

UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL

SCANING OF SELECTION SIGNATURES IN DAIRY
BUFFALO (*Bubalus bubalis*)

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2020

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André Vieira do Nascimento

Orientador: Prof. Dr. Humberto Tonhati

Coorientador: Dr. Diercles Francisco Cardoso

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutor em Genética e Melhoramento Animal.

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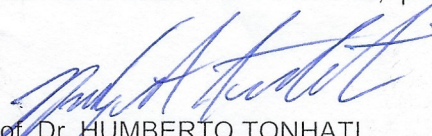
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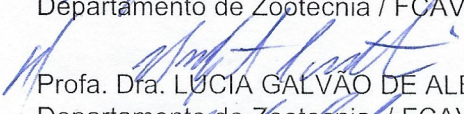
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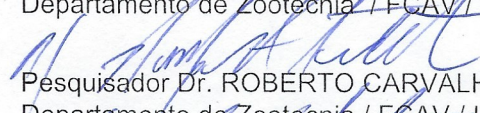
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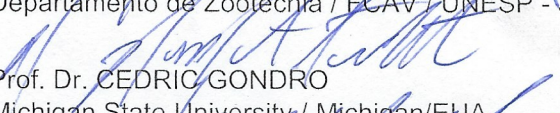
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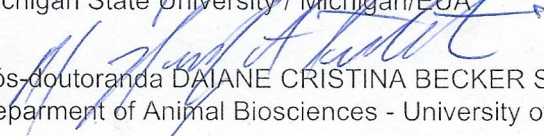
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DADOS CURRICULARES DO AUTOR

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DETECÇÃO DE ASSINATURAS DE SELEÇÃO EM BÚFALOS LEITEIROS (*Bubalus bubalis*)

RESUMO - O objetivo do presente trabalho foi aperfeiçoar o mapa do painel Axiom Buffalo Genotyping (ABG) com mapeamento de SNPs com posição indefinida SNPs e prever a localização genômica de QTL (*Quantitative Trait Loci*) bovinos no genoma bubalino por meio de alinhamento local. Além de identificar assinaturas de seleção em rebanho da raça Murrah, buscando genes e QTL relacionados a aspectos raciais e produtivos da espécie. Foram utilizados 865 animais genotipados com o painel ABG. Todas as sequências das sondas (123.040) foram realinhadas contra o genoma bubalino (UOA_WB_1) usando o BLAST, já as sequências dos QTL bovinos foram alinhadas com o genoma do búfalo usando o *Burrows-Wheeler Aligner*. O controle de qualidade dos genótipos foi realizado com a remoção de marcadores não autossômicos, duplicados, com $Call\ rate_{SNP}$ e $Call\ rate_{Amostra}$ inferiores a 0,95 e 0,90 respectivamente. Para a identificação de assinaturas de seleção por ROH (*Runs of homozygosity*), os seguintes parâmetros foram utilizados: região genômica com comprimento homozigoto maior que 1Mb; mínimo de 30 SNP em homozigose; ao menos 1 SNP por 100 kb; intervalo máximo entre dois SNPs de até 500 kb; e no máximo 1 locus heterozigoto por segmento. A análise de iHS (Integrated Haplotype Score) foi realizada com o software SELSCAN. As médias de $|iHS|$ foram estimadas em janelas de 500 kb, com 250 kb de sobreposição. Sendo consideradas assinaturas de seleção as janelas com valores médios de $|iHS|$ superior a 2,36 (0,1% superior). Foram estimados os níveis de endogamia, onde os coeficientes de endogamia foram calculados a partir da matriz de parentesco genômico (F_{GRM}), pedigree (F_{PED}) e ROH (F_{ROH}). O número de SNP mapeados aumentou de 106.778 no mapa ABG para 116.708 em nosso mapa. As assinaturas de seleção foram identificadas usando o Novo mapa e o mapa ABG. Houve intensificação dos picos de autozigosidade e surgimento de novos no BBU5 e BBU11 quando todos os marcadores no novo mapa foram utilizados. Após alinhamento e controle de qualidade, 63.995 QTL bovinos foram mapeados com sucesso para o genoma do búfalo. Assim, o novo mapa de SNP pode melhorar os resultados das análises genômicas, por meio do aumento de 9.930 SNPs e redução do espaçamento médio do marcador em ~2kb. Devido a homologia entre bovinos e búfalos, as coordenadas dos QTL apresentados neste trabalho, podem ser consideradas potenciais QTL em búfalos, levando à apresentação do primeiro banco de dados de QTL de búfalos. Do total de animais, 350 tiveram ROHs superiores a 10 Mb e comprimento médio por animal de $4,28 \pm 1,85$ Mb. Coeficiente de endogamia utilizando informação de pedigree teve baixa correlação com estimativas genômicas. As estimativas de F_{GRM} foram maiores que as obtidas por meio do F_{PED} e F_{ROH} . Análises baseadas em ROH identificaram assinaturas de seleção nos cromossomos autossômicos 1, 2, 3, 5, 16 e 18, enquanto iHS identificou regiões em assinatura de seleção localizadas nos cromossomos 1, 6, 7, 9, 14 e 23. Ambas análises abrangeram genes e QTL previamente relacionados a características de produção de leite, reprodução, morfológica e resposta imune. Os níveis de endogamia nessa população ainda são baixos, mas devem ser gerenciados para evitar perdas futuras devido à depressão por endogamia.

Palavras-chave: Autozigosidade; Murrah; endogamia

SCANNING OF SELECTION SIGNATURES IN DAIRY BUFFALO (*Bubalus bubalis*)

ABSTRACT - The aim of the present work was to improve the map of Axiom Buffalo Genotyping array with determining the positions of ~10 thousand SNPs and to predict the genomic location of QTL (*Quantitative Trait Loci*) cattle in the buffalo genome using local alignment. In addition to identifying signature of selection in a Murrah breed herd, looking for genes and QTL related to racial and productive aspects of the specie. A population of 865 animals genotyped with the Axiom Buffalo Genotyping (ABG) panel was used. All probe sequences (123,040) have been realigned with buffalo genome (UOA_WB_1) using BLAST, however the sequences of bovine QTL were aligned with the buffalo genome using the *Burrows-Wheeler Aligner*. The quality control of the genotypes was carried out with the removal of duplicate non-autosomal markers, with call rates_{SNP} and call rates_{Sample} less than 0.95 and 0.90 respectively. For the detection of signature of selection by ROH (Runs of homozygosity), the following parameters were used: genomic region with homozygous length greater than 1Mb; minimum of 30 SNP in homozygosity; at least 1 SNP per 100 kb; maximum interval between two SNPs up to 500 kb; and no more than 1 heterozygous locus per segment. Integrated Haplotype Score (iHS) analysis was performed with the SELSCAN software. The averages of |iHS| were estimated in 500 kb windows, with 250 kb of overlap allowed. Where selection signatures were the windows with mean values of |iHS| greater than 2.36 (top 0.1%). The inbreeding coefficients were calculated from the genomic relationship matrix (F_{GRM}), pedigree (F_{PED}) and ROH (F_{ROH}). The number of SNP mapped increased from 106,778 in ABG map to 116,708 in our map. We identified signatures of selection using the new map and the ABG map. There was an intensification of peaks of autozygosity and the appearance of new ones in the BBU5 and BBU11 when we used all the markers on the new map were. After alignment and quality control, 63,995 cattle QTL were successfully mapped to the buffalo genome. Thus, the new SNP map can improve the results of genomic analyzes, by increasing 9,930 SNPs and reducing the average marker spacing by ~ 2kb. Due to the homology between cattle and buffaloes, the coordinates of the QTL presented in this work can be considered potential buffalo QTLs, leading to the presentation of the first buffalo QTL database. Of the total animals, 350 had ROHs greater than 10 Mb and average length per animal of 4.28 ± 1.85 Mb. Inbreeding estimates of the pedigree had a low correlation with genomic estimates. The estimates of F_{GRM} were higher compared to F_{PED} and F_{ROH} . Analysis of selection signatures based on ROH highlighted regions on autosomal chromosomes 1, 2, 3, 5, 16 and 18, while iHS identified regions located on chromosomes 1, 6, 7, 9, 14 and 23. The regions of both analyzes were in overlap with genes and QTL previously related to traits of milk production, reproduction, morphology and immune response. Inbreeding levels in this population are still low, but must be managed to avoid future losses due to inbreeding depression.

Keywords: Autozygosity; Murrah; inbreeding

CAPÍTULO 1 - Considerações gerais

1.1. Introdução

Embora a bubalinocultura seja relativamente recente no Brasil, é considerada promissora na pecuária leiteira. Isto ocorre devido à combinação do bom desempenho produtivo e rusticidade, conferindo aos búfalos maior resistência a doenças parasitárias e adaptação às condições climáticas brasileiras (Bernardes, 2007a).

A composição físico-química do leite de búfala favorece a sua conversão em derivados lácteos, como a *mozzarella*, devido ao maior teor de rendimento e valor agregado (Bernardes, 2013; Seno et al., 2007). Assim, contribuindo para a cadeia da indústria brasileira de leite, que teve no ano de 2019 um faturamento de aproximadamente R\$ 33 bilhões (MAPA, 2019).

Apesar da produção leiteira de origem bubalina ser uma atividade de interesse para indústria, práticas de melhoramento genético visando aumentar a produtividade ainda não alcançam proporções como as aplicadas à rebanhos bovinos (BORGHESE; MAZZI, 2005). Este cenário começou a mudar com surgimento de programas de controle leiteiro, avaliação genética e uso de índices de seleção (Seno et al., 2007). Atualmente as técnicas de seleção tradicionais podem ser aliadas a informações genômicas. Nesse sentido, o lançamento do painel de genotipagem para búfalos possibilitou a obtenção de genótipos para 90.000 marcadores, proporcionando que estudos genômicos se tornem tendência para essa espécie (Ghoreishifar et al., 2018; Iamartino et al., 2017).

Devido à baixa qualidade da montagem do genoma bubalino da versão lançada em 2013 (Williams et al., 2017), o mapa dos SNPs do painel bubalino foi desenvolvido com base no genoma de referência bovino (UMD v3.1). Isto acarretou em dificuldades para análises dependentes do posicionamento dos marcadores no genoma (Zimin et al., 2009). Em 2018 um novo genoma de referência do búfalo foi publicado, o que possibilitou a atualização das posições dos marcadores para a espécie (Low et al., 2019). Entretanto, com a atualização do mapa, SNPs que antes possuíam posições definidas, passaram a ter coordenada desconhecida para o genoma bubalino, ocasionando perda de densidade e conseqüentemente menor cobertura do genômica.

Entre as análises genômicas que podem se beneficiar da atualização do *assembly* bubalino, está a identificação de assinaturas de seleção. Análises de assinaturas de seleção permitem identificar regiões que foram afetadas em resposta à domesticação, adaptação e características produtivas devido à redução de diversidade genética em localidades genômicas específicas, afetadas pela seleção (Ghoreishifar et al., 2018; Kim et al., 2013; Zavarez et al., 2015; Zhang et al., 2015). Essas informações podem contribuir para o planejamento de esquemas mais eficientes de programas de melhoramento genético (Mokhber et al., 2018).

1.2. Objetivos

Aperfeiçoar o mapa do painel Axiom Buffalo Genotyping com a determinação das posições de SNPs não mapeados e predizer a localização genômica de QTL (*Quantitative Trait Loci*) bovinos no genoma bubalino por meio de alinhamento local. Além de identificar assinaturas de seleção em rebanho da raça Murrah, buscando genes e QTL relacionados a aspectos raciais e produtivos da espécie.

1.3. Revisão de literatura

Os búfalos pertencem a espécie *Bubalus bubalis*, originários da Ásia, e foram introduzidos no Brasil no final do século XIX pela ilha do Marajó no estado do Pará, cujo rebanho inicial foi composto por pouco mais de 200 animais (Bernardes, 2007a; Marques, 1998). As características produtivas e adaptativas contribuíram para o aumento mundial dos rebanhos bubalinos, que teve o crescimento médio anual de 43% entre os anos de 1980 a 2005, enquanto rebanhos bovinos cresceram 11% no mesmo período (Bernardes, 2007a).

Atualmente são estimados 200 milhões de cabeças de búfalos no mundo. De acordo com Bernardes (2007a) o Brasil detém o maior rebanho do ocidente, distribuídos principalmente na região Norte do país. No Brasil o Ministério da Agricultura Pecuária e Abastecimento (MAPA, 2019) estima que são 1.390.066 bubalinos, porém esse dado é conflitante com os divulgados pela ABCB (Associação Brasileira dos Criadores de Búfalos) e pela FAO (Organização das Nações Unidas) que relatam a existência de aproximadamente 3 milhões de animais (Bernardes, 2007a; Borghese, 2005).

Os búfalos apresentam temperamento dócil, maior rusticidade, resistência a doenças, maior taxa de fertilidade (~80%) e adaptabilidade ambiental comparado aos bovinos. Desta forma, esses animais possuem desenvolvimento satisfatório em áreas desfavoráveis à pecuária bovina, tornando-se uma alternativa para pequenos produtores (Bernardes, 2006, 2007a). Estas particularidades são provavelmente resquícios da adaptação dos bubalinos aos ambientes onde as populações foram estabelecidas e domesticadas.

No território nacional, são reconhecidas pela ABCB três raças de búfalos do rio (Murrah, Mediterrâneo e Jafarabadi) (*Bubalus bubalis bubalis*) e uma de búfalos de pântano (Carabao) (*Bubalus bubalis carabanensis*) (ABCB, 2020). As variedades de búfalos no Brasil possuem também suas particularidades com relação ao número cromossômico, sendo que animais da raça Carabao possuem $2n=48$ e búfalos de rio $2n=50$.

Dentre os bubalinos mencionados, a raça Murrah é nacionalmente uma das principais produtoras de leite, além de ser amplamente difundida em países da Ásia e Europa (Kumar et al., 2019), fato que a torna economicamente relevante devido à

crescente demanda por leite e seus derivados (Borghese, 2010). De acordo com a Organização das Nações Unidas (FAO, 2019), 15% da produção mundial de leite é oriunda de rebanhos de búfalos, ocupando o segundo lugar entre as demais espécies produtoras, ficando apenas atrás dos bovinos (81%).

A produção total de leite no Brasil em 2019 ocupou a terceira colocação da produção pecuária com aproximadamente R\$ 33 bilhões e 24 bilhões de litros de leite (MAPA, 2019). Embora não se tenha registros do montante referente ao leite bubalino, Bernardes (2007a) estimou que os rebanhos de búfalos produzem aproximadamente 92 milhões de litros de leite por ano, onde 45 milhões são processados e geram 18,5 toneladas de produtos lácteos. Além da conhecida *mozzarella* de búfala, outros produtos oriundos do leite de búfala são: ricota, doce de leite, provolone, iogurte e outros queijos regionais (Bernardes, 2007b).

Em bovinos, práticas visando o melhoramento produtivo são costumeiras, o que contrasta com a realidade dos bubalinos, que ainda não desfruta de uma evolução a níveis produtivos na mesma proporção, isso pode ser devido à seleção artificial ser pouco empregada (Borghese; Mazzi, 2005). Entretanto, a capacidade de produção leiteira da espécie bubalina interessa a indústria de laticínios, pois esta possui maior teor de sólidos totais em comparação ao leite bovino, proporcionando rendimento superior na elaboração de produtos lácteos (Tonhati et al., 2008). Além disso, possuem características benéficas a saúde humana, como por exemplo, maior concentração de proteína no leite e menores taxas de colesterol (Bernardes, 2007a, 2006; Pirondi et al., 2019).

1.3.1. Montagem do genoma Bubalino

O desenvolvimento de novas metodologias de sequenciamento e montagem do genoma de espécies de interesse zootécnicos, como os bubalinos, permitiram a obtenção de informações de milhares de pares de bases, permitindo a associação de regiões genômicas com características de relevância econômica (Furlan et al., 2007). Os métodos de montagem podem ser classificados em duas categorias: genoma-dependente ou genoma-independente (*de novo*), este último foi utilizado pelo consórcio internacional do genoma de Búfalos d'água (*International Water Buffalo*

Genome Consortium), que depositou em 2013 a montagem completa do genoma do búfalo de rio (UMD_CASPUR_WB_2.0) (Williams et al., 2017; Zimin et al., 2013).

A disponibilidade do genoma do búfalo foi fundamental para construção do painel *Axiom Buffalo Genotyping*, para a espécie bubalina, que foi lançado em 2014. Esse painel contém 123.029 sondas capazes de genotipar 90.000 SNP (do inglês, *Single Nucleotide Polymorphism*). Embora o painel possibilitasse a realização de análises genômicas, este apresentava limitações intrínsecas, principalmente devido ao mapeamento dos SNPs ser referenciado no genoma *Bos taurus* (UMD v.3.1) (Iamartino et al., 2017).

Os bovinos e bubalinos compartilham alta similaridade genômica, entretanto, apresentam discrepâncias na organização cromossomal, sendo o número de cromossomos diferentes. Enquanto a espécie bovina tem 29 pares autossômicos (BTA) os búfalos possuem 24 cromossomos autossômicos (BBU). Porém os braços cromossômicos apresentam homologia entre as espécies, de modo que, o BBU1 corresponde aos BTA1 e BTA27; BBU2 equivale aos BTA2 e BTA23; BBU3 aos BTA8 e BTA19; BBU4 aos BTA5 e BTA28 e por fim o BBU5 equivale aos BTA16 e BTA29. Os demais possuem apenas um cromossomo correspondente (Amaral et al., 2008).

Desta forma, a localização dos marcadores com base no genoma bovino não representa a exata posição para o búfalo, dificultando análises que requerem o posicionamento dos marcadores, como assinaturas de seleção, corridas de homozigose (ROH, do inglês, *Runs of homozygosity*) e estudos de associação genômica ampla (GWAS).

A fim de contornar problemas com a localização de marcadores, em 2019 foi publicado uma nova montagem do genoma bubalino (UOA_WB_1), que possui 25 cromossomos e tamanho de 2,66 Gb. Embora o novo *assembly* não seja livre de lacunas, a utilização do sequenciamento de longas *reads* (*PacBio*) possibilitou uma maior cobertura. De modo que, 19 regiões com tamanho superior a 8 kb, que não haviam sido detectadas pelo sequenciamento de *reads* curtas realizado anteriormente foram identificadas (Low et al., 2019).

1.3.2. Assinatura de Seleção

Alterações na morfologia, fisiologia e hábitos de uma espécie são reflexos da seleção natural e domesticação durante várias gerações, podendo estas mudanças serem acentuadas com emprego da seleção artificial (Driscoll et al., 2009; Flori et al., 2009). Tais processos atuam modificando a composição genética de uma população, fazendo com que extensões específicas do genoma sejam alteradas e apresentem padrões diferentes do restante do DNA, sendo, estas localidades conhecidas como assinaturas de seleção (AS).

Estudos com AS têm permitido compreender os fundamentos genéticos responsáveis pelas diferenças fenotípicas entre espécies/raças (Qanbari; Simianer, 2014). Atualmente, as metodologias empregadas para identificação de AS têm basicamente duas vertentes, uma para detecção dentro de populações e outra para comparação entre populações distintas.

As novas tecnologias de genotipagem possibilitaram a obtenção de alta densidade de informações de polimorfismos, o que proporcionou maior cobertura do genoma por reduzir o espaçamento entre marcadores. O surgimento de ferramentas genômicas destinadas a animais de interesse zootécnico permitiu detectar assinaturas de seleção nestas espécies (Flori et al., 2009; Ma et al., 2015; Rubin et al., 2010).

Atualmente é possível identificar regiões genômicas sob seleção em búfalos com aproximadamente 90.000 SNPs. Esta densidade é provavelmente suficiente para captar regiões em seleção, pois em comparativo com bovinos que apresenta tamanho do genoma semelhante, a densidade de cerca de 50.000 marcadores foi suficiente para identificar AS (Flori et al., 2009; Gautier; Naves, 2011; Qanbari et al., 2010).

Alguns métodos de análise de AS buscam extensões genômicas que se apresentem em autozigosidade para grande parte dos indivíduos de uma população, ou seja, captam a redução de diversidade genética em determinadas localidades. Segmentos em autozigosidade compreendem uma extensa parcela do DNA que apresenta marcadores consecutivos em homozigose, onde possivelmente esse fragmento foi herdado de um ancestral comum (Gibson et al., 2006).

A distribuição das ROHs no genoma não é uniforme em uma população, podendo assim, serem mais frequentes em determinadas regiões, denominadas ilhas de ROH (Nothnagel et al., 2010; Pemberton et al., 2012). Assim, a utilização de ilhas

de ROH podem identificar AS, devido à redução de diversidade genética em localidades genômicas específicas, afetadas pela seleção (Kim et al., 2013; Zavarez et al., 2015; Zhang et al., 2015).

Adicionalmente, a proporção do genoma inserido em ROHs em relação ao tamanho total do genoma representa o coeficiente de endogamia baseado em informações genômicas (F_{ROH}) (Ferenčaković et al., 2013; McQuillan et al., 2008). A correlação entre o F_{ROH} e o coeficiente de endogamia a partir do pedigree em bovinos se mostra alta (>0.6), corroborando para o uso da informação genômica na estimação deste coeficiente (Ferencakovic et al., 2011; Purfield et al., 2012).

Outro método estatístico capaz de detectar assinaturas de seleção dentro de populações é o escore de integração haplotípica (iHS, do inglês *Integrated Haplotype Score*). O iHS é derivado da metodologia de homozigose do haplótipo estendido (EHH, do inglês *Extended Haplotype Homozygosity*) proposta por Sabeti et al. (2002), que corresponde à probabilidade de que dois haplótipos longos com um mesmo núcleo, selecionados aleatoriamente em uma população, sejam iguais. Para o cálculo da estatística iHS, cada alelo de um SNP é considerado como núcleo e tem valores de EHH estimados considerando diferentes extensões haplotípicas. Os padrões de decaimento de EHH em torno de cada alelo servem para identificar assinaturas de seleção, dado que um alelo selecionado deve apresentar alta frequência, mantendo altos valores de EHH por longas distâncias.

O diferencial destes métodos de análise (ilhas de ROH e iHS) está na capacidade de identificação de assinaturas de seleção de momentos cronológicos distintos, onde as ilhas de ROH são capazes de captar AS de origem antiga, enquanto o iHS capta eventos de seleção em curso.

Vale lembrar que até o presente momento, são escassos os estudos utilizando ferramentas genômicas destinados à detecção de assinaturas de seleção em búfalos. Refletindo a carência de pesquisas para esta espécie relevante para a produção de alimentos.

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CHAPTER 2 - Updating the Axiom buffalo genotyping array map and mapping cattle quantitative trait loci to the new water buffalo genome

ABSTRACT - The objectives of this study were to get the mapping information of SNP markers with unknown position on Axiom Buffalo Genotyping 90k array (ABG array), using the updated map to identify signatures of selection in Murrah buffaloes and map all the cattle QTL from cattle QTL database to a buffalo assembly. For this purpose, all SNPs probe sequences from ABG array were re-aligned against the UOA_WB_1 assembly using BLAST. After checking for compatibility between the map based on our alignments and the ABG array map, we combined them to generate a new map. The intersect of SNPs with map positions in both the ABG array and our map was 93,334. The number of mapped markers increased from 106,778 in the ABG array map to 116,708 on the new map. Signatures of autozygosity were then identified using both the new map and ABG map, based on genomic information from 865 Murrah buffaloes. The results show that there was an intensification of peaks of autozygosity and appearance of new peaks in BBU5 and BBU11 when all markers in the new map were used. Finally, the cattle QTL annotated on the UMD 3.1 assembly were aligned to *Bubalus bubalis bubalis* genome assembly (UOA_WB_1). After alignment and quality control, 63,995 cattle QTL were successfully mapped to the buffalo genome. Thus, we demonstrated that our new map can improve results of genomic analyses since the new map increased the marker density by 9,930 SNPs and reduced the average marker spacing by ~2kb. Furthermore, the mapping of the bovine QTL database to the buffalo genome will be useful for genome-wide association studies in buffalo. Considering homology between cattle and buffalo genome, the buffalo coordinates of cattle QTL can be regarded as potential QTL in buffalo, which may lead to the development of the first buffalo QTL database.

Keywords: QTL map, SNP map, water buffaloes.

2.1. Introduction

When the Axiom Buffalo Genotyping 90k array (ABG) (Thermo Fisher Scientific, Santa Clara, CA) was initially released, a buffalo reference genome was not assembled on chromosomes and the first version of the markers map had its genomic

positions based on the bovine genome reference, UMD 3.1 (Zimin et al., 2009). This was possible due to the homology between the genomes of these species, but cattle have 29 autosomal chromosomes while buffaloes 24 (Amaral et al., 2008). However chromosomal incompatibility was an obstacle to genomic analyzes dependent on the physical position of the markers, such as signatures of selection and genome wide-association study (GWAS), since cattle have 29 autosomal chromosomes while buffaloes have 24.

Following the newly released assembly of the buffalo genome (UOA_WB_1) in 2018, an updated map with the SNPs distributed on 25 buffalo chromosomes was also released (Low et al., 2019). This significant update provided marker positions on the correct species genome. However, it contained a large number of SNPs with unknown position (16,262), making difficult to link genomic regions with known quantitative trait loci (QTL) annotated to the cattle genome.

The massive coverage of the genome by markers is essential for genomic studies, so the greater number of available SNPs increases the genomic coverage due to the reduction of the distance between markers. Another question is about the QTL databases, they are important for research and comparison of studies carried out with different populations and statistical methods, thus contributing to the location of promising regions related to complex traits (Hu et al., 2005).

The objectives of this study were to map buffalo markers without a known position on the ABG SNP map, comparing the results of signatures of selection study based on our new map versus the conventional map, and align the QTL reported for cattle on Cattle QTL database to the buffalo genome to create a water buffalo QTL map file.

2.2. Material and Methods

2.2.1. Mapping unmapped ABG SNP markers to buffalo genome

Figure 1 shows the positions of the SNPs in the different versions of the ABG maps, firstly bovine (UMD3.1) and lately buffalo (UOA_WB_1). It is possible to observe that there is a great number of markers with a known position on the bovine map but unmapped on the buffalo map (zero on the X-axis).

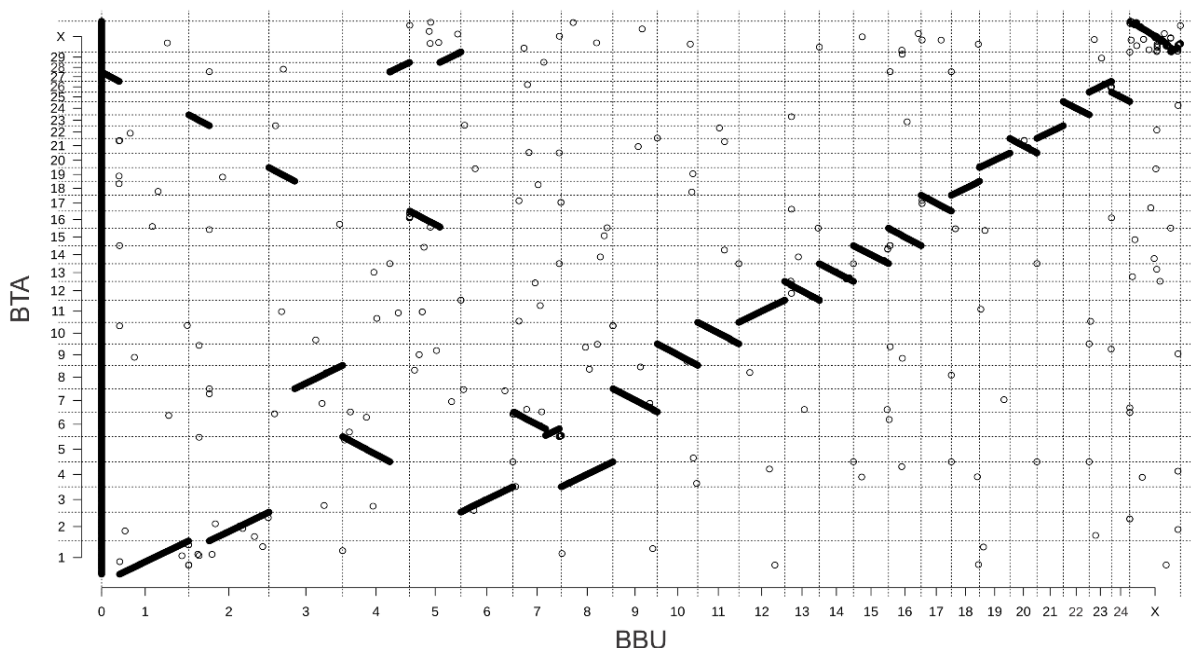


Figure 1. Marker positions on each Axiom Buffalo Genotyping 90k map. On the Y axis we have the positions of the SNPs on the bovine genome (UMD 3.1) and on the X axis the new version of the map on the buffalo genome (UOA_WB_1).

We sought to map these unmapped markers by using their probe sequences, which were 72 base pairs long. To do this, all SNPs probe sequences (123,040) were re-aligned against the buffalo assembly (UOA_WB_1) using BLAST (Madden, 2013) with highly similar sequences parameter i.e. megablast.

Three quality control (QC) criteria were applied on the BLAST results. There were kept: 1) the aligned sequences that had a length of at least 36bp (one half of the total probe length), 2) base pair identity greater than 90%, and 3) the lowest e-value. Aligned probes with duplicate e-value were aligned to the latest bovine genome (ARS-UCD1.2 assembly) (Rosen et al., 2020). Thus, when a sequence aligned with the same minimum e-value in more than one region, only the one that also aligned on a homologous bovine chromosome was considered. In this way, information on homology between bovine and buffalo chromosomes was used to identify the correct position of the probe (Amaral et al., 2008).

In the end, we merged our map with the Axiom Buffalo Genotyping 90k array map to get a new whole map that contained the buffalo coordinates of a greater number

of markers. While merging, if there was a difference between map positions, the conventional one (from ABG array) was maintained.

2.2.2. Autozygosity estimates with a new and conventional map

The New map was used in a selection signature scanning based on runs of homozygosity (ROH), in order to observe the impact on the results in selection signatures study. For this purpose, 865 Murrah dairy buffaloes genotyped with Axiom Buffalo Genotyping 90k array were used. Quality control for the genomic data was applied using Plink v.1.9 (Chang et al., 2015), where non-autosomal, duplicated and with $\text{SNP}_{\text{call rate}} < 0.95$ markers were removed, as well as samples with $\text{Sample}_{\text{call rate}} < 0.9$.

The following parameters were used in the ROH detection: homozygous length greater than 1Mb; minimum of 30 SNPs in homozygosity; on average at least 1 SNP per 100 kb; no gaps longer than 500 kb; and tolerance of 1 heterozygous locus, as described by Zavarez et al., (2015). This procedure was also performed using Plink software.

Autozygosity coefficients were estimated for each SNP, as the proportion of the population which have an ROH comprising that SNP, following Kim et al., (2013):

$$F_{\text{SNP}} = \frac{\sum_{i=1}^N hm_i}{N}$$

where F_{SNP} is the autozygosity of each SNP; hm_i is locus autozygosity status (1 or 0, i.e. in an ROH or not) of i^{th} individual, and N is the total number of individuals.

2.2.3. Mapping cattle QTL to buffalo assembly

A total of 130,407 cattle QTL annotated on the UMD_3.1 assembly were downloaded in the CattleQTLdb r.40 (Hu et al., 2013). Quality control filtering was applied to the data to remove QTL without a position, with a starting position equal to 0, duplicated, and longer than 100bp. This step removed 35,966 QTL (27.5%), and 94,441 QTL remained (50,547 sequences) for the alignment process. Since the most common QTL length in CattleQTL database is 40bp (91.5%), we adjusted the size of windows expanding to both sides until reaching 40bp for 51 short QTL (1-21bp).

The sequences data for *Bos primigenious taurus* (UMD 3.1) and *Bubalus bubalis bubalis* (UOA_WB_1) genomes available on NCBI (NCBI Resource Coordinators, 2018) were used. The BedTools “getfasta” function (Quinlan; Hall, 2010) was applied to obtain the FASTA sequences of each QTL in the bovine genome. After that, all sequences were aligned with the buffalo genome using the Burrows-Wheeler Aligner (BWA) v0.7.17 (Li; Durbin, 2009). Sequences with non-unique alignments and a probability of being wrongly mapped greater than 1% were removed. Afterward, 63,995 (33,160 sequences) cattle QTL were successfully mapped on the buffalo genome (Table 1). This is approximately 50% of the total number of QTL in the CattleQTLdb. Finally, the file was organized by a custom script in R (R Development Core Team, 2013), containing the following information: location in the buffalo genome (chromosome, start and end position), location in the bovine genome, QTL trait, Class of trait (Exterior, Health, Meat and Carcass, Milk, Production and Reproduction), number of QTL in the region, QTL_ID and PubMed_ID.

2.3. Results

2.3.1. Mapping unmapped ABG SNP markers to buffalo genome

A total of 103,264 out of 123,040 SNP probes were successfully mapped to unique locations on the buffalo genome. From these, 9,930 SNPs did not have a position in the ABG map. Therefore, the number of common SNPs with map positions in both the ABG and our map was 93,334. The majority of these, mapped to the exact same position as ABG map (90,928 [97.42%]), whereas 2,389 SNPs (2.56%) mapped to the same chromosome but at a different base pair position (only 16 were more than 5bp apart), and only 17 SNPs (0.02%) mapped to a different chromosome.

The results show that the alignments were comparable to the ABG map and it was able to successfully map the previously unmapped SNP markers. In this way, the number of mapped markers increased from 106,778 (ABG map) to 116,708 in the new map (Table 1 and Figure 2). This led to an increase in marker density by 9,930 SNPs and reduced the average marker spacing by ~2kb. The whole New Map is available in the supplementary material (Supplementary File 1).

Table 1. Number of SNPs with known position and QTL per chromosome for the buffalo genome.

BBU	N. of SNPs (ABG)	N. of SNPs (New Map)	N. de QTL	BBU	N. of SNPs (ABG)	N. of SNPs (New Map)	N. of QTL
1	8,638	9,167	2,275	14	3,364	3,756	1,361
2	7,921	8,463	2,185	15	3,477	3,834	3,239
3	7,304	7,862	1,568	16	3,229	3,655	651
4	6,602	7,344	3,558	17	2,986	3,309	2,111
5	5,104	5,651	1,215	18	2,786	2,972	983
6	5,136	5,424	1,539	19	3,012	3,117	1,906
7	4,809	5,258	7,441	20	2,673	3,015	1,320
8	5,090	5,345	3,390	21	2,602	2,698	534
9	4,401	4,909	1,456	22	2,565	2,832	397
10	3,998	4,522	798	23	2,215	2,324	2,050
11	4,069	4,564	1,199	24	1,803	2,015	1,548
12	4,538	4,777	1,997	X	5,017	5,942	18,708
13	3,439	3,953	566	Total	106,778	116,708	63,995

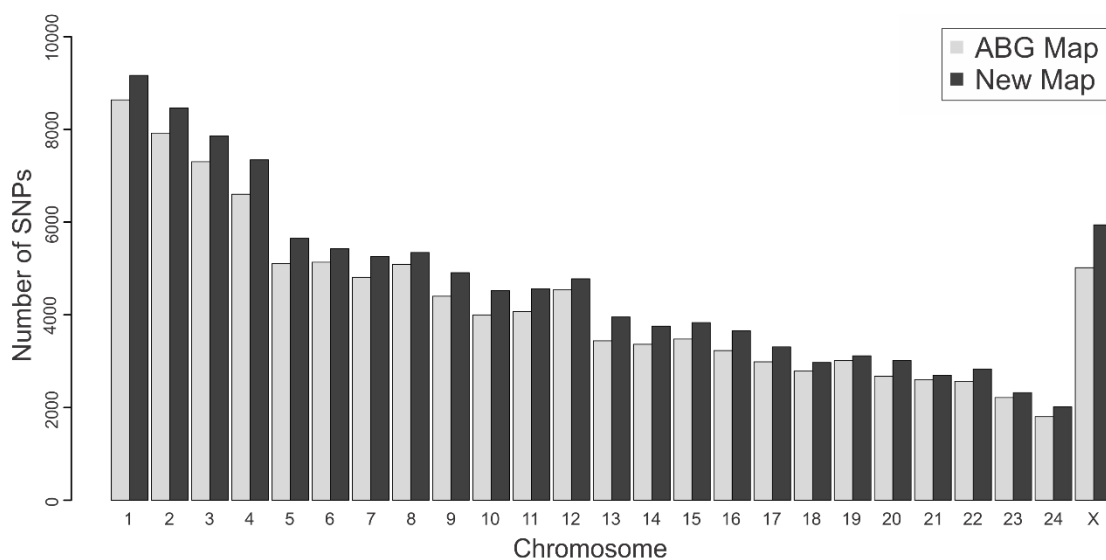


Figure 2. Number of SNPs per chromosomes and per map.

2.3.2. Selection signatures with a new and conventional map

The markers included to create the New map (9,930) had MAF values of varying from zero to 0.5 with an average of 0.17.

The use of the New map improved the results of the ROH analysis through the *Bubalus bubalis* chromosome (BBU). The inclusion of additional markers (4,039 SNPs) provided a greater number of ROHs (increase of 4,041 ROHs) and, consequently, a greater number of genomic regions in autozygosity. We can highlight the BBU1, BBU3, BBU9, BBU16, and BBU18, where there was an intensification of peaks of autozygosity and the appearance of new peaks in BBU5 and BBU11 (Figure 3).

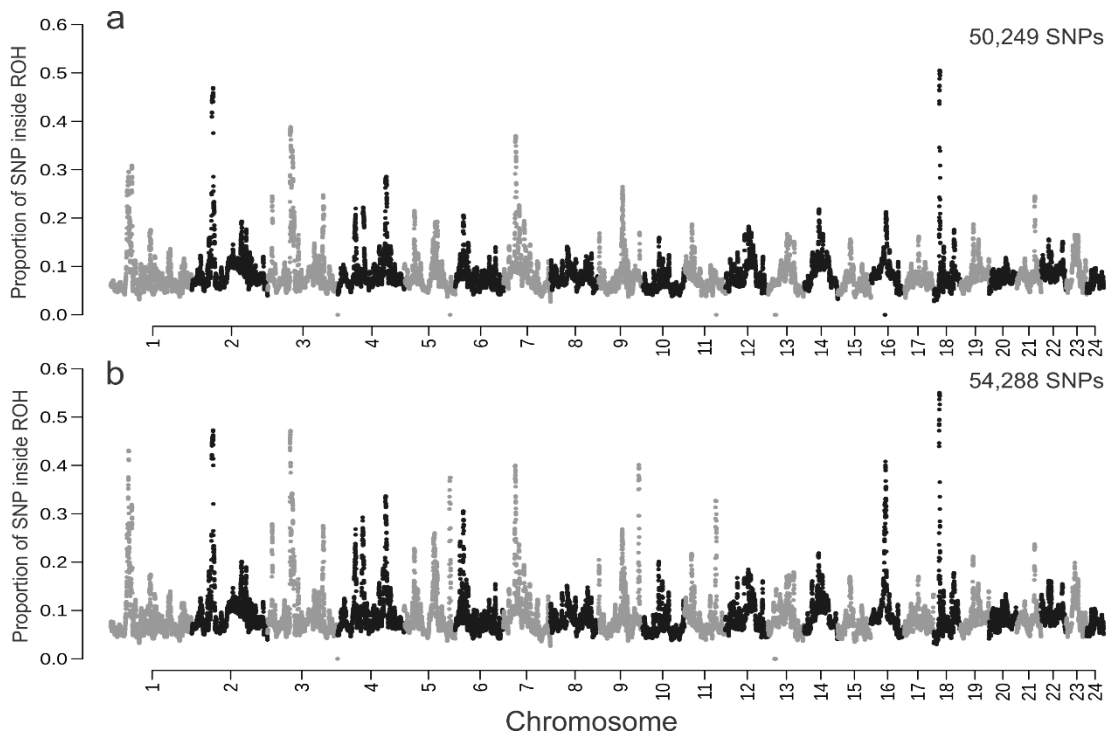


Figure 3. Manhattan plot of genome-wide SNP autozygosity in Murrah buffaloes. (a) results obtained for Axiom Buffalo Genotyping 90k array map and (b) for New map.

2.3.3. Mapping cattle QTL to buffalo assembly

Figure 4 shows the QTL density for each buffalo chromosome, where the highest number of QTL was observed in BBUX (18,708), followed by BBU7 with 7,441. Trait classes with the highest number of QTL were respectively: Reproduction (27,617), milk (20,806), production (6,856), exterior (5,869), health (1,548), and meat

and carcass (1,299). The complete Water Buffalo QTL map file is available in the Supplementary File 2.

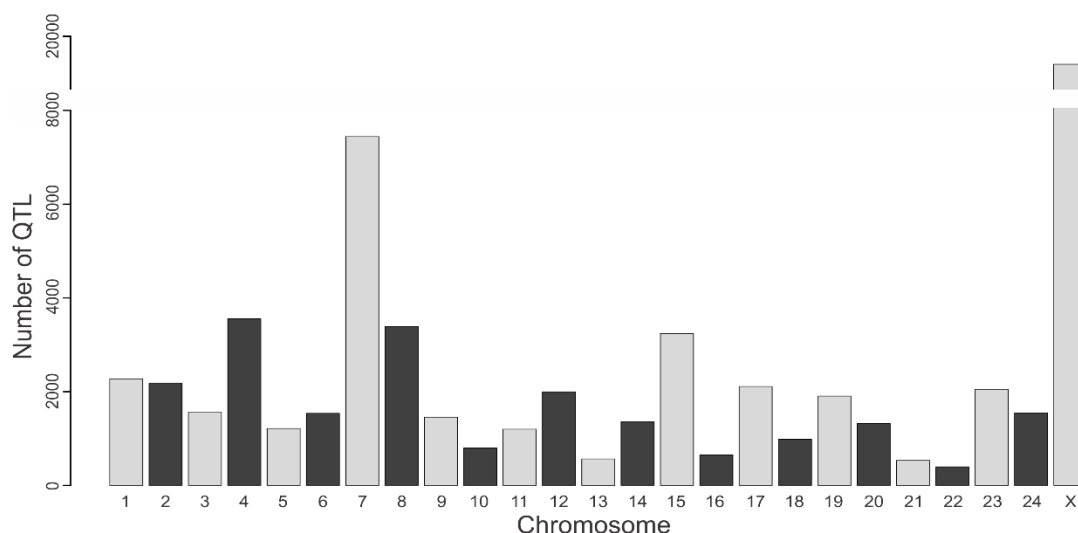


Figure 4. Number of QTL per chromosomes.

2.4. Conclusion

We successfully mapped the unmapped Axiom Buffalo Genotyping 90k array SNP markers, which allowed us to identify two novel ROH regions in a buffalo population of Murrah breed. This implies that using our updated map with improved coverage and a larger number of markers with mapping information can provide better resolution in future buffaloes genomic researches.

We provided the buffalo coordinates of cattle QTL identified in the literature. Considering homology between cattle and buffalo, these coordinates can be considered as a database of putative buffalo QTL. This alignment of the bovine QTL database should be useful for association studies in buffalo and is a first step towards establishing a water buffalo QTL database. Additional researches based on buffalo map locations may help validate these QTL.

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2.6. Supplementary Material

Due to the extension of the marker and QTL maps, they will be available in separate files from the text. The links of the files provided here are for exclusive use for thesis defense, as they will be provided in the future when the work is published in a scientific journal.

Arquivo Suplementar 1. New map marker file with positions determined for the buffalo genome. Available in <
<https://www.dropbox.com/s/6pdhfhhy699g44/Arquivo%20Suplementar%201.txt?dl=0>>.

Arquivo Suplementar 2. QTL map file with genomic coordinates for the buffalo. Available in <
<https://www.dropbox.com/s/5sxzgmfiygz08ow/Arquivo%20Suplementar%202.txt?dl=0>>.

CHAPTER 3 - Inbreeding coefficients and runs of homozygosity islands in Brazilian water buffalos

ABSTRACT - Characterization of autozygosity is relevant to monitor genetic diversity and manage inbreeding levels in breeding programs. Identification of autozygosity hotspots can unravel genomic regions targeted by selection for economically important traits and can help identify candidate genes for selection. In this study we estimated the inbreeding levels of a Brazilian population of Murrah buffaloes undergoing selection for milk production traits, particularly milk yield. We also studied the distribution of runs of homozygosity (ROH) islands and identified putative genes and QTL (Quantitative Trait Loci) under selection. A total of 422 Murrah buffalos were genotyped for 51,611 single nucleotide polymorphisms; 350 of these had runs of homozygosity longer than 10 Mb, indicating the occurrence of inbreeding in the last 5 generations. The mean length of ROH per animal was 4.28 ± 1.85 Mb. Inbreeding coefficients were calculated from the pedigree, genomic relationship matrix, and ROH with estimates varying between 0.242 and 0.035. Inbreeding estimates on pedigree had a low correlation with the genomic estimates. Estimates from the genomic relationship matrix were much higher than those from pedigree or the ROH. Signatures of selection were identified in six genomic regions located on chromosomes 1, 2, 3, 5, 16 and 18 encompassing a total of 190 genes and 174 QTL. Many of the genes (e.g. *APRT* and *ACSF3*) and QTL identified are related to milk production traits, such as milk yield, milk fat yield/percentage and milk protein yield/percentage. Other genes are associated with reproduction and immune response traits as well as morphological aspects of buffalo species. Inbreeding levels in this population are still low but it is increasing due to selection and should be managed to avoid future losses due to inbreeding depression. The proximity of genes linked to milk production traits with genes associated with reproduction and immune system traits suggests the need to include them in the breeding program to avoid negatively impacting them due to selection for the production traits.

Keywords: *Bubalus bubalis*, dairy, Murrah, ROH

3.1. Introduction

The inbreeding coefficient is defined as the probability that a pair of alleles present at a given locus are identical by descent (IBD) (Wright, 1922). Homozygous loci combining two identical by descent alleles are referred to as autozygous. The inbreeding coefficient (F) is commonly used to describe the proportion of autozygosity in the genome of an individual, and it can be estimated from either pedigree (F_{PED}) or genomic data (Ferenčaković et al., 2013a). Characterizing and monitoring autozygosity levels in livestock is relevant to help preserve genetic diversity, ensure long-term sustainability of breeds and for breeding program management decisions (e.g. to reduce incidence of homozygous recessives or mitigate the effects of inbreeding depression) (Zavarez et al., 2015).

The F_{PED} represents the expected value of an individual's inbreeding. A common difficulty to accurately estimate F_{PED} are that pedigree records are often inaccurate, incomplete or shallow with few generations. Other model assumptions needed to calculate F_{PED} may not hold true and can lead to inaccurate results. For example, the assumption that founder animals are unrelated – this is particularly problematic in shallow pedigrees; or that the loci are neutral, which effectively ignores the stochastic effects of recombination and selection (Forutan et al., 2018). An alternative approach to estimate the inbreeding coefficient is through runs of homozygosity (ROH), which are tracts of DNA composed of consecutive homozygous markers (Gibson et al., 2006). ROH can provide reliable estimates of genomic autozygosity (Iacolina et al., 2016) while taking into account stochastic autozygosity variation at the individual level (McQuillan et al., 2008). Through this approach the genomic inbreeding coefficient (F_{ROH}) can be estimated, which is the proportion of the total genome contained within ROH regions (Ferenčaković et al., 2013a; McQuillan et al., 2008).

ROH are not uniformly distributed across the genome of a population and may be more frequent in some regions than in others. These regions with higher frequencies in ROH are referred to as ROH islands (Nothnagel et al., 2010; Pemberton et al., 2012) and are used to identify autozygotic loci. The accumulation of ROH in certain genomic regions is probably due to directional selection pressures which reduce genetic diversity in these regions. This makes the ROH islands particularly informative for the detection of genomic regions under selection (Bertolini et al., 2018;

Kim et al., 2013). ROH islands present in most individuals of a population provide evidence of reduced genetic diversity in these regions that probably resulted from selection events (Kim et al., 2013; Zavarez et al., 2015; Zhang et al., 2015). The identification of regions under selection provides information about the genetic history of a population, such as its origin and migration history. Additionally, in livestock, it is particularly relevant for the detection of genetic variants that influence traits of economic interest (Cardoso et al., 2018; Colli et al., 2018; Mokhber et al., 2018).

This work aims to characterize the distribution of ROH and autozygosity levels in a Brazilian population of Murrah buffalos as well as to identify genomic regions under selection for production traits related to milk yield (MY).

3.2. Material and Methods

3.2.1. Samples and Genotypic Information

The sample group consisted of 422 Murrah dairy buffalos genotyped using the Affymetrix Axiom® Buffalo Genotyping Array (90k SNP).

The marker positions for this array were originally based on the bovine genome assembly but were recently updated to the map coordinates of the newly released buffalo assembly (UOA WB v.1). However, after the update, 16,262 SNP had undefined chromosomal locations on the new map (in comparison, the previous map based on the bovine map had only 48 SNP with unassigned positions). To recover some of these lost markers, the flanking sequences (71 bp) for the unknown markers were aligned with the UOA WB v.1 assembly using BLAST (Basic Local Alignment Search Tool) (Zhang et al., 2000). The following parameters were used to determine the physical positions of the markers: 1) alignment length > 36 bp; 2) initial position < 35 bp < final position; 3) identity > 90%; 4) lowest single e-value. The positions of 9,995 SNP, around 61% of the missing SNP, were recovered by this alignment step and were included in subsequent analyzes.

Quality control of genotypes was performed using the Plink v.1.9 software (Chang et al., 2015), non-autosomal or duplicated markers were removed as well as markers with call rates lower than 0.95. All genotyped animals had a call rate higher than 0.90 and passed quality control.

3.2.2. Identification of ROH

ROH were calculated individually for each buffalo. To be considered a ROH, a genomic region had to have a homozygous length greater than 1Mb; a minimum of 30 SNP in homozygosity; at least 1 SNP per 100 kb; maximum interval between two consecutive markers of up to 500 kb; and a maximum of 1 heterozygous locus in the segment as described by Zavarez et al., (2015). The procedure was also performed using Plink and results stored for downstream analyses in a binary matrix of sample by SNP, where one represents a SNP that is part of an ROH for that particular sample, and zero represents a SNP that is not in a ROH.

3.2.3. Estimation of Inbreeding Coefficients

Genomic autozygosity was calculated as the proportion of the genome included in ROH regions according to the following formula:

$$F_{ROH} = \frac{\sum_{j=1}^n L_{ROH_j}}{L_{Total}}$$

where L_{ROH_j} is the length of the j^{th} ROH; and L_{Total} is the sum of the length of the autosomal chromosomes covered by markers on the chip (2.47 Gb – sum of the number of base pairs between the first and the last SNP on each chromosome) (McQuillan et al., 2008).

The F_{ROH} coefficient of each animal was estimated for 4 ROH classes according to minimum lengths of 1, 4, 8 and 16 Mb (F_{ROH1} , F_{ROH4} , F_{ROH8} , and F_{ROH16} , respectively), representing cumulative autozygosity from events that happened 50, 13, 6 and 3 generations ago, respectively (Ferenčaković et al., 2013b; Zavarez et al., 2015). Another alternative measurement of genomic inbreeding was obtained by subtracting 1 from the diagonal elements of the genomic relationship matrix (GRM) estimated using a base allele frequency of 0.5 (F_{GRM}) (VanRaden, 2008; VanRaden et al., 2011). In addition, F_{PED} was estimated from the pedigree, which contained records of 20,093 animals across 15 generations; F_{PED} inbreeding per generation was also calculated.

3.2.4. Identification of Signatures of Selection

ROH islands were identified from the estimated autozygosity coefficient for each marker. This coefficient is the proportion of individuals in which each SNP is contained in a ROH, and it was calculated according to the following formula proposed by Kim et al., (2013):

$$F_L = \frac{\sum_{i=1}^N hm_i}{N}$$

where F_L is the autozygosity of each SNP; hm_i is the status of autozygosity (1 or 0, i.e. in a ROH or not) of the locus in the i^{th} individual, and N is the total number of individuals.

The thresholds used in this type of analysis are not well defined in the literature, where it is adopted: top 0.01~2.5% SNPs (Avila et al., 2018; Gurgul et al., 2019; Howard et al., 2015; O'brien et al., 2014), autozygosity values greater than 0.45~0.5 (Xu et al., 2019; Zavarez et al., 2015) or standard deviations (Maiorano et al., 2018). Seeking an intermediate threshold, SNP with autozygosity coefficient values greater than 8 standard deviations (P -value = $8.6e^{-6}$) from the mean were considered as signatures of selection.

3.2.5. Exploring Regions in Signatures of Selection

From the center of each ROH island identified, 1 Mb windows were constructed irrespective of island length. Annotated genes close to these regions were extracted from *NCBI's* (National Center for Biotechnology Information) annotation r.101 (NCBI Resource Coordinators, 2018) databases based on the UOA WB v1 assembly of the buffalo genome. BLAST was used to align the signatures of selection regions (see Supplementary Table S1) to the bovine assembly UMD v3.1 to obtain the bovine genome homologs and to identify overlapping QTL described in the *CattleQTLdb* r.38. (Hu et al., 2013).

3.3. Results

3.3.1. Inbreeding

After quality control, 422 samples and 51,611 SNP remained. Results showed that ROH had a minimum, maximum, average and median length equal to 1.00, 129.63, 4.47 ± 6.53 and 2.13 Mb, respectively. Additionally, 350 (83%) animals had at least one segment longer than 10 Mb, indicating the occurrence of inbreeding in the last 5 generations (Howrigan et al., 2011; Zavarez et al., 2015).

The total ROH of 16,900 (>1 Mb) was observed in the population, where 4,316 (25.5%), 2,215 (13.1%) and 871 (5.1%) were the numbers of ROHs with sizes greater than 4, 8 and 16 Mb respectively. Thus, ROHs of 1-4Mb were the most frequent in the population, representing 74.5% (12,584).

The animal with the lowest autozygosity (1.21%) had 13 ROH regions longer than 1 Mb, totaling close to 30 Mb, with segments ranging from 1.12 to 3.36 Mb, and mean of 2.31 ± 0.71 Mb. The most inbred individual (37.2%) had 60 ROH regions for a total autozygosity of 920.82 Mb, with segments varying between 1.47 and 93.46 Mb and a mean of 15.35 ± 21.45 Mb. Overall, the population had 40.05 ± 10.39 ROH on average, and 4.28 ± 1.85 Mb mean length per animal ($7.23 \pm 4.33\%$ of the autosomal genome in autozygosity). Figure 1 shows the variation between animals in total number of ROH and percentage of autozygosity.

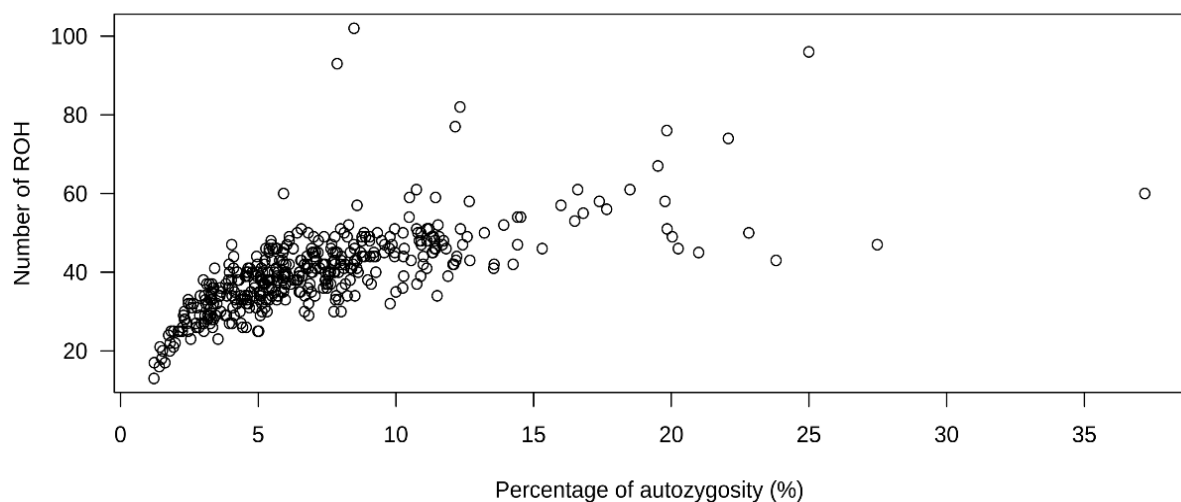


Figure 1. Number of ROH per animal and percentage of the genome in autozygosity.

Table 1 shows the descriptive statistics for the different methodologies used to estimate inbreeding coefficients (F). The F averages estimates per generation are

shown in Supplementary Table S2, where it is possible to observe that an increasing trend occurs over the generations. Pearson correlations among the inbreeding coefficients estimated by different approaches ranged from 0.307 to 0.991 (Figure 2). Results from map dependent (F_{ROH}) and independent (F_{GRM}) methods were highly correlated (> 0.831). However, these estimates were moderate, ranging from 0.307 to 0.41, between the genomic coefficients (F_{ROH} and F_{GRM}) and F_{PED} .

Table 1. Descriptive statistics for estimates of inbreeding coefficients (F).

	F_{ROH1}	F_{ROH4}	F_{ROH8}	F_{ROH16}	F_{GRM}	F_{PED}
Min	0.012	0.000	0.000	0.000	0.125	0.000
Max	0.372	0.348	0.334	0.288	0.477	0.312
Mean	0.072	0.048	0.037	0.022	0.242	0.035
SD	0.043	0.042	0.038	0.031	0.037	0.048

*Classes F_{ROH1} , F_{ROH4} , F_{ROH8} and F_{ROH16} correspond, respectively, to the inbreeding coefficient estimated for ROH with 1, 4, 8 and 16 Mb minimum length. F_{GRM} and F_{PED} are the inbreeding coefficients obtained from the genomic relationship matrix and pedigree, respectively.

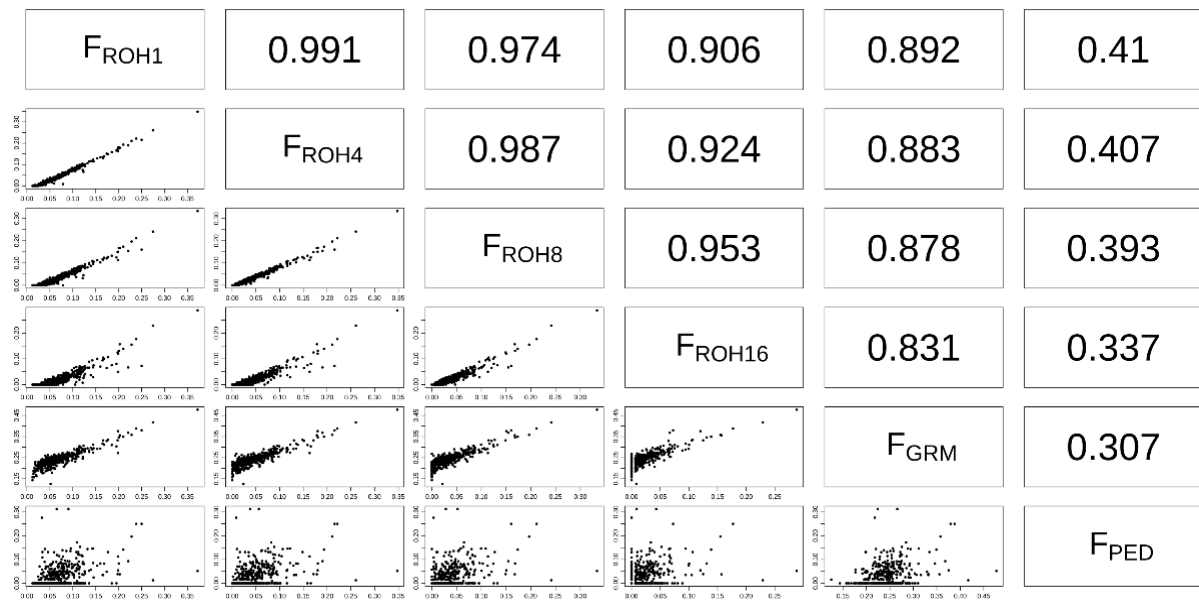


Figure 2. Scatterplots (below the diagonal) and inbreeding coefficient correlations (above the diagonal) estimated for the different approaches.

3.3.2. Signatures of Selection

The analysis of autozygosity levels identified ROH hotspots in six chromosomes (Figure 3): BBU1, BBU2, BBU3, BBU5, BBU16 and BBU18 (BBU for *Bubalus bubalis* chromosome). These regions of selection signatures contained 190 genes (Table 2 and Supplementary Table S3) and 174 bovine QTL (see Supplementary Table S4), including some previously associated with milk yield and milk quality traits, as well as some genes linked to morphological traits.

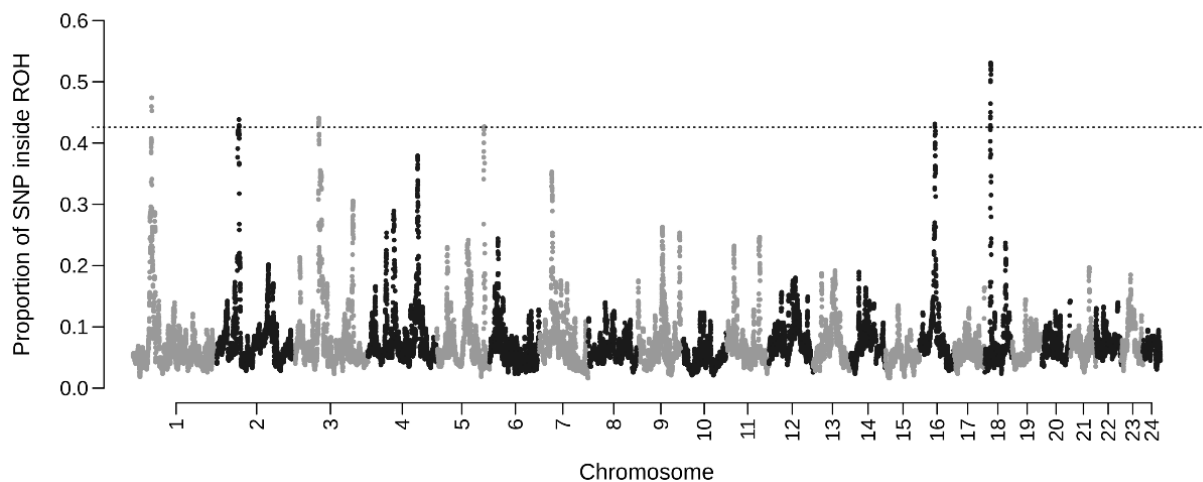


Figure 3. Manhattan plot of genome-wide SNP autozygosity in Murrah buffalos. The dashed line represents the 8 standard deviations threshold.

Table 2. Summary of genes related to phenotypes of economic impact on livestock present in the 1 Mb window from the center of the selection signatures.

BBU	Window position (start-end [bp])	Genes
1	44,484,231-45,484,231	-
2	52,813,756-53,813,756	<i>HERC2, OCA2</i>
3	56,617,686-57,617,686	-
5	114,902,856-115,902,856	<i>PAG4, PAG13, PAG16, PAG17, PAG18</i>
16	36,871,376-37,871,376	-
18	13,611,038-14,611,038	<i>ACSF3, ANKRD11, APRT, CBFA2T3, CDH15, CDK10, CDT1, CHMP1A, CPNE7, DBNDD1, DEF8, DPEP1, FANCA, GALNS, GAS8, MC1R, RPL13, SHCBP1, ORC6, PABPN1L, PIEZO1, RPL13, SHCBP1, SPATA2L, SPATA33, SPIRE2, TCF25, VPS9D1, ZNF276</i>

A gene rich region detected in BBU18 comprises the *ANKRD11*, *DBNDD1*, *FANCA* and *ZNF276* genes, which were also found in other signatures of selection studies of different dairy and beef cattle breeds (Ramey et al., 2013; Zhao et al., 2015). Some of the genes in this region, such as *ANKRD11*, *CBFA2T3*, *CDT1*, *CDH15*, *CHMP1A*, *CPNE7*, *DPEP1*, *DBNDD1*, *DEF8*, *FANCA*, *GALNS*, *ORC6*, *PABPN1L*, *PIEZO1*, *RPL13*, *SHCBP1*, *SPATA2L* and *SPIRE2* have been correlated with mammary gland health and with the immune response trigger to mastitis in cattle (Chen et al., 2015; Ibeagha-Awemu et al., 2016). *ACSF3* and *APRT* genes have been associated with differentiation of the mammary cells and milk fat content (Cánovas et al., 2013; Choudhary et al., 2018). Genes *SPATA33* and *SPIRE2* are related to the meiotic process in mammalian germ cells, impacting the fertility of animals of both sexes (Chen et al., 2013; Pfender et al., 2011). The *PAG* (pregnancy-associated glycoprotein) gene family in BBU5 is related to placentation in ruminants (Santos et al., 2018). In addition to production traits, genes associated with skin pigmentation were also found: *HERC2*, *OCA2*, *CDK10*, *GAS8*, *MC1R*, *TCF25*, *RPL13*, *DBNDD1* and *VPS9D1* (Branicki et al., 2009; Dalziel et al., 2010; Li et al., 2014; Makina et al., 2015; Nigenda-Morales et al., 2018).

The regions of BBU1, BBU3 and BBU16 contain 108 genes, although only 11 currently are annotated and the remaining are denominated as LOC#. However, the corresponding regions in the bovine genome contain 48 reported QTL that are related to reproductive and milk yield traits (Supplementary Table S4). Out of the 174 QTL found in the autozygosity hotspots, approximately, 40% were closely linked to traits important to the dairy industry, such as milk components and yield, reproduction and health (somatic cell score and clinical mastitis) (Table 3).

Table 3. Summary of the QTL related to milk production, health and reproductive traits that were located within 1Mb windows of the ROH islands.

Window position (BBU:start-end [bp])	Window position (BTA:start-end [bp])	QTL Description
1:44,484,231-45,484,231	1:121142-229953	Calving ease (maternal)
1:44,484,231-45,484,231	14:1-1,184,633	305-day milk yield

1:44,484,231-45,484,231	14:1-1,184,633	Age at puberty
1:44,484,231-45,484,231	14:1-1,184,633	Average daily gain
1:44,484,231-45,484,231	14:1-1,184,633	Average daily milk yield
1:44,484,231-45,484,231	14:1-1,184,633	Calving ease
1:44,484,231-45,484,231	14:1-1,184,633	Clinical mastitis
1:44,484,231-45,484,231	14:1-1,184,633	Conception rate
1:44,484,231-45,484,231	14:1-1,184,633	Daughter pregnancy rate
1:44,484,231-45,484,231	14:1-1,184,633	Gestation length
1:44,484,231-45,484,231	14:1-1,184,633	Inseminations per conception
1:44,484,231-45,484,231	14:1-1,184,633	Milk composition*
1:44,484,231-45,484,231	14:1-1,184,633	Milk fat yield/percentage
1:44,484,231-45,484,231	14:1-1,184,633	Milk polyunsaturated fatty acid content
1:44,484,231-45,484,231	14:1-1,184,633	Milk protein yield/percentage
1:44,484,231-45,484,231	14:1-1,184,633	Milk saturated to unsaturated fatty acid ratio
1:44,484,231-45,484,231	14:1-1,184,633	Milk solids percentage
1:44,484,231-45,484,231	14:1-1,184,633	Milk yield
1:44,484,231-45,484,231	14:1-1,184,633	Non-fat milk solids
1:44,484,231-45,484,231	14:1-1,184,633	Non-return rate
1:44,484,231-45,484,231	14:1-1,184,633	Ovulation rate
1:44,484,231-45,484,231	14:1-1,184,633	Reproductive problems
1:44,484,231-45,484,231	14:1-1,184,633	Scrotal circumference
1:44,484,231-45,484,231	14:1-1,184,633	Somatic cell score
1:44,484,231-45,484,231	21:1,227,011-2,103,515	Calving ease
1:44,484,231-45,484,231	21:1,227,011-2,103,515	Gestation length
1:44,484,231-45,484,231	21:1,227,011-2,103,515	Heifer pregnancy
2:52,813,756-53,813,756	2:174416-1121267	Daughter pregnancy rate
2:52,813,756-53,813,756	2:174416-1121267	Stillbirth (maternal)
2:52,813,756-53,813,756	2:174416-1121267	Milk fat yield
2:52,813,756-53,813,756	2:174416-1121267	Somatic cell score
3:56,617,686-57,617,686	19:6,077,996-7,071,120	Calving ease
3:56,617,686-57,617,686	19:6,077,996-7,071,120	Daughter pregnancy rate
3:56,617,686-57,617,686	19:6,077,996-7,071,120	Milk fat yield
3:56,617,686-57,617,686	19:6,077,996-7,071,120	Milk yield

3:56,617,686-57,617,686	19:6,077,996-7,071,120	Somatic cell score
3:56,617,686-57,617,686	19:6,077,996-7,071,120	Stillbirth (maternal)
16:36,871,376-37,871,376	15:47,619,681-48,801,787	Age at second calving
16:36,871,376-37,871,376	15:47,619,681-48,801,787	Clinical mastitis
16:36,871,376-37,871,376	15:47,619,681-48,801,787	Milk fat yield/percentage
16:36,871,376-37,871,376	15:47,619,681-48,801,787	Milk protein yield/percentage
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Birth index
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Calf size
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Calving ease
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Conception rate
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Milk fat/protein yield
18:13,611,038-14,611,038	18:14,024,170-15,076,672	Milk yield

*Milk arachidic acid, capric acid, conjugated linoleic acid, myristoleic acid, palmitic acid, stearic acid and tetracosanoic acid contents and milk fatty acid unsaturated index.

3.4. Discussion

The mean length of ROH for the population ($4,47 \pm 6,53$) and per individual ($4,28 \pm 1,85$) indicate inbreeding starting ~13 generations ago (Ferenčaković et al., 2013a; Zavarez et al., 2015), which coincides with the increased selection pressure for milk yield that stated in the Brazilian population at around that time (Vilela et al., 2019). The mean length of ROH per animal was similar to the length reported in dairy cattle breeds, such as the Italian Brown (~3.9 Mb), Italian Holstein (~3.6 Mb) (Marras et al., 2014) and Gyr (3.17 Mb) (Peripolli et al., 2018). However, it was longer than ROH reported in beef cattle with reported values of 1.3 Mb in Nellore (Zavarez et al., 2015), ~1.9 Mb in Piedmontese, and ~2.2 Mb in the Italian Simmental (Marras et al., 2014). Most animals had long autozygous genomic segments that resulted from the recurrent use of a limited number of sires in the current population, signaling an ongoing inbreeding process (Zavarez et al., 2015).

The mean number of ROH observed ($40,05 \pm 10,39$) was lower than in taurine (54.0 ± 7.2 to 94.6 ± 11.6) and indicine (55.1 ± 10.3) cattle (Marras et al., 2014; Peripolli et al., 2018). Additionally, F_{ROH1} (0,072) was also smaller in comparison to various other cattle breeds (0.083 to 0.156), except for Piedmontese (0.041) and Nellore (0.048) cattle (Ferenčaković et al., 2013a; Marras et al., 2014; Zavarez et al., 2015).

This suggests that even though inbreeding seems to be increasing in the population, it is still on the lower end in comparison to cattle breeds.

The inbreeding coefficients estimated through the genomic relationship matrix (F_{GRM}) were higher than those obtained using the pedigree (F_{PED}) (Table 1), which agrees with the results reported in dairy cattle by VanRaden et al., (2011). This occurs due to the F_{GRM} 's inability to distinguish between homozygosity types, IBD (identity by descent) and IBS (identity by state), in the analyses (Forutan et al., 2018), and also due to the allelic frequency parameter set at 0.5 overestimate the coefficients (Forni et al., 2011); whereas F_{PED} and F_{ROH} estimates are only of the IBD portion of the genome. Despite the large differences observed in the F_{GRM} and F_{ROH} values, these estimators were still highly correlated, regardless of the minimum threshold of ROH lengths. The correlation reported in this study is higher than those value reported in cattle (Bjelland et al., 2013; Zavarez et al., 2015).

Both genomic estimates of F (F_{GRM} and F_{ROH}) presented moderate correlations with F_{PED} . The correlation values ranged between 0.31 – 0.41 (Figure 2) and were lower than those reported in cattle (Purfield et al., 2012; VanRaden et al., 2011). This is probably due to a higher number of pedigree limitations in the buffalo population; the pedigree information available for this study is not very deep and may contain systemic inaccuracies, since it is reported directly by the producers.

Although the values observed in this study are lower than those reported in the literature, Forutan et al., (2018) used simulated data and concluded that F_{PED} does not accurately reflect the true inbreeding rate and becomes even less representative as the number of generations increases. So, higher deviations of F_{PED} from the true inbreeding coefficients may have additionally contributed to further lower the correlation between F_{PED} and the genomic estimates. The ROH approach is considered appropriate to evaluate and compare the history of species and populations for detecting adaptation and selection patterns (Iacolina et al., 2016).

The main selection drive in this buffalo population is to improve the quantity and quality of its milk to produce mozzarella cheese. Selection is based on an index that is calculated based on milk yield, milk fat and protein percentages (Aspilcueta-Borquis et al., 2010). The detection, in this study of QTL associated to milk fat yield/percentage, milk yield and milk protein yield/percentage that are relevant for mozzarella production

is well aligned with the selection criteria used in this population. It is interesting to note that the QTL are located on different chromosomes, a characteristic behavior of quantitative polygenic inheritance phenotypes (Table 3).

In comparison to bovine which has 21,295 genes annotated (protein-coding), the annotation of the buffalo genome is less comprehensive with only 20,801 genes annotated (protein-coding) since the assembly of the buffalo genome (UOA WB v.1) was only released in 2018 whilst the bovine UMD v.3.1 was released a few years earlier in 2014 (NCBI Resource Coordinators, 2018). Due to this incomplete annotation only the signatures of selection signals on chromosomes 2, 5 and 18 had gene symbols available – genes potentially selected in these regions are discussed below.

Pregnancy-associated glycoproteins (PAGs) are proteins secreted by trophoblast cells and can be detected in milk and blood during the gestational period (Klisch et al., 2005; Santos et al., 2018) since PAG levels increase gradually over the period of placental development (day 105 post-insemination) in buffalos (Barbato et al., 2017). In cattle, PAG plays an important role in placental development, pregnancy maintenance, embryo survival and immune modulation (Dosogne et al., 2002; Pohler et al., 2013; Santos et al., 2018). Therefore, it is worth highlighting the PAG gene family as a possible candidate for reproductive traits.

The *APRT* gene found in the vicinity of the signature of selection signal on chromosome 18 may play a role in traits related to milk yield and its components; Choudhary et al., (2018) previously pointed out that the *APRT* gene is present in the metabolic pathways associated with the proliferation and differentiation of mammary stem cells in dairy goats. Additionally, the *ACSF3* gene in the same chromosomal region has been implicated in the regulation of citrate content in milk in Holstein cows which influences the coagulation properties of curd cheese, as well as the flavor and texture of cured cheese (Cánovas et al., 2013). Still in the same region, the genes *FANCA*, *ANKRD11* and *ZNF276* were previously identified in a signatures of selection study (iHS and FST) in Holstein-Friesian cattle selected for milk production (Zhao et al., 2015), which lends support to the results obtained in this work.

Genes related to immune system performance in response to mastitis were also detected. Although buffalos are considered more resistant to mastitis than cows the negative impacts are similar to those observed in cattle herds (Catozzi et al., 2017)

and of significant concern to the industry. The main problems caused by this disease include a high calves mortality rate caused by maternal mastitis, as well as reduced milk yield and changes in milk composition (El-Halawany et al., 2017). Due to the economic impact of mastitis on buffalo herds, it is important to identify genes that can help further our understanding of the genetic mechanisms linked to resistance. Some of the genes within the signature of selection on BBU18 have been previously associated with somatic cell count, which is an important indicator of mammary gland health since during the inflammatory process caused by mastitis the concentration of these cells increases as a response to inflammation (Chen et al., 2015; Sharif; Muhammad, 2008). The Cadherin-15 protein encoded by the *CDH15* gene is used in somatic cell counts in milk as an indirect measurement of the mammary gland sanity, helping to control mastitis in dairy cows (Ibeagha-Awemu et al., 2016). Another gene in the region, *CBFA2T3* has been reported as associated with an autoimmune disease that causes skin depigmentation in humans (vitiligo) (Jin et al., 2016) and the *CDK10* gene has been associated with immune responses (Makina et al., 2015).

Genetic factors associated with coat color are interesting because they are tied not only to breed patterns but also to the adaptive traits related to thermal tolerance, production and animal health (Edea et al., 2017). In this context, the *TCF25* and *GAS8* genes were previously associated with black (coat) pigmentation in sheep (Li et al., 2014) while *VPS9D1* is involved in adaptation mechanisms linked to the coat pigmentation of skunks (Nigenda-Morales et al., 2018). *RPL13* was associated with eye color in humans and, together with *DBNDD1*, associated with coat color in horses (Grilz-Seger et al., 2019). *DBNDD1* was also reported in a signatures of selection study of three breeds of Korean cattle distinguished by coat color, suggesting a relationship between the gene and breed patterns (Kim et al., 2018). *OCA2* identified in the signal on chromosome 2 is also known for its important role in the pigmentation of eyes, hair and skin in humans. On the other hand, *HERC2* is not a pigmentation gene but it has a regulatory role on *OCA2* and has an epistatic relationship with *MC1R* (Branicki et al., 2009; Sulem et al., 2007; Visser et al., 2012).

MC1R was associated with coat color in different species, such as cattle (*Bos taurus* and *Bos indicus*) and goats (Goud et al., 2019; Li et al., 2019). In buffalos, SNP104 of the *MC1R* gene was previously associated with black coat color (Miao et

al., 2010). It should be highlighted that skin pigmentation varies little in buffalos since the predominantly black coloration is an important factor for adaptation to hot and humid environments (Badreldin et al., 1952; Chandra; Bharadwaj, 1969), as those observed in tropical regions.

In a study that aimed to identify genes responsible for infertility in humans and mice, *SPATA33* was found to be a candidate gene associated with male fertility (Chen et al., 2013). The *SPIRE2* gene was also associated with pregnancy quality and fertility in females (Pfender et al., 2011).

The buffalo population used in this study was mainly selected for milk yield. Our results already show strong loss of variation in six genomic regions that were probably largely driven by selection for milk production traits, particularly milk yield.. A study by Ma et al., (2019) pointed out that selection for milk yield cause decrease in immune response and fertility, it can indicate that selection exclusively on milk yield can have a negative impact on these traits. The genes and QTL identified in this study through the ROH lend support to this argument since there is a considerable overlap of various genes and QTL associated with these three categories of traits. Immune response and reproductive traits should be included in the selection program or at least their trends should be monitored to avoid negative impacts on them in the future.

Resistance to mastitis, high value of milk for mozzarella production and high ratios of fat and protein in the milk are hallmark characteristics of the buffalo species (Barros et al., 2018). Many of the genes identified in this study have already been previously reported in bovine studies. This is not unexpected due to the high levels of similarity between bovine and buffalo markers (De Camargo et al., 2015). However, the genes related to these buffalo specific traits that were identified in the signatures of selection regions are suggestive candidates for future research that could be of value not only to buffalo producers but also the cattle industry.

3.5. Conclusions

The average inbreeding coefficient in this buffalo population was lower than reported in cattle populations. However, it is quite high in some animals, which suggests that management of inbreeding levels is warranted to avoid losses due to inbreeding depression in the future.

The methodology used was adequate for the identification of genomic regions in selection signatures that could be useful candidates for future studies to identify causal polymorphisms. The proximity of genes linked to milk production traits with genes associated with reproduction and immune system traits suggests the need to include them in the breeding program or at least monitor their trends to avoid negatively impacting them due to selection for the production traits.

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3.7. Supplementary Material

Supplementary Table S1. Window alignment in selection signatures on buffaloes with the bovine genome.

BBU	Window_start_(bp)	Window_end_(bp)	BTA	Window_start_(bp)	Window_end_(bp)	Query_start_(bp)	Query_end_(bp)
1	44484231	45484231	1	121142	229953	892020	1000001
1	44484231	45484231	14	1	1184633	282399	573453
1	44484231	45484231	21	1227011	2103515	282399	653398
2	52813756	53813756	2	174416	1121267	38915	1000000
3	56617686	57617686	19	6077996	7071120	1049	1000001
5	114902856	115902856	29	38487505	40110297	1	1000001
16	36871376	37871376	15	47619681	48801787	1	1000001
18	13611038	14611038	18	14024170	15074672	1	998365

Supplementary Table S2. Average (standard deviation) of inbreeding coefficients per generation for each estimation method.

Generation	N F_{PED}	F_{PED}	N $F_{GENOMIC}$	F_{ROH1}	F_{ROH4}	F_{ROH8}	F_{ROH16}	F_{GRM}
0	1179	0 (0.0)	4	0.084 (0.079)	0.062 (0.074)	0.043 (0.058)	0.026 (0.038)	0.246 (0.039)
1	739	0 (0.0)	2	0.062 (0.067)	0.044 (0.061)	0.029 (0.042)	0.022 (0.031)	0.24 (0.075)
2	202	0.012 (0.051)	3	0.123 (0.111)	0.097 (0.104)	0.071 (0.078)	0.036 (0.032)	0.293 (0.076)
3	455	0.004 (0.029)	0	-	-	-	-	-
4	785	0.038 (0.062)	0	-	-	-	-	-
5	718	0.039 (0.046)	3	0.075 (0.006)	0.047 (0.009)	0.03 (0.02)	0.024 (0.021)	0.235 (0.033)
6	816	0.035 (0.047)	19	0.059 (0.04)	0.033 (0.04)	0.025 (0.035)	0.016 (0.023)	0.244 (0.033)
7	1240	0.037 (0.046)	24	0.045 (0.026)	0.02 (0.02)	0.015 (0.017)	0.007 (0.012)	0.22 (0.029)
8	2005	0.041 (0.054)	70	0.058 (0.025)	0.035 (0.023)	0.025 (0.022)	0.014 (0.016)	0.235 (0.029)
9	2631	0.052 (0.056)	102	0.072 (0.047)	0.048 (0.047)	0.037 (0.043)	0.023 (0.036)	0.244 (0.041)
10	3305	0.056 (0.051)	64	0.087 (0.045)	0.062 (0.045)	0.048 (0.04)	0.029 (0.035)	0.254 (0.036)
11	2902	0.064 (0.047)	61	0.062 (0.028)	0.039 (0.026)	0.029 (0.024)	0.017 (0.019)	0.232 (0.028)
12	1806	0.061 (0.06)	59	0.089 (0.046)	0.065 (0.046)	0.052 (0.043)	0.033 (0.037)	0.253 (0.04)
13	1196	0.058 (0.054)	9	0.116 (0.062)	0.094 (0.065)	0.08 (0.066)	0.054 (0.061)	0.277 (0.055)
14	113	0.058 (0.052)	0	-	-	-	-	-
15	1	0.06 (-)	0	-	-	-	-	-

Supplementary Table S3. Genes within the 1Mb windows for each region in selection signatures.

GeneID	BBU	Window position (start-end) (bp)	Gene position (start-end) (bp)	Gene	Description
102405569	1	44484231-45484231	44705228-44767444	LOC102405569	ADAMTS-like protein 3
112587489	1	44484231-45484231	44721180-44721326	LOC112587489	small nucleolar RNA SNORA81
112587326	1	44484231-45484231	44773674-44773792	LOC112587326	5S ribosomal RNA
112583779	1	44484231-45484231	44774331-44777606	LOC112583779	uncharacterized LOC112583779
112587497	1	44484231-45484231	44778019-44778137	LOC112587497	5S ribosomal RNA
102413798	1	44484231-45484231	44788679-44817949	LOC102413798	liprin-alpha-1-like
102414470	1	44484231-45484231	44827350-44872784	LOC102414470	frizzled-3-like
102395906	1	44484231-45484231	44889349-44940551	LOC102395906	uncharacterized LOC102395906
112587327	1	44484231-45484231	44932618-44932724	LOC112587327	U6 spliceosomal RNA
102415270	1	44484231-45484231	44967073-45119584	LOC102415270	uncharacterized LOC102415270
112587499	1	44484231-45484231	44986864-44986982	LOC112587499	5S ribosomal RNA
112583790	1	44484231-45484231	45003499-45010700	LOC112583790	uncharacterized LOC112583790
112587513	1	44484231-45484231	45015747-45015865	LOC112587513	5S ribosomal RNA
112577730	1	44484231-45484231	45041434-45050021	LOC112577730	coiled-coil domain-containing protein 3-like
102415042	1	44484231-45484231	45119822-45155893	LOC102415042	olfactory receptor 5W2-like
112583792	1	44484231-45484231	45174495-45176727	LOC112583792	olfactory receptor 5W2-like
112578749	1	44484231-45484231	45185846-45188425	LOC112578749	olfactory receptor 5W2-like
112578764	1	44484231-45484231	45193524-45199541	LOC112578764	olfactory receptor 5W2-like
112583795	1	44484231-45484231	45209870-45212043	LOC112583795	olfactory receptor 5W2-like
112586142	1	44484231-45484231	45220886-45222731	LOC112586142	olfactory receptor 1030-like
102390871	1	44484231-45484231	45242826-45244047	LOC102390871	olfactory receptor 5M8-like
102410494	1	44484231-45484231	45262085-45263774	LOC102410494	olfactory receptor 5M9-like
112578782	1	44484231-45484231	45269620-45272066	LOC112578782	olfactory receptor 1038-like
102406804	1	44484231-45484231	45310435-45337222	LOC102406804	olfactory receptor 5D18-like
112586154	1	44484231-45484231	45344123-45345365	LOC112586154	olfactory receptor 5L1-like
112586167	1	44484231-45484231	45348202-45349838	LOC112586167	olfactory receptor 5D16-like
102393626	1	44484231-45484231	45379714-45435614	CLIC6	chloride intracellular channel 6
102390813	2	52813756-53813756	52820460-52821486	LOC102390813	olfactory receptor 10AG1-like

102390469	2	52813756-53813756	52828206-52829144	LOC102390469	olfactory receptor 10AG1-like
102390141	2	52813756-53813756	52850524-52851509	LOC102390141	olfactory receptor 10AG1-like
102389818	2	52813756-53813756	52863966-52864903	LOC102389818	olfactory receptor 10AG1-like
102389494	2	52813756-53813756	52881680-52882653	LOC102389494	olfactory receptor 5T1-like
102389156	2	52813756-53813756	52884443-52885307	LOC102389156	olfactory receptor 10AG1-like
102416599	2	52813756-53813756	52898597-52899570	LOC102416599	olfactory receptor 5F1-like
102416274	2	52813756-53813756	52907211-52967635	LGSN	lengsin, lens protein with glutamine synthetase domain
102404440	2	52813756-53813756	53003314-53321260	OCA2	OCA2 melanosomal transmembrane protein
112583163	2	52813756-53813756	53129769-53129874	LOC112583163	U6 spliceosomal RNA
112583036	2	52813756-53813756	53322815-53322923	LOC112583036	U6 spliceosomal RNA
102402273	2	52813756-53813756	53335090-53573760	HERC2	HECT and RLD domain containing E3 ubiquitin protein ligase 2
102403460	2	52813756-53813756	53396970-53397871	LOC102403460	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 pseudogene
112583240	2	52813756-53813756	53518795-53518867	TRNAE-CUC	transfer RNA glutamic acid (anticodon CUC)
102401948	2	52813756-53813756	53611208-53661293	NIPA1	NIPA magnesium transporter 1
102400766	2	52813756-53813756	53666128-53687936	NIPA2	NIPA magnesium transporter 2
102400449	2	52813756-53813756	53670322-53670495	LOC102400449	DNA-directed RNA polymerases I, II, and III subunit RPABC4 pseudogene
102400118	2	52813756-53813756	53688405-53764781	CYFIP1	cytoplasmic FMR1 interacting protein 1
102413817	3	56617686-57617686	56943781-56944827	LOC102413817	ubiquitin-conjugating enzyme E2 D1 pseudogene
112583815	3	56617686-57617686	57042713-57085669	LOC112583815	phosphatidylcholine transfer protein-like
102413458	3	56617686-57617686	57115185-57150386	LOC102413458	phosphatidylcholine transfer protein
112583816	3	56617686-57617686	57222648-57231284	LOC112583816	uncharacterized LOC112583816
102412899	3	56617686-57617686	57235523-57265166	LOC102412899	phosphatidylcholine transfer protein
112583481	3	56617686-57617686	57288694-57290338	LOC112583481	NEDD8-conjugating enzyme UBE2F pseudogene
112584098	3	56617686-57617686	57299054-57299138	LOC112584098	small nucleolar RNA Z195/SNORD33/SNORD32 family
102397853	3	56617686-57617686	57312250-57336435	LOC102397853	phosphatidylcholine transfer protein-like
112583817	3	56617686-57617686	57345202-57347952	LOC112583817	uncharacterized LOC112583817
112583818	3	56617686-57617686	57355011-57379332	LOC112583818	phosphatidylcholine transfer protein-like
112583819	3	56617686-57617686	57382999-57385926	LOC112583819	uncharacterized LOC112583819

112584096	3	56617686-57617686	57397779-57397863	LOC112584096	small nucleolar RNA Z195/SNORD33/SNORD32 family
102412952	3	56617686-57617686	57406226-57408566	LOC102412952	NEDD8-conjugating enzyme UBE2F pseudogene
102415089	3	56617686-57617686	57425366-57426677	LOC102415089	NEDD8-conjugating enzyme UBE2F pseudogene
112584097	3	56617686-57617686	57448865-57448949	LOC112584097	small nucleolar RNA Z195/SNORD33/SNORD32 family
102413966	3	56617686-57617686	57463220-57468479	TMEM100	transmembrane protein 100
112585274	5	114902856-115902856	114887546-114904729	LOC112585274	pregnancy-associated glycoprotein 1-like
112585188	5	114902856-115902856	114919945-114929144	LOC112585188	pregnancy-associated glycoprotein 1-like
112585190	5	114902856-115902856	114948967-114966507	LOC112585190	pregnancy-associated glycoprotein 1-like
102399695	5	114902856-115902856	114986076-114994114	LOC102399695	pregnancy-associated glycoprotein 1-like
102396238	5	114902856-115902856	115014825-115024165	PAG16	pregnancy-associated glycoprotein 1
112585193	5	114902856-115902856	115094936-115104080	LOC112585193	pregnancy-associated glycoprotein 1-like
102398144	5	114902856-115902856	115175818-115185499	LOC102398144	pregnancy-associated glycoprotein 1-like
102414026	5	114902856-115902856	115219750-115235463	LOC102414026	pregnancy-associated glycoprotein 1-like
112577671	5	114902856-115902856	115247683-115256525	LOC112577671	pregnancy-associated glycoprotein 1-like
102389426	5	114902856-115902856	115343875-115352932	PAG13	pregnancy-associated glycoprotein 1
112585189	5	114902856-115902856	115417519-115426726	LOC112585189	pregnancy-associated glycoprotein 1-like
102397828	5	114902856-115902856	115464742-115476031	LOC102397828	pregnancy-associated glycoprotein 1-like
102403994	5	114902856-115902856	115496851-115506340	PAG4	pregnancy-associated glycoprotein 1-like
112585192	5	114902856-115902856	115531528-115549575	LOC112585192	pregnancy-associated glycoprotein 1-like
112585275	5	114902856-115902856	115583206-115590443	LOC112585275	pregnancy-associated glycoprotein 1-like
102393774	5	114902856-115902856	115606819-115615912	LOC102393774	pregnancy-associated glycoprotein 1-like
102406102	5	114902856-115902856	115651979-115661284	PAG18	pregnancy-associated glycoprotein 1
102393449	5	114902856-115902856	115747955-115757040	LOC102393449	pregnancy-associated glycoprotein 1
102394099	5	114902856-115902856	115780578-115786180	LOC102394099	pregnancy-associated glycoprotein 1-like
102405429	5	114902856-115902856	115856345-115874235	PAG17	pregnancy-associated glycoprotein 1
102411223	5	114902856-115902856	115895292-115905318	LOC102411223	pregnancy-associated glycoprotein 1-like
102395544	16	36871376-37871376	36871887-36878497	LOC102395544	olfactory receptor 5112-like
102395866	16	36871376-37871376	36890143-36891311	LOC102395866	olfactory receptor 5112-like
102396188	16	36871376-37871376	36900408-36901373	LOC102396188	olfactory receptor 5112-like
102401849	16	36871376-37871376	36914769-36918727	LOC102401849	olfactory receptor 5112-like

102396492	16	36871376-37871376	36926246-36927202	LOC102396492	olfactory receptor 52D1-like
102396802	16	36871376-37871376	36934235-36935191	LOC102396802	olfactory receptor 52D1
112579757	16	36871376-37871376	36935365-36935507	LOC112579757	U4 spliceosomal RNA
102402176	16	36871376-37871376	36939777-36941722	LOC102402176	ubiquilin-3
102397107	16	36871376-37871376	36960643-36963362	UBQLN3	ubiquilin-3
102402491	16	36871376-37871376	36967679-36969945	UBQLNL	ubiquilin like
102403151	16	36871376-37871376	36981642-36982250	LOC102403151	olfactory receptor 52B2-like
102403469	16	36871376-37871376	36997887-36998846	LOC102403469	olfactory receptor 52H1-like
102403787	16	36871376-37871376	37011853-37012794	LOC102403787	olfactory receptor 52H1-like
112579506	16	36871376-37871376	37023100-37024056	LOC112579506	olfactory receptor 52H1
102397431	16	36871376-37871376	37035487-37036580	LOC102397431	olfactory receptor 52H1-like
102404006	16	36871376-37871376	37059470-37060422	LOC102404006	olfactory receptor 52H1-like
102403687	16	36871376-37871376	37071727-37072817	LOC102403687	olfactory receptor 52B6
102397097	16	36871376-37871376	37084038-37102939	TRIM6	tripartite motif containing 6
102413390	16	36871376-37871376	37109948-37110892	LOC102413390	olfactory receptor 52B4-like
102394761	16	36871376-37871376	37116302-37130637	TRIM34	tripartite motif-containing protein 34
102393893	16	36871376-37871376	37135691-37157031	LOC102393893	tripartite motif-containing protein 5
102398669	16	36871376-37871376	37163159-37168928	LOC102398669	tripartite motif-containing protein 5-like
102393048	16	36871376-37871376	37173583-37196100	LOC102393048	tripartite motif-containing protein 5
102393569	16	36871376-37871376	37196109-37210340	LOC102393569	tripartite motif-containing protein 5-like
102398350	16	36871376-37871376	37249663-37250576	LOC102398350	olfactory receptor 51Q1-like
112579371	16	36871376-37871376	37263262-37264216	LOC112579371	endogenous retrovirus group K member 19 Env polyprotein-like
102392712	16	36871376-37871376	37283092-37284033	LOC102392712	olfactory receptor 52H1-like
102392406	16	36871376-37871376	37301501-37302453	LOC102392406	olfactory receptor 52H1-like
102392069	16	36871376-37871376	37311288-37312238	LOC102392069	olfactory receptor 52D1-like
102397388	16	36871376-37871376	37321559-37322536	LOC102397388	olfactory receptor 52E4-like
102397055	16	36871376-37871376	37339090-37340067	LOC102397055	olfactory receptor 52E4-like
102396747	16	36871376-37871376	37354082-37355071	LOC102396747	putative olfactory receptor 52P1
102396442	16	36871376-37871376	37363621-37364604	LOC102396442	putative olfactory receptor 52P1
102391729	16	36871376-37871376	37379938-37380903	LOC102391729	olfactory receptor 52N4

102396140	16	36871376-37871376	37396945-37397920	LOC102396140	olfactory receptor 56B1-like
102391401	16	36871376-37871376	37412331-37413296	LOC102391401	olfactory receptor 52N4-like
102395821	16	36871376-37871376	37427000-37427983	LOC102395821	olfactory receptor 52N4-like
102395504	16	36871376-37871376	37463884-37464849	LOC102395504	putative olfactory receptor 56B2
112579507	16	36871376-37871376	37475547-37476512	LOC112579507	olfactory receptor 52N4-like
112579373	16	36871376-37871376	37488853-37491669	LOC112579373	putative olfactory receptor 56B2
112579374	16	36871376-37871376	37503163-37504122	LOC112579374	putative olfactory receptor 56B2
112579375	16	36871376-37871376	37522630-37523590	LOC112579375	putative olfactory receptor 56B2
102399370	16	36871376-37871376	37530311-37531096	LOC102399370	olfactory receptor 52N5
112579508	16	36871376-37871376	37542902-37543867	LOC112579508	olfactory receptor 52N4-like
112579377	16	36871376-37871376	37553969-37556823	LOC112579377	putative olfactory receptor 56B2
102391058	16	36871376-37871376	37567524-37568483	LOC102391058	putative olfactory receptor 56B2
112579378	16	36871376-37871376	37587057-37588301	LOC112579378	putative olfactory receptor 56B2
112579509	16	36871376-37871376	37593218-37594183	LOC112579509	olfactory receptor 52N5
102408166	16	36871376-37871376	37602092-37603112	LOC102408166	olfactory receptor 52N1
102408509	16	36871376-37871376	37616224-37617186	LOC102408509	olfactory receptor 52N2-like
102411145	16	36871376-37871376	37628883-37629845	LOC102411145	olfactory receptor 52N2-like
102411472	16	36871376-37871376	37635495-37641413	LOC102411472	olfactory receptor 52N2-like
112579765	16	36871376-37871376	37650681-37650787	LOC112579765	U6 spliceosomal RNA
102411809	16	36871376-37871376	37661215-37662180	LOC102411809	olfactory receptor 52N2
102412131	16	36871376-37871376	37679119-37680057	LOC102412131	olfactory receptor 52E4-like
102412450	16	36871376-37871376	37680183-37690589	LOC102412450	olfactory receptor 52E4-like
102412773	16	36871376-37871376	37701185-37702141	LOC102412773	olfactory receptor 52E8-like
102413118	16	36871376-37871376	37713692-37719158	LOC102413118	olfactory receptor 52E8-like
102408830	16	36871376-37871376	37725558-37726487	LOC102408830	olfactory receptor 52E4
102413452	16	36871376-37871376	37743309-37744250	LOC102413452	olfactory receptor 52E5
102409153	16	36871376-37871376	37763217-37764182	LOC102409153	olfactory receptor 56A4-like
102409471	16	36871376-37871376	37776145-37777092	LOC102409471	olfactory receptor 56A3
102409807	16	36871376-37871376	37796752-37797695	LOC102409807	olfactory receptor 56A3-like
102413765	16	36871376-37871376	37810100-37811059	LOC102413765	olfactory receptor 56A3-like
102414090	16	36871376-37871376	37825954-37826901	LOC102414090	olfactory receptor 56A3-like

102410139	16	36871376-37871376	37841812-37842752	LOC102410139	olfactory receptor 56A3-like
102414424	16	36871376-37871376	37848981-37850874	LOC102414424	olfactory receptor 56A4-like
102410474	16	36871376-37871376	37860847-37862341	LOC102410474	olfactory receptor 56A4-like
102410802	16	36871376-37871376	37869425-37870393	LOC102410802	olfactory receptor 56A4-like
102410642	18	13611038-14611038	13571226-13627997	PIEZO1	piezo type mechanosensitive ion channel component 1
102409762	18	13611038-14611038	13640184-13644528	CDT1	chromatin licensing and DNA replication factor 1
102409419	18	13611038-14611038	13644685-13647333	APRT	adenine phosphoribosyltransferase
102408891	18	13611038-14611038	13647982-13663865	GALNS	galactosamine (N-acetyl)-6-sulfatase
102408342	18	13611038-14611038	13663975-13668684	TRAPPC2L	trafficking protein particle complex 2 like
102405316	18	13611038-14611038	13672874-13681497	PABPN1L	poly(A) binding protein nuclear 1 like, cytoplasmic
102406084	18	13611038-14611038	13681881-13735421	CBFA2T3	CBFA2/RUNX1 translocation partner 3
112580222	18	13611038-14611038	13708277-13710440	LOC112580222	uncharacterized LOC112580222
102404462	18	13611038-14611038	13794381-13838903	ACSF3	acyl-CoA synthetase family member 3
102404135	18	13611038-14611038	13848486-13867978	CDH15	cadherin 15
102403794	18	13611038-14611038	13868156-13872790	SLC22A31	solute carrier family 22 member 31
102416150	18	13611038-14611038	13919080-14038129	ANKRD11	ankyrin repeat domain 11
112580223	18	13611038-14611038	13938380-13941603	LOC112580223	uncharacterized LOC112580223
102415830	18	13611038-14611038	14054796-14076136	SPG7	SPG7 matrix AAA peptidase subunit, paraplegin
102403480	18	13611038-14611038	14081692-14083964	RPL13	ribosomal protein L13
112580478	18	13611038-14611038	14082420-14082503	LOC112580478	small nucleolar RNA MBII-202
102402721	18	13611038-14611038	14089978-14104252	CPNE7	copine 7
102402393	18	13611038-14611038	14120733-14133748	DPEP1	dipeptidase 1
102402076	18	13611038-14611038	14142454-14149452	CHMP1A	charged multivesicular body protein 1A
102397650	18	13611038-14611038	14149570-14158978	SPATA33	spermatogenesis associated 33
102397972	18	13611038-14611038	14149981-14170939	CDK10	cyclin dependent kinase 10
102398617	18	13611038-14611038	14167883-14171561	SPATA2L	spermatogenesis associated 2 like
102399905	18	13611038-14611038	14171648-14199829	ZNF276	zinc finger protein 276
102398937	18	13611038-14611038	14174997-14184667	VPS9D1	VPS9 domain containing 1
102400902	18	13611038-14611038	14198246-14237652	FANCA	FA complementation group A
102415488	18	13611038-14611038	14237733-14272027	SPIRE2	spire type actin nucleation factor 2

102397120	18	13611038-14611038	14274363-14295799	TCF25	transcription factor 25
112580225	18	13611038-14611038	14274814-14278005	LOC112580225	uncharacterized LOC112580225
102403160	18	13611038-14611038	14298290-14310277	MC1R	uncharacterized LOC102403160
102400358	18	13611038-14611038	14315560-14329420	DEF8	differentially expressed in FDCP 8 homolog
102401737	18	13611038-14611038	14331008-14334907	CENPBD1	CENPB DNA-binding domain containing 1
102402068	18	13611038-14611038	14335012-14355356	LOC102402068	AFG3-like protein 1
102397220	18	13611038-14611038	14357053-14365294	DBNDD1	dysbindin domain containing 1
102398184	18	13611038-14611038	14365194-14402508	GAS8	growth arrest specific 8
112580226	18	13611038-14611038	14377906-14384486	LOC112580226	uncharacterized LOC112580226
102400019	18	13611038-14611038	14384855-14390977	LOC102400019	5-hydroxyisourate hydrolase
102396899	18	13611038-14611038	14471220-14512036	SHCBP1	SHC binding and spindle associated 1
102396594	18	13611038-14611038	14573709-14601342	VPS35	VPS35 retromer complex component
102396289	18	13611038-14611038	14601479-14610173	ORC6	origin recognition complex subunit 6

Supplementary Table S4. List of QTL within 1Mb windows for each region in selection signatures.

BBU:start-end (bp)	BTA:start-end (bp)	QTL	Class	QTL ID	PubMed ID
1:44484231-45484231	1:121142-229953	Calving ease (maternal)	Reproduction	106485	27328805
2:52813756-53813756	2:174416-1121267	Body weight (yearling)	Production	66835	19966163
2:52813756-53813756	2:174416-1121267	Body weight gain	Production	66864	19966163
2:52813756-53813756	2:174416-1121267	Dressing percentage	Meat and Carcass	102180	27329851
2:52813756-53813756	2:174416-1121267	Daughter pregnancy rate	Reproduction	106907	27209127
2:52813756-53813756	2:174416-1121267	Udder cleft	Exterior	125920	18565942
2:52813756-53813756	2:174416-1121267	Daughter pregnancy rate	Reproduction	39029	21831322
2:52813756-53813756	2:174416-1121267	Stillbirth (maternal)	Reproduction	39030	21831322
2:52813756-53813756	2:174416-1121267	PTA type	Production	39031	21831322
2:52813756-53813756	2:174416-1121267	Udder attachment	Exterior	39032	21831322
2:52813756-53813756	2:174416-1121267	Milk fat yield	Milk	39033	21831322
2:52813756-53813756	2:174416-1121267	Length of productive life	Production	39034	21831322
2:52813756-53813756	2:174416-1121267	Udder height	Exterior	39035	21831322
2:52813756-53813756	2:174416-1121267	Rump width	Production	39036	21831322
2:52813756-53813756	2:174416-1121267	Somatic cell score	Health	39037	21831322
2:52813756-53813756	2:174416-1121267	Udder cleft	Exterior	39038	21831322
2:52813756-53813756	2:174416-1121267	Udder depth	Exterior	39039	21831322
2:52813756-53813756	2:174416-1121267	Lean meat yield	Meat and Carcass	36959	25273628
2:52813756-53813756	2:174416-1121267	Meat color L*	Meat and Carcass	36368	25149327
1:44484231-45484231	14:1-1184633	Milk fat yield	Milk	5050	10430929
1:44484231-45484231	14:1-1184633	Milk yield	Milk	3507	10430929
1:44484231-45484231	14:1-1184633	Milk protein yield	Milk	3509	10430929
1:44484231-45484231	14:1-1184633	Milk fat percentage	Milk	3510	10430929
1:44484231-45484231	14:1-1184633	Milk protein percentage	Milk	3511	10430929
1:44484231-45484231	14:1-1184633	Non-return rate	Reproduction	3525	17179535
1:44484231-45484231	14:1-1184633	Structural soundness (legsfeetpenisand prepuce)	Exterior	3526	17179535
1:44484231-45484231	14:1-1184633	Rump width	Production	3527	17179535
1:44484231-45484231	14:1-1184633	Somatic cell score	Health	13240	12416823
1:44484231-45484231	14:1-1184633	Bovine respiratory disease susceptibility	Health	57558	26020874
1:44484231-45484231	14:1-1184633	Subcutaneous fat	Meat and Carcass	31027	12926773
1:44484231-45484231	14:1-1184633	Intramuscular fat	Meat and Carcass	12029	14510671

1:44484231-45484231	14:1-1184633	Average daily milk yield	Milk	10585	18650297
1:44484231-45484231	14:1-1184633	Sirloin weight	Meat and Carcass	11492	19555501
1:44484231-45484231	14:1-1184633	Sirloin fat depth	Meat and Carcass	11493	19555501
1:44484231-45484231	14:1-1184633	Inseminations per conception	Reproduction	11531	18298935
1:44484231-45484231	14:1-1184633	Conception rate	Reproduction	11532	18822099
1:44484231-45484231	14:1-1184633	Reproductive problems	Reproduction	11533	18822099
1:44484231-45484231	14:1-1184633	Body condition score	Exterior	11534	18822099
1:44484231-45484231	14:1-1184633	Body energy content	Production	11535	18822099
1:44484231-45484231	14:1-1184633	Blood glucose level	Health	11536	18822099
1:44484231-45484231	14:1-1184633	Blood non-esterified fatty acid level	Health	11537	18822099
1:44484231-45484231	14:1-1184633	Cumulative effective energy balance	Production	11539	18822099
1:44484231-45484231	14:1-1184633	Milk capric acid content	Milk	11647	20105547
1:44484231-45484231	14:1-1184633	Milk myristoleic acid content	Milk	11648	20105547
1:44484231-45484231	14:1-1184633	Milk palmitic acid content	Milk	11649	20105547
1:44484231-45484231	14:1-1184633	Milk palmitoleic acid content	Milk	11650	20105547
1:44484231-45484231	14:1-1184633	Milk stearic acid content	Milk	11651	20105547
1:44484231-45484231	14:1-1184633	Milk arachidic acid content	Milk	11652	20105547
1:44484231-45484231	14:1-1184633	Milk conjugated linoleic acid content	Milk	11653	20105547
1:44484231-45484231	14:1-1184633	Milk tetracosanoic acid content	Milk	11654	20105547
1:44484231-45484231	14:1-1184633	Milk fatty acid unsaturated index	Milk	11655	20105547
1:44484231-45484231	14:1-1184633	Height (yearling)	Production	11706	19820053
1:44484231-45484231	14:1-1184633	Carcass weight	Meat and Carcass	28727	15537759
1:44484231-45484231	14:1-1184633	Conformation score	Exterior	28728	24361557
1:44484231-45484231	14:1-1184633	Muscle pH	Meat and Carcass	28729	18254735
1:44484231-45484231	14:1-1184633	Cooking loss	Meat and Carcass	28730	22035000
1:44484231-45484231	14:1-1184633	Non-fat milk solids	Milk	18999	21881942
1:44484231-45484231	14:1-1184633	Non-fat milk solids percentage	Milk	19002	21881942
1:44484231-45484231	14:1-1184633	Milk solids percentage	Milk	19003	21881942
1:44484231-45484231	14:1-1184633	Age at puberty	Reproduction	20719	18650300
1:44484231-45484231	14:1-1184633	Subcutaneous rump fat thickness	Meat and Carcass	20720	22497268
1:44484231-45484231	14:1-1184633	Longissimus muscle area	Meat and Carcass	20723	10375215
1:44484231-45484231	14:1-1184633	Milk saturated to unsaturated fatty acid ratio	Milk	20044	22114848
1:44484231-45484231	14:1-1184633	Milk polyunsaturated fatty acid content	Milk	20045	22114848
1:44484231-45484231	14:1-1184633	305-day milk yield	Milk	20076	17582132

1:44484231-45484231	14:1-1184633	Marbling score	Meat and Carcass	65365	14677852
1:44484231-45484231	14:1-1184633	Udder balance	Exterior	3475	12605852
1:44484231-45484231	14:1-1184633	Body weight (weaning)	Production	10954	19422365
1:44484231-45484231	14:1-1184633	Height (mature)	Production	10953	20477797
1:44484231-45484231	14:1-1184633	Immunoglobulin G level	Health	66189	21138580
1:44484231-45484231	14:1-1184633	Average daily gain	Production	2633	15537758
1:44484231-45484231	14:1-1184633	Meat color a*	Meat and Carcass	28734	24361557
1:44484231-45484231	14:1-1184633	Meat color b*	Meat and Carcass	28735	24361557
1:44484231-45484231	14:1-1184633	Drip loss	Meat and Carcass	28736	24361557
1:44484231-45484231	14:1-1184633	Calving ease	Reproduction	10956	17179535
1:44484231-45484231	14:1-1184633	Body weight (birth)	Production	10955	15537758
1:44484231-45484231	14:1-1184633	Fat thickness at the 12th rib	Meat and Carcass	10957	10764062
1:44484231-45484231	14:1-1184633	Gastrointestinal nematode burden	Health	12022	19254385
1:44484231-45484231	14:1-1184633	Dressing percentage	Meat and Carcass	6280	19422365
1:44484231-45484231	14:1-1184633	Yield grade	Meat and Carcass	1333	12350008
1:44484231-45484231	14:1-1184633	Tick resistance	Health	9917	17894560
1:44484231-45484231	14:1-1184633	Clinical mastitis	Health	3177	11845286
1:44484231-45484231	14:1-1184633	beta-carotene concentration in fat	Meat and Carcass	20663	22451137
1:44484231-45484231	14:1-1184633	Fat color in carcass	Meat and Carcass	20664	22451137
1:44484231-45484231	14:1-1184633	Gestation length	Reproduction	5374	19016677
1:44484231-45484231	14:1-1184633	Carcass length	Meat and Carcass	6281	19422365
1:44484231-45484231	14:1-1184633	Connective tissue amount	Meat and Carcass	13172	20826071
1:44484231-45484231	14:1-1184633	Bone weight	Meat and Carcass	6294	19422365
1:44484231-45484231	14:1-1184633	Behavior	Exterior	1496	11447250
1:44484231-45484231	14:1-1184633	Veterinary treatments	Production	5012	19389971
1:44484231-45484231	14:1-1184633	Body weight (mean)	Production	1731	15537759
1:44484231-45484231	14:1-1184633	Stature	Exterior	4613	10575619
1:44484231-45484231	14:1-1184633	Abnormal flavor intensity	Meat and Carcass	4833	18254735
1:44484231-45484231	14:1-1184633	Lung percentage	Meat and Carcass	12156	20477785
1:44484231-45484231	14:1-1184633	Height (200 days)	Production	6264	19422365
1:44484231-45484231	14:1-1184633	Residual feed intake	Production	4435	17709790
1:44484231-45484231	14:1-1184633	Beef flavor intensity	Meat and Carcass	4834	18254735
1:44484231-45484231	14:1-1184633	Rump angle	Exterior	1592	16230715
1:44484231-45484231	14:1-1184633	Body weight (slaughter)	Production	4514	17347893
1:44484231-45484231	14:1-1184633	Quality grade	Meat and Carcass	20000	22179692

1:44484231-45484231	14:1-1184633	Myristic acid content	Meat and Carcass	20599	22223295
1:44484231-45484231	14:1-1184633	Palmitic acid content	Meat and Carcass	20600	22223295
1:44484231-45484231	14:1-1184633	Arachidonic acid content	Meat and Carcass	20601	22223295
1:44484231-45484231	14:1-1184633	Silverside weight	Meat and Carcass	6282	19422365
1:44484231-45484231	14:1-1184633	Height (400 days)	Production	6265	19422365
1:44484231-45484231	14:1-1184633	Meat weight	Meat and Carcass	6298	19422365
1:44484231-45484231	14:1-1184633	Teat placement - front	Exterior	3715	10984164
1:44484231-45484231	14:1-1184633	Udder attachment	Exterior	3716	10984164
1:44484231-45484231	14:1-1184633	Udder composite index	Exterior	3717	10984164
1:44484231-45484231	14:1-1184633	Body weight (600 days)	Production	6268	19422365
1:44484231-45484231	14:1-1184633	Percentage decrease in PCV up to day 100 after challenge	Health	10527	12805560
1:44484231-45484231	14:1-1184633	Udder cleft	Exterior	1593	16230715
1:44484231-45484231	14:1-1184633	Daughter pregnancy rate	Reproduction	1695	16167984
1:44484231-45484231	14:1-1184633	Body weight (yearling)	Production	10967	20477797
1:44484231-45484231	14:1-1184633	Twinning	Reproduction	1720	16026340
1:44484231-45484231	14:1-1184633	Foot angle	Exterior	1590	16230715
1:44484231-45484231	14:1-1184633	Ovulation rate	Reproduction	1416	15265069
1:44484231-45484231	14:1-1184633	Udder width	Exterior	1696	16167984
1:44484231-45484231	14:1-1184633	Dry matter intake	Production	4365	17709790
1:44484231-45484231	14:1-1184633	Rear leg placement - side view	Exterior	4924	9891277
1:44484231-45484231	14:1-1184633	Dairy capacity composite index	Exterior	4947	9891277
1:44484231-45484231	14:1-1184633	Scrotal circumference	Reproduction	10975	20477797
1:44484231-45484231	14:1-1184633	Body weight (mature)	Production	10977	20477797
16:36871376-37871376	15:47619681-48801787	Clinical mastitis	Health	164756	30269158
16:36871376-37871376	15:47619681-48801787	Body weight gain	Production	68719	19966163
16:36871376-37871376	15:47619681-48801787	Age at second calving	Reproduction	139160	28852499
16:36871376-37871376	15:47619681-48801787	Milk yield	Milk	121830	25128478
16:36871376-37871376	15:47619681-48801787	Milk fat yield	Milk	121831	25128478
16:36871376-37871376	15:47619681-48801787	Milk fat percentage	Milk	121832	25128478
16:36871376-37871376	15:47619681-48801787	Milk protein percentage	Milk	121833	25128478
16:36871376-37871376	15:47619681-48801787	Tridecylic acid content	Meat and Carcass	19626	24156620
16:36871376-37871376	15:47619681-48801787	Residual feed intake	Production	14647	19749024
16:36871376-37871376	15:47619681-48801787	Dry matter intake	Production	14680	19749024
16:36871376-37871376	15:47619681-48801787	Body weight (birth)	Production	31599	24612794

16:36871376-37871376	15:47619681-48801787	Shear force	Meat and Carcass	20796	22497286
18:13611038-14611038	18:14024170-15074672	Conception rate	Reproduction	107162	27209127
18:13611038-14611038	18:14024170-15074672	Milk protein yield	Milk	103399	27287773
18:13611038-14611038	18:14024170-15074672	Calving ease (maternal)	Reproduction	49521	21831322
18:13611038-14611038	18:14024170-15074672	Milk fat yield	Milk	49522	21831322
18:13611038-14611038	18:14024170-15074672	Milk yield	Milk	49523	21831322
18:13611038-14611038	18:14024170-15074672	Net merit	Production	49524	21831322
18:13611038-14611038	18:14024170-15074672	Milk protein yield	Milk	49525	21831322
18:13611038-14611038	18:14024170-15074672	Calving ease	Reproduction	49526	21831322
18:13611038-14611038	18:14024170-15074672	Residual feed intake	Production	35232	25257854
18:13611038-14611038	18:14024170-15074672	Calving ease	Reproduction	30541	22888914
18:13611038-14611038	18:14024170-15074672	Calf size	Reproduction	30546	22888914
18:13611038-14611038	18:14024170-15074672	Calf size	Reproduction	15195	21183059
18:13611038-14611038	18:14024170-15074672	Birth index	Reproduction	15196	21183059
18:13611038-14611038	18:14024170-15074672	Calving ease	Reproduction	15197	21183059
18:13611038-14611038	18:14024170-15074672	Feed conversion ratio	Production	35226	25257854
18:13611038-14611038	18:14024170-15074672	Birth index	Reproduction	30537	22888914
3:56617686-57617686	19:6077996-7071120	Milk fat yield	Milk	103256	27287773
3:56617686-57617686	19:6077996-7071120	Calving ease (maternal)	Reproduction	49992	21831322
3:56617686-57617686	19:6077996-7071120	Daughter pregnancy rate	Reproduction	49993	21831322
3:56617686-57617686	19:6077996-7071120	Stillbirth (maternal)	Reproduction	49994	21831322
3:56617686-57617686	19:6077996-7071120	PTA type	Production	49995	21831322
3:56617686-57617686	19:6077996-7071120	Udder attachment	Exterior	49996	21831322
3:56617686-57617686	19:6077996-7071120	Milk fat yield	Milk	49997	21831322
3:56617686-57617686	19:6077996-7071120	Net merit	Production	49998	21831322
3:56617686-57617686	19:6077996-7071120	Length of productive life	Production	49999	21831322
3:56617686-57617686	19:6077996-7071120	Udder height	Exterior	50000	21831322
3:56617686-57617686	19:6077996-7071120	Calving ease	Reproduction	50001	21831322
3:56617686-57617686	19:6077996-7071120	Somatic cell score	Health	50002	21831322
3:56617686-57617686	19:6077996-7071120	Udder depth	Exterior	50003	21831322
3:56617686-57617686	19:6077996-7071120	Milk yield	Milk	25645	22449276
1:44484231-45484231	21:1227011-2103515	Body weight (birth)	Production	146260	29178833
1:44484231-45484231	21:1227011-2103515	Calving ease	Reproduction	144155	29178833
1:44484231-45484231	21:1227011-2103515	Gestation length	Reproduction	146330	29178833
1:44484231-45484231	21:1227011-2103515	Heifer pregnancy	Reproduction	119782	27494397

5:114902856-115902856	29:38487505-40110297	Average daily gain	Production	102044	27136002
5:114902856-115902856	29:38487505-40110297	Growth index	Production	102045	27136002
5:114902856-115902856	29:38487505-40110297	Margaric acid content	Meat and Carcass	19759	24156620
5:114902856-115902856	29:38487505-40110297	Udder swelling score	Exterior	106760	27335091
5:114902856-115902856	29:38487505-40110297	Shear force	Meat and Carcass	20833	22497286

CHAPTER 4 - Identification of footprints of ongoing selection in dairy Murrah buffaloes

ABSTRACT - Selected regions can provide information for evolutionary processes and validation of genomic extensions involved in metabolic pathways related to characteristics of economic interest. The objective of this work was to use the Integrated Haplotype Score (iHS) statistics to search for signals of ongoing selection in Murrah buffaloes, and to assess candidate genes and known QTL of traits of economic interest in the regions under selection. The sample group consisted of 865 dairy buffalos genotyped using the Axiom Buffalo Genotyping Array. iHS analysis was performed with the SELSCAN v. 1.2.0. Thus, the averages of iHS were estimated in 500Kb windows, with 250 Kb of overlap allowed. Selection signatures were the windows with mean values of $|iHS|$ greater than 2.36 (top 0.1%). Windows of 1 Mb were used for each region in selection signatures. After quality control, 865 samples and 54,288 SNPs remained. Six regions were highlighted as in selection signatures, located in chromosomes 1, 6, 7, 9, 14, and 23. The selection signature regions encompassed 93 genes and 450 QTL, including 42 genes related to milk yield, concentration of fat and protein in milk, health of the mammary gland and reproduction. We can highlight that of the total QTL found, 403 (89%) were related to milk class. Our results shed light in genomic regions putatively targeted by the ongoing selection in dairy buffaloes, hence associated to the milk production. However, it is necessary to validate the SNPs in the indicated regions, relevant step for developing Murrah buffalo selection strategies for dairy traits.

Keywords: *Bubalus bubalis*, dairy trait, iHS, water buffalos

4.1. Introduction

Buffaloes are docile in temperament, resistant to disease, highly fertile (~80%) and adapted to harsh environments compared to cattle. In this way, buffaloes are able to develop in unfavorable areas to cattle raising, traits that attract the interest of ranchers in herds of buffaloes (Bernardes, 2007; Naveena and Kiran, 2014).

Buffalo's milk has higher concentrations of fat and protein than cow's milk, in addition to the proportions of constituents, such as minerals, that contribute to the characteristic flavor of products such as mozzarella cheese (Salman et al., 2014). Buffalo milk is the second most consumed in the world, and due to its economic relevance, the understanding of the biological processes involved in milk production can contribute to selection strategies (Afzal et al., 2007; De Camargo et al., 2015). In buffalo herds in Brazil, the average for milk yield at 305 days increased significantly in a decade, from 1,340.40 kg to 2,039.04 kg (Sesana, 2008).

Morphological, physiological and habits changes of a species are reflections of natural selection and domestication for several generations, and these changes can be accentuated with the use of artificial selection (Qanbari and Simianer, 2014). In livestock, selected regions can provide information for evolutionary processes and validation of genomic extensions involved in metabolic pathways related to characteristics of economic interest (Hayes et al., 2008; O'brien et al., 2014).

Selection events modify the genetic composition of a population, printing different patterns in specific regions of the genome, so-called selection signatures (Qanbari; Simianer, 2014). Integrated Haplotype Score (iHS) is a EHH (Extended Haplotype Homozygosity) based-approach (Sabeti et al., 2002), developed for detecting selection signatures (Voight et al., 2006), and specially skilled for the detection of incomplete sweep (ongoing selection). It computes the standardized log-ratio of the integral of the observed EHH decay in the ancestral allele for the integral of the observed EHH decay in the derived allele of each SNP.

Extreme positive and negative scores indicate selection driven by the ancestral and derived allele, respectively. However, absolute values of iHS may be used to highlight sites undergoing selection independently of the state of favorable allele (Enard et al., 2014), as has been widely done in livestock studies (Kemper et al., 2014).

The objective of this work was to use the iHS statistics to search for signals of ongoing selection in Murrah buffaloes, then to assess candidate genes and known QTL of traits of economic interest in the regions under selection.

4.2. Material and Methods

4.2.1. Samples and Genotypic Information

The sample group consisted of 865 Murrah dairy buffalos genotyped using the Axiom Buffalo Genotyping Array (90k SNP). A New marker map (described in Chapter 2) improving the commercial map with the position of 9,930 SNPs, was applied.

Quality control of genotypes was performed using the Plink v.1.9 software (Chang et al., 2015), where non-autosomal or duplicated markers were removed as well as markers with Call Rates_{SNP} lower than 0.95. All genotyped animals had a Call Rates_{SAMPLE} higher than 0.90 and passed quality control.

4.2.2. Imputation and Phasing

The missing genotypes were imputed, and then the haplotypic phase inference was performed for each chromosome, using EAGLE v2.4.1 (Loh et al., 2016). SHAPEIT v2.r904 (Delaneau et al., 2012) was used to convert EAGLE output files to variant call format (VCF).

4.2.3. Identification of Signatures of Selection

The iHS scores were computed with the SELSCAN (version 1.2.0) (Szpiech and Hernandez, 2014). SNPs with minor allele frequency (MAF) lower than 1% were not included in this analysis due to iHS estimate not admitting monomorphic markers.

The iHS compares the decay of EHH of the ancestral allele in relation to the alternative allele, with negative values indicating selection for the ancestral allele and positive for the alternative allele. In this study, an allele of each locus was arbitrarily declared ancestral. This decision was made because the ancestral alleles of each locus in buffalo are not known. In addition, because an allele can be favorable regardless of whether it is ancestral or alternative in animal populations, the use of absolute values of iHS is most commonly used (Cardoso et al., 2018; Utsunomiya et al., 2013).

EHH_c values were calculated for both alleles of each SNP until 5Mb extension, with the following formula:

$$EHH_c(x_i) = \sum_{h \in H_c(x_i)} \frac{\binom{n_h}{2}}{\binom{n_c}{2}}$$

where, c is one of the alleles represented in each SNP (A or B); i represents the order of the marker adjacent to the core SNP (x_0); $H_c(x_i)$ groups all possible existing haplotypes containing the allele c , in the extension of locus x_0 to the n^{th} SNP adjacent to x_0 (eg. $c = A$ and $i = 1$ $H_c(x_i) = \{AA, AB\}$; $c = B$ and $i = 1$ $H_c(x_i) = \{BA, BB\}$); n_h the observed number of $h \in H_c(x_i)$ type haplotypes; and n_c the total of extended haplotypes that contains the nucleus allele c , therefore it is equal to the frequency of c in the population.

The EHHc integral (iHHc) was estimated for each core allele, considering the physical distance between the haplotype markers. Unstandardized iHS values were calculated according to the following formula:

$$\text{unstandardized } iHS = \ln \left(\frac{iHH_1}{iHH_0} \right)$$

where iHH_1 is iHH of the ancestral allele and iHH_0 is iHH of the alternative allele.

The unstandardized iHS values of the SNPs were subdivided into 20 bins according to the frequency of the allele classified as ancestral and standardized as follows:

$$iHS = \frac{\ln \left(\frac{iHH_1}{iHH_0} \right) - E_p \left[\ln \left(\frac{iHH_1}{iHH_0} \right) \right]}{SD_p \left[\ln \left(\frac{iHH_1}{iHH_0} \right) \right]}$$

where E and SD are, respectively, the mean and standard deviation of each bin p .

Thus, the averages of absolute iHS values ($|iHS|$) were estimated in 500Kb windows, with 250 Kb of overlap. Windows with mean values of $|iHS|$ greater than 2.36 (top 0.1%) were treated as selection signatures.

4.2.4. Exploring Regions in Signatures of Selection

Windows of 1Mb (250 kb upstream and 250 kb downstream of the significant windows) were used for each region in selection signatures. Annotated genes in

intersection with these regions were extracted from *NCBI's* (National Center for Biotechnology Information) annotation r.101 (NCBI Resource Coordinators, 2018) databases based on the UOA WB v1 assembly of the buffalo genome. The map obtained in Chapter 2 was used to search for QTL, where the positions of bovine QTL were predicted for the buffalo genome by alignment.

4.3. Results and Discussion

After quality control, 865 samples and 54,288 SNPs remained. A total of 9,763 windows (500Kb) were obtained for all autosomal chromosomes. Figure 1 shows the distribution of $|iHS|$ averaged to 500Kb windows through the autosomal genome. Six regions were highlighted as in selection signatures, located in BBU1 (BBU, *Bubalus bubalis* chromosome), BBU6, BBU7, BBU9, BBU14 and BBU23.

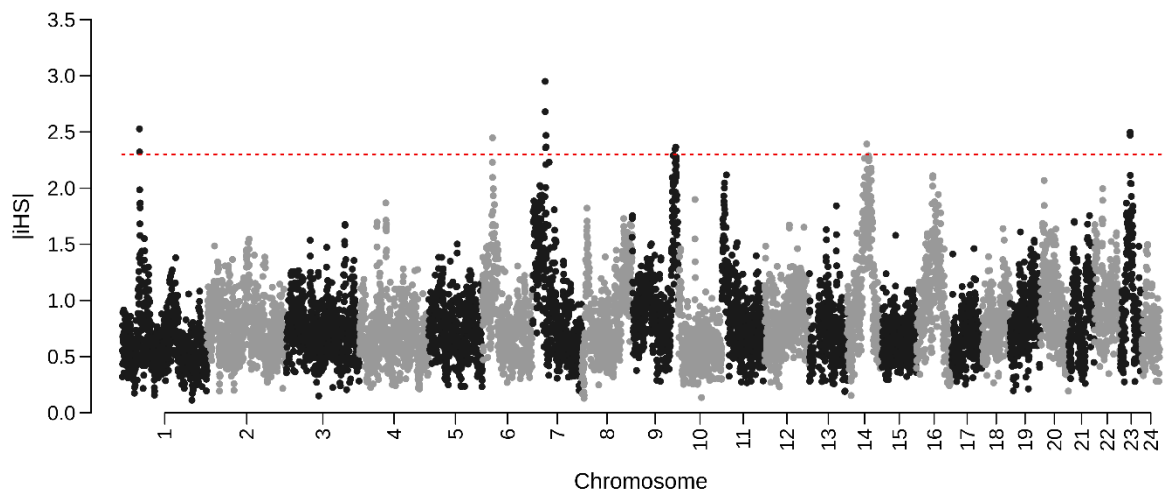


Figure 1. Manhattan plot of genome-wide for $|iHS|$ average in 500Kb windows. The dashed line represents the 0.1% threshold.

The selection signature regions encompassed 93 genes and 450 QTL (Supplementary Table 1 and Supplementary Table 2), including 42 genes (Table 1) related to milk yield, concentration of fat and protein in milk, health of the mammary gland and reproduction. Indicative that possibly these regions are under directional selection. We can highlight that of the total QTL found, 403 (89%) were related to milk class.

Table 1. Summary of genes related to phenotypes of interest present in the 1 Mb window from the center of the selection signatures.

Window position (BBU:start-end [bp])	Genes
1:40500000-41500000	<i>CSMD1</i>
6:24000000-25000000	<i>SPAG17, TBX15</i>
7:29000000-30000000	<i>ADAMTS3, AFM, AFP, ALB, ANKRD17, TRNAC-GCA</i>
9:103750000-104750000	<i>ABHD8, ANKLE1, ANO8, BST2, CHERP, CPAMD8, DDA1, HAUS8, MYO9B, NR2F6, PGLS, PLVAP, SLC27A1, SLC35E1, TMEM221, USE1, USHBP1</i>
14:46250000-47250000	<i>ARMC4, MKX, PCSK2, RAB18</i>
23:23250000-24250000	<i>AS3MT, BORCS7, CALHM1, CALHM2, CALHM3, CNNM2, NEURL1, NT5C2, PCGF6, PDCD11, SH3PXD2A, TAF5</i>

4.3.1. Milk

The *PDCD11* gene acts on the mammary gland and is associated with milk yield from Murrah buffaloes (Deng et al., 2019). In this line, *USE1* was identified in an analysis that compared milk yield in different populations of Holstein cows (Bai et al., 2016). The role of *PGLS* and *NEURL1*, although little explored, has been linked to dairy production (Baik et al., 2009; Iso-Touru et al., 2016).

Fat influences the content of total solids in milk and is capable of modifying sensory characteristics of dairy products (Pignata et al., 2014). Thus, *AS3MT*, *CNNM2*, *BORCS7*, *NEURL1*, *SH3PXD2A*, *CALHM1*, *CALHM3*, *CALHM2* and *NT5C2* were associated with concentration of fatty acids in milk in cattle (Palombo et al., 2018). We can highlight *SLC27A1*, this gene belongs to the family of proteins that transport fatty acids and is related to an increase in the percentage of protein in milk (Kuling). In addition, *SLC27A1* has been reported as a candidate for fat yield and fat percentage in milk in Holstein (Lv et al., 2011; Ordovas et al., 2006).

ANKRD17, *TRNAC-GCA* and *ADAMTS3* were associated with the lactose percentage in the milk of Fleckvieh cattle (Costa et al., 2019). *RAB18* is a member of a family of regulatory proteins for endocytosis, exocytosis and vesicle transport,

important processes in the secretion of milk and development of the mammary gland (Finucane et al., 2008; Lin et al., 2019; Reinhardt and Lippolis, 2008).

4.3.2. Health

In addition to traits linked to milk production, QTL related to immune response (clinical mastitis and somatic cell score), health and udder morphology were detected. Beecher et al. (2010) reported a decrease in milk yield and fat concentration in animals with a high somatic cell score. The metabolic processes involved in the defense against inflammation of the mammary gland involve immune, epithelial and endothelial cells (Rinaldi et al., 2010).

AFM was related to the concentration of α -tocopherol in Holstein. The decrease in postpartum α -tocopherol levels is associated with the prevalence of mastitis (Haga et al., 2018). *ALB* is associated with the innate immune system, and its expression is regulated in response to mastitis (Shamay et al., 2005). *NR2F6* and *CNNM2* are linked with resistance to inflammation of the mammary glands in cattle (Cai et al., 2018; Lutzow et al., 2008).

CHERP is involved in intracellular calcium homeostasis in human cells and humoral response (O'Rourke et al., 2003). *ANKLE1* is associated with the immune response to viruses and bacteria in humans and *DDA1* related to severe cases of ovarian cancer (Jamin and Wiebe, 2015; Liu et al., 2017). The *TMEM221* and *ABHD8* genes are linked to breast cancer susceptibility in women (Jacinta-Fernandes et al., 2020; Lindström et al., 2016). In cattle, *ABHD8* expression is stimulated in response to postpartum inflammatory processes Holstein (Moisá et al., 2017).

The expression of *SLC35E1* was observed in mammary glands in humans and goats, where it was related to the metabolism of oligosaccharides in colostrum, which provides passive immunity to progeny (Crisà et al., 2016), a fundamental adaptive trait for the health of calves (Gupta et al., 2019). *CPAMD8* and *BST2* are involved with the innate immune system in cattle (Armstrong; Quigley, 1999; Takeda et al., 2012).

4.3.3. Reproduction

Qureshi and Ahmad (2008) reported that buffaloes with moderate milk production have superior reproductive performance than cows with higher or lower

production, suggesting a relationship between production and reproduction traits. This may explain the identification of QTL (daughter pregnancy rate, sperm motility, reproductive efficiency and stillbirth (maternal)) and genes related to reproductive traits.

PCSK2 is associated with the fertility traits in Holstein (Leyva-Corona et al., 2018). *ARMC4* is related to the primary ciliary dyskinesia disease in humans, affecting sperm motility causing infertility (Boaretto et al., 2016), also affecting reproduction in Nelore heifers (Costa et al., 2015). Knockout from *CSMD1* causes infertility in rats, due to the reduction in ovarian quality and ramifications of breast vessels (Lee et al., 2019). Inactivation of *SPAG17* causes infertility in males, affecting sperm motility (Zhang et al., 2019).

In buffaloes, *AFP* gene was related to ovulation capacity, so that overexpression causes negative regulation of the hormone GnRH (antiestrogenic), which causes anovulation (Kumar et al., 2019). In humans, high levels of *TAF5* expression were observed in oocytes, and this gene can be considered a possible indicator of oocyte quality (Di Pietro et al., 2008).

HAUS8 is associated with cell proliferation of ovarian tissues and *MYO9B* expressed in germ cells in cows (Reyes et al., 2015; Schütz et al., 2018). *USHBP1* acts on cells responsible for follicle maturation and *ANO8* in the regulation of the estrous cycle in cattle (Hatzirodos et al., 2014; Kommadath et al., 2011).

TBX15 is related to embryonic implantation in heifers (Peñagaricano et al., 2013). *PLVAP* expression is regulated by the establishment of pregnancy in dairy cows (Evans et al., 2012). *PCGF6* and *MKX* are associated with embryonic development in humans and mice (Anderson et al., 2006; Santanach et al., 2017), as well as *AS3MT*, a candidate for successful embryonic development influenced by feeding in cattle (Salehi et al., 2016).

Our results shed light in genomic regions putatively targeted by the ongoing selection in dairy buffaloes, hence associated to the milk production. However, it is necessary to validate the SNPs in the indicated regions. Fundamental step for developing Murrah buffalo selection strategies for dairy traits.

4.4. Conclusions

The methodology used was able to identify genomic regions potentially affected by selection in at buffalo population. The presence of genes and QTL related to characteristics of economic importance such as milk production, milk components and reproduction, suggests that these differentiated genomic patterns have been established due to contemporary selection for productive improvements in the studied population.

4.5. References

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4.6. Supplementary Material

Supplementary Table S1. Genes within the 1Mb windows for each region in selection signatures.

GeneID	BBU	Window position (start-end) (bp)	Gene position (start-end) (bp)	Gene	Description
102397335	1	40500000-41500000	40664564:42730963	CSMD1	CUB and Sushi multiple domains 1
102415518	6	24000000-25000000	23955865:24081286	TBX15	T-box transcription factor 15
112585816	6	24000000-25000000	24213188:24213294	LOC112585816	U6 spliceosomal RNA
112585475	6	24000000-25000000	24387165:24400246	LOC112585475	uncharacterized LOC112585475
112585771	6	24000000-25000000	24590821:24605502	LOC112585771	RNA-binding protein 12-like
102408053	6	24000000-25000000	24721795:24722643	LOC102408053	40S ribosomal protein S6 pseudogene
102409457	6	24000000-25000000	24847250:25117959	SPAG17	sperm associated antigen 17
102411166	7	29000000-30000000	28985766:29022519	LOC102411166	alpha-fetoprotein-like
102410619	7	29000000-30000000	29029715:29050636	AFM	afamin
102394555	7	29000000-30000000	29061237:29082552	AFP	alpha fetoprotein
102394611	7	29000000-30000000	29090319:29108103	ALB	albumin
112586113	7	29000000-30000000	29155436:29179611	LOC112586113	ferritin light chain pseudogene
102394937	7	29000000-30000000	29231072:29398713	ANKRD17	ankyrin repeat domain 17
102395897	7	29000000-30000000	29402334:29414119	LOC102395897	cytochrome c oxidase assembly protein COX18, mitochondrial
102396414	7	29000000-30000000	29452393:29453113	LOC102396414	CDKN2AIP N-terminal-like protein
112586293	7	29000000-30000000	29654650:29654717	LOC112586293	small nucleolar RNA SNORD42
112586347	7	29000000-30000000	29853303:29853374	TRNAC-GCA	transfer RNA cysteine (anticodon GCA)
102411211	7	29000000-30000000	29867497:30146985	ADAMTS3	ADAM metalloproteinase with thrombospondin type 1 motif 3
102410493	9	103750000-104750000	103697749:103804653	EPS15L1	epidermal growth factor receptor pathway substrate 15 like 1
102411060	9	103750000-104750000	103811811:103836908	CALR3	calreticulin 3
102412258	9	103750000-104750000	103836948:103860193	C9H19orf44	chromosome 9 C19orf44 homolog
102411940	9	103750000-104750000	103840432:103841512	LOC102411940	60S ribosomal protein L12 pseudogene
102411611	9	103750000-104750000	103859590:103878961	CHERP	calcium homeostasis endoplasmic reticulum protein
102415455	9	103750000-104750000	103883578:103902278	SLC35E1	solute carrier family 35 member E1
112587003	9	103750000-104750000	103904540:103945357	MED26	mediator complex subunit 26
102412598	9	103750000-104750000	103961155:103974366	SMIM7	small integral membrane protein 7

102413250	9	103750000-104750000	103975647:104002645	TMEM38A	transmembrane protein 38A
102412925	9	103750000-104750000	103990791:103991581	LOC102412925	DNA-directed RNA polymerases I and III subunit RPAC2-like
102413584	9	103750000-104750000	104038505:104120194	NWD1	NACHT and WD repeat domain containing 1
102414564	9	103750000-104750000	104131400:104175331	SIN3B	SIN3 transcription regulator family member B
102415129	9	103750000-104750000	104177684:104180607	F2RL3	F2R like thrombin or trypsin receptor 3
102415794	9	103750000-104750000	104181914:104282245	CPAMD8	C3 and PZP like alpha-2-macroglobulin domain containing 8
112587004	9	103750000-104750000	104281073:104295028	LOC112587004	uncharacterized LOC112587004
102415454	9	103750000-104750000	104338289:104363336	HAUS8	HAUS augmin like complex subunit 8
102416664	9	103750000-104750000	104363536:104471780	MYO9B	myosin IXB
112587150	9	103750000-104750000	104391078:104391181	LOC112587150	U6 spliceosomal RNA
112587005	9	103750000-104750000	104419433:104423211	LOC112587005	uncharacterized LOC112587005
102389443	9	103750000-104750000	104473518:104476515	USE1	unconventional SNARE in the ER 1
112587006	9	103750000-104750000	104476961:104480076	LOC112587006	uncharacterized LOC112587006
102389754	9	103750000-104750000	104480100:104482572	OCEL1	occludin/ELL domain containing 1
102390530	9	103750000-104750000	104484559:104495105	NR2F6	nuclear receptor subfamily 2 group F member 6
102390863	9	103750000-104750000	104498970:104508176	USHBP1	USH1 protein network component harmonin binding protein 1
102392424	9	103750000-104750000	104511286:104517227	BABAM1	BRISC and BRCA1 A complex member 1
102391427	9	103750000-104750000	104518127:104523044	ANKLE1	ankyrin repeat and LEM domain containing 1
102393176	9	103750000-104750000	104524851:104532152	ABHD8	abhydrolase domain containing 8
102393494	9	103750000-104750000	104533800:104535208	MRPL34	mitochondrial ribosomal protein L34
102393813	9	103750000-104750000	104537656:104547786	DDA1	DET1 and DDB1 associated 1
102416112	9	103750000-104750000	104549751:104560690	ANO8	anoctamin 8
102394142	9	103750000-104750000	104562586:104563933	LOC102394142	uncharacterized LOC102394142
102394360	9	103750000-104750000	104563764:104567459	GTPBP3	GTP binding protein 3, mitochondrial
102394669	9	103750000-104750000	104573317:104592137	PLVAP	plasmalemma vesicle associated protein
102394995	9	103750000-104750000	104598277:104606093	CCDC194	coiled-coil domain containing 194
102395297	9	103750000-104750000	104606447:104609243	BST2	bone marrow stromal cell antigen 2
112587007	9	103750000-104750000	104609347:104610928	LOC112587007	uncharacterized LOC112587007
102395641	9	103750000-104750000	104611973:104615290	LOC102395641	bone marrow stromal antigen 2
112587133	9	103750000-104750000	104637311:104639267	LOC112587133	uncharacterized LOC112587133
102395963	9	103750000-104750000	104654716:104658929	MVB12A	multivesicular body subunit 12A
102396269	9	103750000-104750000	104665861:104673326	TMEM221	transmembrane protein 221
102416442	9	103750000-104750000	104680369:104685022	NXNL1	nucleoredoxin like 1

102396571	9	103750000-104750000	104699177:104741265	SLC27A1	solute carrier family 27 member 1
102397079	9	103750000-104750000	104745814:104754834	PGLS	6-phosphogluconolactonase
102415434	14	46250000-47250000	46052543:46295514	PCSK2	proprotein convertase subtilisin/kexin type 2
112578919	14	46250000-47250000	46549894:46588852	LOC112578919	uncharacterized LOC112578919
102389678	14	46250000-47250000	46672089:46723333	LOC102389678	ankyrin repeat domain-containing protein 26
112578891	14	46250000-47250000	46742361:46744727	LOC112578891	patched domain-containing protein 3-like
102390668	14	46250000-47250000	46753144:46757762	LOC102390668	patched domain-containing protein 3-like
102388909	14	46250000-47250000	46800358:46829218	RAB18	RAB18, member RAS oncogene family
102390337	14	46250000-47250000	46841270:46851217	LOC102390337	adenine phosphoribosyltransferase pseudogene
112578920	14	46250000-47250000	46865417:46867504	LOC112578920	uncharacterized LOC112578920
102415919	14	46250000-47250000	46931068:47004266	MKX	mohawk homeobox
102414927	14	46250000-47250000	47070155:47232293	ARMC4	armadillo repeat containing 4
112578817	14	46250000-47250000	47217376:47228614	LOC112578817	uncharacterized LOC112578817
102414362	14	46250000-47250000	47232519:47315813	LOC102414362	uncharacterized LOC102414362
102397576	23	23250000-24250000	23244478:23258318	BORCS7	BLOC-1 related complex subunit 7
102398096	23	23250000-24250000	23263128:23281173	AS3MT	arsenite methyltransferase
102396411	23	23250000-24250000	23313032:23499322	CNNM2	cyclin and CBS domain divalent metal cation transport mediator 2
112581631	23	23250000-24250000	23387749:23388315	LOC112581631	ubiquitin-40S ribosomal protein S27a pseudogene
112581630	23	23250000-24250000	23403092:23404836	LOC112581630	small kinetochore-associated protein-like
102395469	23	23250000-24250000	23504103:23603586	NT5C2	5'-nucleotidase, cytosolic II
112581659	23	23250000-24250000	23639292:23639398	LOC112581659	U6 spliceosomal RNA
102395147	23	23250000-24250000	23654457:23666604	INA	internexin neuronal intermediate filament protein alpha
102394609	23	23250000-24250000	23674522:23706738	PCGF6	polycomb group ring finger 6
102393977	23	23250000-24250000	23735233:23749969	TAF5	TATA-box binding protein associated factor 5
102394306	23	23250000-24250000	23749965:23755209	ATP5MD	ATP synthase membrane subunit DAPIT
102393436	23	23250000-24250000	23755289:23796916	PDCD11	programmed cell death 11
102392897	23	23250000-24250000	23798526:23804079	CALHM2	calcium homeostasis modulator family member 2
102392565	23	23250000-24250000	23806384:23811390	CALHM1	calcium homeostasis modulator 1
102392247	23	23250000-24250000	23827041:23836012	CALHM3	calcium homeostasis modulator 3
102390139	23	23250000-24250000	23846121:23854189	LOC102390139	60S ribosomal protein L6 pseudogene
102390466	23	23250000-24250000	23854085:23929956	NEURL1	neuralized E3 ubiquitin protein ligase 1
102391252	23	23250000-24250000	23931102:24179387	SH3PXD2A	SH3 and PX domains 2A
112581560	23	23250000-24250000	24200450:24204870	LOC112581560	uncharacterized LOC112581560
102416491	23	23250000-24250000	24205893:24247440	STN1	STN1 subunit of CST complex

Supplementary Table S2. List of QTL within 1Mb windows for each region in selection signatures.

BBU:start-end (bp)	BTA:start-end (bp)	QTL	Class	QTL ID	PubMed ID
1:40526390-40526430	27:3215080-3215120	Somatic cell score	Health	32491	25288516
1:40530356-40530396	27:3211194-3211234	Somatic cell score	Health	32490	25288516
1:40662346-40662386	27:3081666-3081706	Reproductive efficiency	Reproduction	63734	26286463
1:41123362-41123402	27:2622980-2623020	Body weight	Production	31752	24733441
1:41125398-41125438	27:2620941-2620981	Body weight	Production	31751	24733441
1:41126272-41126312	27:2620068-2620108	Body weight	Production	31750	24733441
1:41127117-41127157	27:2619222-2619262	Body weight	Production	31749	24733441
1:41131424-41131464	27:2614971-2615011	Body weight	Production	31748	24733441
1:41421097-41421137	27:2339815-2339855	Body weight (mature)	Production	65317	26445451
1:41478791-41478831	27:2279259-2279299	Body weight (mature)	Production	65314	26445451
6:24055764-24055804	3:24336016-24336056	Milk yield	Milk	25395	22449276
6:24888783-24888823	3:25150743-25150783	Milk protein percentage	Milk	105618	27287773
6:24926065-24926105	3:25187102-25187142	Milk protein percentage	Milk	105465	27287773
7:29008752-29008792	6:90331826-90331866	Milk kappa-casein percentage	Milk	109498	27485317
7:29008752-29008792	6:90331826-90331866	Milk glycosylated kappa-casein percentage	Milk	114582	27485317
7:29008752-29008792	6:90331826-90331866	Milk protein percentage	Milk	138036	27760518
7:29008752-29008792	6:90331826-90331866	Milk unglycosylated kappa-casein percentage	Milk	117521	27485317
7:29010692-29010732	6:90328601-90328641	Milk unglycosylated kappa-casein percentage	Milk	117520	27485317
7:29010692-29010732	6:90328601-90328641	Milk protein percentage	Milk	138035	27760518
7:29010692-29010732	6:90328601-90328641	Milk glycosylated kappa-casein percentage	Milk	114581	27485317
7:29010692-29010732	6:90328601-90328641	Milk kappa-casein percentage	Milk	109497	27485317
7:29029742-29029782	6:90311872-90311912	Milk unglycosylated kappa-casein percentage	Milk	117519	27485317
7:29029742-29029782	6:90311872-90311912	Milk glycosylated kappa-casein percentage	Milk	114580	27485317
7:29029742-29029782	6:90311872-90311912	Milk kappa-casein percentage	Milk	109496	27485317
7:29029742-29029782	6:90311872-90311912	Milk protein percentage	Milk	138034	27760518
7:29064832-29064872	6:90277965-90278005	Milk protein percentage	Milk	137979	27760518
7:29065807-29065847	6:90276987-90277027	Clinical mastitis	Health	137417	27760518
7:29066323-29066363	6:90276471-90276511	Clinical mastitis	Health	137416	27760518
7:29081254-29081294	6:90260166-90260206	Milk protein percentage	Milk	137971	27760518
7:29082008-29082048	6:90259408-90259448	Milk protein percentage	Milk	137970	27760518
7:29082969-29083009	6:90258440-90258480	Milk protein percentage	Milk	137969	27760518
7:29087339-29087379	6:90254073-90254113	Milk protein percentage	Milk	137968	27760518
7:29152259-29152299	6:90188952-90188992	Udder height	Exterior	42337	21831322
7:29152259-29152299	6:90188952-90188992	Stature	Exterior	42339	21831322
7:29152259-29152299	6:90188952-90188992	PTA type	Production	42333	21831322

7:29152259-29152299	6:90188952-90188992	Body depth	Production	42330	21831322
7:29152259-29152299	6:90188952-90188992	Foot angle	Exterior	42331	21831322
7:29152259-29152299	6:90188952-90188992	Strength	Exterior	42340	21831322
7:29152259-29152299	6:90188952-90188992	Udder attachment	Exterior	42334	21831322
7:29152259-29152299	6:90188952-90188992	Net merit	Production	42335	21831322
7:29152259-29152299	6:90188952-90188992	Rump width	Production	42338	21831322
7:29152259-29152299	6:90188952-90188992	Rear leg placement - rear view	Exterior	42336	21831322
7:29152259-29152299	6:90188952-90188992	Udder depth	Exterior	42341	21831322
7:29152259-29152299	6:90188952-90188992	Feet and leg conformation	Exterior	42332	21831322
7:29236191-29236231	6:90098101-90098141	Milk kappa-casein percentage	Milk	109874	27485317
7:29236191-29236231	6:90098101-90098141	Milk glycosylated kappa-casein percentage	Milk	114769	27485317
7:29236191-29236231	6:90098101-90098141	Milk unglycosylated kappa-casein percentage	Milk	118405	27485317
7:29254275-29254315	6:90080323-90080363	Milk kappa-casein percentage	Milk	112319	27485317
7:29254793-29254833	6:90079803-90079843	Milk kappa-casein percentage	Milk	112275	27485317
7:29372303-29372343	6:89962869-89962909	Milk protein yield	Milk	35276	20630249
7:29421562-29421602	6:89912176-89912216	Daughter pregnancy rate	Reproduction	106950	27209127
7:29441372-29441412	6:89893457-89893497	Milk unglycosylated kappa-casein percentage	Milk	117957	27485317
7:29441372-29441412	6:89893457-89893497	Milk glycosylated kappa-casein percentage	Milk	115073	27485317
7:29441372-29441412	6:89893457-89893497	Milk kappa-casein percentage	Milk	109889	27485317
7:29450715-29450755	6:89884292-89884332	Milk glycosylated kappa-casein percentage	Milk	116853	27485317
7:29455269-29455309	6:89880521-89880561	Milk unglycosylated kappa-casein percentage	Milk	117956	27485317
7:29455269-29455309	6:89880521-89880561	Milk kappa-casein percentage	Milk	109888	27485317
7:29455269-29455309	6:89880521-89880561	Milk glycosylated kappa-casein percentage	Milk	115072	27485317
7:29474549-29474589	6:89867985-89868025	Milk kappa-casein percentage	Milk	111129	27485317
7:29508446-29508486	6:89833554-89833594	Milk kappa-casein percentage	Milk	112245	27485317
7:29508446-29508486	6:89833554-89833594	Milk unglycosylated kappa-casein percentage	Milk	118843	27485317
7:29540604-29540644	6:89797748-89797788	Milk kappa-casein percentage	Milk	110928	27485317
7:29540604-29540644	6:89797748-89797788	Milk unglycosylated kappa-casein percentage	Milk	118609	27485317
7:29544388-29544428	6:89793984-89794024	Milk unglycosylated kappa-casein percentage	Milk	118557	27485317
7:29544388-29544428	6:89793984-89794024	Milk kappa-casein percentage	Milk	110940	27485317
7:29549099-29549139	6:89786413-89786453	Milk kappa-casein percentage	Milk	111102	27485317
7:29549099-29549139	6:89786413-89786453	Milk unglycosylated kappa-casein percentage	Milk	118316	27485317
7:29659039-29659079	6:89677050-89677090	Milk kappa-casein percentage	Milk	109523	27485317
7:29659039-29659079	6:89677050-89677090	Milk glycosylated kappa-casein percentage	Milk	114918	27485317
7:29659039-29659079	6:89677050-89677090	Milk unglycosylated kappa-casein percentage	Milk	117328	27485317
7:29705111-29705151	6:89626746-89626786	Milk glycosylated kappa-casein percentage	Milk	114552	27485317
7:29705111-29705151	6:89626746-89626786	Milk kappa-casein percentage	Milk	110120	27485317
7:29713115-29713155	6:89617889-89617929	Milk kappa-casein percentage	Milk	110119	27485317

7:29713115-29713155	6:89617889-89617929	Milk glycosylated kappa-casein percentage	Milk	114551	27485317
7:29737940-29737980	6:89596086-89596126	Clinical mastitis	Health	137442	27760518
7:29747964-29748004	6:89586108-89586148	Ketosis	Health	179564	31311492
7:29788225-29788265	6:89546141-89546181	Milk kappa-casein percentage	Milk	112667	27485317
7:29792178-29792218	6:89542634-89542674	Milk unglycosylated kappa-casein percentage	Milk	118866	27485317
7:29792178-29792218	6:89542634-89542674	Milk glycosylated kappa-casein percentage	Milk	116088	27485317
7:29792178-29792218	6:89542634-89542674	Milk kappa-casein percentage	Milk	110283	27485317
7:29817314-29817354	6:89514549-89514589	Milk glycosylated kappa-casein percentage	Milk	115227	27485317
7:29817314-29817354	6:89514549-89514589	Milk kappa-casein percentage	Milk	110250	27485317
7:29826879-29826919	6:89504984-89505024	Milk myristoleic acid content	Milk	126239	28193175
7:29826879-29826919	6:89504984-89505024	Clinical mastitis	Health	137411	21906097
7:29837933-29837973	6:89494372-89494412	Milk kappa-casein percentage	Milk	110164	27485317
7:29837933-29837973	6:89494372-89494412	Milk glycosylated kappa-casein percentage	Milk	114977	27485317
7:29851885-29851925	6:89479533-89479573	Lean meat yield	Meat and Carcass	36807	25273628
7:29855634-29855674	6:89475802-89475842	Milk glycosylated kappa-casein percentage	Milk	114434	27485317
7:29855634-29855674	6:89475802-89475842	Milk kappa-casein percentage	Milk	110425	27485317
7:29861574-29861614	6:89469852-89469892	Milk kappa-casein percentage	Milk	110424	27485317
7:29861574-29861614	6:89469852-89469892	Milk glycosylated kappa-casein percentage	Milk	114433	27485317
7:29866110-29866150	6:89461822-89461862	Milk kappa-casein percentage	Milk	110423	27485317
7:29866110-29866150	6:89461822-89461862	Milk glycosylated kappa-casein percentage	Milk	114432	27485317
7:29869355-29869395	6:89458889-89458929	Milk kappa-casein percentage	Milk	110065	27485317
7:29869355-29869395	6:89458889-89458929	Milk glycosylated kappa-casein percentage	Milk	114423	27485317
7:29909428-29909468	6:89419420-89419460	Milk glycosylated kappa-casein percentage	Milk	115025	27485317
7:29918352-29918392	6:89410851-89410891	Milk kappa-casein percentage	Milk	111165	27485317
7:29918352-29918392	6:89410851-89410891	Milk unglycosylated kappa-casein percentage	Milk	118103	27485317
7:29925344-29925384	6:89403794-89403834	Milk phosphorylated alpha-S2-casein percentage	Milk	120182	27485317
7:29929414-29929454	6:89399716-89399756	Milk phosphorylated alpha-S2-casein percentage	Milk	120181	27485317
7:29936299-29936339	6:89393023-89393063	Milk iron content	Milk	69613	25989905
7:29969227-29969267	6:89359619-89359659	Clinical mastitis	Health	137497	27760518
9:103874115-103874155	7:6413299-6413339	Body weight (yearling)	Production	67870	19966163
9:104014079-104014119	7:6276033-6276073	Body weight (weaning)	Production	67863	19966163
9:104014079-104014119	7:6276033-6276073	Body weight (yearling)	Production	67864	19966163
9:104241405-104241445	7:6036506-6036546	Udder depth	Exterior	42684	21831322
9:104241405-104241445	7:6036506-6036546	Udder cleft	Exterior	42683	21831322
9:104241405-104241445	7:6036506-6036546	Stillbirth (maternal)	Reproduction	42676	21831322
9:104241405-104241445	7:6036506-6036546	Teat placement - rear	Exterior	42681	21831322

9:104241405-104241445	7:6036506-6036546	Feet and leg conformation	Exterior	42677	21831322
9:104241405-104241445	7:6036506-6036546	Udder height	Exterior	42682	21831322
9:104241405-104241445	7:6036506-6036546	Udder attachment	Exterior	42680	21831322
9:104241405-104241445	7:6036506-6036546	PTA type	Production	42678	21831322
9:104241405-104241445	7:6036506-6036546	Teat placement - front	Exterior	42679	21831322
9:104601649-104601689	7:5675601-5675641	Sperm motility	Reproduction	65814	27610941IS U0080
14:46297278-46297318	13:37810997-37811037	Lactation persistency	Production	125425	20412936
14:47087758-47087798	13:37041116-37041156	Reproductive efficiency	Reproduction	63717	26286463
23:23254156-23254196	26:23737132-23737172	Milk myristoleic acid content	Milk	63435	26364108
23:23254156-23254196	26:23737132-23737172	Milk C14 index	Milk	62710	26364108
23:23277222-23277262	26:23760568-23760608	Milk C14 index	Milk	63172	26364108
23:23277222-23277262	26:23760568-23760608	Milk myristoleic acid content	Milk	63270	26364108
23:23316130-23316170	26:23797836-23797876	Milk myristoleic acid content	Milk	62874	26364108
23:23316130-23316170	26:23797836-23797876	Milk C14 index	Milk	62678	25511820
23:23329049-23329089	26:23810765-23810805	Milk fat yield	Milk	99811	27006194
23:23362633-23362673	26:23841827-23841867	Milk C14 index	Milk	63271	26364108
23:23362633-23362673	26:23841827-23841867	Milk myristoleic acid content	Milk	63482	26364108
23:23368401-23368441	26:23847574-23847614	Milk myristoleic acid content	Milk	63614	26364108
23:23368401-23368441	26:23847574-23847614	Milk C14 index	Milk	63201	25511820
23:23374177-23374217	26:23853314-23853354	Milk C14 index	Milk	32617	24858810
23:23386907-23386947	26:23866235-23866275	Milk fat yield	Milk	99883	27006194
23:23388130-23388170	26:23867454-23867494	Milk myristoleic acid content	Milk	62819	25511820
23:23388130-23388170	26:23867454-23867494	Milk C14 index	Milk	62577	25511820
23:23390633-23390673	26:23869981-23870021	Milk C14 index	Milk	32976	25511820
23:23390633-23390673	26:23869981-23870021	Milk myristoleic acid content	Milk	33720	25511820
23:23391013-23391053	26:23870354-23870394	Milk fat yield	Milk	100238	27006194
23:23397393-23397433	26:23876456-23876496	Milk C14 index	Milk	32571	24858810
23:23397393-23397433	26:23876456-23876496	Stearic acid content	Meat and Carcass	107713	27112906
23:23397393-23397433	26:23876456-23876496	Milk myristoleic acid content	Milk	32528	24858810
23:23414314-23414354	26:23892340-23892380	Milk C14 index	Milk	62597	25511820
23:23414314-23414354	26:23892340-23892380	Milk myristoleic acid content	Milk	62846	25511820
23:23423021-23423061	26:23901859-23901899	Milk myristoleic acid content	Milk	33724	25511820
23:23423021-23423061	26:23901859-23901899	Milk C14 index	Milk	32980	25511820
23:23428552-23428592	26:23907660-23907700	Milk myristoleic acid content	Milk	62848	25511820
23:23428552-23428592	26:23907660-23907700	Milk C14 index	Milk	62599	25511820
23:23432009-23432049	26:23910996-23911036	Milk fat yield	Milk	100497	27006194
23:23438165-23438205	26:23917121-23917161	Milk fat yield	Milk	100389	27006194

23:23438759-23438799	26:23917719-23917759	Milk fat yield	Milk	100212	27006194
23:23440293-23440333	26:23919252-23919292	Milk myristoleic acid content	Milk	63609	26364108
23:23440293-23440333	26:23919252-23919292	Milk C14 index	Milk	63304	26364108
23:23440945-23440985	26:23919904-23919944	Milk myristoleic acid content	Milk	63610	26364108
23:23440945-23440985	26:23919904-23919944	Milk C14 index	Milk	63305	26364108
23:23441846-23441886	26:23920807-23920847	Milk fat yield	Milk	100426	27006194
23:23443944-23443984	26:23922916-23922956	Milk myristoleic acid content	Milk	63612	26364108
23:23443944-23443984	26:23922916-23922956	Milk C14 index	Milk	63307	26364108
23:23446159-23446199	26:23925100-23925140	Milk myristoleic acid content	Milk	63465	26364108
23:23446159-23446199	26:23925100-23925140	Milk C14 index	Milk	63209	25511820
23:23446789-23446829	26:23925720-23925760	Milk fat yield	Milk	99738	27006194
23:23446834-23446874	26:23925765-23925805	Milk fat yield	Milk	100846	27006194
23:23447145-23447185	26:23926076-23926116	Milk C14 index	Milk	34519	25511820
23:23447438-23447478	26:23926368-23926408	Milk fat yield	Milk	100247	27006194
23:23447571-23447611	26:23926501-23926541	Milk fat yield	Milk	100251	27006194
23:23447844-23447884	26:23926774-23926814	Milk fat yield	Milk	100249	27006194
23:23447956-23447996	26:23926886-23926926	Milk fat yield	Milk	100256	27006194
23:23448202-23448242	26:23927120-23927160	Milk fat yield	Milk	100258	27006194
23:23448495-23448535	26:23927414-23927454	Milk fat yield	Milk	100259	27006194
23:23448818-23448858	26:23927737-23927777	Milk fat yield	Milk	100300	27006194
23:23449024-23449064	26:23927933-23927973	Milk fat yield	Milk	100269	27006194
23:23449315-23449355	26:23928223-23928263	Milk fat yield	Milk	100271	27006194
23:23449380-23449420	26:23928288-23928328	Milk fat yield	Milk	100246	27006194
23:23449476-23449516	26:23928384-23928424	Milk fat yield	Milk	100275	27006194
23:23449929-23449969	26:23928837-23928877	Milk fat yield	Milk	100277	27006194
23:23450330-23450370	26:23929237-23929277	Milk C14 index	Milk	34520	25511820
23:23450479-23450519	26:23929386-23929426	Milk fat yield	Milk	100278	27006194
23:23450709-23450749	26:23929619-23929659	Milk fat yield	Milk	100274	27006194
23:23450772-23450812	26:23929682-23929722	Milk fat yield	Milk	100273	27006194
23:23450895-23450935	26:23929804-23929844	Milk fat yield	Milk	100281	27006194
23:23450899-23450939	26:23929808-23929848	Milk fat yield	Milk	100294	27006194
23:23451448-23451488	26:23930357-23930397	Milk fat yield	Milk	100305	27006194
23:23451487-23451527	26:23930396-23930436	Milk fat yield	Milk	100309	27006194
23:23451903-23451943	26:23930811-23930851	Milk fat yield	Milk	100310	27006194
23:23453202-23453242	26:23932109-23932149	Milk fat yield	Milk	100434	27006194
23:23456272-23456312	26:23935250-23935290	Milk fat yield	Milk	100473	27006194
23:23457126-23457166	26:23936072-23936112	Milk fat yield	Milk	100481	27006194
23:23458821-23458861	26:23937716-23937756	Milk fat yield	Milk	100503	27006194

23:23460456-23460496	26:23939358-23939398	Milk fat yield	Milk	100505	27006194
23:23460895-23460935	26:23939797-23939837	Milk fat yield	Milk	100510	27006194
23:23464341-23464381	26:23943233-23943273	Milk fat yield	Milk	100517	27006194
23:23464885-23464925	26:23943777-23943817	Milk fat yield	Milk	100518	27006194
23:23465003-23465043	26:23943895-23943935	Milk fat yield	Milk	100520	27006194
23:23466893-23466933	26:23945798-23945838	Milk fat yield	Milk	100528	27006194
23:23466966-23467006	26:23945871-23945911	Milk fat yield	Milk	100522	27006194
23:23467524-23467564	26:23946424-23946464	Milk C14 index	Milk	34399	25511820
23:23467824-23467864	26:23946724-23946764	Milk fat yield	Milk	100523	27006194
23:23468060-23468100	26:23947126-23947166	Milk fat yield	Milk	100982	27006194
23:23468742-23468782	26:23947785-23947825	Milk fat yield	Milk	100524	27006194
23:23468958-23468998	26:23948001-23948041	Milk fat yield	Milk	98922	27006194
23:23471401-23471441	26:23950448-23950488	Milk fat yield	Milk	100527	27006194
23:23472965-23473005	26:23952013-23952053	Milk fat yield	Milk	100526	27006194
23:23481380-23481420	26:23960271-23960311	Milk fat yield	Milk	100830	27006194
23:23481497-23481537	26:23960376-23960416	Milk fat yield	Milk	99388	27006194
23:23482942-23482982	26:23961817-23961857	Milk fat yield	Milk	100243	27006194
23:23483199-23483239	26:23962070-23962110	Milk fat yield	Milk	100504	27006194
23:23487247-23487287	26:23966114-23966154	Milk fat yield	Milk	99799	27006194
23:23487442-23487482	26:23966506-23966546	Milk fat yield	Milk	99797	27006194
23:23494373-23494413	26:23973462-23973502	Milk fat yield	Milk	100699	27006194
23:23497185-23497225	26:23976251-23976291	Milk fat yield	Milk	100692	27006194
23:23497185-23497225	26:23976251-23976291	Milk C14 index	Milk	34158	25511820
23:23497348-23497388	26:23976414-23976454	Milk fat yield	Milk	100696	27006194
23:23497403-23497443	26:23976469-23976509	Milk fat yield	Milk	100701	27006194
23:23498456-23498496	26:23977523-23977563	Milk fat yield	Milk	100694	27006194
23:23498456-23498496	26:23977523-23977563	Milk C14 index	Milk	34182	25511820
23:23499361-23499401	26:23978427-23978467	Milk fat yield	Milk	100689	27006194
23:23500634-23500674	26:23979702-23979742	Milk fat yield	Milk	100693	27006194
23:23503131-23503171	26:23982204-23982244	Milk fat yield	Milk	100609	27006194
23:23503381-23503421	26:23982454-23982494	Milk fat yield	Milk	100593	27006194
23:23503504-23503544	26:23982577-23982617	Milk fat yield	Milk	100612	27006194
23:23503726-23503766	26:23982799-23982839	Milk C14 index	Milk	34164	25511820
23:23505925-23505965	26:23985015-23985055	Milk fat yield	Milk	100633	27006194
23:23506179-23506219	26:23985268-23985308	Milk C14 index	Milk	34165	25511820
23:23508049-23508089	26:23987148-23987188	Milk fat yield	Milk	100648	27006194
23:23508364-23508404	26:23987467-23987507	Milk C14 index	Milk	34183	25511820
23:23508364-23508404	26:23987467-23987507	Milk fat yield	Milk	100656	27006194

23:23508624-23508664	26:23987730-23987770	Milk fat yield	Milk	100647	27006194
23:23509464-23509504	26:23988489-23988529	Milk fat yield	Milk	100657	27006194
23:23509464-23509504	26:23988489-23988529	Milk C14 index	Milk	34184	25511820
23:23509581-23509621	26:23988606-23988646	Milk fat yield	Milk	100652	27006194
23:23509992-23510032	26:23989016-23989056	Milk fat yield	Milk	100653	27006194
23:23510170-23510210	26:23989194-23989234	Milk fat yield	Milk	100646	27006194
23:23510231-23510271	26:23989255-23989295	Milk fat yield	Milk	100530	27006194
23:23510308-23510348	26:23989335-23989375	Milk fat yield	Milk	100636	27006194
23:23510572-23510612	26:23989599-23989639	Milk fat yield	Milk	100631	27006194
23:23510601-23510641	26:23989628-23989668	Milk fat yield	Milk	100628	27006194
23:23510601-23510641	26:23989628-23989668	Milk C14 index	Milk	34159	25511820
23:23510682-23510722	26:23989709-23989749	Milk fat yield	Milk	100629	27006194
23:23511066-23511106	26:23990093-23990133	Milk fat yield	Milk	100810	27006194
23:23511327-23511367	26:23990362-23990402	Milk fat yield	Milk	100626	27006194
23:23511360-23511400	26:23990395-23990435	Milk fat yield	Milk	100627	27006194
23:23511360-23511400	26:23990395-23990435	Milk C14 index	Milk	34160	25511820
23:23512128-23512168	26:23991158-23991198	Milk fat yield	Milk	100658	27006194
23:23512610-23512650	26:23991640-23991680	Milk fat yield	Milk	100663	27006194
23:23512822-23512862	26:23991849-23991889	Milk fat yield	Milk	100679	27006194
23:23512951-23512991	26:23991977-23992017	Milk fat yield	Milk	100685	27006194
23:23513512-23513552	26:23992540-23992580	Milk fat yield	Milk	100690	27006194
23:23513801-23513841	26:23992834-23992874	Milk fat yield	Milk	100705	27006194
23:23513926-23513966	26:23992959-23992999	Milk fat yield	Milk	100717	27006194
23:23516122-23516162	26:23995142-23995182	Milk fat yield	Milk	100797	27006194
23:23516266-23516306	26:23995287-23995327	Milk fat yield	Milk	100798	27006194
23:23516267-23516307	26:23995288-23995328	Milk fat yield	Milk	100804	27006194
23:23521001-23521041	26:24000034-24000074	Milk C14 index	Milk	34161	25511820
23:23521001-23521041	26:24000034-24000074	Milk fat yield	Milk	100913	27006194
23:23522081-23522121	26:24001114-24001154	Milk fat yield	Milk	100865	27006194
23:23522179-23522219	26:24001212-24001252	Milk fat yield	Milk	100672	27006194
23:23526045-23526085	26:24005086-24005126	Milk fat yield	Milk	100827	27006194
23:23526097-23526137	26:24005138-24005178	Milk fat yield	Milk	100824	27006194
23:23526120-23526160	26:24005161-24005201	Milk fat yield	Milk	100801	27006194
23:23526502-23526542	26:24005541-24005581	Milk fat yield	Milk	100087	27006194
23:23529435-23529475	26:24008487-24008527	Milk fat yield	Milk	100244	27006194
23:23531084-23531124	26:24009926-24009966	Milk fat yield	Milk	99229	27006194
23:23536513-23536553	26:24015351-24015391	Milk myristoleic acid content	Milk	33837	25511820
23:23536513-23536553	26:24015351-24015391	Milk C14 index	Milk	34067	25511820

23:23537641-23537681	26:24016478-24016518	Milk fat yield	Milk	99207	27006194
23:23538230-23538270	26:24017067-24017107	Milk fat yield	Milk	99197	27006194
23:23540424-23540464	26:24019269-24019309	Milk fat yield	Milk	99242	27006194
23:23543545-23543585	26:24022393-24022433	Milk C14 index	Milk	33757	25511820
23:23543545-23543585	26:24022393-24022433	Milk myristoleic acid content	Milk	33838	25511820
23:23549690-23549730	26:24028569-24028609	Milk fat yield	Milk	99208	27006194
23:23558960-23559000	26:24037893-24037933	Milk myristoleic acid content	Milk	62695	25511820
23:23558960-23559000	26:24037893-24037933	Milk C14 index	Milk	62630	25511820
23:23564066-23564106	26:24040479-24040519	Milk fat yield	Milk	99257	27006194
23:23564910-23564950	26:24041323-24041363	Milk fat yield	Milk	99262	27006194
23:23567090-23567130	26:24043505-24043545	Milk fat yield	Milk	99259	27006194
23:23577881-23577921	26:24054018-24054058	Milk fat yield	Milk	100649	27006194
23:23583840-23583880	26:24059984-24060024	Milk C14 index	Milk	34010	25511820
23:23586835-23586875	26:24062957-24062997	Milk C14 index	Milk	34011	25511820
23:23596068-23596108	26:24073094-24073134	Milk fat yield	Milk	98383	27006194
23:23599185-23599225	26:24076125-24076165	Milk C14 index	Milk	34012	25511820
23:23600289-23600329	26:24077258-24077298	Milk fat yield	Milk	98283	27006194
23:23602041-23602081	26:24079073-24079113	Milk C14 index	Milk	34013	25511820
23:23604428-23604468	26:24081815-24081855	Milk C14 index	Milk	34014	25511820
23:23607994-23608034	26:24085522-24085562	Milk C14 index	Milk	34015	25511820
23:23620001-23620041	26:24099748-24099788	Milk myristoleic acid content	Milk	63665	26364108
23:23620001-23620041	26:24099748-24099788	Milk C14 index	Milk	63605	26364108
23:23639996-23640036	26:24119349-24119389	Milk fat yield	Milk	98291	27006194
23:23653154-23653194	26:24130377-24130417	Myristoleic acid content	Meat and Carcass	107794	27112906
23:23658111-23658151	26:24135635-24135675	Milk fat yield	Milk	98293	27006194
23:23764419-23764459	26:24240854-24240894	Milk C14 index	Milk	63664	26364108
23:23848639-23848679	26:24325400-24325440	Milk C14 index	Milk	63052	25511820
23:23848639-23848679	26:24325400-24325440	Milk myristoleic acid content	Milk	63130	25511820
23:23871415-23871455	26:24348230-24348270	Milk myristoleic acid content	Milk	33568	25511820
23:23871415-23871455	26:24348230-24348270	Milk C14 index	Milk	33192	25511820
23:23879942-23879982	26:24356792-24356832	Milk myristoleic acid content	Milk	62575	25511820
23:23879942-23879982	26:24356792-24356832	Milk C14 index	Milk	62499	25511820
23:23886396-23886436	26:24362981-24363021	Milk fat yield	Milk	99421	27006194
23:23891662-23891702	26:24368340-24368380	Milk fat yield	Milk	100160	27006194
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23:23893530-23893570	26:24370206-24370246	Milk fat yield	Milk	99616	27006194
23:23894197-23894237	26:24370874-24370914	Milk fat yield	Milk	99957	27006194
23:23894989-23895029	26:24371672-24371712	Milk fat yield	Milk	99902	27006194

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23:23899322-23899362	26:24376003-24376043	Milk fat yield	Milk	99914	27006194
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23:23900697-23900737	26:24377374-24377414	Milk fat yield	Milk	99918	27006194
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23:23925209-23925249	26:24399883-24399923	Milk fat yield	Milk	100129	27006194
23:23925537-23925577	26:24400211-24400251	Milk fat yield	Milk	99689	27006194
23:23925708-23925748	26:24400383-24400423	Milk fat yield	Milk	100098	27006194
23:23926589-23926629	26:24401283-24401323	Milk fat yield	Milk	100110	27006194
23:23926980-23927020	26:24401674-24401714	Milk fat yield	Milk	99685	27006194
23:23927129-23927169	26:24401823-24401863	Milk fat yield	Milk	100108	27006194
23:23927527-23927567	26:24402221-24402261	Milk fat yield	Milk	100191	27006194
23:23927790-23927830	26:24402484-24402524	Milk fat yield	Milk	100113	27006194
23:23930059-23930099	26:24404760-24404800	Milk fat yield	Milk	99692	27006194
23:23931990-23932030	26:24406691-24406731	Milk fat yield	Milk	100122	27006194
23:23932315-23932355	26:24407016-24407056	Milk fat yield	Milk	100123	27006194
23:23932967-23933007	26:24407668-24407708	Milk fat yield	Milk	100130	27006194
23:23933157-23933197	26:24407858-24407898	Milk fat yield	Milk	100132	27006194
23:23933767-23933807	26:24408472-24408512	Milk fat yield	Milk	100139	27006194
23:23936968-23937008	26:24411662-24411702	Milk fat yield	Milk	100398	27006194
23:23937120-23937160	26:24411813-24411853	Milk fat yield	Milk	99677	27006194
23:23937189-23937229	26:24411882-24411922	Milk fat yield	Milk	100430	27006194
23:23937233-23937273	26:24411926-24411966	Milk fat yield	Milk	100144	27006194
23:23939635-23939675	26:24414306-24414346	Milk fat yield	Milk	99768	27006194
23:23943320-23943360	26:24417960-24418000	Milk C14 index	Milk	34025	25511820
23:23944443-23944483	26:24419057-24419097	Milk fat yield	Milk	99765	27006194

23:23947967-23948007	26:24422584-24422624	Milk fat yield	Milk	99769	27006194
23:23948206-23948246	26:24422823-24422863	Milk fat yield	Milk	99764	27006194
23:23949094-23949134	26:24423642-24423682	Milk fat yield	Milk	99759	27006194
23:23950095-23950135	26:24424643-24424683	Milk C14 index	Milk	34026	25511820
23:23955624-23955664	26:24430175-24430215	Milk fat yield	Milk	100622	27006194
23:23958177-23958217	26:24432738-24432778	Milk C14 index	Milk	34023	25511820
23:23984736-23984776	26:24458992-24459032	Milk C14 index	Milk	34033	25511820
23:24003866-24003906	26:24477942-24477982	Milk C14 index	Milk	34031	24858810
23:24003866-24003906	26:24477942-24477982	Milk myristoleic acid content	Milk	32530	24858810
23:24005033-24005073	26:24479111-24479151	Milk C14 index	Milk	34017	25511820
23:24016673-24016713	26:24490719-24490759	Milk C14 index	Milk	34020	25511820
23:24024271-24024311	26:24498406-24498446	Milk C14 index	Milk	34016	25511820
23:24033098-24033138	26:24507233-24507273	Milk C14 index	Milk	34022	25511820
23:24054821-24054861	26:24530099-24530139	Milk myristoleic acid content	Milk	63407	26364108
23:24054821-24054861	26:24530099-24530139	Milk C14 index	Milk	33132	25511820
23:24056459-24056499	26:24531743-24531783	Milk myristoleic acid content	Milk	63409	26364108
23:24056459-24056499	26:24531743-24531783	Milk C14 index	Milk	32613	24858810
23:24059195-24059235	26:24534460-24534500	Milk C14 index	Milk	33135	25511820
23:24059195-24059235	26:24534460-24534500	Milk myristoleic acid content	Milk	63410	26364108
23:24061269-24061309	26:24536536-24536576	Milk myristoleic acid content	Milk	63411	26364108
23:24061269-24061309	26:24536536-24536576	Milk C14 index	Milk	33136	25511820
23:24061771-24061811	26:24537038-24537078	Milk C14 index	Milk	32629	24858810
23:24061875-24061915	26:24537142-24537182	Milk myristoleic acid content	Milk	63412	26364108
23:24061875-24061915	26:24537142-24537182	Milk C14 index	Milk	62740	25511820
23:24065369-24065409	26:24540589-24540629	Milk C14 index	Milk	62741	25511820
23:24065369-24065409	26:24540589-24540629	Milk myristoleic acid content	Milk	63413	26364108
23:24069070-24069110	26:24544288-24544328	Milk myristoleic acid content	Milk	63416	26364108
23:24069070-24069110	26:24544288-24544328	Milk C14 index	Milk	33141	25511820
23:24075417-24075457	26:24550671-24550711	Milk myristoleic acid content	Milk	63418	26364108
23:24075417-24075457	26:24550671-24550711	Milk C14 index	Milk	63106	25511820
23:24077891-24077931	26:24553144-24553184	Milk C14 index	Milk	34027	25511820
23:24128977-24129017	26:24604279-24604319	Milk fat yield	Milk	99897	27006194
23:24154710-24154750	26:24630101-24630141	Milk fat yield	Milk	100262	27006194
23:24161422-24161462	26:24634779-24634819	Milk C14 index	Milk	34271	25511820
23:24168113-24168153	26:24642153-24642193	Milk fat yield	Milk	99650	27006194
23:24173322-24173362	26:24647394-24647434	Milk fat yield	Milk	100155	27006194
23:24249393-24249433	26:24740110-24740150	Milk C14 index	Milk	63292	26364108
23:24249393-24249433	26:24740110-24740150	Milk myristoleic acid content	Milk	63376	26364108

