

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 Greenland 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 Utqiagvik (formerly Barrow), Alaska, rather than a single large BCB stock. Another study using the same 10 microsatellites suggested that animals harvested from Utqiagvik 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 Utqiagvik 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 stock-structure 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. 6µL of DNA at concentrations up to 50ng/µL 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 marker 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 pre-amplification 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

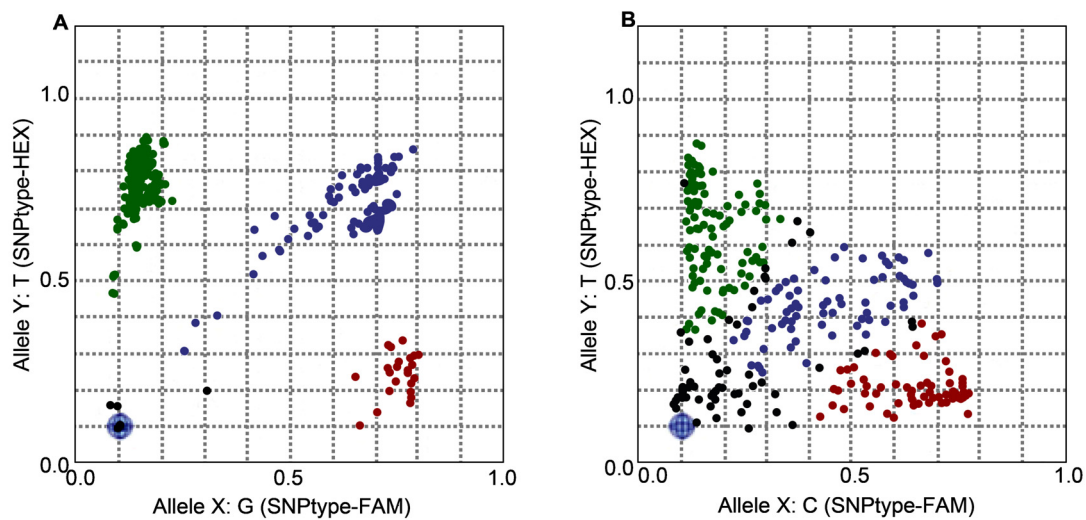


Fig. 1: Examples of genotype calling for a clean locus (A) and a locus that was disregarded based on a lack of distinction among genotypes (B). Each dot represents fluorescence values for one individual. Green and red dots represent the two different homozygous genotypes. Blue dots represent heterozygous 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	ALLELE	ASPL_SEQ	ASP2_SEQ	LSP_SEQ	STA_SEQ	Accession number of genome/ transcriptome resource	Contig number	Position in contig/sequence
Bmys25	CT	CCTTCAGGCTCTCATCTACCCAG	CCTTCAGGCTCTCATCTACCCAA	CAGAACAGATGCAGCCCA	TTGCACTGAGGTTTCCCAATC	As above	comp179499_c0_seq1	138
Bmys28	GT	ACCAGTGTCTAGAAAAGCAAGG	ACCAGTGTCTAGAAAAGCAAGT	CCCGGGCTGGTCTTCA	AGAGAACATCTCACTGAGTTTCACA	As above	comp313642_c0_seq1	246
Bmys29	CG	CAGGGAAACACAACCTTTTCTAAAAC	CAGGGAAACACAACCTTTTCTAAAAG	GTGCTCAAGCTGGACTGCTA	CTTGTAAGACTTTCGCAAGG	As above	comp315937_c0_seq1	1087
Bmys3	CT	GAGCTTAAGTTATTTCCCAAGGC	AGAGCTTAAGTTATTTCCCAAGGT	CCTGGCTCAAGTCTCTGCT	AGAAGGGTAATGAAAAACCAAGGC	As above	comp327063_c0_seq1	1630
Bmys31	AT	GGTAAGACATATGCAAGAAATCTTTACA	GGTAAGACATATGCAAGAAATCTTTACT	TCAGCACTCCCATCATTTGT	TGCCCACTCTGGTAAGACA	As above	comp316968_c0_seq1	1142
Bmys33	GT	AAGAAGTTAACTCTAAGGGCTGGAC	AAAGAAGTTAACTCTAAGGGCTGGAA	TATTTCAATAAAGAAATTAAGACACATTAAC	CAAAATGTCTAAATGAACTATTTCTGAGA	As above	comp320246_c0_seq1	1326
Bmys34	AT	CCTCCCAATCTGAATCATGA	CCTCCCAATCTGAATCATGT	CCACTGGCTATCACTCCAGA	CTGATAACAGTTTCTCATCTGC	As above	comp326581_c0_seq1	350
Bmys39	AG	ACTACAGAACTCTCTCTTTTCT	CTACAGAACTCTCTCTTTTCC	TTTGATTGGTAAAGCAACATTCAGAATAAGACT	TTCGAAAATTAACCATCACTACAGCA	As above	comp376947_c0_seq1	2222
Bmys4	CG	ATGCAATGCACCTTGGGATGAC	ATGCAATGCACCTTGGGATGAG	AGAAAATATTAGTCTTGGTATAAATAGGCATTACA	AGCAAGAAAAATGCAATGCACT	As above	comp351765_c0_seq1	2617
Bmys40	CT	CTTCATGTGTACAGGGCC	CTTCATGTGTACAGGGCGT	TGTACTCCCAACCCGAGT	AGGACAGGGCAGCAA	As above	comp474479_c2_seq5	478
Bmys41	AG	CTTTCAGGGCTCCCCCA	CTTTCAGGGCTCCCCCG	AGGTCTCAACGCCCTGCCA	GGGACACCGTCTCTCC	As above	comp376947_c0_seq1	1068
Bmys43	GT	GGACACTCTGGGATGTGACG	GGACACTCTGGGATGTGACT	GGGACACTCTGGGATGTGACG	TCCTGACTCTTGAAGCTGG	As above	comp473931_c5_seq17	1320
Bmys44	AC	CCTCGCCCTCTAATGACCTT	CCTCGCCCTCTAATGACCTG	TGGTCCACTGGACAGAGGC	GAAGGAATATGGCGTCTCTC	As above	comp462020_c0_seq5	821
Bmys51	AG	TTGGTTGCCCTAGATTTACTGCT	TTGGTTGCCCTAGATTTACTGCC	AGGCAGGTCAAGAAAGTACAATCC	GTTTGGCAGGGGCACCT	As above	comp454422_c0_seq1	944
Bmys7	CT	TGCAAGAACTTGAATAAATAATAGCTGTG	TTTTCAGAACTTGAATAAATAATAGCTGTA	CTGCTAAATTTTCAGTAATTTGTATGAGCATT	CTGTGTTGTAATTTTTTGCAAGAACCT	As above	comp391920_c0_seq1	598
Bmys9	CT	CTTTCATGGAATGATGGAGTAACTG	ATCTTTCATGGAATGATGGAGTAACTA	GAGGTGCACAGTATACACACATAGGA	CTGTAAACCATACTGGAATCTTTCA	As above	comp397729_c0_seq1	1591
C5 (#)	GA	GCAAACTCTGAGACCCATTCATCTAG	GCAAACTCTGAGACCCATTCATCTAA	GGCCCAACCCATAGCATCGG	TGTTGCAAAATTTCTAAAGTGAAGCAA	GU066528	N/A	700
CHY (#)	CT	CATAGAACCCTATGAGAACCCAGG	CATAGAACCCTATGAGAACCCAGA	GTTCCGAGGAGGCCA	AGCAGCATCAGACAGAGACA	GU066531	N/A	286
CSF2 (#)	GC	AGCTCAGTGTACTGCCAC	AGCTCAGTGTACTGCCAG	GTTCCGAGGAGGCCA	CGTGGCTGGTCACTAATAAAG	GU066529	N/A	320
FES (#)	CT	TGTGCTCAGGGGTATTGT	ATGTGCTCAGGGGTATTGT	TGCCCTGTCCCGCC	GGTCAAGGATCTCCAGAT	GU066537	N/A	136
PKM (#)	CG	CCACTTCTTGATTAGAGGACACATG	CCACTTCTTGATTAGAGGACACATC	GGCTTTCCTAAITAGATGGAGCCTGT	CTGTGCTGCCCACTTT	GU066538	N/A	494

Mother/Foetus pairs (part 1)

Sample	BH 108	BH 42b	Bmys 2	28	34	41	C5	Bmy_30270_1768_446	Bmy_18300_4038	BH 34	BH 404	BH 60	Bmys 14b	Bmys 22	Bmys 3_619	Bmys 03606	Bmy_06654_1107_43	Bmy_06654_152_152	Bmy_12670_341	BH 410b	BH 92	Bmys_16528_309	Bmys 44	Bmys 51	CHY	Bmys 31	Bmys 24
0083	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	AC	AA	CC	TT	GG
0083	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	AC	AA	CC	TT	GG
0083F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AC	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	GT	CT	CC	AA	CC	TT	GG
0083F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AC	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	GT	CT	CC	AA	CC	TT	GG
0085	C	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0085F	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0085F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0485	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0485F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0485F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0555	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0555F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0555F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0556	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0556F	C	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0557	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0557F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
0557F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
09KK1	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
09KK1F	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15B24	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15B24F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15B24F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1589	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1589F	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1589F	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15N1	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15N1F	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
15N1F	CC	GG	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1685	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1685F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
1685F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
16H1	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
16H1F	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
16H1F	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
9588	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
9588F	C	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
9685	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
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9685F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
9986	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
9986F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
96811	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG
96811F	CC	GT	GG	GG	GG	GG	GG	AG	TT	AA	AA	AA	AA	GG	CT	GG	GT	AA	GG	GG	TT	CT	CC	AA	CC	TT	GG

Mother/Foetus pairs (part 2)

Sample	Bmy_04322_72	BH382	Bmy_18001_1857	Bmy_06147_437	Bmy_14843_1856	Bmy_06377_150	Bmy_04604_4249	C5F2	Bmys7_639	Bmy_03506_639	Bmy_04117_681	BH42a	BH387-2	Bmy_13463_324	Bmy_05005_2391	Bmy_05573_143	Bmy_11209_216	Bmys4	Bmys33_333	Bmy_15448_333	Bmy_16681_523	BH395	Bmy_15464_273	FES	Bmys9_72	Bmy_18902_72	
00B3	TT	CT	GG	AA	CT	GG	GA	AG	CC	AG	AG	GG	TA	TA	CC	TT	TT	GG	CT	CT	AA	AA	TC	CC	CT	TT	AG
00B3	TT	CT	GG	AA	CT	GG	GA	AG	CC	AG	AG	GG	TA	TA	CC	TT	TT	GG	CT	CT	AA	AA	TC	CC	CT	TT	AG
00B3F	CT	CC	GG	AA	TT	GG	GG	GG	GC	AG	AG	GG	TA	TA	CC	TT	TT	GG	GT	GT	AA	AA	TT	CC	CT	TT	AA
00B3F	CT	CC	GG	AA	TT	GG	GG	GG	GC	AG	AG	GG	TA	TA	CC	TT	TT	GG	GT	GT	AA	AA	TT	CC	CT	TT	AA
00B5	TT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
00B5F	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
04B5F	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
04B5F	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0555	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0555F	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0555F	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
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0556F	TT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0557	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0557F	TT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
0557F	TT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
09K1	CT	CC	GG	AA	CT	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
09K1F	CT	CC	GG	AA	CT	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
15B24	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
15B24F	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
15B24F	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	TT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	GG
15B9	CC	CC	GG	AA	TT	GG	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
15B9	CC	CC	GG	AA	TT	GG	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
15B9F	CT	CC	GG	AA	TT	GG	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
15N1	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AA	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
15N1F	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AA	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16B5	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16B5	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16B5F	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16B5F	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16H1	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16H1	CT	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16H1F	CC	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
16H1F	CC	CC	GG	AA	CC	CC	AA	AG	CC	GG	AG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
95B8	CC	CC	GG	AA	CT	GG	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
95B8F	CT	CC	GG	AA	CT	GG	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
96B5	CT	CC	GG	AA	CT	GG	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
96B5F	CC	CC	GG	AA	CT	GG	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
99B6	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
99B6F	CC	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
96B11	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG
96B11F	CT	CC	GG	AA	CC	CC	GA	GG	CC	AG	GG	AA	GT	TT	CC	TT	TT	GG	CT	CT	AA	AA	CC	CC	CT	TT	AG

Appendix 3.

Scaffolds from which the second group of SNPs were derived.

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