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Evaluation of runs of homozygosity and genomic inbreeding in Holstein cattle from Colombia

Evaluación de tramos de homocigosidad y consanguinidad genómica en bovinos Holstein de Colombia

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Highlights:
Our results shown lowest LD extension levels related with other Holstein populations.
Coefficients based on GRM and SNP may be useful estimators of individual autozygosity.
Genes related with production, reproduction and adaptation were found.

Abstract

Traditional selection programs for dairy cattle, based on quantitative principles, have worked well and allowed strong selection processes in the world over many decades. The objectives of this work were to estimate linkage disequilibrium (LD) levels at varying SNPs densities, to evaluate the effective population size of Holstein cattle, to characterize runs of homozygosity (ROH) distribution through Holstein cattle from Nariño and, to estimate and compare inbreeding coefficient (F) based on genomic markers information, runs of homozygosity (FROH), genomic relationship matrix (FGRM), and excess of homozygous (FSNP). After quality control, the dataset used was composed of 606 Holstein animals and 22200 SNP markers. PLINK program was used to identify LD, Ne, ROH segment and FROH and FSNP, FGRM was calculated with BLUPF90 family of programs. The average of r2 in all chromosomes was 0.011, the highest r2 was found in BTA3 (0.0323), and the lowest in BTA12 (0.0039). 533 ROH segments were identified in 319 animals; findings obtained in this study suggest that on average 0,28% of Holstein genome is autozygous. Total length of ROH was composed mostly of small segments (ROH1-4Mb and ROH4-8Mb). These segments accounted for approximately 96%, while larger ROH (ROH>8Mb) were 3.37% of all ROH detected. Inbreeding averages FROH, FSNP and FGRM methodologies were 0.28%, 3.11% and 3.36% respectively. The Pearson's correlation among these different F values was: 0.49 (FROH-FSNP), 0.25 (FROH-FGRM), 0.22 (FSNP-FGRM). The distribution of ROH shared regions identified on 19 autosome chromosomes, cover a relevant number of genes inside these ROH. Our result evidenced lowest LD extension levels compared with other Holstein populations; inbreeding results suggest that FGRM and FSNP may be useful estimators of individual autozygosity in Holstein from Colombia. Genes related with production and reproduction were found, but the most important are the two that may be related to adaptation to Colombian high

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tropics. This work is a pioneer and be the starting point for programs of genetic improvement and genomic population studies in the country and mainly in high tropic areas where the dairy breeds have an important production.
Key words Dairy cattle. Genomic. Linkage disequilibrium. Population.

Resumen

Los programas de selección tradicionales para ganado lechero, basados en principios cuantitativos, han funcionado bien y han permitido grandes procesos de selección en el mundo durante muchas décadas. Los objetivos de este trabajo fueron estimar los niveles de desequilibrio de ligamiento (LD) en diferentes densidades de SNPs, evaluar el tamaño efectivo de la población de ganado Holstein, caracterizar tramos de homocigosidad (ROH) distribuidas a través de ganado Holstein de Nariño y, estimar y comparar coeficiente de consanguinidad (F) basado en información de marcadores genómicos, tramos de homocigosidad (FROH), matriz de relación genómica (FGRM) y exceso de SNP homocigotos (FSNP). Después del control de calidad, el conjunto de datos utilizado estaba compuesto por 606 animales Holstein y 22200 marcadores SNP. Se utilizó el programa PLINK para identificar LD, Ne, segmentos de ROH, FROH y FSNP, FGRM se calculó con la familia de programas BLUPF90. El promedio de r2 en todos los cromosomas fue de 0.011, el r2 más alto se encontró en BTA3 (0.0323) y el más bajo en BTA12 (0.0039). Se identificaron 533 segmentos de ROH en 319 animales; los hallazgos obtenidos en este estudio sugieren que, en promedio, el 0,28% del genoma de Holstein es autocigoto. La longitud total de ROH se compuso principalmente de pequeños segmentos (ROH1-4Mb y ROH4-8Mb). Estos segmentos representaron aproximadamente el 96%, mientras que los ROH más grandes (ROH > 8Mb) representaron el 3.37% de todos los ROH detectados. Los promedios de consanguinidad de las metodologías FROH, FSNP y FGRM fueron 0.28%, 3.11% y 3.36% respectivamente. La correlación de Pearson entre estos diferentes valores de F fue: 0.49 (FROH-FSNP), 0.25 (FROH-FGRM), 0.22 (FSNP-FGRM). La distribución de las regiones compartidas de ROH identificadas en 19 cromosomas autosómicos cubre un número importante de genes dentro de estos ROH. Nuestro resultado evidenció niveles más bajos de extensión de LD en comparación con otras poblaciones Holstein; los resultados de la endogamia sugieren que FGRM y FSNP pueden ser útiles estimadores de autozigosidad individual en Holstein de Colombia. Se encontraron genes relacionados con la producción y reproducción, pero los más importantes son los dos que pueden estar relacionados con la adaptación al trópico alto colombiano. Este trabajo es pionero y será el punto de partida para los programas de mejoramiento genético y estudios de población genómica en el país y principalmente en áreas del trópico alto donde las razas lecheras tienen una producción importante.
Palabras-clave: Desequilibrio de ligamiento. Ganado lechero. Genómica. Población.

Introduction

Traditional selection programs for dairy cattle, based on quantitative principles, have worked well and allowed strong selection processes in the world over many decades. However, more effective selection and breeding strategies may result from the incorporationof molecular information (Goddard & Hayes, 2007). Linkage disequilibrium (LD) is defined as the non-random associations between alleles at different loci within a population, showing great importance in the context of QTL mapping (Quantitative Trait Loci) and estimation of population parameters (Tenesa et al., 2007). The extent of LD varies between populations becauseit is influenced by their evolutionary history, effective population size (Ne) (Nielsen, 2005; Slatkin, 2008), and non-genetic factors like marker ascertainmentbias (Pritchard & Rosenberg, 1999; Smith & Kuhner, 2009). Effective population size determines optimal selection pressures (Rexroad & Vallejo, 2009), since populations with small sizes are limited by the need to control in breeding

(Mastrangelo et al., 2014), which represents one of the most important problems in dairy cattle. Inbreeding is a consequence of mating among closely related individuals, and could result in detrimental effects on performance and fitness of the progeny (Bjelland, Weigel, Vukasinovic, & Nkrumah, 2013). Since it was developed by Wright (1922), inbreeding coefficient has mainly been estimated from the pedigree information, but now can be calculated using three approaches: the first by examining identical by state (IBS) information, marker by marker, estimating it through diagonal elements of the genomic relationship matrix (GRM) (VanRaden, 2008); the second based on excess SNP homozygosity in PLINK (Keller, Visscher, & Goddard, 2011; Purcell et al., 2007); and other based on runs of homozygosity (ROH), which was proposed by (Gibson, Morton, & Collins, 2006) for cattle. Nowadays, F estimated from ROH (FROH) is considered to be the most powerful method to detect inbreeding effects amongs everal alternative estimates of inbreeding (Keller et al., 2011; McQuillan et al., 2008). Therefore, to estimate F using ROH is particularlyappealing, as the number of inbreeding generations and the history of recent selection events can be inferred from the extend and frequency of ROH regions (Purfield, Berry, McParland & Bradley, 2012). ROH are contiguous lengths of homozygous genotypes present in an individual due to parents transmitting identical haplotypes to their offspring (Purfield et al., 2012). Although, ROH from high-through put genotyping analyses have been studied extensively in humans, these estimates are recently explored incattle, particularly in local breeds and in different livestock species (Ferenčaković, Sölkner, & Curik, 2013; Pertoldi et al., 2014; Purfield et al., 2012; Silió et al., 2013). Such regions could harbor genes targeted by selection, and some of these genes have even become fixed.

The extent of genome-wide LD, genomic Ne, and inbreeding by ROH have not been assessed in Colombian high tropic dairy breeds yet. Holstein cattle from Colombia were described in previous studies by Mejía, Hernández, Rosero, & Solarte-Portilla (2015), Solarte-Portilla and Zambrano-Burbano (2012), Zambrano-Burbano, Eraso-Cabrera, Solarte-Portilla, & Rosero-Galindo (2012), but no structural or population details have been found for the cattle breeds living in this area. To show the historical and selection events to which Holstein of high tropic of Nariño were submitted, and check this population inbreeding by molecular methods with greater reliability, the aim of this study was: i) to estimate linkage disequilibrium levels at varying distance of panel, ii) to evaluate effective population size of Holstein cattle from Nariño, iii) to characterize ROH distribution through Holstein cattle from Nariño and, iv) to estimate and compare the inbreeding coefficient (F) based on genomic markers information (F_{ROH}), (F_{GRM}) and (F_{SNP}).

Material and Methods

Animals and genotyping

The animals in study belong to a project called *Selection Research Using Genomic and Polygenic Models for the Genetic Improvement of Dairy Cattle in High Tropic of Nariño*, financed with resources of Sistema General de Regalías (SGR) in cooperation with Cooperativa de Lacteos de Nariño and executed by Animal Production Research Group of Universidad de Nariño, cod BPIN No. 2013000100091.

A total of 619 Holstein animals born in Nariño, southwestern Colombia were studied. These animals were chosen randomly proportionally to the herd, those herds enrolled in the Nariño breeding program. Blood samples were collected following the ethical guidelines established by the University of Nariño Animal welfare. DNA extraction from blood was done using Ultraclean bloodspin kit (MoBio Laboratories Inc, Carlsbad, California, USA). DNA was stored at -20 °C until genotyping. Total DNA from selected individuals was submitted to GeneSeek Genomic Profiler LD v3 (http://

www.neogen.com/GeneSeek/) for genotyping. Data quality control was performed using PLINK software (Purcell et al., 2007). Only SNPs located on autosomes were considered for further analyses, we excluded SNP assigned to X chromosome. Moreover, the following filtering parameters were adopted to exclude certain loci and to generate the pruned input file: i) SNP with call rate <95%, and ii) animals with more than 5% of missing genotypes were removed. Finally, 606 samples and 22200 SNP markers were retained for analyses.

Linkage disequilibrium analysis

Linkage disequilibrium was calculated as pairwise r^2, which relies on allele phase information at gametic level and it can vary between 0 and 1. Considering two marker loci (A and B), each one with two alleles (A1, A2, B1, and B2) the allelefrequency in the population can be denoted as P_{A1}, P_{A2}, P_{B1} and P_{B2} and haplotypes frequency with allele 1 at marker locus A, and allele 1 at locus B, for example, denoted P_{A1B1} (Hill & Robertson,1968). For each population, LD values between all SNP pairs of all chromosomes were binned according to pairwise physical distances into intervals starting from 0 up to 5Mb. Average values of r^2 were calculated for each bin (25-50Kb, 50-100Kb, 100-500Kb, 0,5-1Mb, 1.5Mb). The r^2 was obtained by PLINK program v1.90 (Purcell et al., 2007), command ld, and considered in the calculation of r^2 statistic for all pairs of SNPs in each chromosome by R Studio software (http://www.r-project.org/). In large databases, r^2 equal to 1 indicates complete LD assuming recombination events; r^2 equal to 0 means that SNPs are in equilibrium, so there is no LD (Hill & Robertson, 1968). LD decay was analyzed for two different distances between SNP pairs, defined as 5kb and 5Mb using r^2 values. To show the average trend in LD decay, r^2 was calculated for all possible SNP comparisons included within different size windows, i.e. 5kb, 10kb, 50kb, and 100 kb, dependingon the distance between markers. The trend in LD decay for each breed was plotted through the whole genome and by chromosome (BTA).

Estimation of effective population size

Several methods are available for characterizing conservation degree of livestock breeds genetic variation, and the most favored one for practical purposes is Ne (Hall, 2016). The relationship between effective population sizes (Ne), recombination rate, and DL (r^2) without mutation was used to infer effective population size (Sved, 1971). It was estimated considering each SNP pair located within 100Mb of the same chromosome, with physical distances between SNP converted to genetic distances, using default values 1Mb = 1cM (Jiang, Wang, Moore, & Yang, 2012; Qanbari et al., 2010).

Detection of runs of homozygosity

ROH segments were identified using PLINK software (Purcell et al., 2007) considering the sliding window approach with specified homozygosity for contiguous SNPs. Parameters and thresholds applied to define ROH were (i) a sliding window of 40 SNPs across the genome; (ii) the proportion of homozygous overlapping windows was 0.05; (iii) the minimum length of ROH was set to 1000kb; (iv) the maximum gap between consecutive homozygous SNPs was 1000kb; (v) a density of one SNP per 120kb; and (vi) a maximum of two SNPs with missing genotypes and up to zero heterozygous genotype were allowed in ROH, since the chip used has less density than other reports, the parameters were modified a bit to be able to properly identify the ROH (Peripolli et al., 2018; Purfield et al., 2012; Rodríguez-Ramilo, Elsen & Legarra, 2019; Rodríguez-Ramilo, Fernández, Toro, Hernández, & Villanueva, 2015). Three different classes of ROH were used considering length classes 1 - 4Mb, 4-8Mb and > 8Mb.

Genomic inbreeding coefficients

The inbreeding coefficient (F) based on genomic information was computed in different ways. Three types of inbreeding coefficients were taken into account: i) from the diagonal of genomic relationship matrix (matrix G); ii) based on the excess of SNPs in homozygous obtained as expected value of population deviation; iii) applying runs of homozygosity.

G matrix was built in BLUPF90 family of programs (Misztal et al., 2015) according to VanRaden, (2008). Diagonal elements of G matrix represent the relationship of the animal with itself, thus, it was used to assess genomic inbreeding coefficient (FGRM). In order to obtain positive values, the Z matrix was centered in zeros VanRaden (2008). The second method used to estimate F genomic (FSNP) is based on the excess of homozygosity and the value obtained as a deviation from the expected homozygous for the population, (Keller et al., 2011). This analysis was calculated whit a command het in PLINK v1.90 (Purcell et al., 2007). Finally, genomic inbreeding coefficients based on ROH (FROH) were estimated for each animal according to Bjelland et al. (2013):

$$F_{ROH} = \frac{\Sigma_j \, \text{length}(ROH_j)}{L}$$

where j is the number of ROH identified for each individual and L is the total length of the genome (2.612.820kb) (Zimin et al., 2009). For each animal F_{ROH} ($F_{ROH1\text{-}4Mb}$, $F_{ROH4\text{-}8Mb}$) was calculated based on ROH distribution of two minimum different lengths.

In addition, Pearson's correlation analyses between different inbreeding coefficients were performed to assess association strength between different estimates (Williams, 1996).

Gene prospection in shared ROH regions, identification of genes

The shared ROH regions can be defined as genomic regions with reduced genetic diversity and, consequently, high homozygosity around the selected locus that might harbor targets of positive selection and are under strong selective pressure (Pemberton et al., 2012). These regions are common among individuals in which homozygous regions were identified. The ROH segments shared by more than 15% of the population individuals was considered common regions. The Map Viewer of bovine genome UMD3.1.1 was used for identification of genes in ROH regions, available at National Center for Biotechnology Information (Map Viewer - http://www.ncbi.nlm.nih.gov/mapview/) and to interpret the list of candidate genes for segments more likely to represent homozygosity by descent.

Results and Discussion

619 individuals representing the Holstein cattle of Nariño tropic were genotyped with panel of 26K in the present study. Table 1 shows statistical analyses from linkage disequilibrium for chromosomes, in complete panel. The square correlation of alleles at two loci (r^2) is considered the most robust measure of LD (Badke, Bates, Ernst, Schwab, & Steibel, 2012). The average r^2 was 0.011, the highest value was found in BTA3 (0.0323), and the lowest in BTA12 (0.0039). BTA1 was the one with the highest amount of SNP (1073) and the lowest was BTA27 (418). The mean r^2 for SNPs separated by distance bins 5 to 10kb, 10 to 25kb, 25 to 50kb, 50 to 100kb, 100 to 500kb, 0.5 to 1Mb and 1 to 5Mb on each autosomal chromosome are shown in Figure 1. Greater LD were observed only at small distances between pairs of SNPs. To the distance from 5 to 10 kb in different

chromosomes was high for BTA9 and BTA2, being 0.220 and 0.210 respectively; for bin of 25 to 50kb was high for BTA9 and BTA7, with values of 0.165 and 0.119, respectively. To the distance 50 to 100kb was higher for BTA9 (0.114) and low for BTA12 (0.020). With increasing distances r^2 was reduced, in this population average LD for distance bins 5 to 10kb, 10 to 25kb, 25 to 50kb, 50 to 100kb, 100 to 500kb, 0.5 to 1Mb, and 1 to 5Mb were 0.096, 0.074, 0.071, 0.059, 0.040, 0.035 and 0.023, respectively (Figure 1). At distances after 5Mb, r^2 was similar for all pairs SNPs. Linkage disequilibrium levels (Figure 2), show that LD decreases with increasing distances, being useful up to a distance of 100kb. In general, the mean LD declined rapidly with increasing distance between SNP to a very low level, as other author comment (Kukučková, Moravčíková, Trakovická, & Kadlečík, 2016; Toro Ospina et al., 2019). Rincon, Lopez, & Echeverri (2018) studied Holstein population in Colombian tropic with Bovine LD Illumina chip, found a linkage disequilibrium between markers (r^2<0.1) similar to our study. Otherwise, Qanbari et al. (2010) with samples of 810 German Holstein-Friesian cattle genotyped by Illumina Bovine SNP50K BeadChip found a mean r^2 value of 0.30 in pairwise distances of <25kb, and it dropped to 0.20 at 50-75kb, which is higher than our study. Also, Goddard, Hayes, McPartlan, & Chamberlain (2006) reported mean r^2 of 0.73 for SNP separated by 10 kb for Holstein cattle in Australia. De Roos, Hayes, Spelman, & Goddard (2008) studied LD in Holstein-NZL and Angus-AUS breeds at distances of 70 to 75kb, with mean r^2 of 0.10 and 0.24, while other studies found results in Bos Taurus animals in which LD show drastic decay after 60kb, 100kb, 500kb, 1Mb, where r^2 was decreasing from 0.16, 0.11, 0.08, and 0.06, respectively (O'Brien et al., 2015; Porto et al., 2014). Sargolzaei, Schenkel, Jansen, & Schaeffer (2008), conclude that r^2 ideal level of LD for genome-wide studies was 0.2, extended over short distances in Holstein cattle in North America. Nevertheless, previous studies have shown that it is difficult to compare LD obtained in different populations due to factors that can affect LD level and extent estimates, such as different sample sizes, LD measures, marker types and densities of panels, ascertainment bias, and recent and historical population (selection practices, demographics) (Kukučková et al., 2016; Pritchard & Przeworski, 2001). In general, values reported in this study are very low compared with other Holstein studies in the world, which may be due to a high diversity associated with a founder effect, taking into account that the Holstein breed was achieved by absorption in this tropical zone, in addition to chip density, since this Holstein population is more diverse than indicine breeds. Based on the above, our results suggest that a higher density chip is recommended for future evaluations of genome-wide association studies.

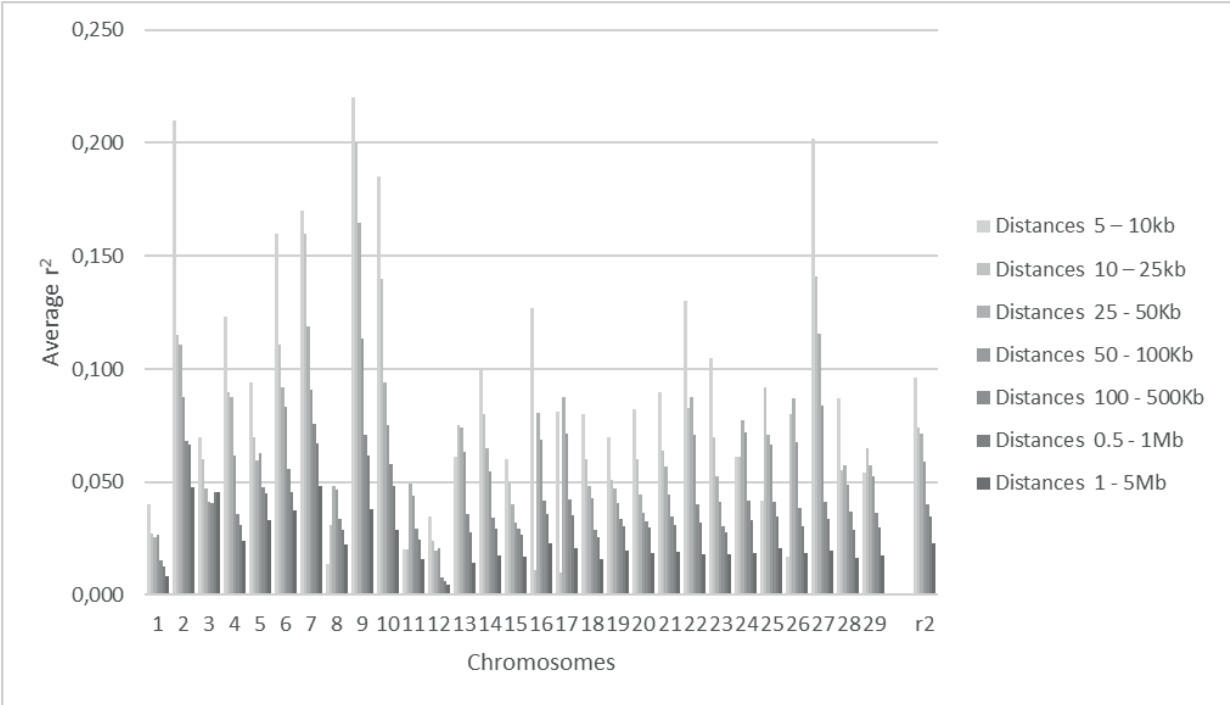

Figure 1. Statistical analysis of chromosome-wise average r^2 values when increasing evaluated physical distances between SNPs with 26K panel in Holstein population from Colombia.

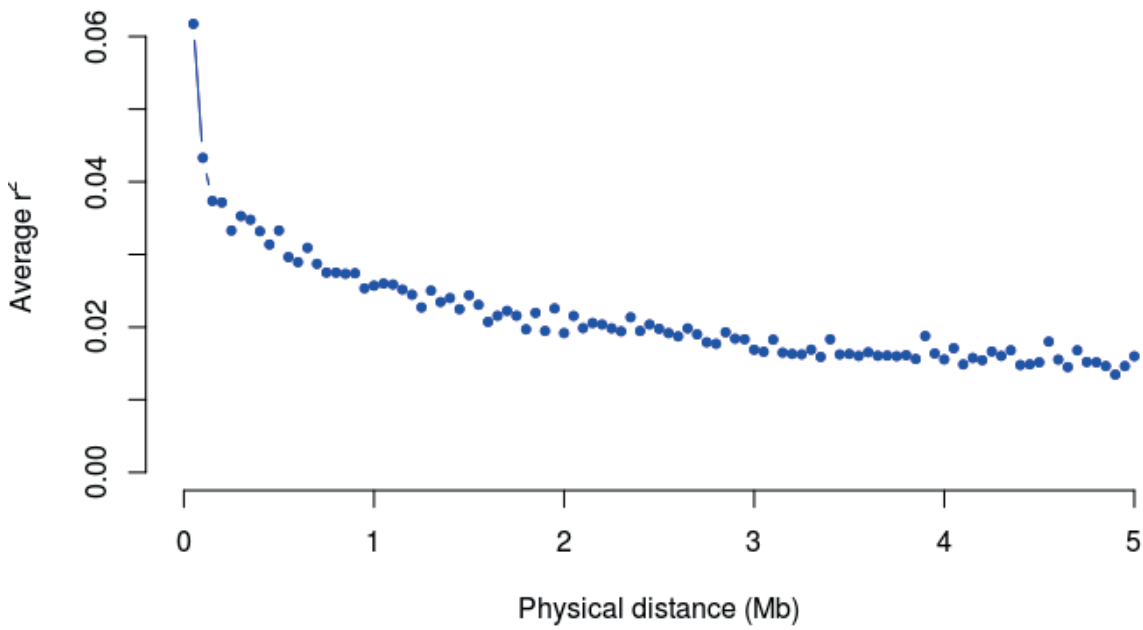

Figure 2. Extent of r^2 as a function of inter-marker distance in Holstein populations.

Table 1
Statistical information from linkage disequilibrium analyzes of SNP with panel of 26K in Holstein population from Nariño

Chromosome	Mb[a]	Number of SNP	r^{2} [b]
1	54.95	1073	0.0047
2	50.12	840	0.0197
3	42.84	803	0.0323
4	43.91	721	0.0111
5	40.87	888	0.0211
6	41.36	818	0.0240
7	40.54	699	0.0213
8	40.45	729	0.0120
9	38.09	710	0.0112
10	36.88	701	0.0112
11	38.98	803	0.0071
12	33.26	693	0.0039
13	30.26	762	0.0061
14	31.12	821	0.0066
15	29.56	763	0.0067
16	29.19	703	0.0092
17	27.68	668	0.0081
18	24.69	717	0.0070
19	23.72	710	0.0065
20	24.87	677	0.0076
21	25.47	662	0.0082
22	23.01	544	0.0077
23	18.51	545	0.0089
24	23.55	570	0.0085
25	15.88	457	0.0111
26	18.91	478	0.0090
27	16.95	418	0.0096
28	16.35	460	0.0080
29	19.21	550	0.0083

[a]Mb. Distances between SNP pairs in Megabases. [b]r^{2}. Correlation coefficient of linkage disequilibrium.

LD structure can be used to provide insights into the evolutionary history of populations (Biegelmeyer, Gulias-Gomes, Caetano, Steibel, &Cardoso, 2016), this is related to historical effective population size (Ne), and in this study the strength of LD at different genomic distances between loci (r^{2}) was used to estimate Ne. After analyzing the 606 animals and 23184 markers, Ne observed for that population was 212 animals. Our results are also higher than Maiwashe et al. (2006) who estimated Ne of 137 and 108, for Holstein and Jersey respectively; and Decker et

al. (2012), and Weigel and Lin, (2002) who found Ne values of 39, 38 and 94 animals, for Holstein, Jersey, and Angus respectively. On the other hand, Magalhães Araújo da Silva et al. (2016) reported an effective number of founders and ancestors of 473 and 471 animals respectively in Brazilian Holstein. Our results show that Holstein of high tropics of Nariño had a reasonable effective population sizemay be due to the founding effect of creole breeds and the recent introduction of new animals of the Holstein breed and to presence of high admixture within other breeds, intense selection and probably wide spread use of artificial insemination and the use of relatively few elite sires after 1970 (Hayes, Bowman, Chamberlain, & Goddard, 2009; Makina et al., 2015). Also, the elevated effective population size of Holstein in Nariño compared with other Holstein could be implicated as the key source of short extend LD in livestock populations (Hayes, Visscher, McPartlan, & Goddard, 2003). The Holstein population of Colombia was produced generally by absorption of creole, therefore diversity may be a little higher and theremay even be introgression of other breeds in the process of establishing Holstein herds in Nariño and in Colombia, and that maybe are not still stable, what makes it different to the pure Holstein of the world.

In this study, 533 runs of homozygosity (ROH) segments across 319 animals were identified. Our results showed that ROH size ranged from tens of kb to Mb and varied among individuals. The average number of ROH per animal was 1.67, the mean ROH length was 4.39Mb, and the longest segment was 31.9Mb in length (average 51.82SNPs/ROH). Overall, small sized ROH were most common. The average percentage of chromosome coverage by ROH was evaluated and the highest average was observed on chromosome 5 (26,17%), andchromosome 15 had the lowest percentage coverage by ROH with only 0.077%. On average, 0.2807% of the Holstein population genome had coverage by ROH. Descriptive statistics of ROH number and length by classes is given in Table 2. Total length of ROH was composed mostly of small segments ($ROH_{1\text{-}4Mb}$ and $ROH_{4\text{-}8Mb}$). These segments accounted for approximately 96%, while larger ROH ($ROH_{>8Mb}$) were 3.37% of all ROH detected. Which indicates old selection and little recent selection despite selection programs that are very new and do not appear to have significant effects on the population. The greatest number of ROH per chromosome was described on BTA5, however, results in taurine breeds have evidenced the highest number of ROH on BTA1 (Mastrangelo et al.,2016; Purfield et al., 2012). Similar results were reported in Gyr, with the longest ROH with 5.85Mb in length, Peripolli et al. (2018) and Kim et al. (2013) in Holstein on BTA8 with 108.97Mb and 87.13Mb, and in Cinisara cattle breed Mastrangelo et al. (2016) showed 112.65Mb for the longest ROH, taking into account that these studies were done with a higher density panel than the one used for our analyzes. The fact of evaluating a lower density panel than the one normally used, made us modify the parameters more flexibly for the conformation of ROH inthis population, capturing a greater number of ROH that reveal the evolutionary history of the breed in this Colombian region. During the last few decades, genetic selection of Holstein cattle for its improvement has had adverse effects on fertility, production, and immunology (VanRaden et al.,2004), which is probably related to ROH. Kim et al. (2013) supposed that the most frequent haplotypes in high ROH regions affect milk production when considering that highest ROH were discovered in commercial Holsteins from U.S. Indeed, in selected Holsteins a frequent haplotype contributing to ROH changes is related with genetic improvement of yield traits. Recently, the primary objective of selection in Holsteins has been changing from higher yield to other economic traits such as net merit, productive life, and fertility using genomic predictions (VanRaden et al., 2009),

which will change genomic features of ROH even further in the future. ROH could be considered the optimal measure for inbreeding based on genomic information (Curik, Ferenčaković, & Sölkner, 2014). Identification of ROHs described herein not onlyprovide information about specific regions that could be related to selection events,but also has been exploited to improve the accuracy of inbreeding coefficient estimatesand to assess relation traits that could be present in the population.

Table 2
Distributions of runs of homozygosity (ROH) by class of length

Class	Number of ROH	Percent	Mean length	Standard deviation
$ROH_{1\text{-}4Mb}$	240	45	2.901	0.819
$ROH_{4\text{-}8Mb}$	275	51	5.198	0.923
$ROH_{>8Mb}$	18	3.37	11.893	5.68

In this study, we derived inbreeding coefficients based on the excess of SNPs in homozygous (FSNP) and on the diagonal of genomic relationship matrix (FGRM), and compared them based on runs of homozygosity (FROH), to test for a correlation between these coefficients and to verify if ROH, SNP or GRM analysis can be a useful tool for estimating the recent inbreeding in Holstein cattle maintained in high tropics of southwestern Colombia. The summary of genomic inbreeding coefficients statistics for the 606 animals remaining after quality control are presented in Table 3. Inbreeding averages for FROH, FSNP and FGRM methodologies were 0.28%, 3.11% and 3.36% respectively. The mean of inbreeding estimated from the diagonal of genomic relationship matrix (matrix G) method show a little higher value than the mean of inbreeding coefficients based on the excess of SNPs in homozygous and runs of homozygosity (ROH). These results are consistent with results reported by other authors but in inbreeding based on pedigree, for example, Sørensen, Sørensen and Berg (2005) where inbreeding average levels of Holstein in Denmark were 3.4%, and for Jersey were 3.1%; and Weller and Ezra (2005) found results lower than ours (1.7%) with pedigree from Holstein herds in Israel. On the other hand, authors like Kim et al. (2013) reported mean of inbreeding coefficient based on pedigree and genomic methods in Holstein (3.4 and 7.4%, respectively), and Thompson, Everett & Hammerschmidt (2000) calculated average inbreeding coefficients of 4.2% and 4.6% in Holstein and Jersey respectively. The low value of the inbreeding coefficient indicates the high diversity in this population, in addition to the high effective number of populations estimated in this study. Pearson's correlation was used to assess relationships between F coefficients. FSNP was more correlated with FROH (0,49) indicating that these approaches could be equivalent in terms of identifying the most and the least-inbred animals. However, the correlations between FGRM and FSNP were the lowest correlation (0.22), and FGRM and FROH was (0.25). There are several authors who propose FROH as the best way to calculate inbreeding, if it is suggested that this is the best way to find inbreeding (FROH), then inbreeding in the Holstein population of Nariño is very low (Bjelland et al., 2013). VanRaden, O'Connell, Wiggans, & Weigel (2011) explain that genomic relationship matrix was expected to be a better indicator of relatedness between individuals,

nevertheless, Forutan et al. (2018) determined that FROH was the genomic method that provides an accurate measure of relatedness, and is a better indicator of the true level of inbreeding. The strategy considered, using information from genomic relationship matrix (GRM), is an effective way to provide a more precise estimate of the extent of relation among individuals, allowing a more effective reduction in inbreeding levels (Hayes et al., 2009; Pryce, Hayes & Goddard, 2012; VanRaden, 2008). Some authors explain that inbreeding values estimated from pedigree data tend to underestimate homozygosity by descent among individuals, indicating that pedigree data suffer from inaccuracies of assumed parentage unless confirmed by DNA typing (Scraggs et al., 2014).

Table 3.
Descriptive statistics of genomic inbreeding coefficients in Holstein from high tropics of Nariño

F value	Mean	Standard deviation	Min	Max
F_{GRM} [a]	0.0336	0.0416	0.00000	0.4649
F_{SNP} [b]	0.0311	0.0343	0.00010	0.2519
F_{ROH} [c]	0.2807	0.0268	0.00004	0.1379

[a]F_{SNP}: Inbreeding coefficient based on excess homozygosity; [b]F_{GRM}: Inbreeding coefficient based on genomic kinship matrix; [c]F_{ROH}: Inbreeding coefficient based on homozygous runs.

Finally, we identified genes related to traits of economic importance in the 51 shared regions, located in several autosome chromosomes (BTA 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 15, 16, 17, 18, 19, 21, 25, 26 and 29), it covers a relevant number of genes inside these ROH shared regions. 51 common shared regions were found, containing 58 genes. These genes related with immune system, reproductive process and milk quality, and respective biological pathways are listed in Table 4, being chromosomes 3 and 5 the two that lodges the greater amount of genes (7), and chromosomes 11, 15, 18, 21, 25 and 26 with a single gene. NCBI were used to obtain general information and a broad functional insight into the set of genes.

Table 4
Chromosome (Chr), position and genes of ROH identified segments that play an important role in dairy cattle

Chr[a]	Physical Position (bp)[b]	Gene Symbol
1	115533109 – 135995337	*IL-20RB, PPP2R3A, SLC35G2-TMEM22, STAG1, PIK3CB, P2RYfamily*
2	127333 – 136386853	*MECR, CD52, E2F2, HMGN2, IFI6, CALCRL*
3	380257 – 121374825	*CD58, CSF1, CYM, PROK1, ATP1A, MUCIN1, FCGR3A*
4	76859 – 119462290	*GRB10, ZPBP, CALCR, CACNA2D1, TRPV6, EPHA1*
5	52990011 – 58841085	*APON, NACA, CYP27B1, PTGES3, CDK2, HDAC2, RPSA*
6	2213487 – 119394736	*MAD2L1, ABCG2, NCAPG,GNRHR, CSN3*
7	53313 – 35532135	*SCL27A1, SCL27A6*
8	147573 – 113227939	*HPGD, TLR4*
10	2137438 – 104136694	*DIO2, TSHR*
11	103326092 – 106987165	*LHX3*
15	23024837 – 24238959	*DRD2*
16	22789059 – 27629566	*CAPN2, TGFB2, TLR5*
17	71737909 – 74948921	*MAPK1, VPREB1*
18	60019327 – 65640960	*KIR2DS1*
19	54488129 – 58258281	*RPTOR, CBX2, CBX4, CBX8, ITGB4, LGALS3BP, SOCS3*
21	56370577 – 60175026	*DICER1*
25	34399979 – 39781392	*ZP3*
26	97421 – 51680135	*FANK1*
29	38921550 – 41480547	*GSTP1, IGF2, PC*

[a]Chr: chromosome. [b]bp: base pair.

Shared regions included genes reported in previous studies, listed in Table 5. We found eleven genes related to milk; protein phosphatase 2, regulatory subunit B''alpha (PPP2R3A), which provides a mechanism for calcium regulation (Davis, Yan, Martinez, & Mumby, 2008); mitochorial trans-2-enoyl-CoA reductase (MECR) is a key protein in mitochondrial fatty acid synthesis (Heimer et al., 2016); Chymosin (CYM) is an enzyme normally used as "rennet" which is well-known because it has properties on milk coagulation (cheese production) (Bansal, Fox, & McSweeney, 2008; Palmer et al., 2010; Ramírez & Ayala, 2014; Sandra, Alexander, & Dalgleish, 2007); Mitogen-activated protein

kinase 1 (MAPK1) which is involved in regulation of milk synthesis and in milk protein synthesis mechanisms (Lu, Li, Huang, & Gao, 2012); mTOR complex 1 (RPTOR) regulatory associated protein comprises protein synthesis that occurs in the mammary epithelium (Burgos, Kim, Dai, & Cant, 2013), and suppressor of cytokine signaling 3 (SOCS3), which expression was found in cytoplasm and nucleus of dairy cow mammary epithelial cells, implicated in milk protein and fatty acid synthesis (Huang et al., 2013). Also, same authors validated that SOCS3 inhibited β-casein gene expression and cell proliferation as an inhibitor of JAK2/STAT5a signaling pathway in dairy cow mammary epithelial cells. Kappa (κ) casein gene (CSN3) plays an important role in cheese production technology (e.g., stabilization of casein micelles), and in physiological processes such as cytotoxic and antibacterial activities important for disease resistance (Chen et al., 2008; Matin & Otani, 2002). The ‘‘solute carrier family 27 member 1’’ protein (SLC27A1) is a member of the fatty acid transport protein family (FATP), candidate gene for milk production traits in Chinese Holstein cattle, Lv et al. (2011) provide evidence that the C allele have potential effects on milk yield trait. Solute carrier family 27, isoform A6 (SLC27A6) can be used to select for cattle producing milk with lower concentrations of saturated fatty acids (SFA) and higher concentrations of unsaturated fatty acids (UFA) (Nafikov et al., 2013).

Table 5
Role of genes identified in ROH segment in chromosomes of Holstein cattle

Trait	Chr	Genes	Author
Immune system	1	*IL-20RB*	(Wahl et al., 2009)
		TMEM22	(Dobashi et al., 2009)
		PIK3CB	(Li et al., 2018)
		P2RY family	(Seo et al., 2014)
	2	*IFI6*	(N. Parker & Porter, 2004)
	3	*ATP1A1*	(Liu et al., 2012)
		CD58	(Dustin, 1997)
		PROK1	(Kisliouk et al., 2007)
		MUCIN1	(P. Parker et al., 2010)
		CSF1	(Chitu & Stanley, 2006)
		FCGR3A	(Robledo et al., 2012)
	4	*CACNA2D1*	(Deb et al., 2014; Yuan et al., 2011)
		FPHA1	(Kang et al., 2018)
	5	*CYP27B1*	(Nelson et al., 2010)
	6	*ABCG2*	(Wei et al., 2012)
	8	*TLR4*	(Astakhova et al., 2009; Goldammer et al., 2004)
	16	*TLR5*	(Seabury et al., 2007)
	18	*KIR2DS1*	(Dobromylskyj & Ellis, 2007

continue

continuation

Reproduction	2	*CD52*	(Michalková et al., 2010)
		CALCRL	(Hayashi et al., 2013)
	3	*PROK1*	(Kisliouk et al., 2007)
		CSF1	(Sherr et al., 1988)
	4	*GRB10*	(Ruddock et al., 2004)
		CALCR	(Kamano et al., 2014)
		TRPV6	(Sprekeler et al., 2012)
	5	*APON*	(O'Bryan et al., 2004)
		CDK2	(Yamauchi et al., 2003)
		HDAC2	(Beyhan et al., 2007)
		NACA	(Beatrix et al., 2000)
		PTGES3	(Arosh et al., 2002; Flisikowski et al., 2010; Ulbrich et al., 2009)
	6	*RPSA*	(Ishiwata et al., 2003a)
		MAD2L1	(Ishiwata et al., 2003b)
		NCAPG	(Cole et al., 2014)
		GNRHR	(Derecka et al., 2010; Yang et al., 2011)
	8	*HPGD*	(von Hof et al., 2017)
	16	*TGFB2*	(Hatzirodos et al., 2011)
	17	*MAPK1*	(Gao et al., 2014)
		VPREB1	(Ekman et al., 2012)
	19	*CBX2*	(Ruddock-D'Cruz et al., 2008)
		ITGB4	(Zhao et al., 2015)
		LGALS3BP	(Okumu et al., 2011)
	21	*DICER*	(Burrola-Barraza et al., 2011; Lei et al., 2010)
	25	*ZP3*	(Kanai et al., 2007; Suzuki et al., 2015; Zhang et al., 2017)
	26	*FANK1*	(Hwang et al., 2005)
	29	*IGF2*	(Fagundes et al., 2011)
Milk quality	1	*PPP2R3A*	(Ahn et al., 2007; Davis et al., 2008)
	2	*MERC*	(Heimer et al., 2016; Miinalainen et al., 2003)
	3	*CYM*	(Okigbo et al., 1985; Palmer et al., 2010; Sandra et al., 2007)
	6	*CSN3*	(Chen et al., 2008)
	7	*SCL27A1*	(Lv et al., 2011; Ordovas et al., 2008)
		SCL27A6	(Nafikov et al., 2013)
	17	*MAPK1*	(Lu et al., 2013)
	19	*RPTOR*	(Burgos et al., 2013)
		SOCS3	(Huang et al., 2013)
	29	*GSTP1*	(Rao et al., 2013)
		PC	(Velez & Donkin, 2005; White et al., 2011)
Adaptation	29	*GSTP1*	(Rao et al., 2013)
		PC	(White et al., 2012)

Two genes related with milk production and adaptation were found. Milk production in cattle is very susceptible to high ambient temperature due to their weak ability to tolerate heat, which has strongly restricted the development of dairy industry in tropical regions and caused considerable economic loss. Rao, Ramesha, Barani, Chauhan, & Basavaraju (2013) found that animals with pattern a in glutathione S-transferase pi 1 (GSTP1) gene had better lactation length and yield compared to animals with pattern b. Also, GSTP1 plays a central role in the detoxification of ROS (reactive oxygen species), namely, a positive role under heat stress in controlling cellular toxicants and to alleviate its destructive effect on cattle. Velez and Donkin (2005) indicate that bovine Pyruvate carboxylase (PC) promoter 1 is responsive to fatty acids in dairy cattle and conclude in their studies that PC imply a greater capacity for enhanced metabolism of lactate, alanine, and other amino acids, and to increase protein turnover during feed restriction; and White, Koser, & Donkin (2012) suggest that there are unique characteristics of bovine PC promoters that may contribute to physiological response to thermal stress.

On the other hand, Solute carrier family 35 member G2 - (transmembrane protein 22) (SLC35G2-TMEM22), localized at the cytoplasmic membrane in mammalian cells, is involved in cell growth of renal cell carcinoma, caused significant reduction of cancer cell growth (Dobashi et al., 2009), also the TMEM173 gene was related to activation of innate response in heat-stress serum and normal Holstein cows (Huang et al., 2014). Liu, Zhou, Li, Cui ,& Wang (2010) suggested that bovine ATP1A1 (ATPase Na+/K+ transporting subunit alpha 1) may play an important role in heat resistance and could be considered as a DNA marker for bovine anti-heat stress trait in marker-assisted selection, as well as related with immune system, Liu, Xu, Gao, & Sun (2012) found that ATP1A1 gene polymorphism, may act as a physical indicator of mastitis resistance, because this system plays an important role in osmoregulation of milk.

In the same way, in this study we found 18 genes involved with immune response. Wahl et al. (2009) show that Interleukin 20 receptor subunit beta (IL-20Rb) regulates inflammatory genes such as chemokines, proteases, and defenses in the skin. Interferon alpha inducible protein 6 (IFI6), generates diverse cellular and physiological states involving antiviral, apoptotic, anti-proliferative, antitumor, and immune-modulatory activities (Parker & Porter, 2004). The T lymphocyte glycoprotein CD2, and its ligand CD58 (CD58 molecule), play an important role in stabilizing adhesion between T cells, these are closely related members of the immunoglobulin superfamily (Crosby, Yatko, DerSimonian, Pan, & Edge, 2004; Dustin, 1997). Colony-stimulating factor-1 (CSF-1) may be an important modulator of placental development (Sherr, Roussel & Rettenmier, 1988), it plays roles in innate immunity, cancer, and inflammatory diseases (Chitu & Stanley, 2006). Kisliouk et al. (2007) reported that members of Prokineticin 1 (PROK1) family induce migration of monocytes and secretion of pro-inflammatory cytokines from macrophages. Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) is an enzyme with a critical factor regulating immune function. Toll like receptor 5 (TLR5) plays an important role in the recognition of invading pathogens and modulation of innate immune responses (Seabury, Cargill & Womack, 2007; Werling, Piercy, & Coffey, 2006). Pre-B lymphocyte 1 (VPREB1), which in the absence of its expression, suggests a decline of b lymphopoiesis in adult cattle (Ekman, Ilves, & Iivanainen, 2012). The last gene involved in immune response is (KIR2DS1) killer cell immunoglobulin-like receptor, two domains; short cytoplasmic tail, 1, are the major functional natural killer (NK) cell receptors in humans, in cattle KIR genes encode three Ig-domain KIRs (Dobromylskyj & Ellis, 2007). Phosphatidylinositol 3 kinases (PI3K), It has

been found to play an important role in the defense mechanism of Streptococcus uberis (S. uberis) known as mastitis causing pathogen (Li et al., 2018). Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (CACNA2D1) gene is considered to be an important noncytokine candidate gene influencing mastitis (Deb et al., 2014). Eph A-ephrin A1 (EPHA1) system is a positive factor in the increase and maintenance of epithelial cells in mammary glands of cows; the signaling system contributes to development, remodeling, and functionality of normal mammary glands and could overcome mastitis in cows and other mammals (Kang et al., 2018). Fc-g receptors (FCGR) play a role in the response to rituximab in autoimmune -receptor III a (FCGR3A) diseases and is a very important factor of immune system (Robledo et al., 2012).

Genes involved in reproduction system like luteolysis, maternal recognition, embryonic and placental development, implantation and pregnancy were also found in several chromosomes: CD52 molecule (CD52), colony stimulating factor 1 (CSF1) and prokineticin 1 (PROK1), ovarian and testicular apolipoprotein N (ApoN), nascent polypeptide-associated complex alpha subunit (NACA), prostaglandin E synthase 3 (PTGES3), cyclin dependent kinase 2 (CDK2), histone deacetylase 2 (HDAC2) and ribosomal protein SA (RPSA), transforming growth factor beta 2 (TGFB2), in mitogen-activated protein kinase 1 (MAPK1), five genes in BTA19 chromobox 2, 4, and 8 (CBX2, CBX4, CBX8), integrin subunit beta 4 (ITGB4), galectin 3 binding protein (LGALS3BP), dicer1, ribonuclease III (DICER1), insulin like growth factor 2 (IGF2), the zona pellucida (ZP3), 15-Hydroxyprostaglandin dehydrogenase (HPGD), and gonadotrophin releasing hormone (GnRH) receptor gene.

Results of genes found in regions of homozygosity related to traits of economic importance in livestock can confirm some of the results of random pairings implemented in the region for many years, presenting that Holstein of southwestern Colombia have genes of good reproductive, immunological and productive quality, in addition, adaptation genes to the tropics that will be inherited to the offspring, so they have a better performance in the high tropic region of Nariño.

Conclusion

The Ne of the Colombian Holstein population is greater than some studies reported for the breed, suggesting the existence of an important diversity in Holstein from Colombian high tropics, what agrees with medium to low values reported of inbreeding in Holstein, this may be related to the history of Holstein in Colombia. Inbreeding results suggest that F_{GRM} and F_{SNP} may be useful estimators of individual autozygosity in Holstein populations from Colombia and may give some insights on pedigree-based inbreeding estimates in cases when animals' pedigree data are unavailable. In addition, genes related with production and reproduction were found, but the most important are the two that may be related to adaptation to the high tropics of Colombia. Finally, the updated information based on Holstein population analyzed with low density chip, appears to show one of the lowest LD extension levels described in studies made on this breed and those proposed for genomic selection, which suggests that the use of much higher SNP density (e.g., 50K) panels for adequate representation of the breed, and for development of future design of a genomic selection program and/or marker-phenotype association studies.

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