Development of a new SNP panel for bowhead whales (*Balaena mysticetus*)

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ABSTRACT

Population genetic research is a critical tool for the conservation and management of marine mammals and other species. The bowhead whale (Balaena mysticetus) is subject to aboriginal subsistence hunting in Alaska, Canada, Chukotka (Russian Federation) and Greenland, where, except for Canada, it is managed by the International Whaling Commission (IWC). Molecular genetic studies support conservation management plans and aid determination of sustainable hunting quotas by providing information on levels of genetic diversity, estimates of census size, effective population size and structure. Because bowhead populations are monitored, including genetic monitoring, in several countries, there is a need for genetic methods that can be consistently used in multiple labs and provide comparable data that can be publicly shared and built upon by successive studies. Here we present a new panel of single nucleotide polymorphisms (SNPs), derived from multiple bowhead populations, which meets these criteria. We describe the use of the Fluidigm SNPtype assay for analysing 69 autosomal, six X-chromosome and one Y-chromosome SNPs. Results indicate the methods herein have high efficacy and low error rates. Furthermore, because SNPs are discrete sequence-based genetic markers, the panel of loci described here can be reliably replicated and is directly comparable across different labs, making this SNP panel more useful than existing microsatellite markers.

KEYWORDS: BOWHEAD WHALE; FLUIDIGM; GENETICS; GENOTYPING; SINGLE NUCLEOTIDE POLYMORPHISMS

INTRODUCTION

Bowhead whales (*Balaena mysticetus*) are divided into four management stocks or populations: 1) the Bering-Chukchi-Beaufort Seas (BCB) stock; 2) the East Canada–West Greenland stock (ECWG); 3) the Okhotsk Sea (OKH) stock; and 4) the East Greenland–Svalbard–Barents Sea stock (EGSB) (George *et al.*, 2021). These stocks are recognised based on migration patterns, geographic distribution, satellite tagging and population genetic analyses (Baird *et al.*, 2021).

The BCB stock, which is the largest of the four stocks, migrates between the Bering and Beaufort Seas during spring and autumn. Along their migratory path the whales pass several villages in Alaska (USA) and Chukotka (Russian Federation) where indigenous people have hunted them for thousands of years (Stoker *et al.*, 1993). During the commercial whaling period from the sixteenth to early-twentieth centuries, all bowhead stocks suffered severe population declines and possible genetic bottlenecks. The BCB population was extensively hunted

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from c.1850 to the early 1900s and it is estimated that only 1,000 whales remained once commercial whaling ceased (George *et al.*, 2021). This stock has made a remarkable comeback and was estimated to number 16,820 whales in 2011 (Givens *et al.*, 2016; 2021).

The ECWG stock is currently the second largest, with an abundance estimate accepted of 6,446 individuals (Doniol-Valcroze *et al.*, 2015). This stock is thought to have some degree of gene flow with the BCB stock, and the degree of population differentiation between them is low but significant (Givens *et al.*, 2010; Alter *et al.*, 2012; Morin *et al.*, 2012).

The OKH and EGSB stocks are much smaller than the other stocks. The most recent estimate of the size of the OKH stock is about 218 (Cooke *et al.*, 2017) and 318 for EGSB (Boertmann *et al.*, 2015; Hansen *et al.*, 2018).

Today both the BCB and ECWG (in Greenland) stocks are subject to aboriginal harvests regulated by the IWC which sets quotas for each managed hunt. The hunts of ECWG bowheads in Canada are managed collaboratively between the Canadian government and regional Wildlife Management Boards, while harvests in Canada are considered by the IWC when setting quotas for Greenland (Suydam *et al.*, 2021). When establishing aboriginal quotas, the IWC considers multiple lines of evidence, including data from mathematical modeling of the population, overall health of the population, connectedness of populations, estimates of population size and trend, in addition to the number of requested takes. Information from population genetics is used to understand genetic diversity, gene flow, relatedness, levels of interbreeding, historical demography and population structure. The IWC aims to provide quotas which lead to sustainable harvests while also providing for the nutritional and cultural needs of indigenous hunters and communities. A clear understanding of stock structure, including population size and connectedness, is therefore important for accurately estimating the impacts of human-induced mortality.

Single Nucleotide Polymorphisms (SNPs) have successfully been used in evolutionary and population genetic studies across a wide variety of organisms, including non-model organisms (Helyar *et al.*, 2011) and samples collected noninvasively (Kraus *et al.*, 2015). In baleen whales, SNPs have been identified for several cetacean species, including bowheads (Morin *et al.*, 2010; Baird *et al.*, 2015; 2016); sperm whales (*Physeter macrocephalus*) (Morin *et al.*, 2007); humpback whales (*Megaptera novaeangliae*) (Schmitt, 2013); gray whales (*Eschrichtius robustus*) (DeWoody *et al.*, 2017); minke whales (*Balaenoptera acutorostrata*) (Malde *et al.*, 2017); and killer whales (*Orcinus orca*) (Moura *et al.*, 2014).

SNPs are directly analysed by sequence, an advantage over methods used to analyse microsatellites. Microsatellites have typically been analysed based on estimation of fragment size, which only infers the number of repeats at the locus, but next generation sequencing methods do offer the ability to directly analyse them by sequence. Compared to microsatellites, SNP genotyping can be more easily reproduced and directly compared among different labs with consistent results, which means a public database can be established and built upon by successive studies. Bowhead genetic studies are conducted in Russia, USA, Canada, Norway, among others. Having an inexpensive and repeatable method where comparable data can be shared across these labs would be invaluable.

Morin *et al.* (2012) evaluated the relative statistical power of SNPs and microsatellites. They concluded that a panel of 29 phased and unlinked bowhead SNP loci (derived from a total of 42 SNPs, some of which were closely linked) provide similar power to a panel of 22 microsatellites for detecting low levels of differentiation ($F_{sT} = 0.005-0.030$) among bowhead populations when sample sizes were at least n = 20 per population. The microsatellite panel performed better when used for estimates of effective population size (N_e) and assignment tests.

Previous studies of bowhead population genetics have used: mitochondrial DNA (mtDNA) control region alone (Pastene *et al.*, 2004; LeDuc *et al.*, 2008); multiple mtDNA loci (Phillips *et al.*, 2012); a panel of 42 linked and unlinked SNPs (a total of 29 unlinked and phased loci) (Morin *et al.*, 2012); a panel of 22 focal microsatellite loci (e.g., the microsatellite primers derived from bowheads) (Givens *et al.*, 2010); and a panel of 10 microsatellite loci comprised of focal and non-focal loci (Jorde *et al.*, 2007; Givens *et al.*, 2004). Some of the earliest studies purported to find evidence for potential sub-structuring of the BCB stock. For example, Jorde *et al.* (2007), using 10 microsatellite loci from 117 whales, found temporal pulses of whales that were significantly less related than

whales during other time intervals. The authors suggested there could be multiple stocks with different migration times passing Utqiaġvik (formerly Barrow), Alaska, rather than a single large BCB stock. Another study using the same 10 microsatellites suggested that animals harvested from Utqiaġvik and St. Lawrence Island (SLI; in the Bering Sea) were genetically distinct (Givens *et al.*, 2004). Finally, analysis of mtDNA control region haplotype frequencies has indicated significant differences between whales harvested at Utqiaġvik in the spring and autumn (Pastene *et al.*, 2004). But when a new panel of 22 microsatellites was developed using bowheads as the focal species from which the loci were generated, along with a larger sample size of whales compared to previous studies, the above patterns disappeared (Givens *et al.*, 2010). Likewise, the difference between spring and autumn migrants disappeared with a larger mtDNA sample size (LeDuc *et al.*, 2008). In the time since the above studies were conducted, substantially more bowhead tissue samples have been collected. This has resulted in the availability of larger sample sizes, especially for the BCB population (Baird *et al.*, 2017), which is hunted in Alaska and Chukotka, and the ECWG population hunted in Canada and Greenland (Frasier *et al.*, 2020).

With the rapid advance of molecular technologies, new and better approaches are available to test stockstructure hypotheses. We therefore aimed to: 1) generate a larger SNP panel compared to previous studies, designed from bowheads; and 2) develop an approach capable of producing high-throughput data usable and repeatable by multiple laboratories to test hypotheses about population structure applicable for management and conservation.

MATERIALS AND METHODS

Sampling

Tissue samples (skin and/or spleen) from BCB whales were obtained during aboriginal hunts, biopsies from tagged whales, and whales found dead. ECWG and OKH samples were obtained from biopsies. Bowhead whale DNA was extracted using a Qiagen DNeasy blood and tissue kit (Qiagen Inc.).

We used five plates of samples. Each plate contained 95 samples and one negative control, for a total of 475 samples analysed. 6μ L of DNA at concentrations up to 50ng/ μ L was added to each reaction (there is a preamplification process for the Fluidigm method, so relatively low DNA concentrations are generally acceptable). Our samples ranged in quality due to the sample age and method of collection. Samples from newer harvested bowheads were generally higher quality than older or biopsied samples. Our sampling included 22 pairs of duplicated samples in addition to 16 pairs of mothers and fetuses. Samples from BCB, ECWG and OKH were included in this study. We genotyped all 475 whales to examine them for quality and consistency. Here we focused on the replicated samples and mother/fetus pairs in drawing conclusions about the repeatability and reliability of our panel of SNPs. Population level analyses of the total dataset will be reported elsewhere.

Single nucleotide polymorphism (SNP) selection

The SNPs for our panel were derived from the following sources: 1) previously published bowhead SNPs (Morin *et al.*, 2010; Bickham *et al.*, 2013); 2) bowhead genome; and 3) transcriptome data (Keane *et al.*, 2015; see also *http://www.bowhead-whale.org/*).

Previously identified autosomal SNPs from Morin *et al.* (2010) used in our panel were derived from BCB and ECWG whales. These data were acquired from GenBank (for loci described in Morin *et al.*, 2010), and our own data on X- and Y-linked loci (Bickham *et al.*, 2013). Several of the available autosomal SNPs were described from single amplicons (Morin *et al.*, 2010), which placed some SNPs close to one another (sometimes less than 30 base pairs (bp) apart). To avoid linkage between autosomal SNPs, we selected only one SNP per amplicon. Our evaluation of each locus included noting position of the SNP in the bowhead genome (Keane *et al.*, 2015); proximity of a given SNP to any other known SNPs; proximity of the SNP to repeated genetic elements that might make amplification difficult; and whether the SNP was a base substitution or an insertion/deletion. A list of all SNPs can be found in Appendix 1.

New SNPs derived from the bowhead transcriptome data (Keane *et al.*, 2015) were selected from a larger pool of candidate SNPs described in Baird *et al.* (2014), where SAMTools and mPileUp (Li *et al.*, 2009) were used

to identify likely variable nucleotides, verified by examining the sequence reads. In our final panel we selected only one SNP per contig.

New SNP loci derived from the bowhead genome data were obtained by scanning individual genome scaffolds for heterozygous positions. This process ensured minimal linkage disequilibrium between SNP loci because no more than one SNP per scaffold was selected.

Our SNPs were labelled according to the method used to identify them. The first group was labelled according to the genome scaffold from which they are derived; for example, locus Bmy_03499_297 is from scaffold 03499 and the SNP is at position 297 within the scaffold. The second group of new SNPs was labelled 'Bmys' followed by a number, and these loci were derived from the transcriptome. The scaffolds from which the second group of SNPs were derived can be found in Appendix 3. All other SNPs were described by Morin *et al.* (2010) and Bickham *et al.* (2013); we have maintained the same labelling schemes used in these studies.

Our methods of selecting SNPs from different sources ensured that the final SNP panel contained loci with variable positions derived from the BCB stock and ECWG stock. Morin *et al.* (2010) amplified SNP loci from up to 17 bowheads (from eastern Russia, St. Lawrence Island, northern Alaska, and eastern Canada; BCB and ECWG stocks). Loci described in Bickham *et al.* (2013) were derived from 26 Alaska bowheads (Barrow and Kaktovik; BCB stock). The bowhead genome sequence was derived from a female bowhead from west Greenland (ECWG stock). The bowhead transcriptome data were derived from two bowheads from Alaska (BCB stock) (Keane *et al.*, 2015). The selection scheme for our SNP panel was designed to minimise ascertainment bias across the panel (McTavish *et al.*, 2015).

We selected SNPs that could be used in future studies to estimate current and historical effective population size and examine whether there is genetic interchange between defined stocks. Therefore, the SNPs we used derive from chromosomes with different inheritance patterns, and thus different mutation rates, including autosomes, the X-chromosome and Y-chromosome. These markers can be used in combination with mtDNA sequence data to achieve a more complete picture of evolutionary history than by using any single maker type alone. The X- and Y-chromosome markers also aid in sex identification, which is frequently unknown in studies using biopsy methods.

Primer design for Fluidigm SNP genotyping

We tested multiple platforms for genotyping, including TaqMan on a real time thermal cycler (see Morin *et al.*, 2010), High Resolution Melting Analysis, and Fluidigm SNPtype genotyping. Baird *et al.* (2015) describe the pros and cons of each method, including cost. Ultimately, we selected the Fluidigm SNPtype method as recommended by DeWoody *et al.* (2017). Fluidigm's D3 Assay Design primer software requires a minimum 60bp flanking sequence both upstream and downstream of the desired SNP. Indels could not be greater than 10bp. A desired SNP could not be within 30bp of another variable locus. The flanking sequence was submitted to Fluidigm for quality control and primers were designed using the Fluidigm software. Fluidigm SNPtype assay primers were designed for 96 SNPs as described above (final loci listed in Appendix 1).

The Fluidigm SNPtype assays contain three types of primers: Allele-Specific Primers (ASP), a Specific Target Amplification (STA) primer, and a Locus-Specific Primer (LSP). The STA and LSP primers were used in a preamplification to increase quantity of the target locus for the subsequent allele specific amplification step. Next, the LSP and labelled ASP primers were used in a round of amplification. The fluorescently labelled alleles were then read using Fluidigm's integrated fluidic circuits (IFCs) read on their Biomark platform (Fluidigm has recently changed to Standard BioTools Inc.).

Quality control of Fluidigm SNP genotyping

Fluidigm's BioMark SNP Genotyping Analysis software was used initially to automatically call genotypes (Fig. 1). Editing was then conducted by eye. We discarded any locus with no clear distinction among genotypes.

We conducted several tests of quality control of the SNP data. First, we submitted duplicate samples on different plates to ensure identical calls were obtained for each duplicate. We also submitted mother/fetus pairs to determine whether each fetus had at least one allele to match the mother's alleles at each locus. Finally, we



Fig. 1: Examples of genotype calling for a clean locus (A) and a locus that was disregarded based on a lack of distinction among genotypes (B). Each dot represents fluorescence values for one individual. Green and red dots represent the two different homozygous genotypes. Blue dots represent heterozygous genotypes. Black dots represent no template controls and gray dots are samples with no calls (their signal is too weak or ambiguous).

included samples used by Morin *et al.* (2010) to determine whether they matched calls for loci used from their studies. Samples duplicated in both this study and Morin *et al.* (2010) included whales with the identifiers of 02G2, 02S2, 02S5, 01S3, 96B18, 05B29, 96B8 and 96B16.

We calculated the error rate (e) of genotype calls using 22 pairs of duplicate samples. This was done using the formula e = m/[d(s)]. In this case, m is the total number of mismatches between each replicate sample (including errors of differing genotypes called and errors based on no amplification in one sample); d is the total number of loci per replicate sample (in this case, the 69 autosomal loci that passed quality control; see below); and s represents the total number of replicated samples (Doyle et al., 2016).

RESULTS

SNP loci

The panel of SNP loci included 89 autosomal loci, one Y-chromosome locus and six X-chromosome loci (96 total loci). Of these 96 loci, 69 autosomal loci, one Y-chromosome locus and six X-chromosome loci were high-enough quality to be part of our final dataset. The high-quality loci are detailed in Appendix 1. Results below include data from only the autosomal loci. Sex chromosome SNP data will be used in future studies.

Any samples yielding less than 95% of calls across all loci were discarded from the database. 18 of the 475 samples were discarded. The final set of samples contained 27 from ECWG, 28 from OKH and the rest from BCB.

Genotypes for the 22 duplicate pairs of samples can be found in Appendix 2. Of these pairs, 20 had identical genotypes (excluding cases where a SNP locus did not amplify for one replicate). We observed six cases where there was one no-call out of the 69 autosomal loci evaluated for replicate samples. One duplicate pair contained three loci with no-calls. There were also two duplicate pairs that had at least three loci where genotypes differed.

Of the loci that passed our quality standards (produced clear distinction among genotypes), the SNPs originally used by Morin *et al.* (2010) showed complete genotype matches with our Fluidigm SNPtype assays for the same samples.

Sixteen mother/fetus pairs were genotyped (Appendix 2). Fourteen of these fetuses contained at least one allele from the mother at every locus. The remaining two fetuses had one mismatched locus (i.e., they did not contain at least one allele present in the mother at one locus). Potential causes for these mismatches could include sample degradation or imprecise priming. These causes will be explored in future studies.

Apart from the purposefully duplicated samples described above, there were five other instances of two samples having identical genotypes. Those pairs with identical genotypes were: 05H3/05H5, 05H4/05H7, 09KK1/

09KK1F, 08B7/10B4 and 99B6/99B6F. All of these pairs have identical mtDNA haplotypes except 08B7/10B4. The remaining pairs were collected in the same season. Two represent mother/fetus pairs; it is possible they represent a mix-up of samples, cross-contamination or samples from a single whale having been given more than one sample number. The 08B7/10B4 pair could have been a mix-up of samples, but not during harvest/sampling (they were collected in 2008 and 2010 respectively). They may have been mixed up during DNA extraction or genotyping. These samples will be run again in the future to test this hypothesis. Except for these sample pairs, all other samples of the total 475 have unique genetic fingerprints across the autosomal loci.

Lastly, we verified our 475 samples, including BCB, ECWG and OKH whales, to ensure all autosomal loci described here are variable in multiple individuals, which confirms these loci are polymorphic within populations.

Error rates

For the error rate calculation, there were 22 pairs of replicates (44 samples in total). We observed a total of 22 differences between replicates, including errors of commission and omission (no call). Therefore, we calculated a genotyping error rate (*e*) of 0.007 or 0.7%.

Data accessibility

All primer sequences and data analysed for replicate samples are contained in the appendices.

DISCUSSION

We have shown Fluidigm SNPtype analysis to be a reliable and repeatable method for genotyping bowheads. Loci used in this study derive from previously published genome and transcriptome data (Baird *et al.*, 2015, Keane *et al.*, 2015), which means they are not anonymous.

Due to the protected status of marine mammals, most sampling of bowheads uses minimally invasive techniques, yielding relatively small samples and little DNA (except samples collected from the BCB harvest). Moreover, the tissues and DNA itself cannot easily be shared among labs in different countries due to the Convention on the International Trade of Endangered Species (CITES), creating the need for repeatable methods that can be used in different labs throughout the world. The methods used here have been shown to be reliable when used on low-yield samples (Kraus et al., 2015). We have demonstrated here that our SNP data can easily be replicated using the same or different genotyping methods. For example, we replicated genotypes at 19 SNP loci for eight whales reported in Morin et al. (2010). Their analysis was done in a different lab with a different method of analysis. Primers for our study were developed independently and may be different from those used by Morin *et al.* (2010). Moreover, the error rate (e = 0.007 or 0.7%) reported here is low, again emphasising the reproducibility of this method. For comparison, a study (Carroll et al., 2018) which focused on evaluating genomic techniques using minimally invasive sampling found the average reported error rate for Fluidigm SNP genotyping in real world studies is about 1%, compared to 1.4% for Ampliflour, 9–25% for MassARRAY and 0.01% for GT-seq platforms. Microsatellite sequencing is subject to various errors, such as allelic dropout and false alleles. Depending on the quality of the DNA sample, the allelic dropout error rate ranges from 3.9% to 13.7% (Carroll et al., 2018).

The newly derived SNP loci we describe here increase the number of high-quality SNPs available for population genetic analysis of bowhead whales. The panel consists of 76 loci (69 autosomal and seven sex-chromosomal loci), and here we have demonstrated the autosomal loci to be reliable markers. This number of SNPs should be sufficient to detect differentiation among bowhead stocks (Morin *et al.*, 2012), especially when combined with the large sample sizes that will comprise the SNP database when we eventually genotype all bowhead samples harvested or biopsied to date.

The combination of SNP data with the existing mtDNA database will increase the power to examine the intricacies of population genetics of bowhead whales. Our ability to fingerprint individual whales will lead to future studies that can address issues such as the timing of migration of family groups and support identification of previously tagged whales.

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SNP_NAME ALI	LELE ASP1_SEQ	ASP2_SEQ	LSP_SEQ	STA_SEQ	Accession number of genome/ transcriptome resource	Contig number	Position in contig/sequence
*BH402 (#) /	AC AGAACTATAATTTTTAGCTTGGGAAATTTGAGT 3G ATGGCTCATTGGAGGAAATGAAAC	GAACTATAATTTTTTAGCTTGGGAAATTTGAGG CATGGCTCATTGGAGGAAATGAAAG	CAGCCAGCATGGCCCC TCCTGCCATCATTACATCAA	AGAATCACATTATATGAGAAAAAGTTGAGAGAA GCAGAGACTTGGAATAAATTCAGCAT	GU066523 PRJNA194091 or	N/A scaffold_183	56 202179
*115 DOOT 1 2007 1					www.bownead-wnale.org	007 FL-33	54754
* 1/6621X9420 * X non 3120C	GI UIGIAGIGALITACCACACUCAAAAU	CGTGTCTAGGAAGTCATTCGTTTTG	GGICCIGCACCIAIGIGAGIGI TGTGTTTCCTGGTCCTTAACAAAAGTATGTAATATA	CAGCAAGTICTTAATACATTTAGCACTTC CACAGCCTTTTTAAAATGTCAGGTTTT	As above As above	scarrold_183 scaffold_183	2146/b 213388
*X polv 3120d (C TTTAAAATGTCAGGTTTTTCGTGTGTCTAGG	AAAATGTCAGGTTTTTCGTGTCTAGC	ACAAAAGTATGTAATATACTTAAATTTTAAAAACGAATGACT	TCACAGCCTTTTAAAATGTCAGGT	As above	scaffold 183	213403
*X_poly_3892 (2A TGTCTTGGTTCATTTTTCTCTCAATGATC	TITTGTCTTGGTTCATTTTTCTCTCAATGATA	ACAGCTAAAAGAGACAAGGTTGGGA	TCATATTTGGGGTTTTTGTCTTGGT	As above	scaffold_183	214140
*Y_poly_1660 /	4G GTAACCAAAAACTGTTTGTTTGTCACATT	GTAACCAAAAACTGTTTGTTTGTCACATC	TGCCCAGAGCTTTTATTTCTTATACCAAGA	TGTAACCAAAAACTGTTTGTTTGTCAC	As above	scaffold_183	105432
BH108 (#) [(C/-] GCCCAGCTGAGCACAGG	CCCA GCT GAG CACAGC	CATGTGACTCAGCAGGTTGGG	AGCTCAAGTGTCCCCATCTT	GU066517	N/A	91
BH34 (#)	AC CCGGGCATTTGTGCAACT	CCGGGCATTTGTGCAACG	ACAGTCACTGAACATCCTGCCT	AACCGGGCATTTGTGCAA	GU066508	N/A	251
BH382 (#)	CT ACATTGTGATTTGCTGTTCTTTCCC	AACATTGTGATTTGCTGTTCTTTCCT	TGCATGCATCTTGGAGGAGTGA	GTGAGTTCCCTGGTGATTATTATTAGAA	GU066519	N/A	279
BH387-2 (#)	TA ACAAAGGAGATGTCAGGGGTT	CACAAAGGAGATGTCAGGGGTA	CCAGCAGTCAAG GAACTGGC	CATAGCTTAGGCACAAAGGAGAT	GU066520	N/A	50
BH395 (#)	TC CAGTACCTCCACAGACCTCAT	CAGTACCTCCACAGACCTCAC	CCTCACGCCTCTCGGG	CCCATTTCGGTGTCCCC	GU066521	N/A	158
BH404 (#)			AGIAAGCAAIGGCIAIAIGAAICAGGAACA	IGACGIGAGCCCIGGAAG	GU066524	N/A	316
BH410D (#)	GI AICIIGGICCICAAAIGCIICAAIIG	GIAIAICI IGGI CCI CAAAI GCI I CAATTI GCTTTGTGGAAAACATACCAAAI GCI I CAATTI	CCGGGAACIACIGGIIAGICCA	CCAGIAGIICAACAIAGIGCIIIGIA GCTTTGTGGAAACATACCAAACAA		N/A	200
BH42a (#) (() (GCAAGCTACACTCAGTCCTCAAA	CUCCUMULTIAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	GGTTTGCGGAGAGCACA	GUD66509	A/N	330 46
BH42b (#) ((3T GGAACATGGTAGGCAGAACAC	GGAACATGGTAGGCAGAGAACAA	CTCCCAGCTCTCCCTGGT	TGGGAACATGGTAGGCAGA	GU066510	N/A	180
BH43 (#) (T TGCTACTGCTCACCAAAGACG	TGCTACTGCTCACCAAAGACA	TGCTTGTGGAGCTTCTGTGGTA	AGATGCACAGACAGAAGAATT	GU066511	N/A	377
BH60 (#)	TC GACAAGTAAAGTGACATGGCCTT	GACAAGTAAAGTGACATGGCCTC	CAGGCATTTCATACCTGCTGGG	AGTTTCAGGCACCGGGA	GU066513	N/A	148
BH92 (#)	rg ctgggttttttgcacttcagagt	CTGGGTTTTTTGCACTTCAGAGG	TCCTCTAAAATGAATATCCTGAGGTTAAAAAACAAAGA	GATGGTGTTTGAGTCTCTATGTTCC	GU066515	N/A	354
Bmy_03499_297	CG GAACCTCAACCACCATGGC	TGAACCTCAACCACCATGGG	AGCAATAGCCTTGGTACCCCTG	CGGAACATGGAGGAGTCCTAT	PRJNA194091 or	03499	297
					www.bownead-wnale.org		000
Bmy_03506_639 /		ACICCA ICCI II ICCI I ICCI ICCC	GCAGCAGAAAAIAGGACIIICCIAAGC	CCIGITITIGITCALCIGCITCAC	As above	03506	639
4 610_0000_0191	NG GGAAGGAILUULAGIILULAGA	GGAAGGAILUUUUAGIILUUAGG	GGGTCAGIGAIGAIGICCICA	AAAGG I GGACCCAG AAGGA CCA ATTTA GCTTTCTAG TTCTTGG	As above		619
, 100-1110-100	T CONTROCTORYCOCON				Avode 20	(1110	100
Bmv 04604 4249	ve gaagatetttaaggatggctcaatgaata	AGGATGTTTAAGGATGGCTCAATGAATG	TGAGATCATCAAGGGTGCATGTCT	ATCTTATGGCCTCTTCAGGGA	As above	04604	4249
Bmv 05005 2391 (CT GCCACCTGTGCGGGTGTAC	GCCACCTGTGCGGGTGTAT	GCTGTAGCGCTGCCCG	CACAGACCAGCCCTGC	As above	05005	2391
Bmy 05337 152 (CG GCCAGGGAGCTGGAGAC	GCCAGGGAGCTGGAGAG	CCGGCTGGGCTCAGGA	TGAAGGAACTATTTGCCATGGAAG	As above	05337	152
Bmy 05573 143 (CAGAAGAGCATCCTTTTGTGGATAC	ACAGAAGAGCATCCTTTTGTGGATAT	ACAACATAAAGTTTTTGTCAGCTTCATCTTCA	TGACTTCTCAAGAGAAGACAGAAGA	As above	05573	143
Bmy_06147_437 +	VG GATTGGGAAAAATAAAAGACCCATCCA	ATTGGGAAAAATAAAGACCCATCCG	CATGGGCTTCTGTGGGCAA	CATGATTGGGAAAAATAAAAGACCCAT	As above	06147	437
Bmy_06377_150 (CONTRATGACGCTCACGGC	TCATGATGACGCTCACGGG	GGCCACTTCTCGTCCCAGA	CCCTGAGTGAGGCCCTT	As above	06377	150
Bmy_06654_1107 /	4G CCTTCCCCTTGAACAGGTGT	CTTCCCCTTGAACAGGTGC	CCAGTTCCTCTTCCCGGCC	ACCTTCTGACCCCAGATGTC	As above	06654	1107
Bmy_11209_216 (CT CTTGGCGTGCTCTGTGTAG	GCTTGGCGTGCTCTGTGTAA	AATCCGGGACGCCGTCA	CGTCCATGGCAGTCACAG	As above	11209	216
Bmy_12670_341 /	AG CGCTGTGGAATTTCTTCCTGGA	GCTGTGGAATTTCTTCCTGGG	CGGAGAGACCTAAGCTCGCA	CCGTAATGCTGCACACGC	As above	12670	341
Bmy_13463_324 (CT CCTGGGCCTGTCCCAC	CCTGGGCCTGTCCCAT	GTCCTCGTTGATGTTGCAGCT	CCAGCCTGCGAGACC	As above	13463	324
Bmy_14843_1856 (CT CCAGAGTACTTTCACATGATTATGGACC	CCAGAGTACTTTCACATGATTATGGACT	CCTGGTCGCTGGCAATTACA	AACAGTGGTGAAATAAGAACAACCA	As above	14843	1856
Bmy_15448_333 (CT GGAATTGCGCTCTCGGC	AGGAATTGCGCTCTCGGT	GGCCGGACCCTGCAGTA	GAGGCGACCGACGA	As above	15448	333
Bmy_15464_273	CT 6GAIAAIGIIGAACAIGCIIIIGCC	IGGAIAAIGIIGAACAIGCIIIIGCI	GCATTITITICCACTITICCTTAGTIGCTG	TGGITAATGGCGTTTCAATGGA	As above	15464	273
Bmy_16528_309		CCAGAGGAIGCAGACAIAGAIIIAAAAGAI GAGCTCTCAAAATCACAACCAATGTAC	AAAGAG HILUCAA IGITITITAGI UTUUGIT	IGALAGI LAI ULAGALI I ULU CATTEANEAN SATEATEGEA		62201 10221	309
Bmv 18001 1857 4	VG CACCAGTACCCACTTGCCA	ACCAGTACCCACTTGCCG	TCTTGCGGCTGACTACCCG	GGAGGCTGCTCAAGCAC	As above	18001	1857
Bmy_18300_4038 (CTGTCGTCCTCCACCTCG	ACTGTCGTCCACCTCA	GGAGCTTGGCGTGCACT	GGCCCTTGGCAGGACT	As above	18300	4038
Bmy_18709_446 (CT GGGGAAGAGCCAGGTACC	AGGGGAAGAGCCAGGTACT	GCATGGTCCCCATGTAAGTGC	CGCCTCAAGGAAATCATGGA	As above	18907	446
Bmy_18857_278 (3T TGTGACATAATGGCTGCCTACTG	TGTGACATAATGGCTGCCTACTT	TGTTCTCTTCGCTGATCTTGCCA	GTCTTCGAATTCCTCTGTAAGATGT	As above	18857	278
Bmy_18902_72 +	AG ATAAACTTCCCCACTGATACACCTT	AAACTTCCCCACTGATACACCTC	GCCTAAGTTGGACCCCAACG	CCAGGGCAGATGTGGCA	As above	18902	72
Bmy_30270_1768	AG GTTTACCCCAGGCTCTCAGA	GTTTACCCCAGGCTCTCAGG	GCTCCTCAGGGGCCTTGT	CCACTCCTCTTCCCTTGG	As above	30270	1768
Bmys13	AG AGGGTTTGGACTTGACACCAA	AGGGTTTGGACTTGACACCAG	CAGTGCAGGTGTAATTTACACAACAGT	GTCACCTTCTACAGCACATTCA	As above	comp408701_c0_seq1	1023
Bmys14b [/	4/-] [6]6664116A46C11CCA6611	IG IGGAT IGAAGCI I CCAGGI C	GGAACIA ICCI GA IGAACAI GAI I ACI AAAGCA	GICICCATICATGATGITCCCAT		comp409439_c0_seq1	1064
Bmys1/ Bmys10	AG IGICCCIAIIAICIGIGIIIIICAACIIGI	GICCCIALIAICIGIGIIIICAACIIGC	GUUU LUUULI GAALAAAA LUGGA ATTEGA AGGGGGA GT	GETTCACCAGGGAACEAAATTTGGG GGTTCACTCAAGGGAACEAAATTTGGG	As above As above	comp464619_c3_seq1.	2549 5001
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Bmys2 Bmys21	GI GGGAAGGIIICCIGGC	GGGAAGGIIICCICCIGGA CTTECTEATECCACAAAGGG	ΕΙ ΙΘΕΕΕΕΕΑΑΕΙ ΙΘΘΕΕΕΕ ΤΓΛΟΛ 6ΤΑΤΤΤΤΘΑΤΑΛΘΕΓΕΑ 6Α6 ΑΤΕΑΛΟΤ	GU I AAG I AGACAGI UI CUAI GGG TGC ATTTTGTTGTTTTTGC	As above As above	comp1/b62_co_ceg2	1944 576
Bmvs22 k	16 AGAGCTCATCTCTGACAAGTTTTCTT	AGAGCTCATCTCTGACAAGTTTTCTC	AGGCTGCTCCTCAGTTAAGATTAAAA	GGTTTTTGCTGGCTACCAGA	As above	comp668158 c0 seq1	1145
Bmys23 (CG GGGAGTAACTGTCCCACTTGG	GGGAGTAACTGTCCCACTTGC	CAGCCTCCCCGGGAAGT	TTCTTTCCTGGGTTCTAGGAGG	As above	comp1099809_c0_seq;	561
Bmys24 (CG CTTCTGTCCCTCTGCTGTGTATC	TTCTGTCCCTCTGCTGTGTATG	GCCTCAAGGCCTCCGC	GCTGGGCATGTGAACCAA	As above	comp169488_c0_seq1	392

Appendix 1

List of SNP loci used in this study. Primer sequences are also given, read in a 5' to 3' direction. ASP1 and ASP2 = Allele-Specific Primers; LSP = Locus-Specific Primers; STA = Specific Target Amplification Primers. Loci with an asterisk (*) indicate sex chromosome loci that were not used in the autosomal analyses presented here. Loci with a hash sign (#) indicate markers that were first reported in Morin *et al.* (2010).

SNP_NAME	ALLELE ASP1_SEQ	ASP2_SEQ	LSP_SEQ	sta_seq	Accession number of genome/ transcriptome resource	Contig number	Position in contig/sequence
Bmys25	CT CCTTCACGTCTTCATCTACCCAG	CCTTCACGTCTTCATCTACCCAA	CAGAACAAGATGCAGCCCCA	TTGCACTGAGGTTTCCCCAATC	As above	comp179499_c0_seq1	138
Bmys28	GT ACCAGTGTCTCTAGAAAAGCAAAGG	ACCAGTGTCTCTAGAAAAGCAAAGT	CCCGGGGCTGGTCTTCA	AGAGAACATCTCACTGAGTTTTCACA	As above	comp313642_c0_seq1	246
Bmys29	CG CAGGGGAAACACAACTITTTTCTAAAAC	CAGGGGAAACACAACTTTTTTCTAAAAG	GTGCTCAGCTGGGACTGCTA	CTTG GTA AAGA CTTT CG CAGGG	As above	comp315937_c0_seq1	1087
Bmys3	CT GAGCTTAAGTTATTTCCCCCAAGGC	AGAGCTTAAGTTATTTCCCCCAAGGT	CCTGGGCTCAAGTCCTGTCT	AGAAGGGTAATGAAAAACCAAGAGC	As above	comp327063_c0_seq1	1630
Bmys31	AT GGTAAGACATTATGCAAAGAAATCTCTTACA	GGTAAGACATTATGCAAAGAAATCTCTTACT	TCAGCACTCTCCCATCATTGTGT	TGCCCACTCTTGGTAAGACA	As above	comp316968_c0_seq1	1142
Bmys33	GT AAGAAGTTAACTCTTAAGGGTCTGGAC	AAAGAAGTTAACTCTTAAGGGTCTGGAA	TATTTCAATAATAAGAATTACTAAGACACATTACCTTAAC	CAAAATGTTCTAAATGTAAACTATTTCCTGAGA	As above	comp320246_c0_seq1	1326
Bmys34	AT CCTCCCCAATCTCTGAATCATTGA	CCTCCCCAATCTCTGAATCATTGT	CCACTGGCTCTATCACTTCCAGA	CTCGATAACAGGTTTCTCATCTGC	As above	comp326581_c0_seq1	350
Bmys39	AG ACTACAGCAAACTCTTCCTCTTTTCT	CTACAGCAAA CTCTTCCTCTTTTCC	TTTGATTGGTAAAAGCAAACATTCAGAATAAGACT	TTCTGAAAATTAACCATCACTACAGCA	As above	comp376347_c0_seq1	2222
Bmys4	CG ATGCAATGCACTTTGGGGATGAC	ATGCAATGCACTTTGGGATGAG	AGAAAATATTAGTCCTTGGTATAAATAGGCATTCACA	A G C A A G A A A A A T G C A A T G C A C T	As above	comp351765_c0_seq1	2617
Bmys40	CT CTTCATGTGTACAGGGCGC	CCTTCATGTGTACAGGGCGT	TGTACTTCCCAACCCGCAGT	AGGAGCAGGCAGCAA	As above	comp474479_c2_seq5	478
Bmys41	AG CTTTCAGGGCTCCCCCA	CTTTCAG GGCTCCCCG	CCATCAGGCTCCACGGAGG	GG GACACCGTCCTTCCC	As above	comp462020_c0_seq5	1068
Bmys43	GT GGACACTCTGGGGATGTGACG	GGACACTCTGGGATGTGACT	AGGTCTCAACGCCTGCCA	TCCTGACTCCTTGAAGCTGG	As above	comp473931_c5_seq17	1320
Bmys44	AC CCTCGCCCTCTAAATGACCTT	CCTCGCCCTCTAAATGACCTG	TGGTTCCACTGGACAGAGGC	GAAGGAATATGGGCGTTCCTC	As above	comp454422_c0_seq1	821
Bmys51	AG TTGGTTGCCCTAGATTTACTGCT	TGGTTGCCCTAGATTTACTGCC	AGG CAG GT CAGG AAGT AC CATCC	GTTTGGGCAGGGCACTT	As above	comp461138_c6_seq4	944
Bmys7	CT TGCAAGAACCTTGAATAAAATATAGCTGTG	TTTTGCAAGAACCTTGAATAAAATATAGCTGTA	CTGCTAAATTTTCAGTAATTTGTTATGCAGCATT	CTGTGTTGTAATTTTTGCAAGAACCT	As above	comp391920_c0_seq1	598
Bmys9	CT CTTTCATGGAATGATGTGGGGGTATAACTG	ATCTTTCATGGAATGATGTGGGGGTATAACTA	GAGGTGCACAGTATACACAACATACGA	CCTGTAAACCATACTGGAATCTTTCA	As above	comp397729_c0_seq1	1591
C5 (#)	GA GCAAACTCTGAGACCCATTCAATCTAG	GCAAACTCTGAGACCCATTCAATCTAA	GAGGCTAACTATGTTCTACATGATTTCAGAAGT	TGTTGCAAAATTCTTAAAGTGAAGCAAA	GU066528	N/A	700
CHY (#)	CT CATAGAACCTCATGAGGAACCAGG	CATAGAACCTCATGAGGAACCAGA	GGCCAACCCAATAGCATCGG	AGCAGCATCAGACAGAGACA	GU066531	N/A	286
CSF2 (#)	GC AGCTCACGTGACTGCCAC	AGCTCACGTGACTGCCAG	GTTGCGCAGGAGGCCA	CGTGGCTGGTCAGCTAATAAAG	GU066529	N/A	320
FES (#)	CT TGTGCCTTCAGGGGTATTGC	ATGTGCCTTCAGGGGTATTGT	TGCCCTGTCCCCGCC	GGTCAGGGATCTCCCAGAT	GU066537	N/A	136
PKM (#)	CG CCACTTTCTTGATTTAGAAGGACACATG	CCACTTTCTTGATTTAGAAGGACACATC	GGCTTTCCTAATTAGATGGAGCCTGT	CTGTGCTGGCCCACTTT	GU066538	N/A	494

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6 BH 9	F	F	F	F	F	F	F	F	ΤG	TG	F	F	F	F	F	F	99	99	ΠG	DI	D	TG	91	91	= =	: 2	F	F	F	IG	D	D	IG	F	F	F	F		: =	F	51 TG) .
0 BH	F	F	GT	GT	GT	GT	GT	GT	99	99	GT	GT	99	99	99	99	GT	99	5 0	3 19	F	99	99	F	F	F	F	F	Ħ	GT	GT	19 19	5 5	GT	999)))						
Bmy_7 1267	99	99	99	99	99	99	99	99	AG	AG	AG	AG	99	99	99	99	99	99	AG	AG	99	99	99	99	5 6	AG AG	AG	99	99	99	99	99	99	99	99	AG	AG	999	3 9	99	99)))
Bmy_ 05331	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	90	90	99	99	99	99	59	3 9	99	99	99	99	99	99	99	99	99	99	99	99	3 9	99	99 99);
Bmy_ 06654 1107	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AA	AA	AG	AG	AA	AA	AG	AG	AA	AA	AA	AA	AA A	A A	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA AA	A A	AA	AA AA	1111
Bmys 43	GT	GT	F	F	F	F	GT	GT	GT	GT	F	F	GT	GT	F	F	GT	GT	F	F	GT	GT	99	GT	= =	= =	Þ	F	F	GT	GT	F	F	GT	GT	GT	GT	╞╞	5	GT	GT 61	;
Bmy_ 03606 _619	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	00	99	99	99	99	99	5 6	3 8	0 0	99	99	99	99	99	99	99	99	99	00	99	3 8	99	999	;;
Bmys 3	ե	Ե	ե	ե	F	F	ե	ե	Ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	ե	5 I	5 t	55	ե	ե	ե	ե	ե	ե	ե	F	F	ե	5	եե	55	ե	FF	-
Bmys 22	99	90	AG	AG	AA	AA	AA	AA	90	99	99	90	AG	AG	AA	AA	AG	AG	AG	AG	AG	ЯG	99	0	S AG	e e	AA	99	99	AA	AA	AA	AA	AG	AG	AA	A	BA AG	8 ¥	AA	99	;;;
Bmys 14b	AA	AA	AA	AA	-A	-A	-A	-A	-A	-A	I	;	1	1	AA	A	AA	AA	-A	-A	AA	AA	Ą	AA	4 <	{ ₹	AA	-A	-A	AA			:	-A -4	C							
BH 60	F	F	F	F	TC	TC	F	F	F	F	F	F	F	F	F	F	TC	1C	F	F	ប្រ	TC	F	10	= =	: =	F	F	F	TC	TC	F	F	F	F	F	F	╞╞	: =	F	FF	-
BH 404	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	A	AA V	{ }	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA 4	{ }	AA	AA AA	[
BH 34	AA	AA	AC	AC	AA	AA	AA	AA	AA	AA	AA	AA	AA	A	AA	AC	A 4	{ {	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	A	A 4	{ {	AA	AC)								
Bmy_ 18300 _4038	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F		0	= =	: ⊨	F	F	F	F	F	F	F	F	F	F	F	╞╞	=	2	╞╞	
PKM	g	ÿ	8	20	90	ÿ	ប្ល	2	ប្ល	20	8	ខ	S	ដ	2	S	ÿ	g	g	g	ÿ	g	ម្ល	ÿ	3 5	3 8	8	2	20	20	ប្ល	ÿ	S	ÿ	ÿ	2	ខ	88	3 8	g	99 99	;;;
Bmy_ 03499 297	8	2	ÿ	g	2	2	ÿ	g	g	g	99	90	S	8	ÿ	g	ដ	ដ	g	g	ដ	S	ខ	2	3 5	3 8	2	g	g	2	ដ	ÿ	g	ប្ល	ដ	S	ដ	88	3 8	ខ	85	;;
Bmys 29	99	99	90	99	ÿ	ÿ	90	99	g	g	99	99	90	99	90	90	99	99	99	99	99	90	99	90	5 0	3 8	00	99	99	99	99	99	99	99	99	99	00	9 9	3 3	g	99	;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
Bmys 21	99	90	90	99	99	99	99	99	90	99	99	90	90	90	99	90	90	90	90	90	99	99	99	00	5 6	3 8	0 0	99	99	99	99	99	99	90	90	99	0 0	99	3 8	99	99	;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
Bmys 13	AA	AA	AA	AA	AA	AA	AG	AG	AG	AG	AA	AA	A	AA	AG	AG	AG	AG	AA	AA	AG	AG	AA	A	AA V	g B	AG	AA	AA	AA	AA	AA	AA	AA	AA	AG	AG	AA 4	g B	AG	AA AA	2
BH43		СТ		ст	20	ប្ល		CT	ប	20	8	ខ	S	ដ	2	S	ដ	ដ	Я	ដ	ប្ល	ы	5	CT	5 t	5 8	8	СТ	СТ	20	ប្ល	ប្ល	20	F	F	F	E	եե	5 8	Я	85	ç
Bmy_ 18709 _446	F	F	F	F	F	F	F	F	F	F	F	F	сı	CT	F	F	СТ	сı	F	F	F	F	F	E	= =	: 5	F	F	F	F	F	F	F	F	F	F	F	55	5 =	Þ	FF	-
3my 80270 _1768	AG	AG	AG	AG	AG	AG	AG	AG	ЪG	AG	AG	AG	90	90	AG	ЯG	ЪG	ЪG	AG	ЪG	99	99	99	9	S S S	D D D	AG	AG	ЯG	AG	ЪG	ЪG	AG	AG	ЪG	ЯG	AG	9 U	2 9	99	96 AG	,
- ຫຼັງ ອ	99	90	99	99	90	99	90	90	99	99	90	99	90	99	99	90	90	90	ВA	ВA	99	90	99	99	5 0	3 9	00	99	99	99	99	99	99	99	90	ВA	ВA	9 9	3 9	90	99	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
3mys 41	99	99	90	90	90	99	99	90	90	90	99	99	90	90	AA	AA	90	99	99	99	99	90	g	99	9 U	S A	90	99	90	90	99	99	90	AG	AG	AG	AG	999	3	99	99	3
34 E	F	F	F	F	F	F	AT	AT	F	F	⊨	F	F	F	F	F	Ħ	F	⊨	F	АТ	АТ	⊨∣	E	= =	: =	F	АТ	AT	F	F	F	F	F	Ħ	F	F	╞╞	: =	F	FF	-
3mys E 28	GT	GT	99	90	F	Þ	Þ	F	GT	GT	99	99	90	99	F	F	90	90	GT	GT	GT	GT	90	9	5 6	5 ⊨	Þ	GТ	GT	GT	GT	99	90	99	90	GT	GT	⊨⊧	: =	F	GT	5
E nys 2	99	99	99	99	GT	GT	99	99	GT	GT	99	99	90	99	GT	GT	99	99	GT	GT	99	90	99	99	99	g g	GT	99	99	99	99	99	99	99	99	99	90	99	30	99	99	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
142b Br	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	GT	99	99	GT	GT	GT	GT	۔ ب ور	81	GT	99	99	GT	55	55	GT	GT	5							
H 108 BF	ٺ	ٺ	20	U U	20	2	20	20	2 C	cc	ٺ	ٺ	S	S	20	S	S	S	ٺ	ٺ	ٺ	ٺ	ٺ	: ن		, U	U U	20	CC	ٺ	ٺ	с С	c	ٺ	ٺ	ٺ	ٺ	ບ ເ	ن ر	ٺ		رر رر
nple BH	33	33	33F	33F	318	318	33	ŝ	35F	35F	(K1	ĆK1	SF	SF	S7F	SZF	320	320	51	51	32	32	36	92	319 710	324F (324F	39	66	35	35	35F	35F	11	11	41F	41F	312	315	315	14 F	1
San	OOE	00	00	OOE	02E	02E	02E	02E	04E	04E	05	051	055	05	05	055	07E	07E	08(08(085	085	14(141	15	15E	15E	15 E	156	16	16	16	16	16	16	16	16	971 975	14E	14E	151	i

Genotypes of replicated samples and mother/foetus pairs used in quality control analysis. Only the genotypes from the 69 autosomal loci are included here, because only those loci were used in the analysis.

Appendix 2

	Bmy_ 18902 _72	AG	A A A	AA	99	99	99	99	AG	AG	99	9 0	5 0	00	99	99	99	ЪG	AG	99	99	AG	AG	РG	9 U V	3 8	РG	AG	AG	AG	00	50	ט פיפ	5 9	000	AG AG	AG	99	99	AG AG
	Bmys 9	F	╞╞	F	F	F	Ъ	ե	F	F	F	ŧ	5 t	5 🗆	F	F	F	ե	ե	F	F	F	F	F	E E	= =	F	F	ե	b	E I	=	= =	: =	: =	: 5	Ъ	F	F	⊧⊧
	FES	СТ	ե Ե	сŢ	ပ ပ	CC	22	ບ ບ	ບ ບ	5	20	85	5 5	; ;;	20	20	S	с С	20	ບ ບ	ບ ບ	S	2	2	25	35	20	CC	S	2	2	58	3 5		20	3 8	20	CC	5	55
	Bmy_ 15464 _273	C	88	2	Ċ	C	С	5	C	C	8	8 ‡	: =	: 5	С	Þ	F	CT	CT	ប	8	F	Þ	С	58	3 8	сı	СТ	F	Þ	51	51	5 t	5 =	: =	: 5	CT	20	8	55
	BH 395	TC	2 E	F	Þ	F	TC	TC	TC	TC	ខ	8 =	: =	: 2	TC	TC	TC	TC	TC	ТC	TC	F	F	F	⊧⊧	= 2	TC	TC	ប្ល	S	8	58	3 5	3 5	2 2	5 2	TC	F	F	2 D
	Bmy_ 16681 _523	AA	A A	AA	AG	AG	AA	AA	AA	AA	ВĞ	9 U V		AA	AA	AG	AG	AA	AA	AA	AA	ЯG	AG	A	A S	¥ ¥	AA	AA	AA	AA	¥:	AA :	AA V		E A	Ą	AA	AG	AG	AG AG
	Bmy_ 18857 _278	GT	5 ⊨	Þ	Þ	Þ	F	Þ	Þ	Þ	Þ	╞╞	: =	: =	F	Þ	F	99	99	Þ	Þ	GТ	GT	F	⊧⊧	= =	Þ	F	F	Þ	⊨⊧	= 5	5 5	5 =	: =	: ⊨	Ħ	GT	GT	⊨⊨
	Bmys 19	AA	A A	AA	AG	ЪG	AG	AG	AA	AA	Ą	A 4		AA A	A	99	99	AA	AA	99	99	AG	AG	99	00	AG AG	AG	AG	A	AA	¥:	A d	אק אק		300	AG 20	AG	AG	AG	AG AG
	Bmy_ 15448 _333	Ь	╘╘	F	ե	ե	F	F	20	20	F	E t	5 t	5 🗆	F	F	F	F	F	F	F	ե	ե	ե	5‡	= =	ե	Ъ	ե	ե	51	5	= =	: =	: =	: =	F	Ъ	ե	╞╞
	Bmys 33	GT	55	GТ	F	F	F	F	F	F	F	= =	: =	: =	F	GT	GT	F	F	F	F	F	F	GT	5	= =	F	F	F	F	E I	=	= =	: =	: =	: =	F	F	F	╞╞
	Bmys 4	90	5 5	90	ប	2	20	ប្ល	ប	8	99	99	3 5	3 8	8	2	8	2	8	ប្រ	ប	90	9 C	90	9 <u>9</u>	3 8	90	90	90	90	000	3	ງປ	2	200	3 8	2	00	90	ខទ
	Bmy_ 11209 _216	F	⊨⊨	Þ	Þ	F	F	F	Þ	F	F	╞╞	: =	: =	F	F	F	F	F	F	Þ	F	Þ	F	⊧⊧	= =	Þ	F	F	F	51	51	55	55	5 5	; E	F	F	F	⊧⊧
	Bmy_ 05573 _143	F	⊨⊨	F	F	F	F	F	F	F	F	= =	: =	: =	F	F	F	F	F	F	F	F	F	F	⊧⊧	= =	F	F	F	F	E I	=	= =	: =	: =	: =	Ħ	F	F	⊨⊨
	Bmy_ 05005 _2391	ខ	88	2	ប	2	С	ŋ	Þ	Þ	5	55	3 5	35	С	8	8	2	8	ប	8	2	ខ	2	មដ	55	сı	СТ	8	8	51	51	5 5	5 =	: =	:5	CT	2	8	55
	Bmy_ 13463 _324		Ե Ե	С		CT	CT	ст	ប្ល	8	F	t t	5 5	5 🗆	F	F	F	F	F		CT	F	Þ	IJ	ដ	= =	С	СТ	C	CT	88	51	55	55	5 5	5	СТ	F	F	⊧⊧
	ВН 387-2	TA	AT A	ΤA	Þ	F	ΤA	ΤA	ΤA	ΤA	F	= =	: =	: =	F	Þ	F	F	F	F	Þ	ΤA	ΤA	F	¢	A T	F	F	F	F	E I	=	= =	: =	: =	TA	TA	F	F	⊧⊧
	BH42a	99	999	99	GТ	GT	99	99	GТ	GT	99	9 0	5 0	61	GT	GT	GT	GT	GT	99	99	99	99	F	⊨ ;	38	GТ	GТ	99	99	99	36	5 5	5 9	00	GT 0	GТ	99	99	GT GT
	Bmy_ 04117 _681	AG	AG AG	AG	AA	AA	AA	AA	AA	AA	Ą	A 4		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	A	A S	A A	AA	AA	AA	AA	¥ :	A :	AA V		{ {	Ą	AA	AA	AA	A A
	Bmys 39	AG	9 0 A A	AG	99	99	AG	AG	99	99	ВĞ	9 d		AG	AG	AG	ЯG	99	99	99	99	99	99	99	9 0 9	פ פ אפ	AG	AG	ЯG	AG	99	5	ש פע ע	ע ע	D U	AG	AG	AG	AG	AA AA
	Bmy_ 03506 _639	AG	9 0 A A	AG	AG	ВA	AG	AG	AG	AG	ВĞ	9 d		AG	AG	99	99	AA	AA	99	99	99	99	ЯG	9 U V	פ פ אפ	99	99	99	99	9 d	9 C	ש פע ע		00	AG AG	AG	AG	AG	A G A G
	Bmys 7	S	55	ե	20	C	CC	20	20	20	ե	եչ		3 11	20	20	S	20	00	ե	ե	S	2	2	25	55	22	20	ե	ե	51	58	3 5	3 E	5 5	3 23	CC	CC	20	000
	CSF2	00	5 9	СG	СG	gc	99	99	с С	СC	СС		, .	00	00	50	СC	90	СC	99	99	СC	50	99	9	3 8	22	C	СC	СG	90	990	י פיפ	, c b	200	3 8	CC	gC	СC	22
	Bmy_ 04604 _4249	AG	9 0 0 9	99 99	99 99	99	99	99	AG	AG	99	500	ט ש ע	AG	AG	AG	AG	AA	AA	AG	AG	99	AG	99	00	PA A	AG	AG	AG	AG	AG	9 C U	ט פ פ פ	50	AG AG	90	99	99	99	90 AG
	BH 414	GA	e g	99 99	ВA	ВA	ВA	ВA	99	99	ВA	e d	(⊲ 0	GA G	ВA	AA	AA	ВA	ВA	ВA	ВA	AA	AA	AA	A A	AA AA	AA	AA	AA	AA	GA GA	e d	ט פ פ פ		300	6A GA	ВA	AA	AA	A A A A
	Bmys 17	90 00	9 9 9 9	99	99	99	99	99	99	99	AG	9 U U	200	00	99	99	99	ВĞ	AG	99	99	99	99	99	9 U	פפ שפ	99	99	99	99	990	50	ט פ פיפ	200	30	9 9	99	99	99	AG AG
	Bmys 25	ь	58	8	ყ	8	Ь	ե	ប	8	ե	եե	5 t	5 =	F	8	8	8	8	ខ	ե	F	F	8	8 t	5 5	8	8	8	8	88	88	3 5	βt	5 5	55	ե	Ъ	ե	88
	Bmy_ 06377 _150	99	999	99	g	y	g	ÿ	99	99	g	5		00	99	ÿ	g	99	99	ខ	g	99	90	90	0 U	, 9 9 9 9	99	99	99	99	9 0	5	3 5		300	3 8	2	99	99	000
	Bmy_ 14843 _1856	5	5 =	F	20	C	CC	20	20	20	ե	եչ		3 11	20	20	S	20	C	ե	ե	ե	ե	F	⊨ 8	3 8	F	F	S	20	51	58	3 5		2 12	5	Ъ	CC	20	000
	Bmy_ 06147 437	AA	A A A	AA	AA	AA	AA	AA	AA	AA	Ą			AA	AA	AA	AA	AA	AA		AA		AA	AA	Α d	9 9 9 9	99	90	AA	AA	AA	AA	AA AA		A A A	AG	AG		AA	A A
	Bmy_ 18001 _1857	90 0	999	99	AA	AA	99	99	99	99	99	9 0	200	00	90	99	90	AG	AG	99	99	99	AG		000	ט פ ט פ	99	90	99	99	99	90	ט פיפ	5 0	300	9 0	90	99	99	AG AG
s (part 2)	BH 382	C	58	20	20	CC	C C	20	20	20	2	55		3 0	20	20	S	20	20	20	20	20	2	2	85	5 8	20	CC	20	20	200	5 5	., L		3 13	5	СТ	CC	20	22
d Sample:	Bmy_ 04322 _72	F	= 5	сŢ	ст	CT	CT	ŋ	8	8	F	⊨ t	5 5	; ⊨	F	CT	сı	F	F	С	СТ	F	Þ	С	ե	55	8	8	СŢ	CT	51	51	5 t	5 5	3 8	88	8	Þ	Þ	ԵԵ
Replicate	Sample	00B3	00B3 00B3F	00B3F	02B18	02B18	02B3	02B3	04B5F	04B5F	05KK1	05KK1	DESE	0557F	05S7F	07B20	07B20	08G1	08G1	0852	0852	14B6	14B6	15B19	15819	15B24F 15B24F	15B9	15B9	16B5	16B5	16B5F	16B5F	16H1	16H1F	16H1F	97B12	97B12	14B15	14B15	15N1 15N1

AA T AA G GG AA AA GG GG GG AA AA GG GG GG AA AA AG GG GG AA AG GG GG GG AA AA AG GG GG AA AA GG </th <th>38 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619</th> <th>18-300 Bmys Bmys 0-3606 KM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619</th> <th>03499 18300 Bmys Bmys 03606 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619</th> <th>Bmys 03499 18300 03606 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619</th> <th>Bmys Bmys 03499 bmyz bmyz bmys 21 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys _ 03606</th> <th>Bmy_ Bmy_ 1300 13605 13200 13805 13800 13805 13805</th> <th>Bmy_ Bmy_ D3005 D3605 <thd3605< th=""> D3605 <thd3605< th=""> <</thd3605<></thd3605<></th> <th>bmybmybmysbmysbmysbmysbmysbmysbmysbmysbmysbmysbmysbmys03606 1. 1466 BH43 13 21 29 _297 PKM _4038 BH34 BH404 BH60 14b 22 Bmys3619</th> <th>ыту_ ыту_ btrny_ btrny 030270 18709 btrnys btrnys btrnys 03606 107018709 btrnys btrnys 03409 11768 _446 btr43 13 21 29 _297 PtKM _4038 btrl 34 btrl 404 btrl 60 14b 22 btrnys3 _619</th> <th>etiny_ etiny_ 30270 18709 Bmys Bmys Bmys 03499 18300 Bmys Bmys 03606 C5 _1768 _446 BH43 13 21 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys3 _619</th> <th>Bmv Bmv Bmv<th>Bmy_ Bmy_ Bmy Bmy</th></th>	38 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619	18-300 Bmys Bmys 0-3606 KM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619	03499 18300 Bmys Bmys 03606 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619	Bmys 03499 18300 03606 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys 3 _619	Bmys Bmys 03499 bmyz bmyz bmys 21 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys _ 03606	Bmy_ 1300 13605 13200 13805 13800 13805 13805	Bmy_ D3005 D3605 D3605 <thd3605< th=""> D3605 <thd3605< th=""> <</thd3605<></thd3605<>	bmybmybmysbmysbmysbmysbmysbmysbmysbmysbmysbmysbmysbmys03606 1. 1466 BH43 13 21 29 _297 PKM _4038 BH34 BH404 BH60 14b 22 Bmys3619	ыту_ ыту_ btrny_ btrny 030270 18709 btrnys btrnys btrnys 03606 107018709 btrnys btrnys 03409 11768 _446 btr43 13 21 29 _297 PtKM _4038 btrl 34 btrl 404 btrl 60 14b 22 btrnys3 _619	etiny_ etiny_ 30270 18709 Bmys Bmys Bmys 03499 18300 Bmys Bmys 03606 C5 _1768 _446 BH43 13 21 29 _297 PKM _4038 BH 34 BH 404 BH 60 14b 22 Bmys3 _619	Bmv Bmv <th>Bmy_ Bmy_ Bmy Bmy</th>	Bmy_ Bmy
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SNP name	Contig	Site
Bmys2	comp176650_c0_seq1	1,944
Bmys3	comp327063_c0_seq1	1,630
Bmys4	comp351765_c0_seq1	2,617
Bmys7	comp391920_c0_seq1	598
Bmys9	comp397729_c0_seq1	1,591
Bmys13	comp408701_c0_seq1	1,023
Bmys14	comp409439_c0_seq1	1,064
Bmys17	comp464619_c3_seq11	2,549
Bmys19	comp472663_c7_seq1	5,091
Bmys21	comp462235_c0_seq2	546
Bmys22	comp668158_c0_seq1	1,145
Bmys23	comp1099809_c0_seq1	561
Bmys24	comp169488_c0_seq1	392
Bmys25	comp179499_c0_seq1	138
Bmys28	comp313642_c0_seq1	246
Bmys29	comp315937_c0_seq1	1,087
Bmys31	comp316968_c0_seq1	1,142
Bmys33	comp320246_c0_seq1	1,326
Bmys34	comp326581_c0_seq1	350
Bmys39	comp376347_c0_seq1	2,222
Bmys40	comp474479_c2_seq5	478
Bmys41	comp462020_c0_seq5	1,068
Bmys43	comp473931_c5_seq17	1,320
Bmys44	comp454422_c0_seq1	821
Bmys51	comp461138_c6_seq4	944

Appendix 3. Scaffolds from which the second group of SNPs were derived.