	G10L
3200	0.076875	MU51
3200	0.443437	MU50
3200	0.295312	G10B
3200	0.133125	G10C
3200	**0.031875**	G10O
3200	0.210313	G10X
3200	0.795000	G1D
3200	0.852812	G1A
3200	0.618125	G10J
3200	0.672188	MU15
3200	0.749062	MU09/MU10
3200	0.374063	MU09/MU23
3200	0.343750	MU09/MU59
3200	0.142813	MU09/MU05
3200	0.760000	MU09/G10L
3200	0.564688	MU09/MU51
3200	0.961250	MU09/MU50
3200	0.144375	MU09/G10B
3200	0.703438	MU09/G10C
3200	0.239687	MU09/G10O
3200	0.144375	MU09/G10X
3200	0.511250	MU09/G1D
3200	0.074375	MU09/G1A
3200	0.369063	MU09/G10J
3200	0.088125	MU09/MU15
3200	0.121875	MU10/MU23
3200	0.689063	MU10/MU59
3200	0.351875	MU10/MU05
3200	0.249062	MU10/G10L
3200	0.719375	MU10/MU51
3200	0.966250	MU10/MU50
3200	0.244375	MU10/G10B
3200	0.100000	MU10/G10C
3200	**0.034688**	**MU10/G10O**
3200	0.605625	MU10/G10X
3200	0.955000	MU10/G1D
3200	0.839688	MU10/G1A
3200	0.476562	MU10/G10J
3200	0.192188	MU10/MU15
3200	**0.017500**	**MU23/MU59**
3200	**0.022812**	**MU23/MU05**
3200	0.052500	MU23/G10L
3200	**0.003750**	**MU23/MU51**
3200	0.061875	MU23/MU50
3200	0.084062	MU23/G10B
3200	0.168437	MU23/G10C
3200	0.097187	MU23/G10O
3200	0.054062	MU23/G10X
3200	0.488438	MU23/G1D
3200	0.098437	MU23/G1A
3200	**0.000625**	**MU23/G10J**
3200	**0.029063**	**MU23/MU15**
3200	0.273438	MU59/MU05
3200	0.203125	MU59/G10L
3200	0.150938	MU59/MU51
3200	0.959375	MU59/MU50
3200	0.055938	MU59/G10B
3200	0.340000	MU59/G10C
3200	**0.012500**	**MU59/G10O**
3200	0.100312	MU59/G10X
3200	0.464375	MU59/G1D
3200	0.142500	MU59/G1A
3200	0.336562	MU59/G10J
3200	**0.026875**	**MU59/MU15**
3200	0.162812	MU05/G10L
3200	0.692500	MU05/MU51
3200	0.873125	MU05/MU50
3200	0.105625	MU05/G10B
3200	0.496563	MU05/G10C
3200	0.096875	MU05/G10O
3200	0.326562	MU05/G10X
3200	0.245938	MU05/G1D
3200	0.171563	MU05/G1A
3200	0.662500	MU05/G10J
3200	**0.045625**	**MU05/MU15**
3200	0.292812	G10L/MU51
3200	0.835938	G10L/MU50
3200	0.196250	G10L/G10B
3200	0.439375	G10L/G10C
3200	0.061562	G10L/G10O
3200	0.237813	G10L/G10X
3200	0.389688	G10L/G1D
3200	0.125938	G10L/G1A
3200	0.479063	G10L/G10J
3200	0.120938	G10L/MU15
3200	0.413750	MU51/MU50

3200	0.285313	MU51/G10B
3200	**0.015000**	**MU51/G10C**
3200	**0.005625**	**MU51/G10O**
3200	0.085625	MU51/G10X
3200	0.341250	MU51/G1D
3200	0.470000	MU51/G1A
3200	0.321875	MU51/G10J
3200	**0.026875**	**MU51/MU15**
3200	0.804688	MU50/G10B
3200	0.630625	MU50/G10C
3200	0.105625	MU50/G10O
3200	0.596250	MU50/G10X
3200	0.302500	MU50/G1D
3200	0.475625	MU50/G1A
3200	0.653125	MU50/G10J
3200	0.084062	MU50/MU15
3200	0.180000	G10B/G10C
3200	0.055625	G10B/G10O
3200	0.261250	G10B/G10X
3200	0.381563	G10B/G1D
3200	0.072813	G10B/G1A
3200	0.458125	G10B/G10J
3200	**0.017812**	**G10B/MU15**
3200	0.074375	G10C/G10O
3200	0.539375	G10C/G10X
3200	0.651250	G10C/G1D
3200	0.433750	G10C/G1A
3200	0.352500	G10C/G10J
3200	0.068750	G10C/MU15
3200	**0.001250**	**G10O/G10X**
3200	0.142187	G10O/G1D
3200	0.113750	G10O/G1A
3200	0.080625	G10O/G10J
3200	**0.001563**	**G10O/MU15**
3200	0.705000	G10X/G1D
3200	0.193438	G10X/G1A
3200	0.437812	G10X/G10J
3200	0.145625	G10X/MU15
3200	0.731875	G1D/G1A
3200	0.214062	G1D/G10J
3200	0.438437	G1D/MU15
3200	0.355312	G1A/G10J
3200	0.103750	G1A/MU15
3200	0.200000	G10J/MU15

The preserving obtion:

Runs	Prob	Locus combination
3200	0.754687	MU09/MU10
3200	0.937813	MU09/MU23
3200	0.330000	MU09/MU59
3200	0.142187	MU09/MU05
3200	0.765000	MU09/G10L
3200	0.587187	MU09/MU51
3200	0.959375	MU09/MU50
3200	0.148438	MU09/G10B
3200	0.721562	MU09/G10C
3200	0.700313	MU09/G10O
3200	0.138437	MU09/G10X
3200	0.500625	MU09/G1D
3200	0.072500	MU09/G1A
3200	0.380937	MU09/G10J
3200	0.097812	MU09/MU15
3200	0.580313	MU10/MU23
3200	0.688750	MU10/MU59
3200	0.346875	MU10/MU05
3200	0.246875	MU10/G10L
3200	0.735313	MU10/MU51
3200	0.964375	MU10/MU50
3200	0.236875	MU10/G10B
3200	0.103125	MU10/G10C
3200	0.144063	MU10/G10O
3200	0.600625	MU10/G10X
3200	0.964688	MU10/G1D
3200	0.834063	MU10/G1A
3200	0.468438	MU10/G10J
3200	0.193750	MU10/MU15
3200	0.121875	MU23/MU59
3200	0.208437	MU23/MU05
3200	0.416875	MU23/G10L
3200	0.050625	MU23/MU51
3200	0.414062	MU23/MU50
3200	0.535312	MU23/G10B
3200	0.640625	MU23/G10C
3200	0.966250	MU23/G10O
3200	0.305625	MU23/G10X
3200	0.958438	MU23/G1D
3200	0.564688	MU23/G1A
3200	**0.019375**	**MU23/G10J**
3200	0.259062	MU23/MU15
3200	0.260625	MU59/MU05
3200	0.208750	MU59/G10L
3200	0.153125	MU59/MU51
3200	0.962500	MU59/MU50
3200	0.062500	MU59/G10B
3200	0.340000	MU59/G10C
3200	0.065625	MU59/G10O
3200	0.102813	MU59/G10X
3200	0.452188	MU59/G1D
3200	0.137500	MU59/G1A
3200	0.360312	MU59/G10J
3200	**0.024688**	**MU59/MU15**
3200	0.146563	MU05/G10L
3200	0.671250	MU05/MU51
3200	0.877188	MU05/MU50
3200	0.102813	MU05/G10B
3200	0.497500	MU05/G10C
3200	0.396250	MU05/G10O
3200	0.320000	MU05/G10X
3200	0.230937	MU05/G1D
3200	0.173750	MU05/G1A
3200	0.659687	MU05/G10J
3200	0.053750	MU05/MU15
3200	0.307188	G10L/MU51
3200	0.828750	G10L/MU50

3200	0.196250	G10L/G10B
3200	0.430312	G10L/G10C
3200	0.307188	G10L/G10O
3200	0.231563	G10L/G10X
3200	0.364063	G10L/G1D
3200	0.127500	G10L/G1A
3200	0.475313	G10L/G10J
3200	0.122188	G10L/MU15
3200	0.411562	MU51/MU50
3200	0.306875	MU51/G10B
3200	**0.014687**	**MU51/G10C**
3200	**0.039688**	**MU51/G10O**
3200	0.090938	MU51/G10X
3200	0.330000	MU51/G1D
3200	0.480625	MU51/G1A
3200	0.323125	MU51/G10J
3200	**0.031250**	**MU51/MU15**
3200	0.792188	MU50/G10B
3200	0.632812	MU50/G10C
3200	0.395313	MU50/G10O
3200	0.605313	MU50/G10X
3200	0.304063	MU50/G1D
3200	0.485625	MU50/G1A
3200	0.655937	MU50/G10J
3200	0.096875	MU50/MU15
3200	0.187500	G10B/G10C
3200	0.216250	G10J/MU15
3200	0.255937	G10B/G10O
3200	0.264062	G10B/G10X
3200	0.379063	G10B/G1D
3200	0.067812	G10B/G1A
3200	0.444063	G10B/G10J
3200	**0.011875**	**G10B/MU15**
3200	0.233750	G10C/G10O
3200	0.531875	G10C/G10X
3200	0.651250	G10C/G1D
3200	0.438750	G10C/G1A
3200	0.345313	G10C/G10J
3200	0.073750	G10C/MU15
3200	**0.009687**	**G10O/G10X**
3200	0.473438	G10O/G1D
3200	0.406562	G10O/G1A
3200	0.305937	G10O/G10J
3200	**0.017500**	**G10O/MU15**
3200	0.699688	G10X/G1D
3200	0.191562	G10X/G1A
3200	0.437500	G10X/G10J
3200	0.150000	G10X/MU15
3200	0.752500	G1D/G1A
3200	0.211562	G1D/G10J
3200	0.418750	G1D/MU15
3200	0.338125	G1A/G10J
3200	0.094687	G1A/MU15

Appendix 7. DNA profiles (n=115) of the Kamchatka brown bear population

The individual ID (ID), gender (F=female, M=male), genotype at each locus (both alleles denoted in basepairs separated by /), number of samples of each individual (samples) and at which hair trap station (S1-S16) the individual bear was sampled. If the individual bear was sampled at more than one station, all stations are listed.

ID	gender	G1D	G10B	MU05	MU09	MU15	G1A	G10L	MU10	MU23	MU50	MU51	MU59	G10C	G10O	G10X	G10J	samples	stations
KamO1	F	129/133	106/120	115/131	102/110	096/118	177/187	190/194	145/145	174/174	122/130	146/146	238/250	155/165	201/203	174/182	111/121	3	S1
KamO2	M	133/133	106/122	115/129	104/122	114/116	177/183	180/184	147/147	170/174	126/138	148/148	238/248	157/167	195/207	180/182	117/117	1	S1
KamO3	M	133/133	112/118	117/131	114/122	096/122	177/185	184/194	147/147	168/174	122/128	148/148	238/238	155/159	201/201	180/182	099/121	2	S1
KamO4	F	131/133	118/120	113/117	114/124	110/118	185/187	174/184	145/147	176/176	122/126	146/150	242/246	155/155	201/201	182/182	111/117	2	S1, S5
KamO5	F	131/133	098/112	113/129	114/120	114/118	183/189	180/180	145/147	174/176	110/128	146/148	242/252	155/159	201/203	182/182	099/099	8	S2
KamO6	F	133/133	106/112	115/119	110/122	096/118	185/185	174/174	145/147	170/174	110/134	146/148	232/252	157/161	201/203	176/182	099/115	1	S2
KamO7	M	129/133	112/118	113/115	104/110	112/114	177/187	184/184	147/147	174/174	128/130	146/150	228/252	155/155	201/201	182/182	115/121	2	S2
KamO8	F	131/133	112/112	113/123	104/120	114/118	183/187	180/184	143/145	168/174	110/130	144/148	242/252	155/159	201/201	182/182	099/123	2	S2
KamO9	F	131/133	112/120	127/131	112/122	110/114	177/177	184/188	143/143	168/174	130/134	142/150	234/250	157/157	201/203	174/184	099/099	1	S2
KamO10	M	131/131	112/120	115/117	110/124	096/116	177/189	180/184	143/145	168/168	110/130	148/150	244/244	155/157	201/201	178/182	117/117	1	S2
KamO11	F	129/131	112/118	113/133	104/110	116/118	185/187	190/194	141/147	170/174	126/130	146/150	238/248	155/165	201/201	182/182	099/117	7	S3, S4
KamO12	F	127/131	106/114	115/117	110/122	116/116	185/185	174/202	145/147	174/176	126/128	142/144	250/250	155/161	201/203	182/182	115/121	1	S3
KamO13	F	129/131	112/112	113/115	104/112	114/114	187/187	180/184	143/145	168/174	130/130	146/148	252/252	159/167	201/201	180/182	117/123	5	S3
KamO14	M	133/133	116/118	113/115	110/110	114/116	177/187	174/184	145/145	174/174	132/132	146/148	238/248	155/159	201/203	182/182	117/117	2	S3
KamO15	F	129/133	112/112	113/115	104/110	114/114	177/187	184/186	145/147	168/178	110/130	144/148	238/252	159/167	201/201	182/182	099/117	1	S3
KamO16	F	127/129	106/112	115/117	104/122	096/114	177/187	174/184	145/147	174/176	110/130	142/146	232/252	157/159	201/203	180/182	117/119	2	S3
KamO17	M	127/127	106/114	117/119	112/122	116/116	177/185	174/194	145/147	176/176	124/128	142/144	250/250	161/167	203/207	182/196	119/121	1	S3
KamO18	M	129/133	108/114	117/131	114/122	110/110	177/177	190/202	143/151	168/178	110/134	146/148	244/246	155/157	201/201	182/184	117/121	1	S3
KamO19	M	131/133	112/112	113/127	104/112	116/116	177/181	180/186	147/147	174/178	130/134	148/150	244/246	155/157	195/201	178/178	099/111	2	S4
KamO20	M	129/131	112/120	127/129	110/114	116/118	177/185	184/192	147/147	174/174	122/122	150/150	232/242	157/161	201/201	182/184	099/117	3	S4, S5
KamO21	F	127/131	112/120	115/131	112/122	096/116	183/189	188/196	147/147	170/174	128/130	146/148	242/248	155/157	201/201	180/184	099/099	1	S4
KamO22	M	131/133	106/118	127/133	114/124	114/116	183/187	190/202	143/147	170/172	128/134	146/150	248/248	155/157	201/207	184/196	099/115	7	S4
KamO23	F	131/133	106/114	115/123	122/122	096/118	183/185	180/186	143/143	174/176	130/134	142/146	250/252	155/155	201/203	184/184	115/117	3	S4
KamO24	F	129/133	098/112	115/117	104/110	114/122	177/185	184/184	143/147	170/174	126/134	146/150	250/252	157/161	201/203	174/182	099/117	5	S4
KamO25	F	133/133	098/116	113/117	104/110	114/122	185/187	174/184	143/145	170/174	132/134	146/148	238/250	155/161	201/203	174/182	099/117	1	S4
KamO26	F	131/133	114/118	113/117	118/120	096/096	189/189	174/178	147/147	170/174	128/130	144/148	238/238	155/155	201/201	178/184	099/117	4	S4
KamO27	F	127/133	106/106	113/117	110/122	096/116	177/177	194/194	145/147	176/176	128/132	146/148	232/232	155/157	201/203	182/182	117/119	1	S4
KamO28	M	131/133	112/118	133/135	114/114	112/114	177/187	184/184	143/147	172/178	122/130	146/146	242/250	155/155	201/201	174/184	121/123	2	S4, S7
KamO29	F	129/133	118/120	115/123	110/122	116/116	183/185	182/194	145/147	168/174	126/130	144/146	238/244	153/157	201/201	182/184	099/115	1	S4
KamO30	F	131/133	112/112	115/115	114/124	110/116	185/185	174/174	143/145	174/174	110/128	146/148	232/238	155/161	201/201	176/182	115/117	2	S4
KamO31	F	129/133	118/120	127/131	104/112	116/118	177/185	180/194	143/145	174/176	122/128	144/146	246/246	155/155	207/207	182/196	115/121	3	S4, S7
KamO32	M	129/139	106/108	117/125	104/114	116/118	185/189	174/180	143/143	174/176	130/130	146/148	238/238	155/161	195/201	174/174	115/121	7	S4, S7
KamO33	F	131/133	114/118	115/133	110/120	096/116	185/189	180/202	147/147	170/174	126/130	144/146	250/252	155/157	201/201	182/196	099/121	1	S4
KamO34	M	131/133	106/106	115/133	114/114	114/116	183/185	180/190	147/147	168/174	122/128	146/150	244/248	157/157	201/207	182/184	099/099	1	S5
KamO35	F	129/131	112/118	113/133	110/120	116/120	181/183	174/184	145/147	174/176	128/130	144/148	242/250	155/157	201/203	182/194	115/117	7	S5
KamO36	F	127/131	106/112	113/117	104/122	116/116	185/187	194/198	145/147	174/174	122/132	144/146	232/250	155/161	203/203	174/192	115/119	1	S5
KamO37	M	133/135	106/112	117/117	120/122	116/116	177/185	194/194	147/147	174/174	110/134	142/150	250/250	155/157	203/207	182/196	115/117	1	S5

ID	gender	G1D	G10B	MU05	MU09	MU15	G1A	G10L	MU10	MU23	MU50	MU51	MU59	G10C	G10O	G10X	G10J	samples	stations
KamO38	M	127/133	106/118	115/117	110/124	116/122	185/187	184/186	145/145	168/176	126/130	144/148	238/252	155/163	201/203	182/186	101/117	1	S5
KamO39	M	129/131	112/120	115/133	114/116	096/114	185/185	186/194	147/147	168/174	124/130	146/148	232/250	155/159	203/203	176/184	099/099	1	S5
KamO40	F	131/133	112/118	113/127	110/112	112/116	177/187	174/194	147/147	176/176	110/122	146/148	238/244	155/157	201/201	174/182	111/123	5	S5
KamO41	F	129/133	106/118	113/115	102/110	096/118	177/183	184/184	141/145	174/174	122/130	146/150	250/250	155/165	203/203	182/182	099/121	1	S5
KamO42	M	129/129	112/112	115/123	110/112	112/116	187/189	184/188	145/147	170/176	128/134	146/148	250/252	155/157	201/201	182/182	099/115	6	S5, S10
KamO43	F	129/133	106/120	115/117	110/122	114/118	187/189	180/194	143/147	174/174	110/130	146/146	238/252	157/161	201/203	184/184	099/117	5	S5
KamO44	M	129/131	112/112	115/127	114/114	114/116	177/177	174/180	147/149	174/174	110/128	146/148	238/246	155/157	201/203	176/180	099/115	2	S5
KamO45	M	125/133	112/122	117/127	110/120	112/116	183/185	184/188	145/147	174/174	110/134	144/148	234/252	155/157	203/203	182/182	099/117	1	S5
KamO46	M	129/135	106/118	123/123	122/122	096/116	185/189	182/190	147/147	168/174	110/130	148/150	244/252	155/157	201/201	182/182	099/115	2	S5
KamO47	F	133/133	112/114	115/129	110/110	118/118	185/193	174/194	147/147	170/170	134/136	146/148	244/252	155/157	203/203	176/182	099/127	1	S6
KamO48	F	131/133	112/114	113/129	110/110	118/118	185/193	174/174	145/147	170/170	130/134	146/146	234/244	155/155	201/203	182/182	115/127	1	S6
KamO49	M	127/133	106/106	127/127	112/122	110/112	183/183	184/194	147/147	174/176	130/134	146/150	238/242	155/167	201/201	182/196	099/099	5	S7, S10, S11
KamO50	M	131/133	098/112	113/113	104/114	114/118	177/187	184/194	145/147	174/174	122/130	146/148	250/252	157/159	201/203	182/182	099/099	4	S7
KamO51	M	129/133	118/118	117/131	110/114	112/114	185/185	188/192	139/147	174/174	128/130	146/150	242/250	155/161	203/203	176/182	099/117	2	S7
KamO52	F	129/129	106/112	117/127	110/122	096/096	183/183	174/194	147/147	170/174	122/130	146/148	244/246	155/157	201/207	176/182	099/117	7	S7
KamO53	M	129/133	112/120	125/127	104/114	112/114	177/185	184/202	145/147	170/170	128/130	146/146	244/250	155/157	195/201	180/182	099/121	1	S7
KamO54	F	127/133	106/120	113/113	110/124	110/116	185/187	184/198	143/147	168/174	110/134	144/148	244/252	155/155	201/203	176/182	117/117	1	S7
KamO55	F	131/133	106/106	113/127	104/122	096/116	177/185	178/194	143/147	174/174	110/128	146/148	234/242	155/157	201/201	182/184	099/117	10	S8
KamO56	F	133/139	112/118	117/123	110/114	096/116	177/183	184/188	145/147	170/170	126/130	148/148	238/242	155/155	201/201	174/182	099/117	1	S8
KamO57	F	125/129	106/120	113/127	110/114	096/096	177/185	194/194	145/147	174/176	122/128	148/150	242/250	155/155	201/203	174/178	099/121	4	S8, S10, S11
KamO58	M	131/133	114/114	115/123	104/124	116/120	177/187	184/188	143/145	176/176	128/134	146/148	232/234	155/155	195/201	174/182	099/119	10	S8, S10
KamO59	M	129/131	106/118	127/129	110/114	114/116	177/177	186/188	147/147	170/174	134/138	144/148	242/250	157/157	201/203	182/182	115/117	1	S9
KamO60	F	131/131	106/112	117/117	114/128	112/118	183/185	184/192	147/147	176/176	130/130	144/144	238/244	155/155	195/201	180/182	099/115	3	S9
KamO61	M	129/133	106/112	113/115	104/112	096/118	185/187	192/194	143/147	168/174	126/128	144/148	238/252	155/157	203/207	182/184	099/115	7	S9
KamO62	F	133/133	112/112	127/127	110/112	114/118	183/185	184/192	143/147	174/174	122/130	146/148	246/252	155/157	201/201	182/182	099/117	2	S9
KamO63	M	129/135	106/106	117/125	112/124	096/114	185/185	184/194	145/147	174/174	110/122	146/148	234/244	155/157	201/201	176/180	099/099	1	S9
KamO64	F	133/135	098/106	115/117	114/114	112/118	177/177	194/198	139/145	174/176	110/130	144/146	238/250	155/157	203/203	174/182	113/123	1	S10
KamO65	F	133/135	118/120	113/117	104/122	096/096	177/185	180/182	145/147	174/178	122/128	146/150	246/250	155/157	201/201	182/184	115/117	1	S10
KamO66	F	129/131	108/122	115/123	110/122	096/110	183/185	174/180	139/143	168/174	110/122	142/144	242/242	155/155	203/203	176/182	117/123	2	S10
KamO67	M	125/133	112/114	115/131	112/112	112/114	177/183	180/202	143/147	168/170	130/130	142/146	242/252	157/157	201/201	182/196	117/117	2	S10
KamO68	M	127/133	112/112	117/125	114/114	116/116	177/177	190/194	143/147	170/174	128/130	146/146	238/248	155/157	201/201	184/194	099/101	2	S10
KamO69	F	131/133	106/112	115/127	114/124	096/114	177/187	180/194	143/145	170/174	128/134	144/148	242/252	155/155	201/201	176/182	115/117	2	S10
KamO70	M	133/133	106/114	123/127	110/124	114/118	185/187	188/194	143/147	168/178	110/134	146/146	238/244	155/157	195/201	176/182	117/117	1	S10
KamO71	M	125/129	112/114	117/129	110/112	116/116	177/181	180/202	147/147	174/174	126/130	146/148	232/242	155/161	201/203	174/182	115/123	1	S10
KamO72	F	127/133	106/106	113/127	112/114	114/114	177/177	186/202	143/147	168/174	130/134	146/148	244/252	155/157	201/201	182/182	115/117	20	S10, S11, S12
KamO73	M	129/129	114/118	113/113	104/114	116/116	177/185	180/194	145/147	176/176	122/130	148/148	232/242	155/155	201/201	174/182	121/121	1	S10
KamO74	M	129/129	106/118	115/129	122/122	116/118	185/185	184/194	147/149	168/174	124/126	144/146	238/238	155/157	201/207	176/182	117/117	2	S11, S12
KamO75	M	131/131	106/120	127/127	120/120	096/118	179/189	174/194	143/147	174/176	122/134	144/148	238/238	155/155	201/201	182/182	117/117	3	S11
KamO76	M	127/133	106/114	117/119	110/112	096/114	183/185	188/194	145/147	174/174	122/134	146/148	232/250	155/157	201/207	174/182	115/115	2	S11
KamO77	M	133/135	098/106	115/117	102/114	116/118	177/177	194/198	145/147	174/174	128/130	146/148	242/252	155/155	201/203	174/182	117/123	1	S11
KamO78	M	131/133	106/118	115/133	114/124	118/118	185/185	174/194	147/147	168/174	128/134	144/150	250/250	157/163	201/203	182/182	099/117	1	S11
KamO79	F	129/133	106/120	115/123	110/124	116/116	183/189	186/198	143/147	170/170	128/130	148/148	238/252	155/155	201/201	182/184	121/123	2	S11
KamO80	M	133/139	112/118	115/117	102/110	096/112	177/183	190/192	143/147	170/170	122/134	148/148	238/250	155/157	201/201	174/182	099/117	2	S11

ID	gender	G1D	G10B	MU05	MU09	MU15	G1A	G10L	MU10	MU23	MU50	MU51	MU59	G10C	G10O	G10X	G10J	samples	stations
KamO81	M	133/133	108/116	117/117	110/114	112/114	177/185	174/188	147/147	174/178	122/130	146/146	248/250	155/159	201/201	178/182	099/121	3	S12
KamO82	M	131/133	118/118	123/129	104/120	112/118	179/185	188/190	145/147	170/174	128/130	146/148	242/246	155/155	195/201	174/184	099/117	1	S12
KamO83	M	129/133	106/114	113/123	102/110	114/116	177/183	186/190	145/147	170/178	128/130	148/150	252/252	157/157	201/201	182/184	117/123	2	S12
KamO84	F	131/133	114/120	115/129	124/124	114/116	177/189	180/184	143/147	168/170	110/128	142/150	242/244	155/157	201/201	178/178	099/117	2	S12
KamO85	F	129/133	106/120	123/125	104/122	116/116	185/187	174/174	145/147	170/176	128/130	148/148	238/250	155/155	201/201	178/184	099/121	2	S12
KamO86	F	131/135	118/118	115/117	114/122	096/096	177/185	184/198	147/147	170/174	122/130	146/148	238/242	155/159	201/201	182/182	111/117	1	S12
KamO87	F	133/137	098/106	113/113	110/112	114/116	183/189	190/194	145/147	174/178	128/128	146/150	238/244	155/157	201/203	182/194	111/117	2	S12
KamO88	M	127/135	106/106	117/129	114/122	096/096	185/185	174/174	147/147	174/174	122/130	144/146	238/250	155/155	201/201	182/182	115/121	6	S12, S16
KamO89	M	131/133	106/118	113/129	104/110	116/118	177/179	174/184	147/149	172/174	130/132	146/146	242/248	155/157	201/201	174/182	115/127	1	S13
KamO90	M	129/129	118/118	117/133	104/122	114/118	181/185	184/194	141/147	176/178	110/132	146/150	238/242	155/165	201/201	182/184	099/121	1	S14
KamO91	F	127/131	106/106	117/117	110/122	112/118	185/185	184/198	147/147	176/178	130/130	144/144	238/250	155/155	195/201	182/182	115/115	3	S14
KamO92	M	131/131	118/118	115/119	102/122	096/112	177/177	194/198	147/147	170/176	110/128	148/148	242/248	155/155	201/201	182/184	117/121	1	S14
KamO93	M	129/131	106/108	113/129	110/114	096/118	187/187	174/184	145/147	176/176	130/130	144/144	244/246	155/157	201/203	182/182	099/115	3	S14
KamO94	F	131/131	108/118	131/131	102/114	112/116	177/189	178/182	147/147	174/176	128/132	146/146	246/248	155/155	201/201	182/184	099/121	3	S14, S15
KamO95	F	131/133	114/118	117/119	110/124	112/116	177/177	180/194	143/147	174/176	122/128	148/148	234/244	155/157	201/201	184/196	121/121	1	S15
KamO96	F	129/131	116/120	127/127	118/122	096/112	185/189	184/194	145/147	174/174	128/134	146/148	242/246	155/159	201/201	174/182	099/117	2	S15
KamO97	M	125/133	114/118	115/117	110/114	116/118	177/185	174/194	147/147	174/176	122/128	146/148	234/250	161/161	195/201	182/184	121/123	1	S15
KamO98	M	129/135	108/108	113/129	110/110	116/118	183/185	174/174	145/147	176/176	128/130	146/148	238/246	155/157	203/203	194/194	099/119	1	S16
KamO99	F	131/133	112/114	117/129	104/120	096/116	177/183	174/184	143/147	170/174	128/130	148/150	252/252	161/161	201/201	182/184	099/117	1	S16
KamO100	M	131/133	112/114	117/129	110/120	096/096	177/183	174/184	143/147	174/174	128/130	142/150	234/252	161/161	201/201	182/182	099/099	1	S16
KamO101	M	127/135	106/106	117/129	114/122	096/096	181/185	184/198	147/147	174/176	122/130	146/146	238/248	155/159	201/201	182/182	117/121	2	S16
KamO102	F	127/133	106/114	113/115	104/110	096/116	177/183	180/202	143/145	168/170	122/130	148/150	244/252	155/155	201/201	182/184	099/121	2	S16
KamO103	M	133/133	112/114	115/133	110/110	096/110	177/183	180/186	143/145	170/172	122/130	148/148	244/252	155/155	201/201	174/182	099/121	1	S16
KamO104	F	127/133	106/112	117/127	110/114	096/096	177/183	184/186	143/147	170/174	130/134	146/148	242/252	155/155	201/201	182/182	099/121	4	S16
KamO105	M	131/139	112/112	115/117	104/110	112/112	179/183	190/192	143/145	170/170	110/134	148/148	238/250	155/157	201/203	174/182	117/117	1	S16
KamO106	M	000/000	000/000	115/115	104/114	000/000	000/000	174/198	145/147	170/174	110/126	142/146	242/252	000/000	000/000	000/000	000/000	1	S2
KamO107	F	129/133	106/108	117/131	114/122	110/114	177/187	180/202	143/143	174/178	110/110	146/148	238/244	155/161	201/201	184/184	117/117	1	S5
KamO108	M	000/000	106/118	115/133	114/122	000/000	177/183	182/194	145/147	174/174	110/122	146/148	242/252	000/000	201/201	000/000	117/119	1	S10
KamO109	F	131/133	106/112	123/123	110/114	096/116	183/185	174/186	147/147	170/170	128/130	148/150	244/250	157/157	201/201	182/184	099/123	1	S12
KamO110	F	127/131	098/112	115/117	114/124	112/120	177/183	178/192	141/147	174/176	130/130	144/144	244/252	155/155	201/201	180/182	099/121	2	S15
KamO111	M	127/133	098/118	115/115	112/114	000/000	183/185	192/198	145/147	170/176	130/130	000/000	244/250	155/155	201/203	184/184	099/115	1	S1
KamO112	M	000/000	112/114	117/127	112/114	000/000	000/000	174/188	143/145	174/176	110/130	146/146	238/242	155/155	000/000	000/000	115/121	1	S16
KamO113	F	131/131	106/108	123/131	114/114	112/116	177/187	174/194	143/147	170/174	132/134	146/146	242/246	155/157	201/201	182/182	099/117	1	S12
KamO114	M	133/133	098/118	115/129	102/110	096/096	177/177	184/192	147/147	170/170	130/130	146/148	000/000	155/157	201/201	182/182	099/121	1	S6
KamO115	M	000/000	112/114	113/123	120/122	000/000	000/000	192/194	145/147	174/174	128/134	142/148	244/250	000/000	000/000	000/000	000/000	1	S8