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Identification of selection signatures involved in performance traits in a paternal broiler line

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Dissertation presented to obtain the degree of Master in Science. Area: Animal Science and Pastures

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Bachelor of Biotechnology

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

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DEDICATION

I dedicate this work to the people I love.

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“It is our choices that show what we truly are, far more than our abilities.”

J. K. Rowling

SUMÁRIO

RESUMO

Identificação de assinaturas de seleção envolvidas com características de desempenho em uma linhagem paterna de frangos de corte

Seleção natural e artificial causam mudanças em determinadas regiões do genoma, resultando em assinaturas de seleção. Assim, espera-se identificar genes associados às características sob seleção nessas regiões. Assinaturas de seleção podem ser identificadas usando diferentes metodologias, e algumas delas são baseadas na detecção de sequências contíguas de haplótipos homozigotos idênticos por descendência, chamados segmentos de homozigose (ROH), ou na estimativa do índice de fixação (F_{ST}) de janelas genômicas que indicam diferenciação genética. Em nosso estudo, objetivamos identificar assinaturas de seleção em uma linha paterna de frangos de corte e investigar os genes anotados nessas regiões, bem como os fenômenos biológicos envolvidos. Para tal propósito, as análises de ROH e F_{ST} foram realizadas usando dados de sequenciamento completo do genoma (WGS) de vinte e oito aves de duas gerações diferentes. A análise de ROH identificou regiões em homozigose de comprimentos curto e moderado. Analisando os padrões de ROH, foram observadas regiões comumente compartilhadas entre alguns animais e mudanças na abundância e no comprimento da ROH entre as duas gerações. Os resultados também sugerem que dados de WGS apresentam vantagens sobre os dados de SNPchip, porém o número de indivíduos analisados deve ser levado em consideração. A análise baseada em FST revelou diferenciação genética em algumas janelas genômicas. Anotação das regiões consenso de ROH e das janelas F_{ST} identificaram diversos genes, alguns dos quais já foram associados a características de interesse econômico, como *APOB, IGF1, IGFBP2, POMC, PPARG* e *ZNF423*. Análises de enriquecimento dos genes resultaram em termos biológicos de músculo esquelético, proteínas matrilinas, tecido adiposo, hiperglicemia e diabetes, infecções por *Salmonella* e tirosina. Portanto, sugerimos que a seleção ocorrida durante várias gerações na linha TT atuou sobre as regiões que afetam as características de desempenho destes animais.

Palavras-chave: Segmentos de homozigose; Índice de fixação; *Gallus gallus*; Seleção artificial

ABSTRACT

Identification of selection signatures involved in performance traits in a paternal broiler line

Natural and artificial selection cause changes in certain regions of the genome resulting in selection signatures. Thus, is expected to identify genes associated with the traits under selection in such regions. Selection signatures may be identified using different methodologies, and some are based on detecting of contiguous sequences of homozygous identical-by-descent haplotypes, called runs of homozygosity (ROH), or estimating fixation index (F_{ST}) of genomic windows that indicates genetic differentiation. In our study, we aimed to identify selection signatures in a paternal broiler line and to investigate the genes annotated in these regions as well as the biological phenomena involved. For such purpose, ROH and F_{ST}-based analysis were performed using whole genome sequence of twenty eight chickens from two different generations. ROH analysis identified homozygous regions of short and moderate length. Analyzing ROH patterns it was observed regions commonly shared among some animals and changes in ROH abundance and length between the two generations. The results also suggests that WGS outperforms SNPchip data, however the number of individuals analyzed must be properly chosen. F_{ST}-based analysis revealed genetic differentiation in some genomic windows. Annotation of the consensus regions of ROH and F_{ST} windows counted for many genes of which some were previously associated with traits of economic interest, such as *APOB, IGF1, IGFBP2, POMC*, *PPARG,* and *ZNF423*. Overrepresentation analysis of the genes resulted in biological terms of skeletal muscle, matrilin proteins, adipose tissue, hyperglycemia and diabetes, Salmonella infections and tyrosine. Therefore, suggested that ancient and recent selection in TT line acted over regions affecting performance traits

Keywords: Runs of homozygosity; Fixation index; *Gallus gallus*; Artificial selection

1. INTRODUCTION

Currently, Brazil is the second most producer of chicken meat worldwide, with a production of 13.05 million tons in the last year, and the leader in international market of such product, with 4.3 million tons exported in 2017 [1]. Brazilian poultry sector is mainly concentrated in the southern states due its climate conditions allowing better conditions for raising broiler breeds [2].

The current production scenario is in mostly due to advances in genetic improvement of broilers. When comparing broiler lines from the 1950s to the 2000s, there was a reduction in slaughter time to one-third of the time that was needed before, since improved animals achieved slaughter weight faster [3]. This improvement in performance is due to 85-90% of genetic changes in chicken populations, whereas nutrition and management represented only 10 to 15% of it [4]. However, animal genetic improvement still has some challenges and genetic studies of chickens are essential for a better understanding of the biological aspects involved in traits of economic interest [5].

Embrapa Swine and Poultry, a Brazilian National Research Center, has been raising experimental chicken populations under selection since the 1970's. One of these lines is a paternal broiler line called TT, originated from Cornish and White Plymouth Rock breeds [6]. TT line has been under a multi-trait selection process focused on body weight, feed conversion, cut yields, breast weight, abdominal fat, and other traits, since 1992 [6–8]. In partnership with Embrapa, the Animal Biotechnology Laboratory (ESALQ/USP – Brazil) has conducted several genomic studies with these lines, such as association of quantitative trait loci (QTL) with performance traits [9–12]

The recent advances in genomics provided several tools to explore structure, function, regulation and expression of genes [13, 14]. Several studies have approached genomic aspects of livestock species in the past few years [15–20]. One of these approaches is the investigation of selection signatures in populations. Over the generations, natural and artificial selection acts over certain regions of the genome leaving selection signatures [21]. These regions carry genes involved in biological processes that affect the traits under selection pressure. Therefore, there are plenty of methodologies to identify selection signatures across the genome, such as such as extended haplotype homozygosity (EHH) [22], integrated haplotype score (iHS) [23], runs of homozygosity (ROH) [24], and F_{ST} statistics [25].

Runs of homozygosity are contiguous homozygous sequences in the genome, where the two haplotypes are identical-by-descent, what means they were inherited from a common

ancestor [26]. Several authors used this approach to study population structure and traits of interest in livestock species, such as cattle [27, 28], swine [29], poultry [8, 30], and sheep [19]. Another methodology used is the F_{ST} statistics. It is based on the fixation index (F_{ST}), first defined by Wright [31], which measure differences in allele frequencies between populations to infer about the genetic differentiation [32]. Previous studies have reported important selection signatures chickens [15, 16], swine [33] and cattle [18]. Therefore, these two approaches have been useful to detect selection signatures revealing important biological knowledge about them.

1.1. Objectives

To identify and analyze genomic regions under selection pressure in chickens of an experimental broiler line, especially between the periods of the studied populations (TT1998 and TT2007), as well as to investigate the genes of these regions and their respective biological processes.

1.1.1. Specific Objectives

1) Identify runs of homozygosity (ROH) in the individuals of two different generations (7^{th} and 16^{th});
2) Analyze changes occurred in the ROH regions between the 7^{th} and 16^{th} generations;
3) Detect genomic regions with different allele frequency between both populations based on the estimation of fixation index (F_{ST})
4) Identify genes annotated in the candidate selection signatures (ROH regions and FST windows);
5) Perform overrepresentation analysis in order to know the biological terms mainly involved.

1.2. Hypothesis

Artificial selection for traits of economic interest imprints selection signatures on certain regions of the genome containing genes responsible for the control of such traits.

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2. IDENTIFICATION OF SELECTION SIGNATURES INVOLVED IN PERFORMANCE TRAITS IN A PATERNAL BROILER LINE

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ABSTRACT

Background: Natural and artificial selection leads to changes in certain regions of the genome resulting in selection signatures. Thus, it is expected to identify genes associated with the traits under selection in such regions. Selection signatures may be identified using different methodologies, of which some are based on detecting contiguous sequences of homozygous identical-by-descent haplotypes, called runs of homozygosity (ROH), or estimating fixation index (F_{ST}) of genomic windows that indicates genetic differentiation. This study aimed to identify selection signatures in a paternal broiler line and to investigate the genes annotated in these regions as well as the biological phenomena involved. For such purpose, ROH and F_{ST}-based analysis were performed using whole genome sequence of twenty-eight chickens from two different generations.

Results: ROH analysis identified homozygous regions of short and moderate size. Analysis of ROH patterns revealed regions commonly shared among some animals and changes in ROH abundance and size between the two generations. Results also suggest that WGS outperforms SNPchip data avoiding overestimation of ROH size and underestimation of ROH number; however, the number of individuals analyzed must be properly chosen. F_{ST}-based analysis revealed genetic differentiation in some genomic windows. Annotation of the consensus regions of ROH and F_{ST} windows counted for many genes of which some were previously associated with traits of economic interest, such as *APOB*, *IGF1*, *IGFBP2*, *POMC*, *PPARG*, and *ZNF423*. Over-representation analysis of the genes resulted in biological terms of skeletal muscle, matrilin proteins, adipose tissue, hyperglycemia, diabetes, *Salmonella* infections and tyrosine.

Conclusions: Identification of ROH and F_{ST}-based analyses revealed selection signatures in TT line and genes that have important role in traits of economic interest. Changes in the genome of the chickens were observed between the 7^{th} and 16^{th} generations showing that ancient and recent selection in TT line may have acted over genomic regions affecting diseases and performance traits.

Keywords: Runs of homozygosity; Fixation index; F_{ST}; Artificial selection; *Gallus gallus*

2.1. BACKGROUND

Artificial and natural selection of animals lead to changes on particular genomic regions that affect traits of economic interest, as well as traits involved in adaptation to climatic and stress conditions, immune response, and disease resistance [1]. Thus, selection signature regions are printed along the genome as a result of selection pressure. Detecting selection signatures is important for a better understanding of population history and genetic mechanisms affecting phenotypic differentiation in humans, livestock and wild animals [2]. Understanding how selection acts on livestock populations may also benefits breeding programs in order to improve traits of economic interest in these animals, such as some chicken breeds which have been intensively selected for fast growth and muscle development [3]. Detection approaches rely on scanning the genome for regions of homozygosity, as well as on estimating allele or haplotype frequency differences between populations or generations within a population. There are several statistical methods for these analyses, such as extended haplotype homozygosity (EHH) [4], integrated haplotype score (iHS) [5], runs of homozygosity (ROH) [2], and F_{ST} statistics [6].

Runs of homozygosity are regions in the genome containing contiguous homozygous genotypes identical by descent (IBD), i.e. regions where the pairs of alleles are most likely inherited from a common ancestor [2]. Recent studies used this approach to better understand human diseases [7–9], human ancestry [10], and population structure and traits of interest in livestock species, such as cattle [11–14], swine [15], poultry [16, 17], and sheep [18]. The fixation index (F_{ST}), first defined by Wright [19], is a measure that exploits differences in allele frequencies to infer the genetic differentiation between populations or generations [20]. A certain locus under selection pressure changes its frequency over the generations. Thus, high values of F_{ST} indicate candidate selection signatures due to differences in locus frequency among populations or across generations. Previous studies have reported important selection signatures in Virginia [21] and Brazilian broiler and layer chicken lines [22] using this method.

Embrapa Swine and Poultry, a Brazilian National Research Center, has been raising experimental chicken populations under selection since the 1970's. One of these lines is the paternal broiler called TT, which has been under multi-trait selection since 1992 [23]. In partnership with Embrapa, the Brazilian Animal Biotechnology Laboratory (ESALQ/USP) has conducted several genomic studies with broilers. Identification of selection signatures in chicken lines can help understand which regions underwent selection pressure over time and how their biological mechanisms act to express the traits of interest, such as muscle growth and

fat deposition. In this sense, we aimed to investigate selection signatures in TT broiler line by detecting ROH in the 7th and 16th generations, raised in the years of 1998 and 2007, respectively, and estimating F_{ST} statistic between these two generations. The identification of those regions will provide better understanding of natural and artificial selection effects on broiler lines, especially on TT, and may point out candidate genes and biological mechanisms underlying performance traits.

2.2. METHODS

2.2.1. Ethics Statement

This study followed experimental protocols pertinent to animal experimentation with the approval of the Embrapa Swine and Poultry Ethics Committee on Animal Utilization (CEUA) in Concordia, Santa Catarina State, Brazil, on resolution number 011/2010. It followed the rules of National Council of Animal Experimentation Control (CONCEA) in accordance with international guidelines to guarantee animal welfare.

2.2.2. Chicken Population

Chickens used in this study were from a broiler line belonging to the Embrapa Swine and Poultry's breeding program. This line, called TT, was originated from Cornish and White Plymouth Rock breeds, that has been under a multi-trait selection process focused on body weight, feed conversion, cut yields, breast weight, abdominal fat, and other traits, since 1992 [17, 23, 24]. The chickens were raised in open sided poultry houses, receiving commercial broiler diet and water *ad libitum* [17, 24]. Two groups of animals from this line were analyzed, 14 chickens from the seventh-generation raised in the year of 1998 and 14 from the sixteenth-generation raised in 2007. The 7th and 16th generations were hereby renamed as 7G and 16G.

2.2.3. Sequencing and quality control

Whole genome sequencing (WGS) data of 28 chickens were used in this study. Animals were individually sequenced to a minimum coverage of 11.4x using the HiSeq2500 (Illumina) platform, and the alignment of reads was done against the chicken genome assembly

(Gallus_gallus-5.0, UCSC) chicken reference genome using Bowtie2 [25]. Detailed information about library preparation, sequencing, quality control of reads, alignment and SNP and INDEL identification are fully described in Boschiero et al. [22] and Moreira et al. [26]. Variants identified in sexual, mitochondrial, random or unplaced chromosomes were removed from our analysis.

2.2.4. Principal component analysis

Genetic distance between the 28 animals was assessed with a principal component analysis (PCA) using the SNP dataset (n = 9,914,904). The analysis was performed using the SNPRelate package of Bioconductor by means of an in-house script in R.

2.2.5. Identification of runs of homozygosity

The identification of ROH was chosen to obtain information about selection signatures and how they are shared between animals in both generations. Analyses were performed using PLINK v1.9 software [27, 28], which uses a sliding window approach: a window, with a minimum size, slides across the genome, naming a segment if it is in accordance with the parameters established and the threshold of calculated proportion of homozygous windows overlapping each SNP in that segment. The parameters used in the analysis were set based on Ceballos et al. [29] and they are listed in Table 1.

Table 1 PLINK parameters for run of homozygosity (ROH) analysis.

Parameter	Value	Definition
-homozyg-snp	50	Minimum number of SNP required to consider a ROH;
-homozyg-kb	300	Size (Kb) of the sliding window;
-homozyg-density	50	Minimum density required to consider a ROH;
-homozyg-gap	1,000	Maximum size (Kb) between two SNP to be considered in the same ROH;
-homozyg-window-snp	50	Number of SNP present in the sliding window;
-homozyg-window-het	3	Number of heterozygous SNP allowed in a ROH;
-homozyg-window-missing	5	Number of missing calls allowed in a ROH;

-homozyg-window-threshold	0.05	Proportion of overlapping windows that must be called homozygous to define a given SNP as in a homozygous segment.

Dataset of the 28 animals comprised 12,129,994 SNP, and all INDEL were excluded. The parameter *-homozyg-group* was also used to obtain information of the overlapping ROH (pools), i.e., ROH that appeared in at least two animals in the same region of the genome. The output *plink.hom.overlap* shows each ROH of each animal overlapping and their respective union (uROH) and consensus sequences (cROH), besides the genome position, size and number of SNP. We used the consensus ROH (cROH, i.e. a consensus segment of ROH that appeared in a common region in at least two animals) of the pools for annotation and enrichment analysis, to avoid randomly assigned ROH and to represent what is conserved between the animals [30, 31].

2.2.6. Genomic inbreeding coefficients

Individual genomic inbreeding coefficients were calculated based on ROH data (F_{ROH}), as defined by McQuillan et al. [32], to know if there was a difference of inbreeding between the 7G and 16G. F_{ROH} was calculated as:

$$F_{ROH} = \frac{L_{ROH}}{L_{aut}},$$

where L_{ROH} is the total size of ROH in the genome and L_{aut} is the total size of autosomal genome covered by SNP of an individual (933.071 Mb, Gallus_gallus-5.0 chicken reference genome - UCSC).

2.2.7. F_{ST} analysis

This methodology was applied to compare the two generations, i.e. to identify selection signatures by estimating the differences in allele frequency between 7G and 16G. The fixation index was calculated according to Weir and Cockerham's pairwise estimator method [33] using VCFtools v.1.16 software [34], in which SNP and INDEL analyses were run separately, comprising datasets of 9,914,904 SNP and 793,603 INDEL. The same parameters used recently in chickens [22] were applied. Weighted F_{ST} values were calculated using overlapping windows of 20 Kb size sliding by steps of 10 Kb size. Windows with at least 10 SNP or 5 INDEL were

considered, and all negative values were set to zero. Weighted F_{ST} values of the remaining windows were ranked, and those equal or above 0.3 were considered as candidate selection signatures. The software BEDTools [35] was used to check if there was equivalent regions identified in both datasets.

2.2.8. Functional analysis

Functional analysis was performed to identify genes annotated within the candidate selection signature regions identified and, consequently, the biological mechanisms that may be involved with traits of adaptation and performance. Such information was obtained assessing the position (start and end coordinates) of the candidate selection signatures (cROH and F_{ST} windows ≥ 0.3) in the chicken genome database available at BioMart Ensembl genome browser platform (Ensembl Genes release 94, Gallus_gallus-5.0 assembly) [36].

We also assessed the genes annotated in the candidate selection signatures under different perspectives in order to understand the effects of selection on TT line in different periods. First, changes between the 7G and 16G were investigated by assessing genes annotated in specific candidate selection signatures. They were: (i) cROH of regions shared exclusively among animals of the 7G; (ii) cROH of regions shared exclusively among animals of the 16G; (iii) cROH of regions that were shared among at least four animals of the 16G more than animals of 7G; (iv) F_{ST} SNP windows (≥ 0.3); and (v) F_{ST} INDEL windows (≥ 0.3). In addition, we looked for genes annotated in (vi) cROH of regions shared with 12 or more animals (among the 28) to investigate effects of selection in TT line as whole backing to prior periods.

Functional enrichment was performed using MeSH Enrichment and Semantic Analysis, Bioconductor's package [37, 38], in R software [39] to investigate if there was overrepresentation of any biological processes and/ component. For such purpose, datasets of genes annotated in the specific candidate selection signatures previously mentioned (i-vi) were analyzed separately. The *p-value* was adjusted using the Benjamin-Hochberg false discovery rate (FDR) method [40].

2.2.9. Overlapping selection signatures with QTL

The comparison of candidate selection signatures and QTL regions, previously associated with traits of economic interest, supports the role of the selection signatures in the

chicken phenotype construction and evolution and that they unlikely were randomly assigned. In this sense, we investigated if the candidate selection signature regions overlapped with QTL associated with traits of economic interest. The analysis was performed using an in-house script in R considering at least one overlapped base pair, to overlap the regions of all cROH and F_{ST} SNP and INDEL windows against the regions of QTL available at the Chicken QTL database [41], given the positions (chromosome, start and end) of these regions. Particularly, we also analyzed if there was overlap of candidate selection signatures with QTL associated with fat deposition previously identified by Moreira et al. [42] in the TT Reference population, originated from an expansion of TT line in 2007 for genomic studies purpose [17].

2.3. RESULTS

2.3.1. Principal component analysis

Principal component analysis using genomic data (SNP = 9,914,904) revealed a separation between animals of the 7th and the 16th generations (Figure 1).

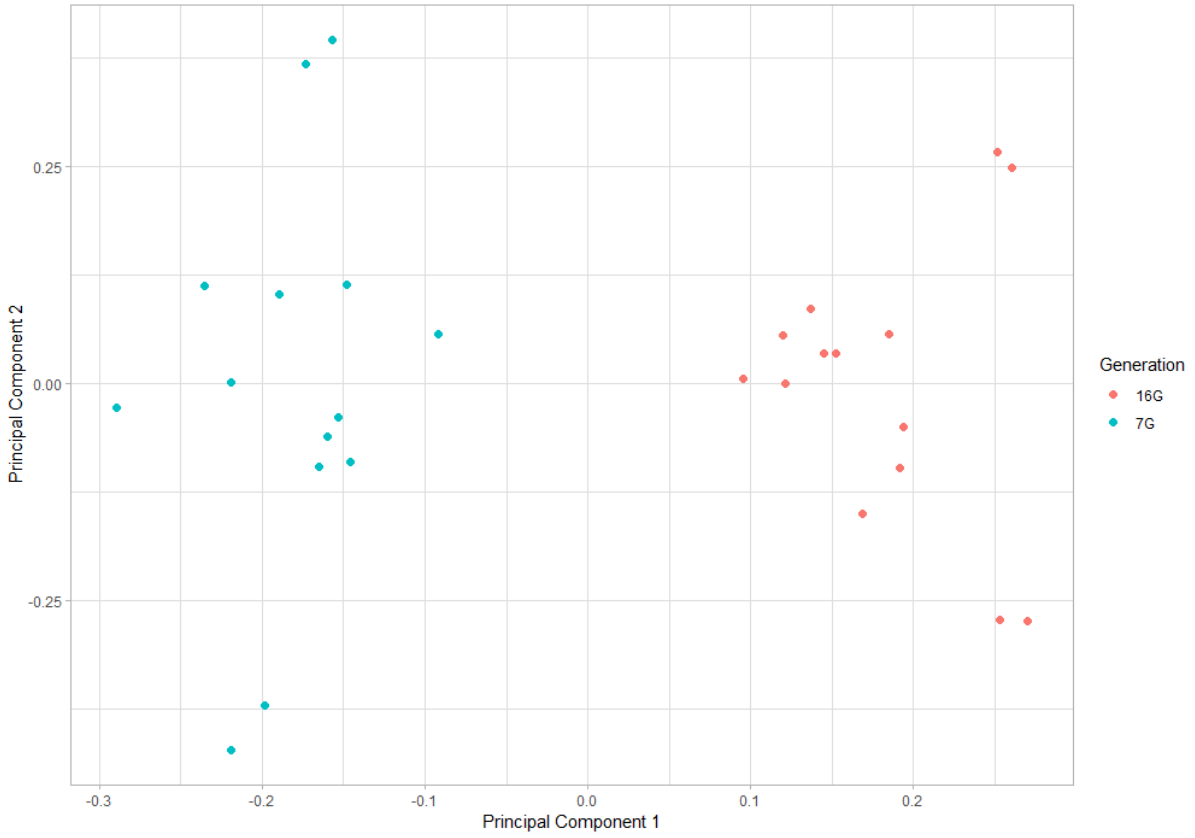

Figure 1 Principal component analysis (PCA) of TT animals' genomic data. PCA revealed a separation between animals of the 7th (blue) and 16th (red) generations.

2.3.2. Runs of homozygosity

Analysis of the sequence information of the 28 animals (14 of the 7G and 14 of the 16G) with PLINK sliding window approach identified 5,721 ROH (1,944 in the 7G animals and 3,777 in the 16G animals). The average number of segments per animal was lower in the 7G (138.9 ROH/animal) than in the 16G (269.8 ROH/animal) (Table 2). The ROH presented small and moderate sizes, ranging between 300 Kb and 4.9 Mb, and most of them had sizes smaller than 1.0 Mb in both generations (1,821 ROH in the 7G animals and 3,120 in 16G animals). A change in the distribution of ROH sizes was also observed between both generations (Figure 2). The proportion of ROH smaller than 1.0 Mb decreased (93.7 % in the 7G to 82.6 % in 16G) while the proportion of ROH with sizes between 1.0 and 2.0 Mb increased (6.1 % in the 7G to 14.7 % in the 16G) as well as ROH bigger than 2.0 Mb (0.3 % in the 7G to 2.7 % in 16G). The average total size of ROH per animal was 73.2 Mb in 7G and 188.6 Mb in 16G and the Figure 3 shows the distribution of the total size in function of the total number of ROH of each animal.

Table 2 ROH features for each animal of 7G and 16G.

IID	Number of ROH[1]	Total size[2] (Kb)	Mean size of segments (Kb)	Individual F_{ROH}
7G_01	130	60492.6	465.328	0.0648
7G_02	212	125062.0	589.913	0.1340
7G_03	131	63732.1	486.504	0.0683
7G_04	61	25416.8	416.668	0.0272
7G_05	65	30014.6	461.763	0.0322
7G_06	85	38303.9	450.634	0.0411
7G_07	183	103302.0	564.491	0.1107
7G_08	148	71381.7	482.309	0.0765
7G_09	209	138608.0	663.195	0.1486
7G_10	219	128120.0	585.024	0.1373
7G_11	94	42055.7	447.401	0.0451
7G_12	175	82420.5	470.974	0.0883
7G_13	188	95302.7	506.929	0.1021
7G_14	44	20107.3	456.985	0.0215
16G_01	245	164355.0	670.839	0.1761
16G_02	241	183336.0	760.729	0.1965
16G_03	254	174906.0	688.608	0.1875
16G_04	280	200919.0	717.567	0.2153
16G_05	268	188023.0	701.579	0.2015
16G_06	318	200111.0	629.281	0.2145
16G_07	256	181422.0	708.681	0.1944
16G_08	290	196574.0	677.843	0.2107
16G_09	289	206456.0	714.381	0.2213

16G_10	283	215635.0	761.963	0.2311
16G_11	275	187931.0	683.386	0.2014
16G_12	254	181634.0	715.095	0.1947
16G_13	242	167875.0	693.697	0.1799
16G_14	282	191333.0	678.484	0.2051

IID = individual identification
[1]Total number of ROH identified in each animal
[2]Total size of autosomal genome covered by ROH

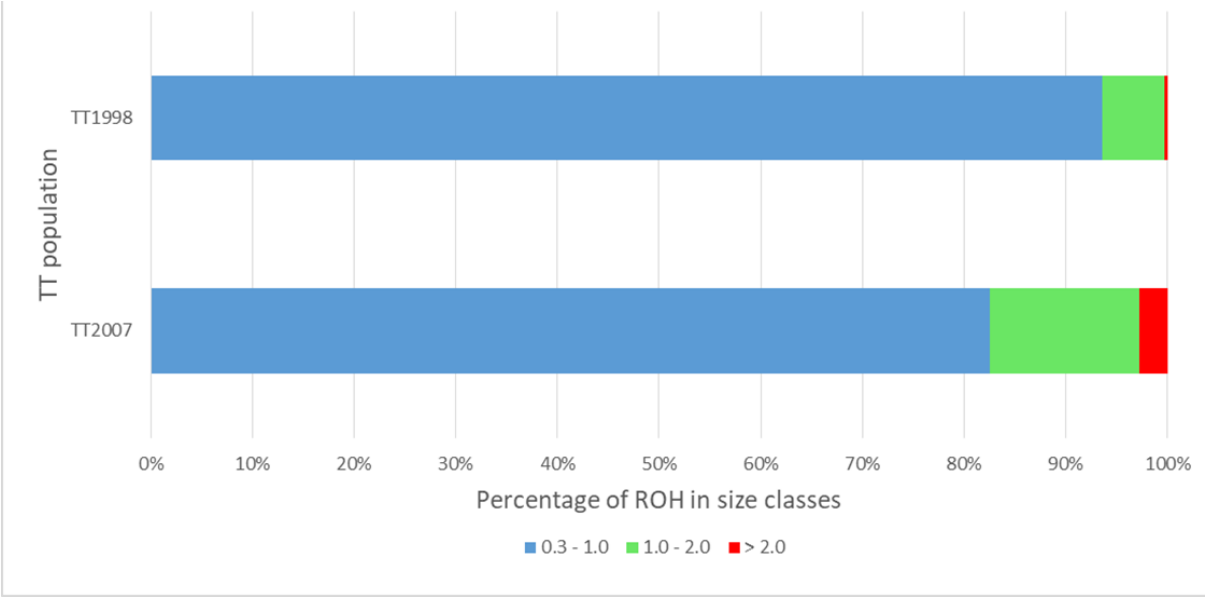

Figure 2 Percentage of ROH in 7G and 16G distributed in size classes. A decrease in the proportion of ROH smaller than 1.0 Mb in 16G is observed, meanwhile the proportions of ROH with sizes above 1.0 MB increased.

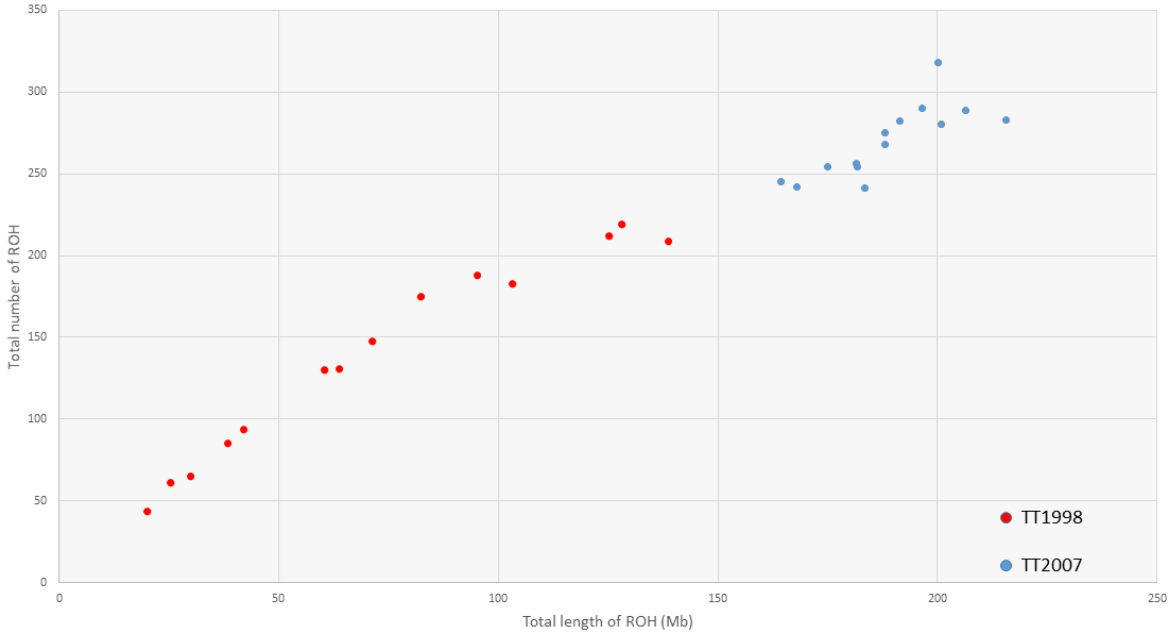

Figure 3 Distribution of the total size of ROH in function of the total number of ROH. Values plotted in red represent 7G individuals and values plotted in blue represent 16G individuals.

ROH were identified in all chromosomes, except GGA16 and GGA30-32 (Table 3), and GGA1 was the chromosome with the greatest number of ROH. Figure 4 represents all ROH, with their proportional sizes, distributed across GGA2 for the 28 animals. Regions where ROH is presented in more than one animal formed a sharing pattern. Figures of other chromosomes are provided as supplementary material. The four longest ROH (> 4 Mb) were located on different macro chromosomes (GGA2: 116,060,874 – 120,088,450; GGA5: 39,097,092 – 43,183,508; GGA3: 25,504,098 – 29,639,462; GGA4: 69,071,022 – 73,960,022). Overlaps of ROH from at least two animals established 1,941 pools. There was one pool of ROH shared between the 28 animals, and it was located in the GGA2 with a consensus sequence of 300.2 Kb (82,146,603 – 82,446,837). Most of the pools consisted in regions shared among two to seven animals (74.4 %). There were 87 regions with ROH commonly shared with at least 12 animals, most of them identified on GGA1.

Table 3 - Summary of runs of homozygosity (ROH), pools of ROH, F_{ST} SNP windows, and F_{ST} INDEL windows by chromosome in TT population.

GGA	Size (Mb)	Number of ROH	Number of pools	Number of F_{ST} SNP windows (≥ 0.3)	Number of F_{ST} INDEL windows (≥ 0.3)
1	196.20	1230	415	53	65
2	149.56	1087	338	28	28
3	111.30	609	228	28	23
4	91.28	582	212	5	4
5	59.83	392	137	6	9
6	35.47	211	77	4	5
7	36.95	267	85	17	21
8	29.96	213	66	1	2
9	24.09	106	39	6	10
10	20.44	106	39	-	2
11	20.22	114	36	1	1
12	19.95	90	41	-	-
13	18.41	107	35	1	2
14	15.60	101	32	-	1
15	12.76	81	32	-	-
17	10.96	72	22	-	-
18	11.05	87	28	1	-
19	9.98	61	16	-	2
20	14.11	54	19	-	-
21	6.86	26	7	-	-
22	4.73	13	4	2	3
23	5.79	21	7	1	-
24	6.28	30	12	-	-
25	2.91	7	1	-	-
26	5.31	9	1	-	-

27	5.66	16	5	-	-
28	4.97	25	6	-	-
33	1.65	4	1	-	-

Figure 4 Genome wide distribution of runs of homozygosity (ROH) in TT population. Size and location of ROH in chromosome 2 for each animal are represented in parallel. Patterns of shared ROH can be observed in some regions of the chromosome. In addition, a higher frequency of ROH in animals of 16G was observed**.**

Regions commonly shared among the animals become more frequent in the 16G. About 79 % of the pools were shared with at least one 16G animal more than 7G's. Table 4 shows how the 1,941 pools are shared among animals of the 7G and 16G, separately; it indicates how many pools are shared among a certain number of animals (0-14) in each generation and the percentage they represent from the total (1,941). For example, considering the 1,941 common regions (pools) identified in TT line, only one region was shared among the 14 animals of the 7G while in the 16G there were eight regions shared among the 14 animals of this generation (Table 4). One of these eight regions was the one shared among the 14 animals of the 7G, the other seven were regions shared with less than 14 animals in the 7G.

Table 4 Distribution of pools according to the proportion of animals that overlap ROH.

Number of animals	7G		16G	
	*Pools (n)	%	Pools (n)	%
0	438	22.57	25	1.29
1	582	29.98	146	7.52
2	432	22.26	372	19.17
3	207	10.66	384	19.78
4	121	6.23	313	16.13
5	65	3.35	246	12.67
6	34	1.75	172	8.86
7	24	1.24	111	5.72
8	18	0.93	66	3.40
9	13	0.67	27	1.39
10	4	0.21	29	1.49
11	0	0.00	22	1.13
12	1	0.05	11	0.57
13	1	0.05	9	0.46
14	1	0.05	8	0.41
Total	1,941	100%	1,941	100%

*Number of pools formed by ROH of 2-14 animals overlapping and their respective percentage for each group.

2.3.3. Genomic inbreeding coefficients

Individual genomic inbreeding coefficients based on ROH were calculated for both groups, 7G and 16G (Table 2). Mean, maximum and minimum individual F_{ROH} for animals of the 7G were 0.0784, 0.1340, and 0.0215, respectively, with a coefficient of variation (CV) of 52.8%. For animals of the 16G, the mean, maximum and minimum individual F_{ROH} were 0.2021, 0.2213, and 0.1761 (CV = 7.4%).

2.3.4. F_{ST} windows

F_{ST} analysis identified 91,638 and 86,404 windows for SNP and INDEL datasets, respectively, after removing windows with less than 10 SNP and five INDEL. The number of markers per window ranged from 10 to 1,562 SNP (average of 216.3 SNP/window) and from

five to 72 INDEL (average of 18.2 INDEL/window). Mean weighted F_{ST} values for SNP and INDEL datasets were 0.040 and 0.038, respectively, while the highest F_{ST} values were 0.598 and 0.555.

Windows with F_{ST} values equal or higher than 0.3 were considered candidate selection signatures. There were 178 windows using SNP dataset (Figure 5) and 154 windows using INDEL dataset (Figure 6) above this threshold value ($F_{ST} \geq 0.3$). Most of these windows were in the macro-chromosomes (Table 3) and approximately 87 % of the INDEL windows overlapped with SNP windows.

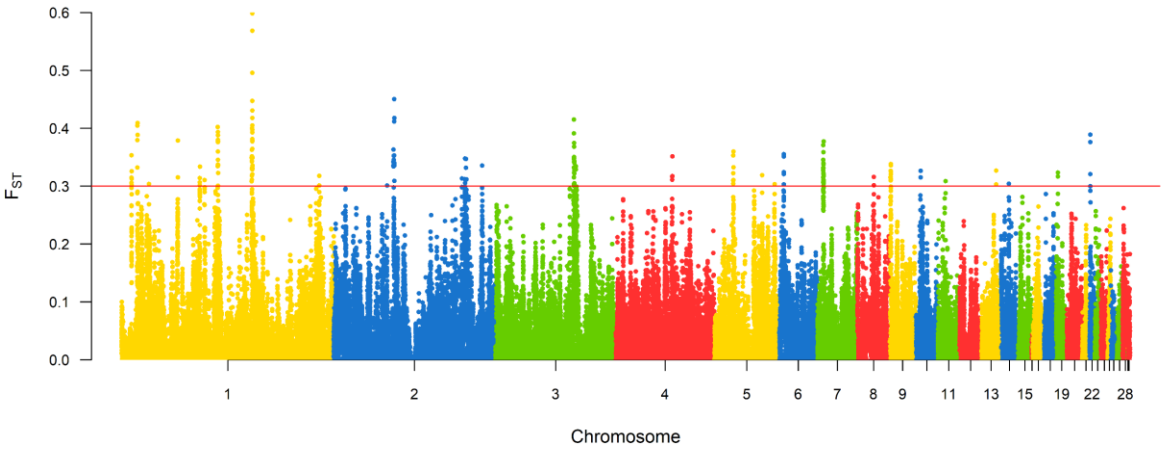

Figure 5 Manhattan plot of genome wide distribution of FST windows for SNP dataset. Red line represents threshold of 0.3, windows above this value were considered candidate selection signature candidates.

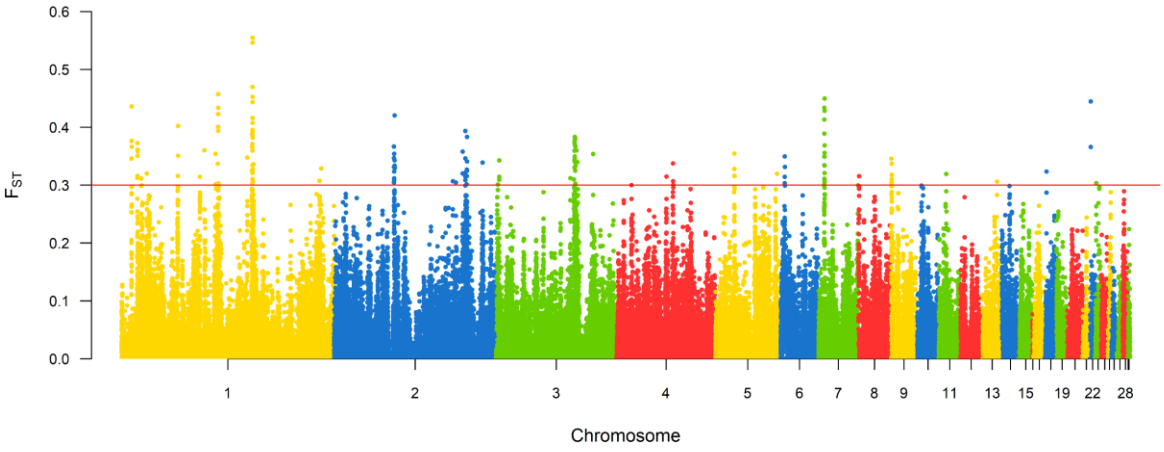

Figure 6 Manhattan plot of genome wide distribution of FST windows for INDEL dataset. Red line represents threshold of 0.3, windows above this value were considered candidate selection signature candidates.

2.3.5. Functional analysis

Annotation analysis using the Ensembl genome browser revealed 5,861 genes annotated in the 1,941 cROH of regions shared among at least two of the 28 animals. Annotation of F_{ST} windows with values equal or higher than 0.3 identified 56 and 60 genes for SNP and INDEL datasets, respectively. Since a great part of SNP windows overlapped with INDEL windows, 37 of these genes were common for both datasets (Supplementary table 1). In addition, about 46.1 % of SNP F_{ST} windows and 37.7 % of INDEL F_{ST} windows overlapped with cROH. Thus, we found 34 genes annotated in cROH in common with genes annotated in FST (SNP and/or INDEL) windows (Supplementary table 1).

Information available at the Biomart Ensembl showed that some of the genes annotated in the selection signature regions play a role in biological processes involved in traits of economic interest in chicken or in other model animals. There were genes involved in lipid metabolic processes, glucose metabolism and homeostasis and adipose tissue development. Other genes were described to be involved in muscle cell differentiation, muscle tissue development, and constituents of skeletal muscle. Moreover, there were a group of genes related to different types of behavior, such as grooming, locomotion, fear response, feeding behavior, aggressiveness, and social, exploration and maternal behaviors. Genes involved in the immune humoral system, differentiation, proliferation, homeostasis and chemotaxis of B cells, and regulation of cytokines production were also annotated in regions of the candidate selection signatures.

Annotation analysis of selection signatures regions that changed between the 7^{th} and 16^{th} generations revealed candidate genes that, consequently, might be involved in biological processes affecting the changes occurred in in such period. These changes were regions that were in homozygosity only in the 7G or in the 16G, regions that were in homozygosity in animals of the 7G but become even more common among animals of the 16G, and regions that occurred differentiation of allele frequencies. In this sense, there were 71 genes annotated in cROH of regions shared exclusively among animals of the 7G and 1,881 genes annotated in cROH of regions shared exclusively among animals of the 16G. Looking at cROH of regions that were shared among at least four animals of the 16G more than animals of the 7G it was revealed 1,318 genes annotated in them. For example, the gene IGF-I (GGA1 55,335,204 – 55,383,631) was annotated in a cROH region (GGA1 55,149,208 – 55,359,089) shared between 7 animals of the 7G and 13 animals of the 16G. In addition, annotation of cROH regions shared

with at least 12 out of the 28 animals showed 232 genes that might be involved in changes due to selection in a period of time previous to the 7th generation.

These genes obtained from annotation of specific candidate selection signatures were used to perform MeSH overrepresentation analysis, with the purpose of having an integrated knowledge of biological processes or components that may be involved in the selection of TT line. MeSH analysis indicates if in a biological category, such as anatomy, diseases or phenomena and processes, there is an overrepresentation of a particular group of genes. In this sense, overrepresentation analysis of these lists of genes resulted in eight different biological terms: 'matrilin proteins', 'skeletal muscle', '*Salmonella* infections in animals', 'adipose tissue', 'cystatins', 'tyrosine', 'pregnancy in diabetics', and 'hyperglycemia'. Table 5 presents each one of these terms and the respective genes that are involved in the term, given a gene list obtained from specific candidate selection signatures used in the analysis.

Table 5 MeSH enrichment analysis of genes annotated in candidate selection signatures.

MeSH term (MeSH ID)	Gene count	*P-value*	BH[1]	Dataset[2]
Matrilin Proteins (D064235)	3	0.00008523	0.01605961	a
Muscle, Skeletal (D018482)	9	0.00037904	0.04927546	a
Salmonella Infections, Animal (D012481)	2	0.02170325	0.02893767	b
Adipose Tissue (D000273)	9	0.00246916	0.0157409	c
Cystatins (D015891)	2	0.00018075	0.01590614	d
	2	0.00019528	0.02089512	e
Tyrosine (D014443)	3	0.00085140	0.03746136	d
Pregnancy in Diabetics (D011254)	1	0.00703107	0.03515533	d
	1	0.00730629	0.03653145	e
Hyperglycemia (D006943)	1	0.01748688	0.04066230	d
	1	0.01816762	0.04223941	e

[1]Benjamini & Hochberg procedure for controlling false discovery rate (FDR) [40].

[2]Analysis considering dataset of: (a) genes annotated in cROH of pools shared between at least 12 animals of both generations; (b) genes annotated in cROH of pools shared exclusively between 7G animals; (c) genes annotated in cROH of pools shared between at least four 16G animals more than 7G's; (d) genes annotated in F_{ST} windows of SNP data (FST ≥ 0.3); and (e) genes annotated in F_{ST} windows of INDEL data (FST ≥ 0.3).

2.3.6. Selection signatures in overlap with QTL in the TT line

As a manner of achieving greater accuracy in the identification of candidate selection signatures, the regions of cROH and F_{ST} SNP and INDEL windows (≥ 0.3) were analyzed if there was overlap with QTL regions associated with traits of economic interest in chickens available at the Chicken QTL database (release 37). About 72.8 % of the 1,941 cROH overlapped with QTL regions (*n* = 2,617). There were also overlap of 60.1 % of the 178 F_{ST} SNP windows and 68.2% of the 154 F_{ST} INDEL windows with QTL regions (*n* = 107 and 105, respectively). These QTL regions were associated with 143 different traits of which some are very important for broilers' breeding program goals such as feed conversion rate, feed intake, average daily gain, body weight, breast muscle weight, and others (Figures 7-9).

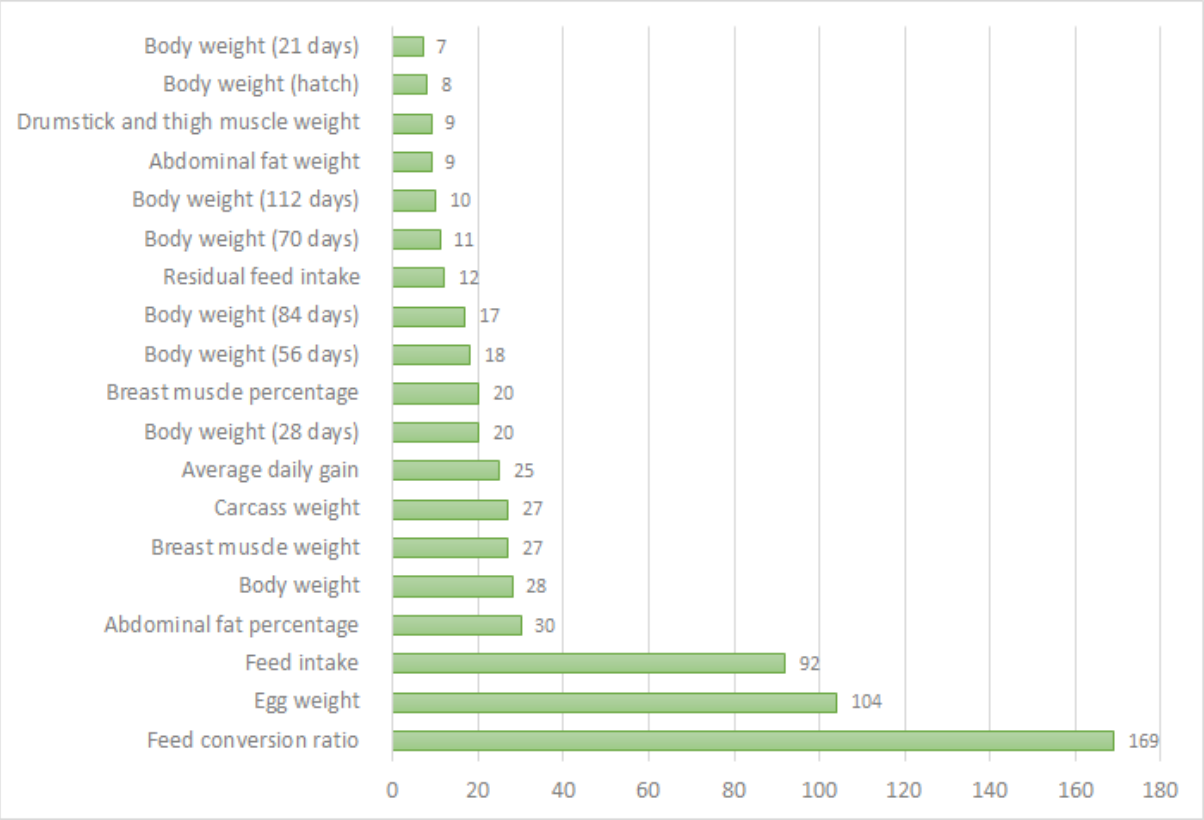

Figure 7 Number of QTL regions associated with traits of economic interest (QTL database) overlapping with ROH regions.

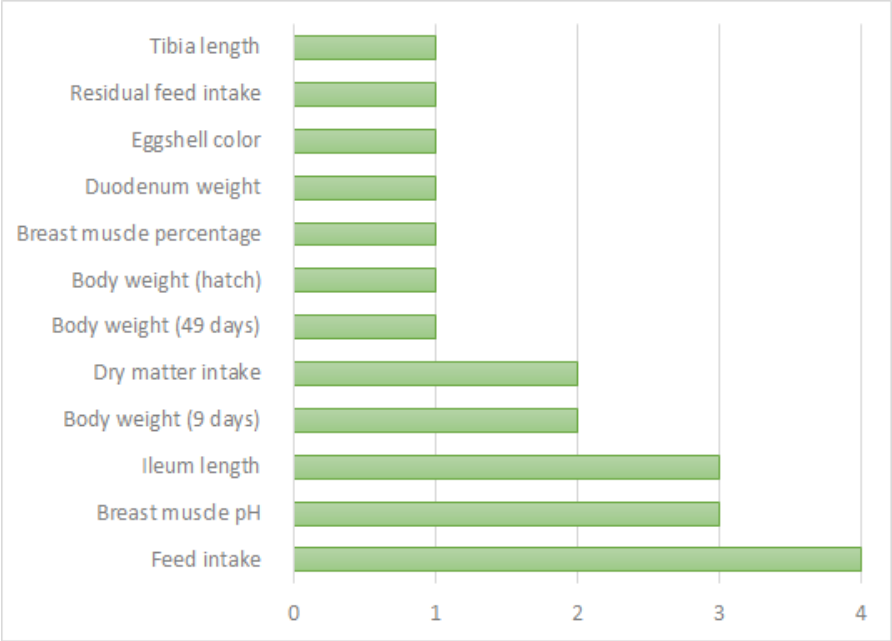

Figure 8 Number of QTL regions associated with traits of economic interest (QTL database) overlapping with FST windows (SNP dataset)

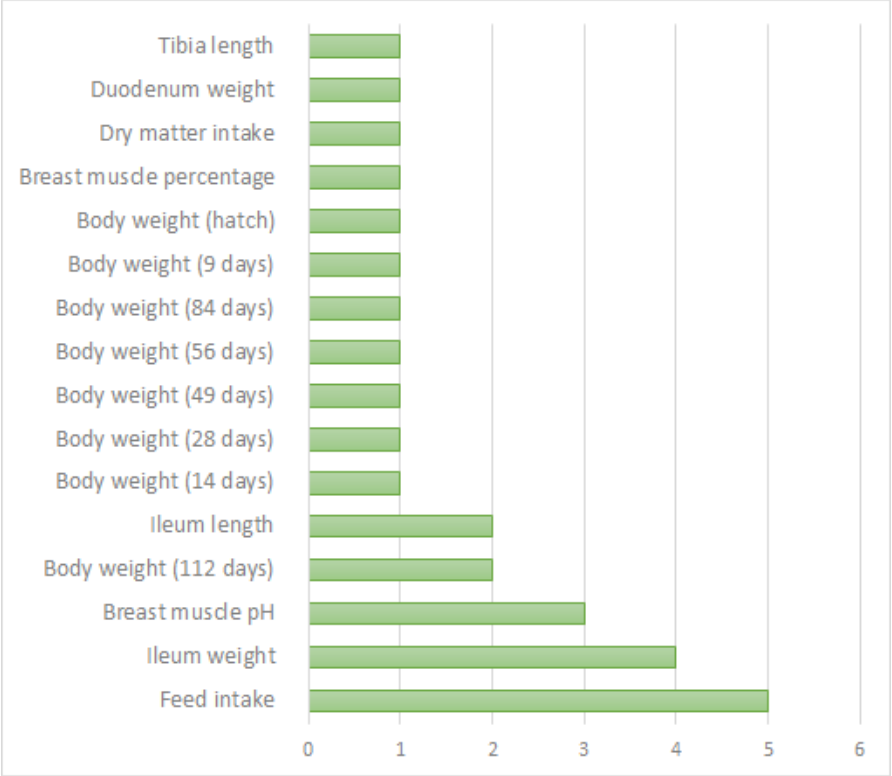

Figure 9 Number of QTL regions associated with traits of economic interest (QTL database) overlapping with FST windows (INDEL dataset)

Particularly, candidate selection signatures (cROH) also overlapped with 10 QTL regions mapped on GGA5, GGA9, GGA10, GGA13, GGA15, and GGA27 chromosomes in

the TT Reference Population for fat deposition traits (Table 6) [42]. There was no overlap of QTL with F_{ST} windows (≥ 0.3) with these QTL.

Table 6 QTLs associated with fat traits in TT Reference Population overlapping with consensus runs of homozygosity (cROH)

Trait (QTL ID)[1]	Chr	QTL position (start-end)[2]	cROH position (start - end)
ABFW (160520)	5	38,000,437 – 38,996-916	38,015,470 – 38,234,917
			38,425,606 – 38,473,340
ABFW (160521)	10	7,000,336 – 7,998,549	6,978,426 – 7,049,244
			7,474,909 – 7,543,996
			7,931,784 – 7,932,642
ABFW (160522)	13	3,002,617 – 3,998,616	3,572,237 – 3,641,314
ABFP (160525)	5	38,000,437 – 38,996-916	38,015,470 – 38,234,917
			38,425,606 – 38,473,340
ABFP (160526)	10	7,000,336 – 7,998,549	6,978,426 – 7,049,244
			7,474,909 – 7,543,996
			7,931,784 – 7,932,642
ABFP (160527)	13	3,002,617 – 3,998,616	3,572,237 – 3,641,314
SKINW (160529)	15	6,000,311 – 6,999,944	6,175,697 – 6,362,475
			6,473,966 – 6,475,580
SKINW (160531)	24	5,000,105 – 5,999,010	4,720,727 – 5,060,139
			5,187,457 – 5,646,905
			5,899,715 – 5,962,715
SKINP (160534)	9	4,000,836 – 4,999,336	4,664,760 – 4,860,555
SKINP (160530)	15	6,000,311 – 6,999,944	6,175,697 – 6,362,475
			6,473,966 – 6,475,580

ABFW = abdominal fat weight, ABFP = abdominal fat percentage, SKINW = skin weight, SKINP = skin percentage
[1]QTLID from QTL chicken database
[2]Positions in the Gallus_gallus-5.0 version of the chicken genome

2.4. DISCUSSION

2.4.1. Runs of homozygosity

ROH studies have mainly addressed human evolution and diseases, conservation and evolution of wild species, and genomic features of livestock animals [2, 7, 13, 17]. The investigation of ROH as selection signatures using two different generations of the TT line provided two important points to explore in our research. First, the identification of important selection signatures that may refer to periods preceding the generations under study. Second, it allowed the comparison of how these selection signatures were shared among the individuals and how they have changed over the generations. Since shared ROH is an indication of regions under selection [18], the consensus regions of ROH (cROH) were used in this study to understand the possible biological consequences of selection in this broiler line.

ROH identified in animals of TT line presented small to moderate sizes, ranging from 300 Kb to 4.9 Mb. Short ROH are most probable to be IBD genomic regions inherited from ancient ancestors indicating long term selection [43]. Over the generations, IBD segments tends to break down due to recombination events by repeated meiosis. Thus, ROH size is associated with the degree of shared parental ancestry and for how long it was passed across generations [32] and, as expected, short ROH regions shared between animals from 7G and 16G encompassed genes associated with traits of interest. It is important to mention that not all short ROH are IBD and a proportion of them may be identical-by-state (IBS) due to genetic drift, as well as population bottlenecks, and therefore some authors recommend to be cautious in attributing these regions as candidate selection signatures [18, 44, 45]. The minimum size of 300 Kb was set in our analysis to detect ROH, considering that the use of WGS data calls ROH with smaller sizes compared to SNPchip data. In this study, we were not able to determine which proportion is attributed to genetic drift; however, strategies were adopted to improve the chances to detect true selection signatures. They were (i) using ROH regions in common with at least two animals, (ii) overlapping these regions with the Chicken QTL database, and (iii) performing MeSH overrepresentation analysis of regions affecting biological processes related to the breeding goal traits. These combined strategies aid in the identification of candidate selection signatures less likely to have arisen from genetic drift.

Marchesi et al. [17] identified ROH in 1,279 chickens of the TT Reference population using a high-density 600K genotyping array data using the same parameters used in the present study, except for the minimum size of ROH and number of heterozygous SNP allowed. The 14 chickens of 16G are some of the parents of TT Reference chickens and, as expected, regions of ROH were commonly shared between both populations. More than 98 % of the cROH identified in animals of the 7G and 16G overlapped with ROH identified in the animals of TT Reference, validating the results of ROH identification in the 28 sequenced animals. However, a higher number of ROH per animal and ROH with smaller sizes were identified in the 28 animals compared to those of TT Reference. We suggest that the reasons for identifying shorter ROH relies on the higher resolution of WGS data in comparison with SNPchip data, leading to an identification of two or more ROH (using WGS data) as a unique ROH. This difference in ROH calling was also observed in another study with feline that used both types of dataset of the same individuals [46].

It is also important to highlight that low coverage WGS data may present higher error rate of variant calling in comparison with SNPchip data and that it may lead to inaccuracy of ROH calling [29]. Thus, we followed parameters based on Ceballos et al. [29], which demonstrated equivalent results to SNPchip data's results, when dealing with low coverage WGS, considering the differences of error rate in variant calling in both types of data. In addition, even the sequencing data of the 28 animals have ≈ 11.4 X of coverage, after filtration (following the same proceedings as in Boschiero et al. [22]), we were able to keep a good accuracy of called variants, what is sustained by Boschiero et al. [22] findings. They performed variant identification using data of the same 14 animals of the 16th we used in ours and they we able to obtain a good accuracy and low false-positive rates in variant calling, including for heterozygous SNP, and they were also able to validate the SNP detected by comparing with two other datasets [22]. Moreover, in order to extend the chances of detecting accurate ROH we opted to investigate consensus regions of ROH, i.e. regions of ROH in common with at least two animals, that might indicate regions under selection [18, 30]. All these observations corroborate with our suggestion that the smaller size and higher number of ROH possibly relies on the better resolution of WGS.

In our study, an increase in ROH abundance was observed, i.e. between the 7^{th} and 16^{th} generations there was an increase on the average number of ROH segments per animal and in the average size of segment per animal (Figures 2 and 3) and these differences may have occurred due to a bottleneck effect. Marchesi et al. [17] estimated the N_e in TT line backing to 200 generations ago and reported a decay in N_e, especially in the last five generations, ranging

from 157 to 113 chickens (N_e of TT Reference Population). Thus, when a population size is reduced, the average of heterozygosity in a certain loci is expected to decline, depending on the N_e [47, 48]. In addition, because of the bottleneck effect, inbreeding may have occurred among families of TT line, which is observed in an increase in the mean genomic inbreeding coefficients of both generations (7^{th} F_{ROH} = 0.078 and 16^{th} F_{ROH} = 0.202). It is worth to mention that, even if mating between close related individuals is avoided, it is possible to occur inbreeding because TT line is a closed population [17].

Furthermore, the percentage of animals sharing a ROH region increased from the 7^{th} to the 16^{th} generation (Table 4). Mastrangelo et al. [18] reported similar observations of an increase in the abundance of ROH in a sheep breed it and suggested that a decrease in the effective population size (N_e) had occurred resulting in recent and historical autozygosity events. Thus, we suggest that the increase in homozygous regions across generations in TT line is consequence of selection pressure over genomic regions that are important to the breeding program's goals jointly to reduction on the N_e and inbreeding. In fact, genes associated with traits of economic interest, such as the *APOB, POMC, PPARG* and other genes (Supplementary table 2), were annotated in regions shared with more 16G animals than with 7G animals, supporting that the regions containing these genes were under selection pressure in the respective period.

2.4.2. F_{ST} windows

An alternative approach applied in this study for identification of selection signatures was the genetic differentiation methodology based on allele frequency differences called F_{ST} statistics. Previous studies have used this method for detecting selection signature in livestock species, such as broilers [14, 21, 22, 49, 50]. Here we compared two groups of animals of TT broiler line, 10 generations distant from each other. The parameters were the same used by Boschiero et al. [22], in the comparison of the TT line against a broiler line. The authors states that windows of 20 Kb allows a finer resolution of the regions in addition to windows with sufficient number of markers, considering that the amount of variants in a window is essential for increasing the power of the analysis [22, 51]. This intent was achieved in our results since we obtained an average of 216 SNP/window and 18 INDEL/window, which were similar to the results obtained by Boschiero et al. [22] with averages of 268 SNP/window and 26 INDEL/window. Furthermore, there was a considerable amount of windows in common

between both datasets, 87 % of overlapping between F_{ST} INDEL and SNP windows, a fact also observed by Boschiero et al. [22].

Estimates of F_{ST} range from zero, meaning no genetic difference between the subpopulations, up to 1.0, meaning complete genetic differentiation [52]. Although there is not a determined threshold to capture regions that indicate genetic differentiation as a candidate selection signature, some authors use a threshold for the top 0.1 % values of F_{ST} [21, 22]. Here we established a threshold value of 0.3 in order to obtain regions that might be in a differentiation process in the TT line. About 0.002 % of the windows had F_{ST} values above this threshold, for SNP and INDEL datasets, and the highest estimated values were 0.598 and 0.555 for SNP and INDEL datasets, respectively. In addition, as discussed by Boschiero et al. [22], combining strategies to detect selection signatures minimizes the occurrence of false positives. Therefore, in our study, some strategies were: (i) perform the analysis with SNP and INDEL datasets; (ii) use a F_{ST} threshold (≥ 0.3) to capture the highest values; (iii) removal of windows with less than 10 SNP or 5 INDEL; (iv) overlapping with QTL database; and (v) to perform functional annotation and enrichment analysis.

2.4.3. Common candidate selection signatures

Besides both types of analyses, ROH and F_{ST}, have different approaches, it was possible to identify common signals of selection. It is strategy that may enforces the possibility of a certain regions be a true selection signature and some authors adopt this strategy in order to obtain finer results. It was possible to observe that F_{ST} windows overlapped with candidate selection signatures identified with the ROH analysis by comparing both regions. Among the genes annotated on them, 34 genes were found in common. Furthermore, the integrated results of biological processes and components shows that both types of analysis provided an insightful overview of what changed in TT line across the generations.

2.4.4. Selection signatures of broiler performance and adaptation

Besides identifying regions in the chicken genome under selection pressure, knowing the genes annotated in these regions and how they biologically act is essential for understanding how the selection signatures contributed to the current phenotype of the evaluated animals. Since TT broiler line is under multi-trait selection since 1992 aiming to improve body weight,

feed conversion, cut yields, breast weight, viability, fertility, and hatchability and to reduce abdominal fat [17, 23], it is expected that genes influencing the performance of these traits are under selection pressure.

Therefore, investigating which genes were annotated in the candidate selection signatures regions identified in both F_{ST} and ROH analysis helps to understand the biological mechanisms that affected the construction and evolution of the phenotype of TT line. In this sense, genes involved with traits of economic interest were identified in these regions. The genes *IGFB2, TGFB2, HOXD9, HOXD10, POMC SPP1, SPP2,* and *IGF1* were some of the genes annotated in the candidate selection signatures of TT line and that were previously found in other selection signatures and associated with performance traits, such as growth, body weight and composition, abdominal fat, organogenesis and feed intake and consumption [22, 50, 53–66]. Furthermore, we identified a group of genes annotated in the selection signatures that are involved with structural constituents, cell differentiation, and development of muscle tissue: ACTC1, AKAP6, ATP2A2, KCNMA1, MYO1B, MYO1C, MYO1E, MYO1F, MYO6, MYO7A, MYO10, MYO16, TPM4, VCL, and V1PR1 [22, 67]. Selection signatures identified in our analysis also indicate regions involved in lipid metabolism and adipose tissue development, encompassing the *ADCY2, AKAP6, APOB, ATPR2, IGFBP2, PLA2R1, PPARG, SCARB1* and *ZNF423* genes [22, 68–77].

Chickens raised in production systems are under several stressful conditions that can meaningfully affect the performance and the immune system of these animals [78, 79]. Stress challenged animals respond by changing their response behavior, metabolic rates, and functioning of cardiovascular and immune systems [78]. Thus, a selective pressure over genomic regions controlling responses to stressor conditions may occur, and the selection signatures identified with the ROH analysis shows a class of genes involved in these aspects: *ACE, BAG1, CACNA1C, ELP2, HSPA8, MOCOS, MRTO4, MYH9, NSUN2, PAX5, PQLC2* and *TRPM8* [16, 17, 80, 81].

2.4.5. Changes in TT line across the generations

TT line is a broiler line under multi-trait selection with emphasis on body weight since 1992 [17], and some studies have shown that this trait has positive genetic correlation with feed conversion and abdominal fat weight [82]. In fact, TT line underwent phenotypic changes of body weight and fat deposition traits. Moreira et al. [42], for example, found that genetic variance of with body weight at 42 days of age, abdominal fat weight, abdominal fat percentage,

skin weight and skin percentage was explained by genetic variants in TT line. Thus, selection pressure acted over genomic regions that affect the traits under direct or indirect selection in the breeding program.

The results of the ROH and F_{ST} analysis indicated candidate selection signatures involved with such traits and that changes occurred during the period between the 7th and 16th generations. First, there were regions of ROH shared exclusively among animals of the 7th but that were not in homozygosity in animals of the 16th generation. As well, regions that were not in homozygosity in animals of the 7th appeared to be in homozygosity in animals of the 16th generation. In addition, certain regions of ROH shared among animals of the 7G become more common among animals of the 16G. For example, the region that encompass the gene *IGF-I* was in homozygosity in 7 animals of the 7G and in 13 animals of the 16G. Yet, F_{ST} analysis showed regions in the genome that were in a process of allele fixation. In these regions, for example, the genes ZNF423 and SPP2 were annotated, which are involved in adipogenesis and growth in chickens, respectively [58, 73]. Because these specific regions may represent changes in TT line, it was interesting to investigate not only the genes annotated on them, but any possible biological process or component in overrepresentation. In this sense, MeSH analysis was performed in order to provide a better and integrated view of the changes [83].

Adipose tissue was in overrepresentation among the genes of these regions what might be expected since selection for growth in broilers, such as TT line, is companied of elevated fat deposition [84]. In addition, overlaps of cROH regions with QTL associated with fat deposition in TT Reference population support that regions affecting these traits were indirectly selected across generations in TT line. Hyperglycemia was also overrepresented and it is possibly a consequence of fat deposition in these animals. The excess of adipose tissue in chickens may lead to a condition similar to the early stage of type 2 diabetes in humans, manifesting hyperglycemia and exogenous insulin resistance [85, 86]. Another overrepresentation was ‘pregnancy in diabetics’, and, as it is known, pregnancy is not a biological mechanism of birds. However, Nadaf et al. [84] discuss that some QTL associated with chicken fatness have genes playing a role in obesity and diabetes in humans, and since MeSH is a tool that comprises animals in general, this association may have be done due to genes with similar functions.

Cystatins was among genes annotated in regions that underwent allele frequency changes during the 7th and 16th generations. Cystatin is a superfamily of reversible competitive inhibitors of cysteine proteases such as calpains, cathepsins, and ficins, and the cystatin system have important roles in protein turnover, antigen presentation and disease immunity [87, 88].

As well, tyrosine was overrepresented among the genes of F_{ST} SNP windows, which is considered a nonessential amino acid in animals [89].

The ROH analysis also provided information about regions that may indicate selection in a period that precedes the studied generations. In these regions there were overrepresentation of genes involved in skeletal muscle and the matrilin proteins, both important for the growth of chickens. Matrilin is a four-member family of proteins composing extracellular matrix of some tissues as cartilage, a connective tissue. They bind to collagen-containing fiber and other matrix constituents and can form oligomers [90]. *MATN3* (matrilin 3), was one of the genes of this family found in the selection signatures in GGA3 and it is a constituent of chicken cartilage [91].

Furthermore, the abundant amount of overlaps between cROH and F_{ST} windows and QTL regions associated with traits of economic interest in broilers, such as feed conversion, feed intake, growth, and abdominal fat, enforces the results of candidate selection signatures involved in performance traits. This fact together corroborates that TT line have been selected for growth and muscle deposition for a long period with a possible consequence of increased fat deposition, and for traits involving proteic turnover, *Salmonella* infections and metabolism of tyrosine.

2.5. CONCLUSION

Regions under selection pressure in a paternal broiler line were investigated in this study. To the best of our knowledge, this was the first study comparing two different generations of a same line, indicating important genomic variations during a period of 10 generations of selection. For such purpose, identification of runs of homozygosity and F_{ST}-based analyses were performed. The use of different approaches allowed looking for selection signatures in different perspectives. First, using ROH analysis, we were able to identify regions that were inherited backing to common ancestors since the beginning of the broiler line origin, how these regions were shared between the animals of both generations, and what has changed in the genetic make-up of the TT line by selection between 1998 and 2007. Second, comparing the two generations, by means of F_{ST}-based analysis, showed regions that were occurring fixation in the population during the period studied. Looking into these different perspectives it was found that selection may have resulted changes in traits such as skeletal muscle, cartilage and adipose tissues, and diseases. In addition, the results support that the use of WGS data offers

advantages over SNPchip data, avoiding underestimation of ROH number and overestimation of ROH size, but the number of individuals analyzed must be taken into account in order to better represent the whole population. Based on the functional analysis of these genes, it was possible to notice that selection signatures were compatible with the selection applied in this paternal line in the last decades. Thus, the investigation of selection signatures provided valuable insights about genes and biological processes of performance, adaptation and disease traits. Importantly, our approach using two different methodologies to analyze two generations of the same line expands the knowledge of selection pressure occurred between both generations, as well as since the beginning of the selection of this line.

2.6. ABBREVIATIONS:

ABFP: abdominal fat percentage; ABFW: abdominal fat weight; CEUA: Ethics Committee on Animal Utilization; CONCEA: National Council of Animal Experimentation Control; cROH: consensus region of runs of homozygosity; EHH: extended haplotype homozygosity; FDR: false discovery rate; F_{ROH}: inbreeding coefficient based on runs of homozygosity; F_{ST}: fixation index; IBD: identical-by-descent; IBS: identical-by-state; iHS: integrated haplotype score; INDEL: insertions and deletions; N_e: effective population size; QTL: quantitative trait loci; ROH: runs of homozygosity; SNP: single nucleotide polymorphism; SKINP: skin percentage; SKINW: skin weight; UCSC: University of California, Santa Cruz; uROH: union region of runs of homozygosity; WGS: whole genome sequencing.

2.7. ETHIC'S APPROVAL

This study followed experimental protocols pertinent to animal experimentation with the approval of the Embrapa Swine and Poultry Ethics Committee on Animal Utilization (CEUA) in Concordia, Santa Catarina State, Brazil, on resolution number 011/2010. It followed the rules of National Council of Animal Experimentation Control (CONCEA) in accordance with international guidelines to guarantee animal welfare.

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doi:10.1016/S0014-5793(97)01126-5.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY MATERIAL A – FIGURES 10, 11, 12 AND 13.

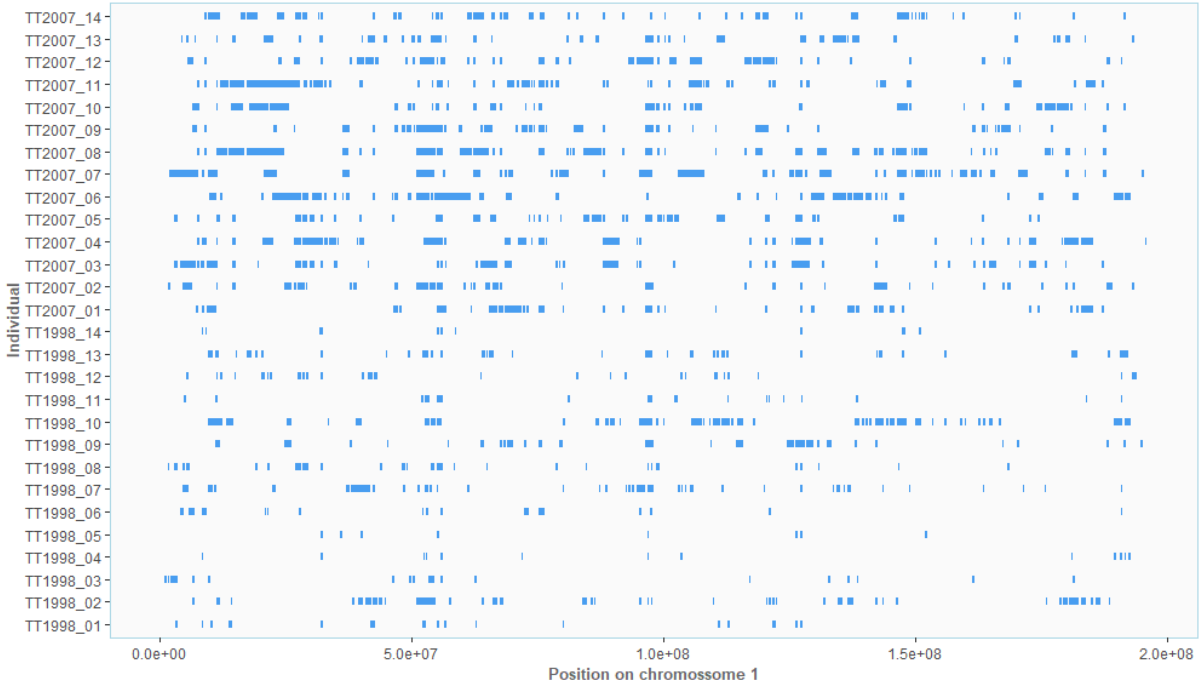

Figure 10 Genome wide distribution of runs of homozygosity on chromosome 1 in TT line.

Figure 11 Genome wide distribution of runs of homozygosity on chromosome 3 in TT line.

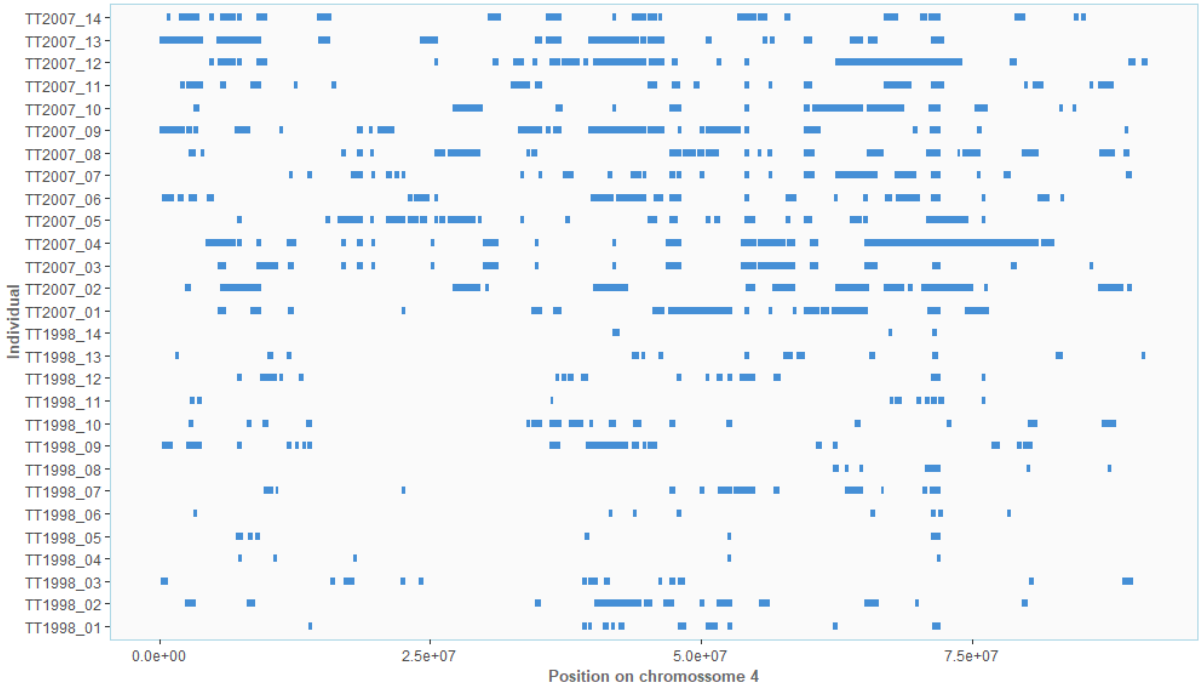

Figure 12 Genome wide distribution of runs of homozygosity on chromosome 4 in TT line.

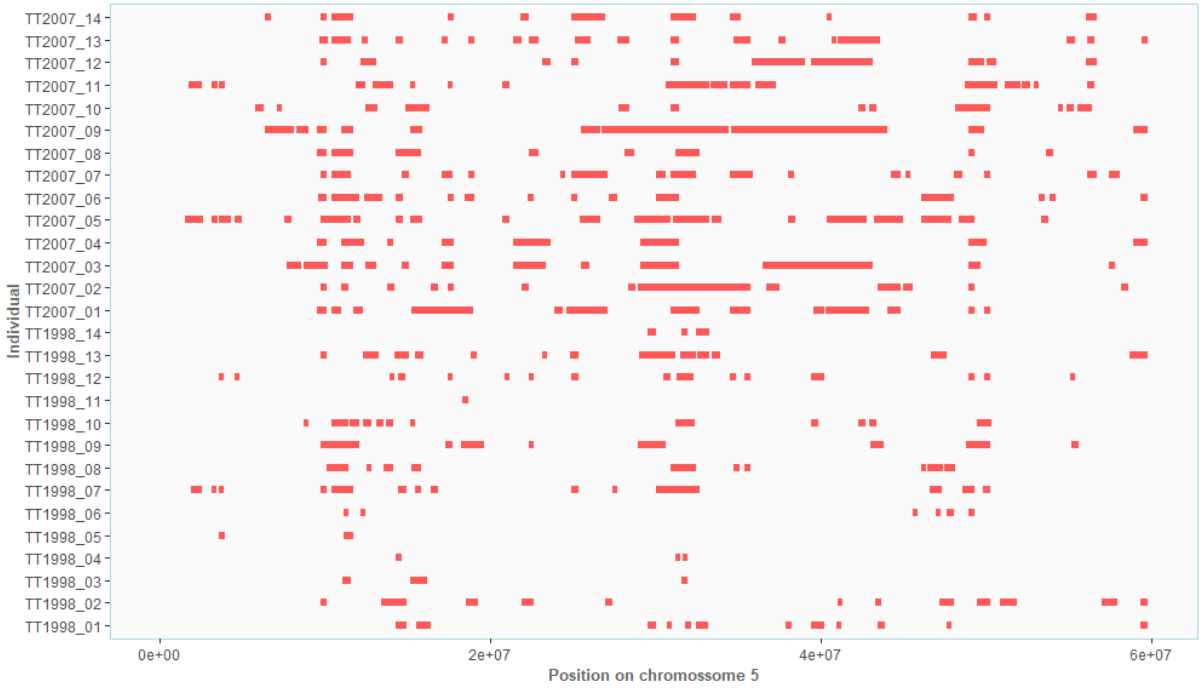

Figure 13 Genome wide distribution of runs of homozygosity on chromosome 5 in TT line.

SUPPLEMENTARY MATERIAL B – FIGURES 14, 15, 16 AND 17.

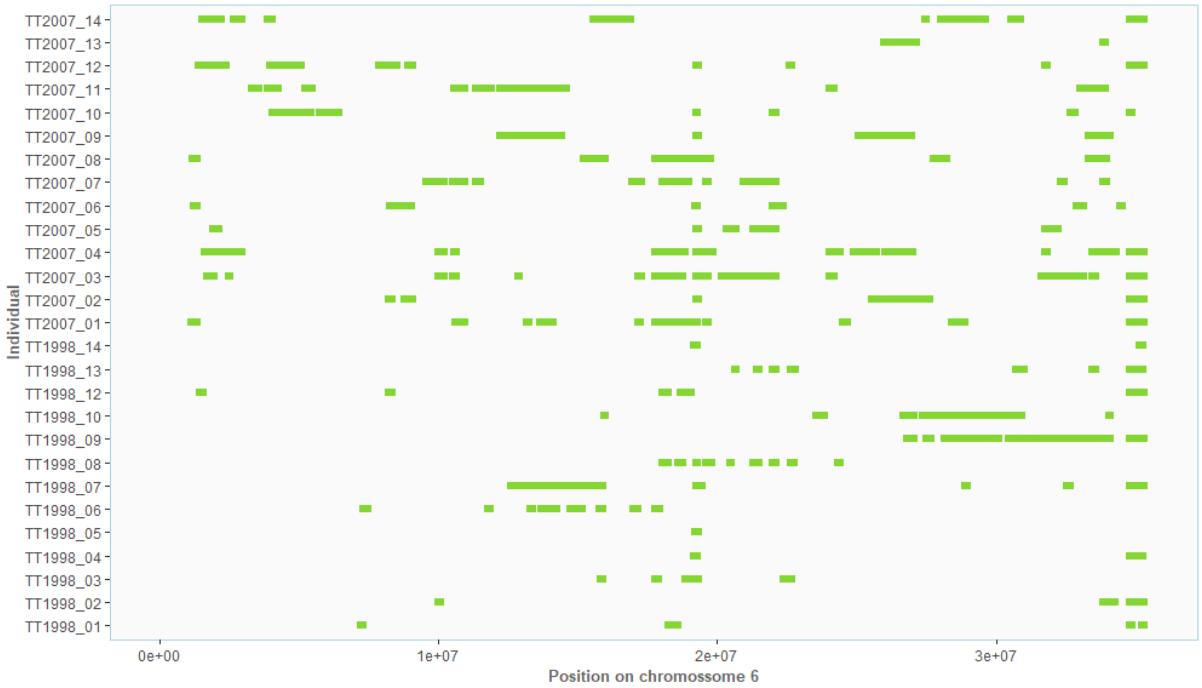

Figure 14 Genome wide distribution of runs of homozygosity on chromosome 5 in TT line.

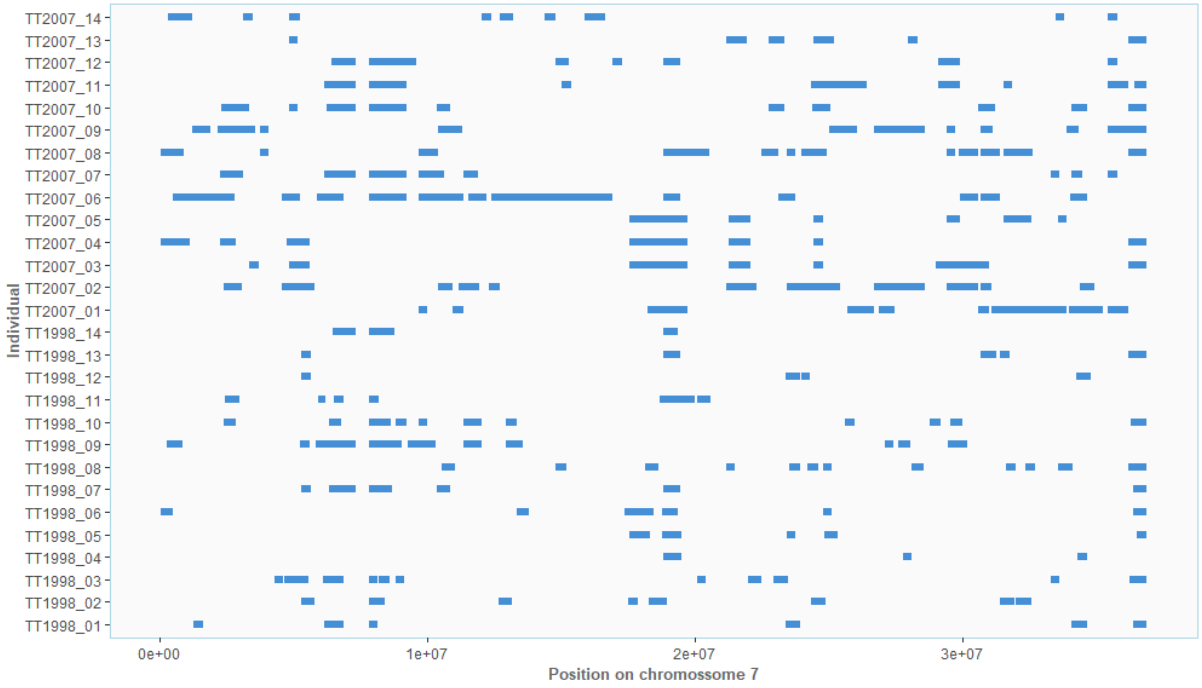

Figure 15 Genome wide distribution of runs of homozygosity on chromosome 7 in TT line.

Figure 16 Genome wide distribution of runs of homozygosity on chromosome 8 in TT line.

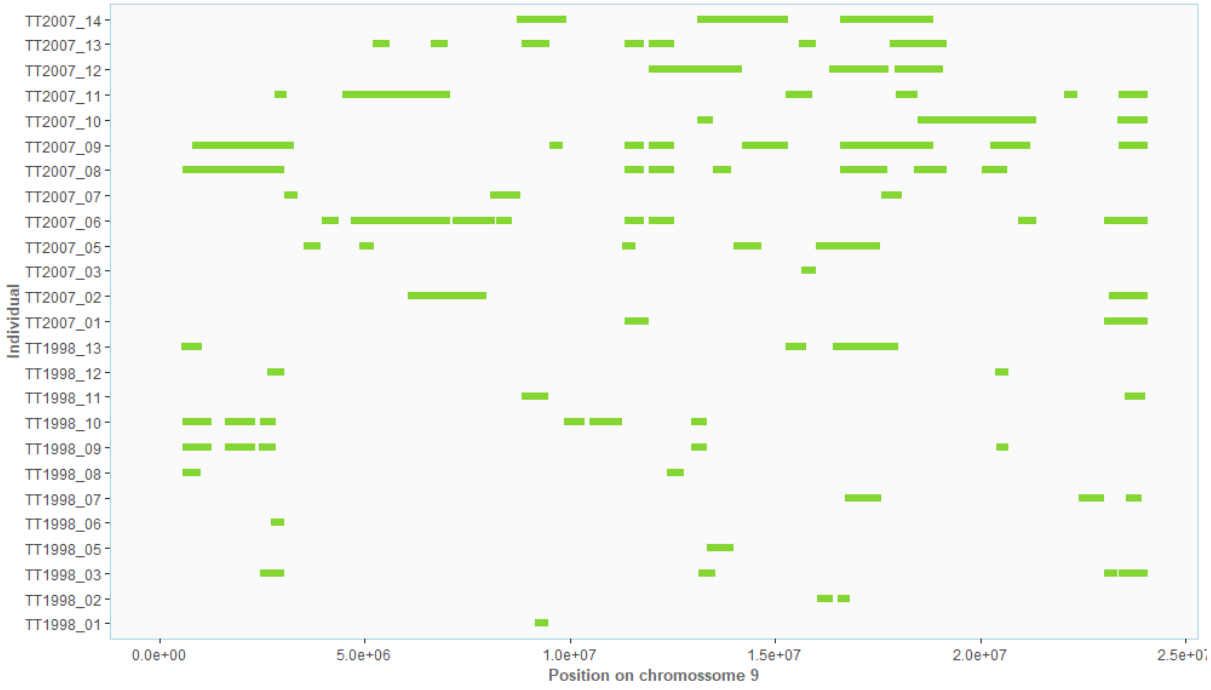

Figure 17 Genome wide distribution of runs of homozygosity on chromosome 9 in TT line.

SUPPLEMENTARY MATERIAL C – FIGURES 18, 19, 20 AND 21.

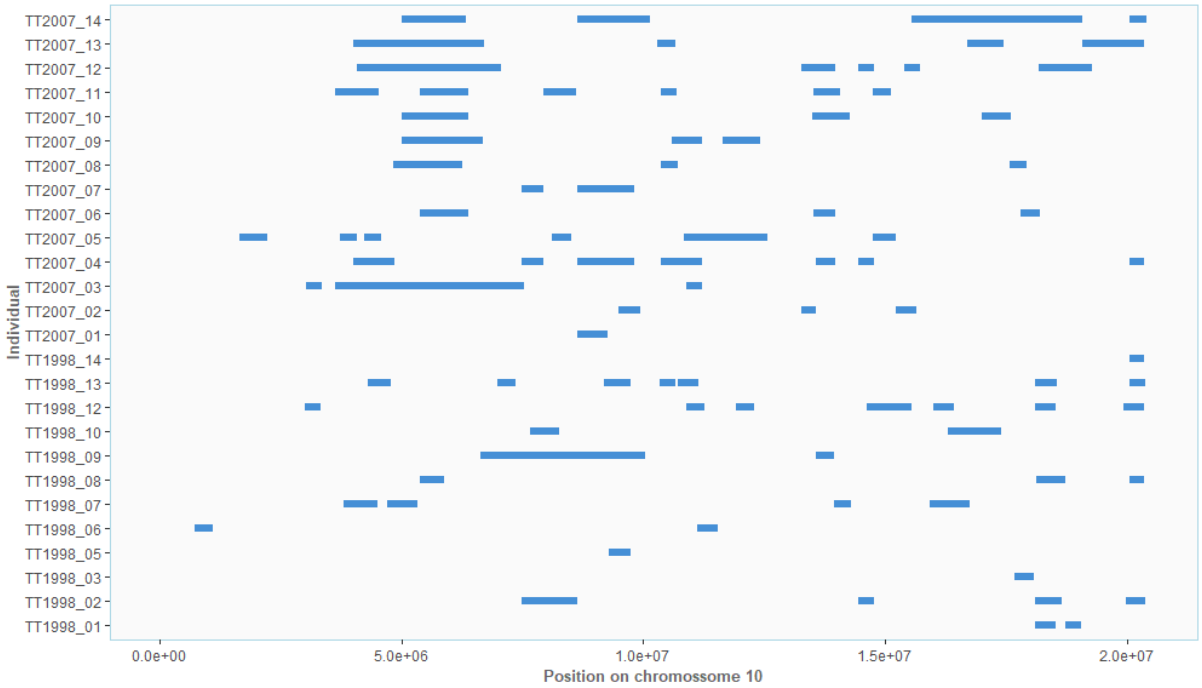

Figure 18 Genome wide distribution of runs of homozygosity on chromosome 10 in TT line.

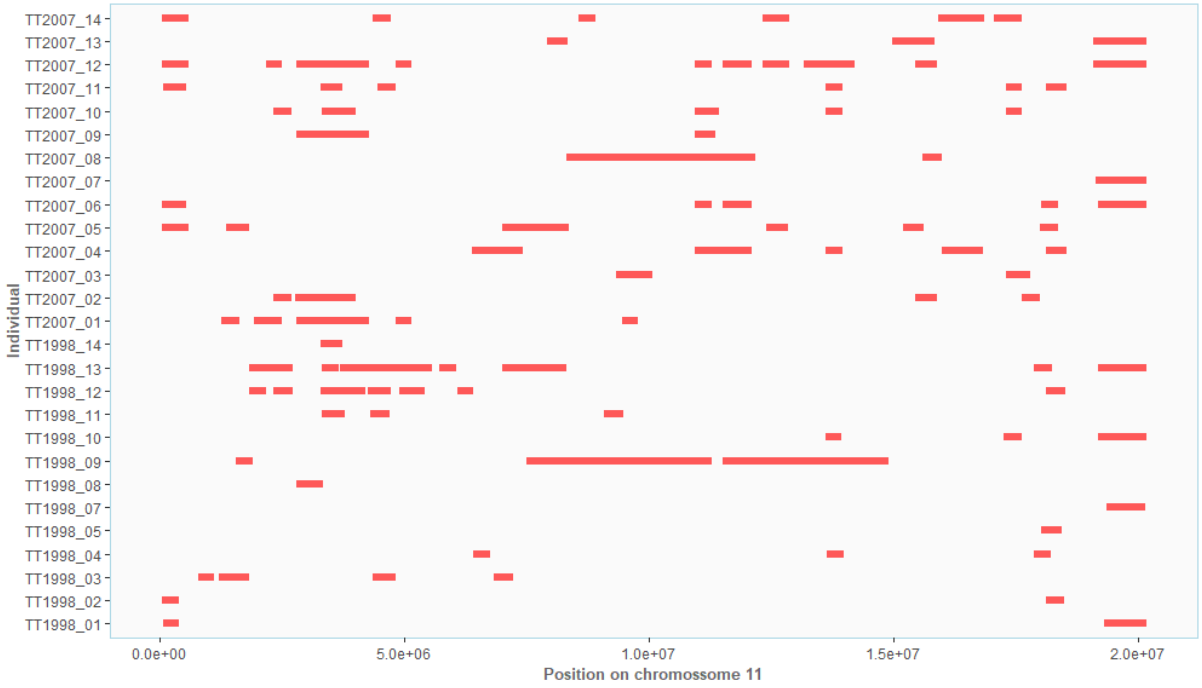

Figure 19 Genome wide distribution of runs of homozygosity on chromosome 11 in TT line.

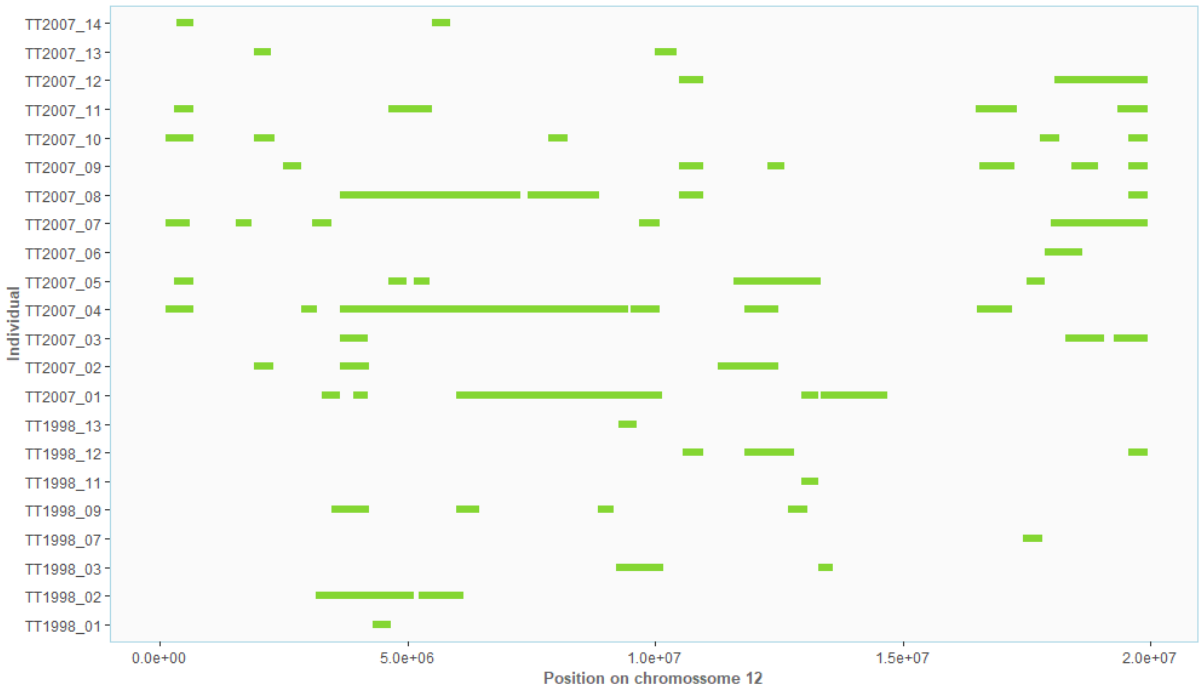

Figure 20 Genome wide distribution of runs of homozygosity on chromosome 12 in TT line.

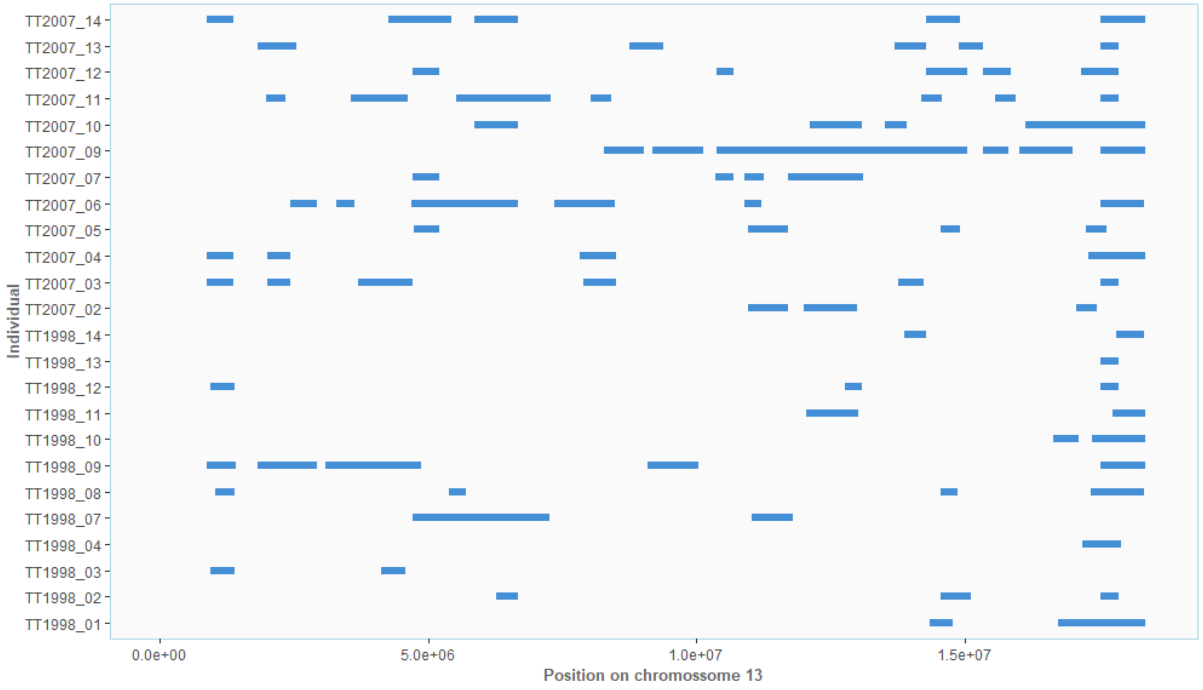

Figure 21 Genome wide distribution of runs of homozygosity on chromosome 13 in TT line.

SUPPLEMENTARY MATERIAL D – FIGURES 21, 22, 23 AND 24.

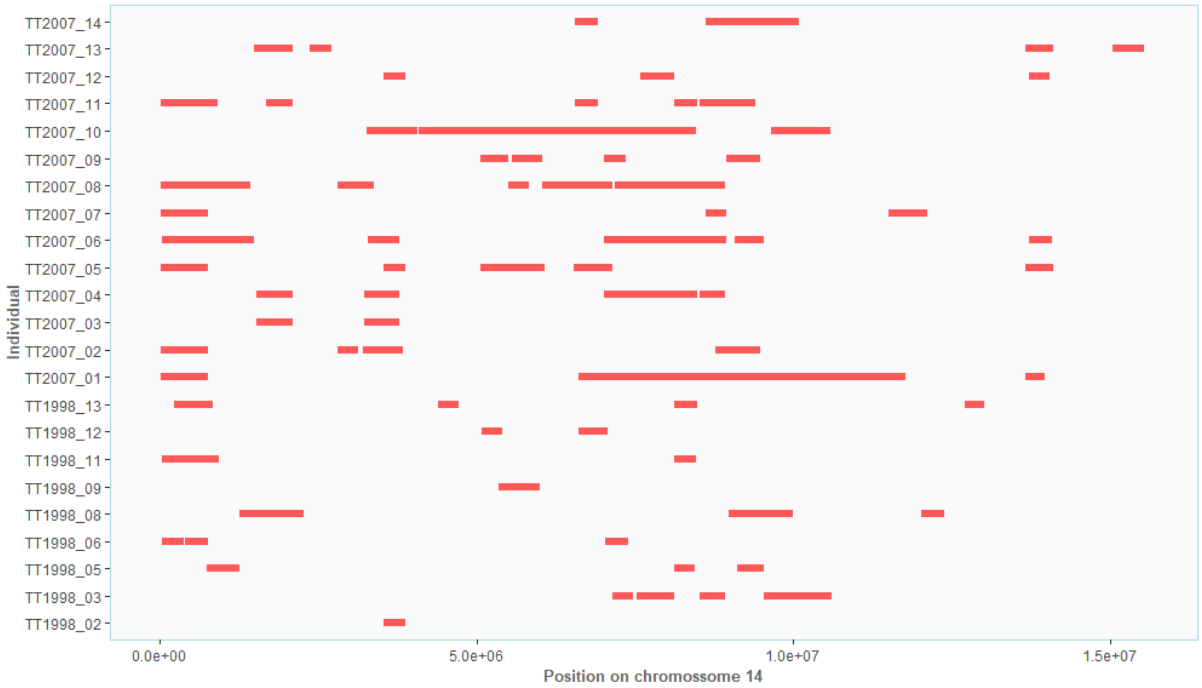

Figure 21 Genome wide distribution of runs of homozygosity on chromosome 14 in TT line.

Figure 22 Genome wide distribution of runs of homozygosity on chromosome 15 in TT line.

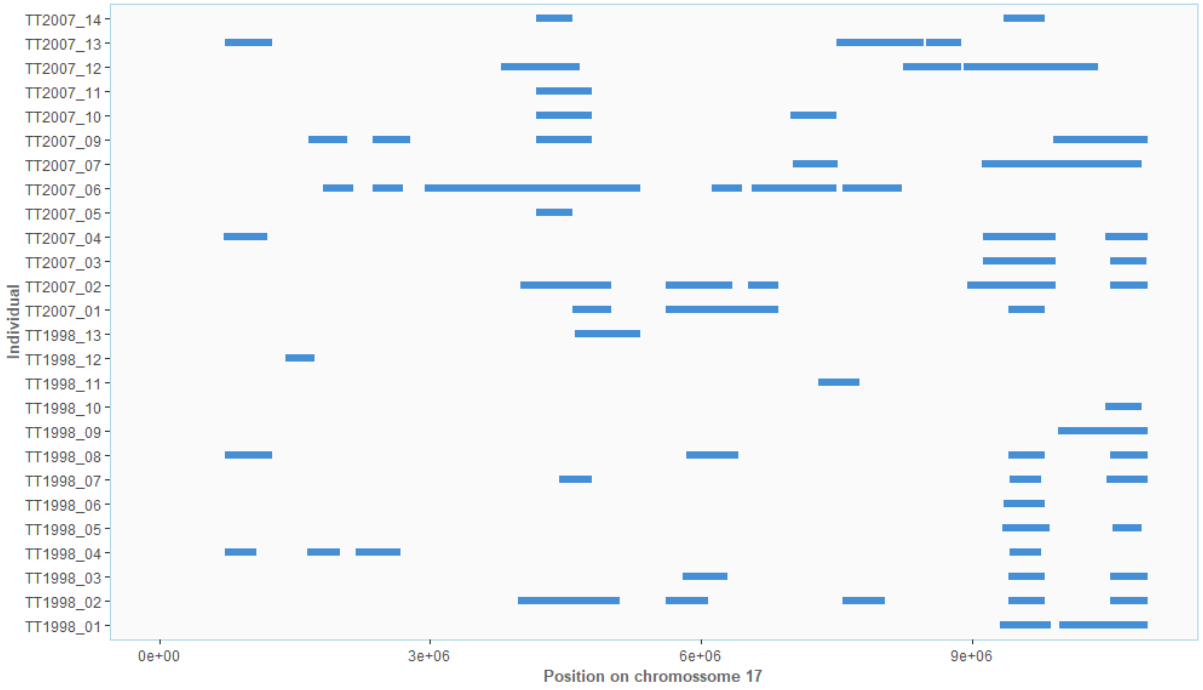

Figure 23 Genome wide distribution of runs of homozygosity on chromosome 17 in TT line.

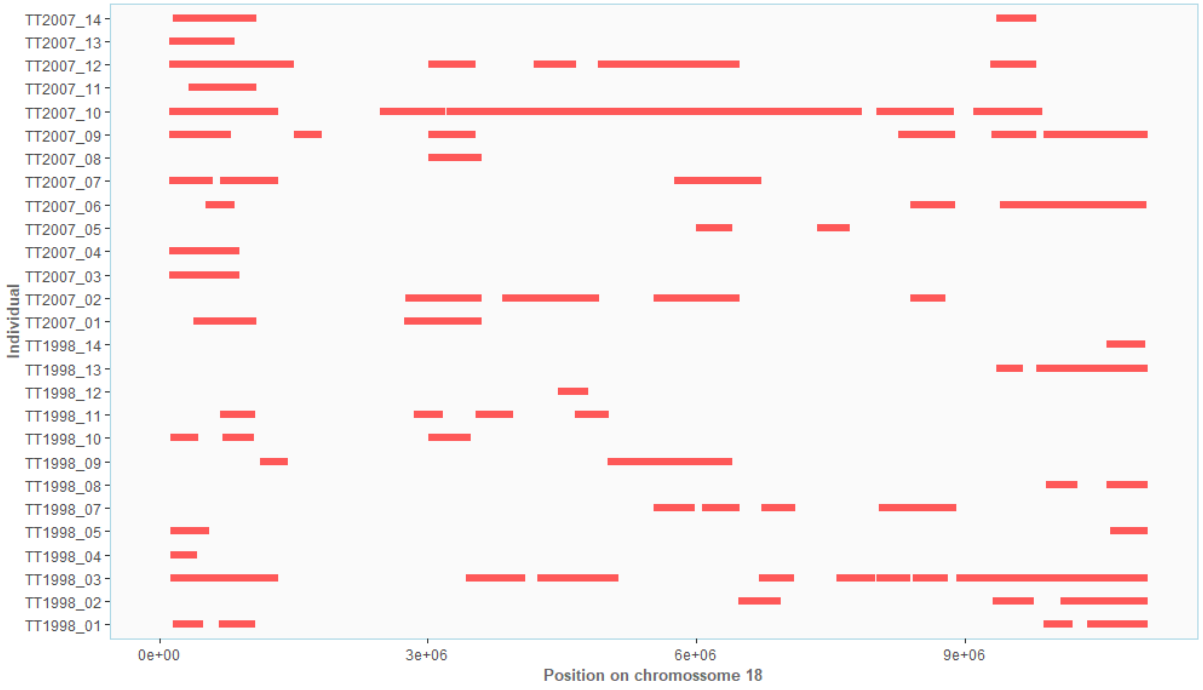

Figure 24 Genome wide distribution of runs of homozygosity on chromosome 18 in TT line.

SUPPLEMENTARY MATERIAL E – FIGURES 25, 26, 27 AND 28.

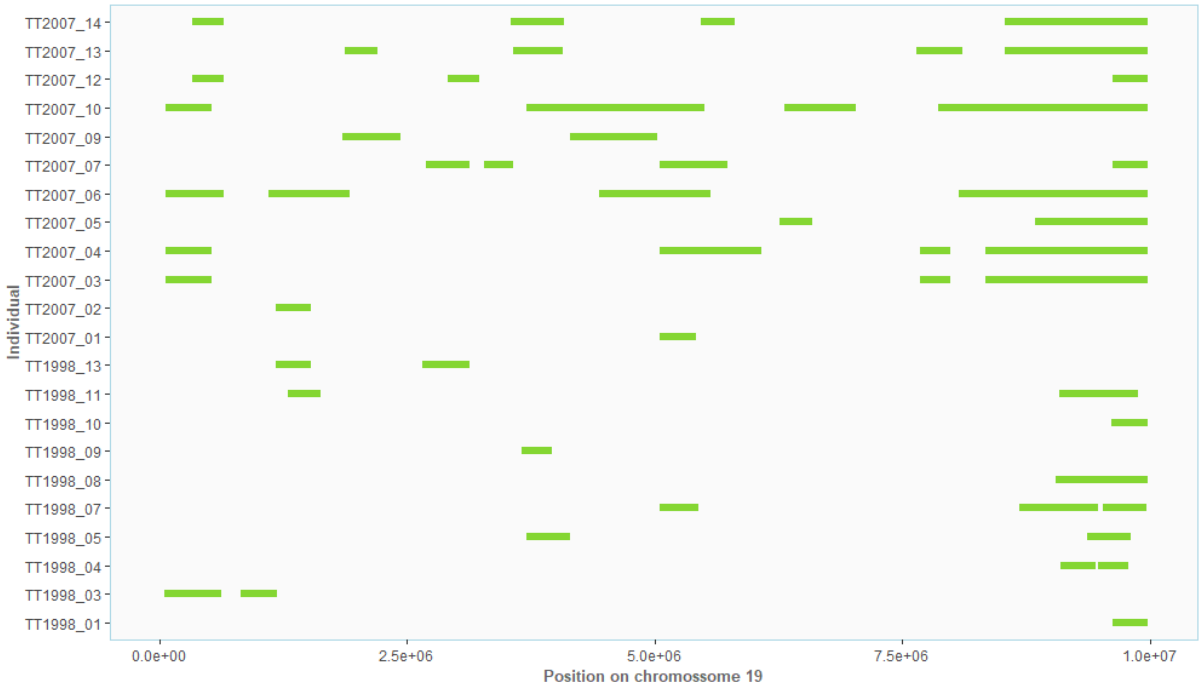

Figure 25 Genome wide distribution of runs of homozygosity on chromosome 19 in TT line.

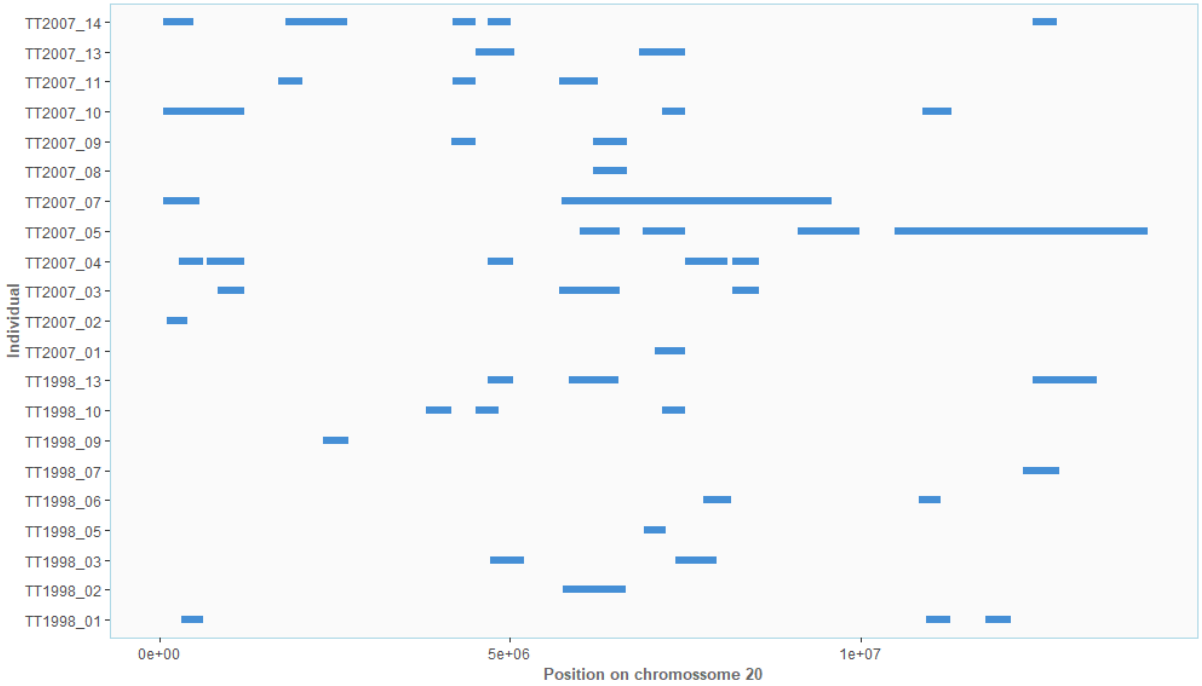

Figure 26 Genome wide distribution of runs of homozygosity on chromosome 20 in TT line.

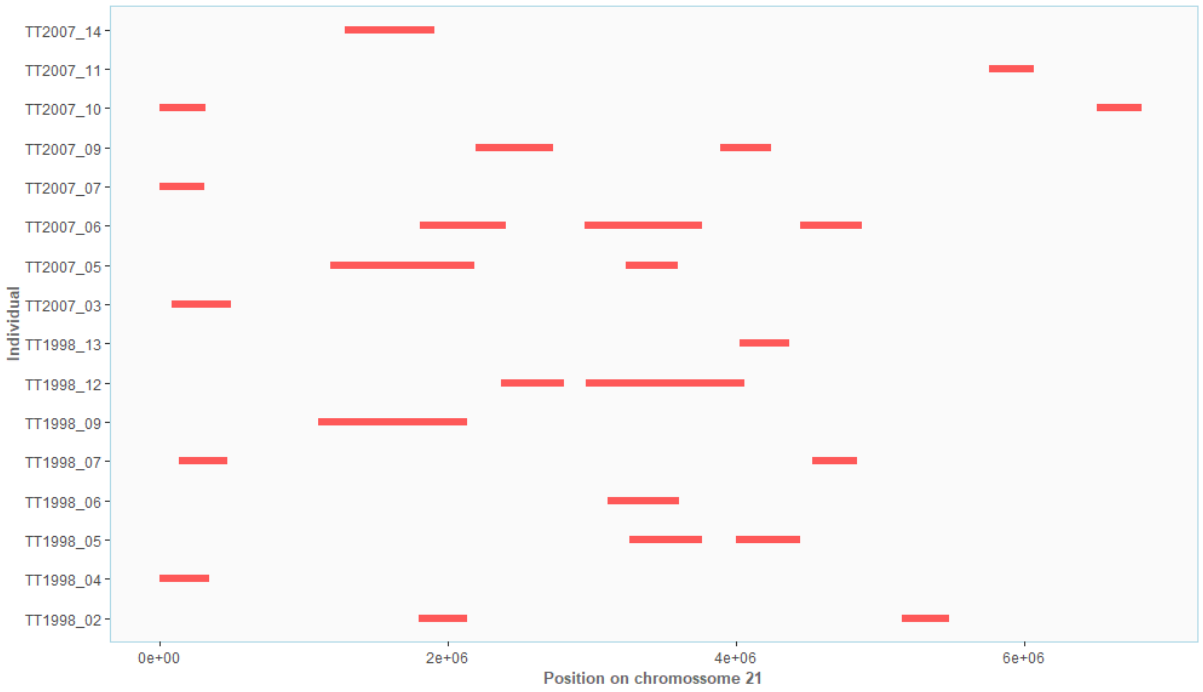

Figure 27 Genome wide distribution of runs of homozygosity on chromosome 21 in TT line.

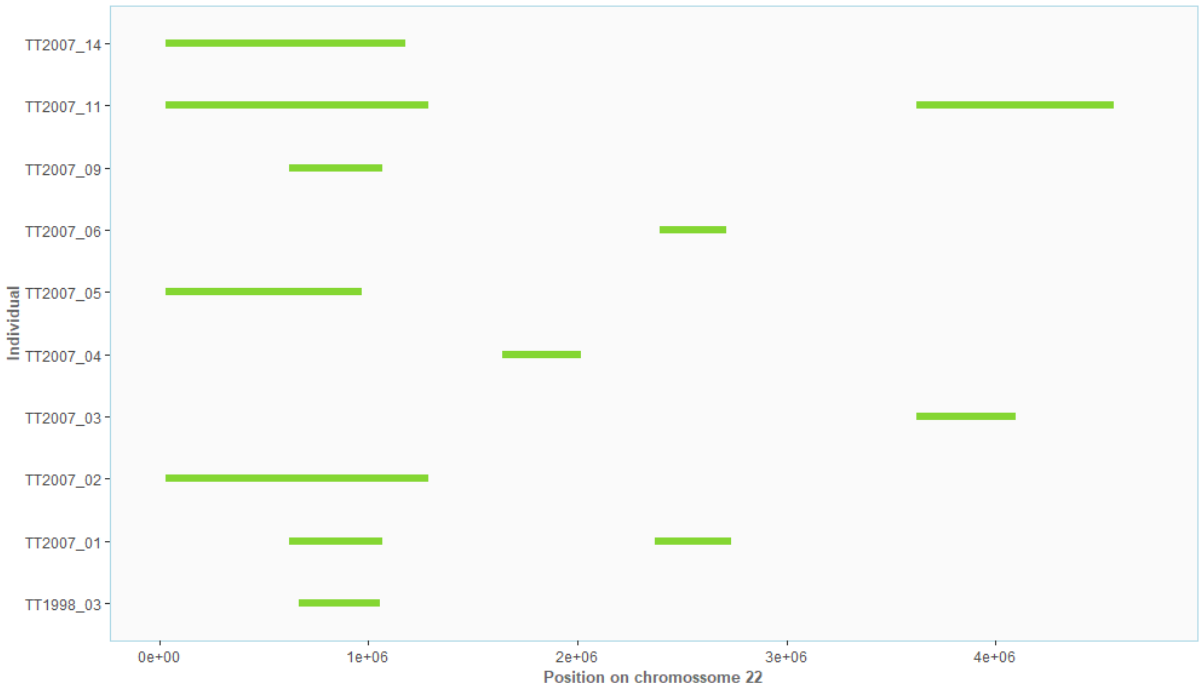

Figure 28 Genome wide distribution of runs of homozygosity on chromosome 22 in TT line.

SUPPLEMENTARY MATERIAL F – FIGURES 29, 30, 31 AND 32.

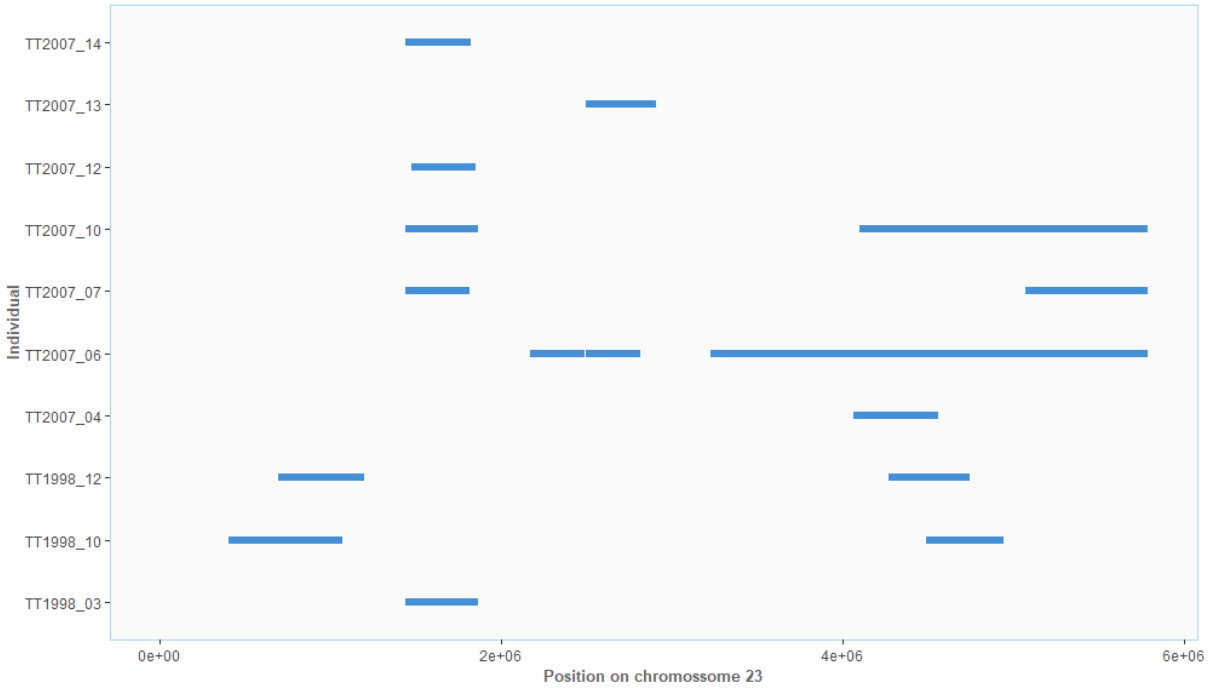

Figure 29 Genome wide distribution of runs of homozygosity on chromosome 23 in TT line.

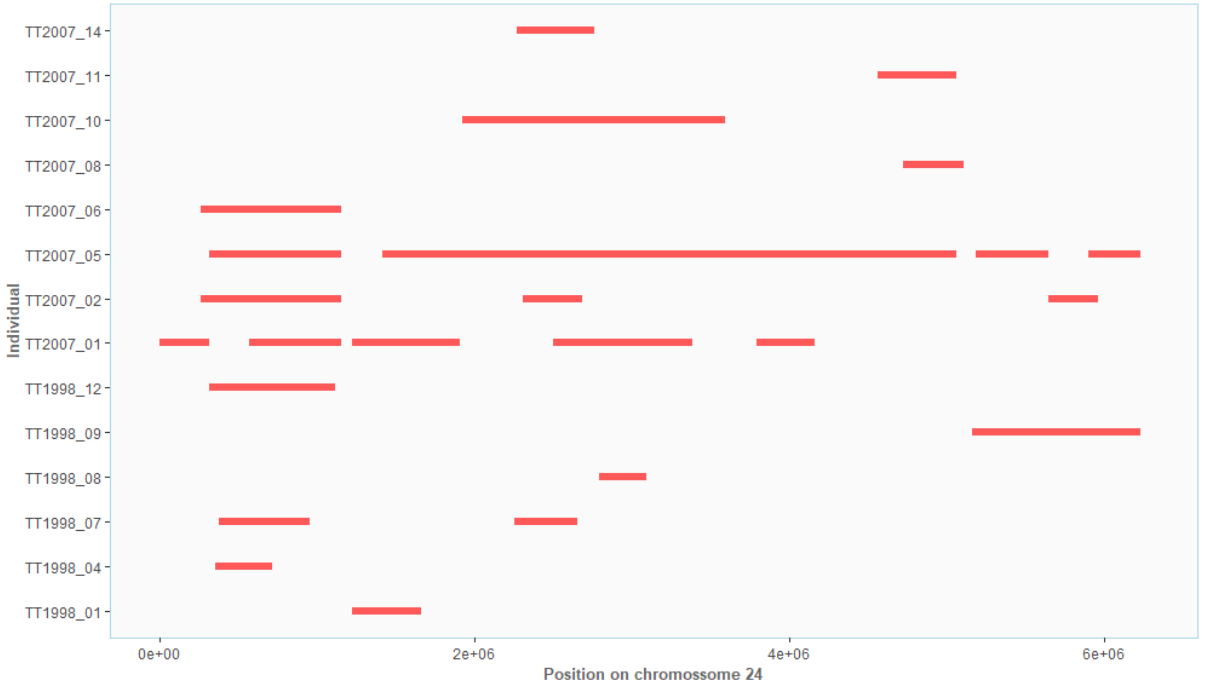

Figure 30 Genome wide distribution of runs of homozygosity on chromosome 24 in TT line.

Figure 31 Genome wide distribution of runs of homozygosity on chromosome 25 in TT line.

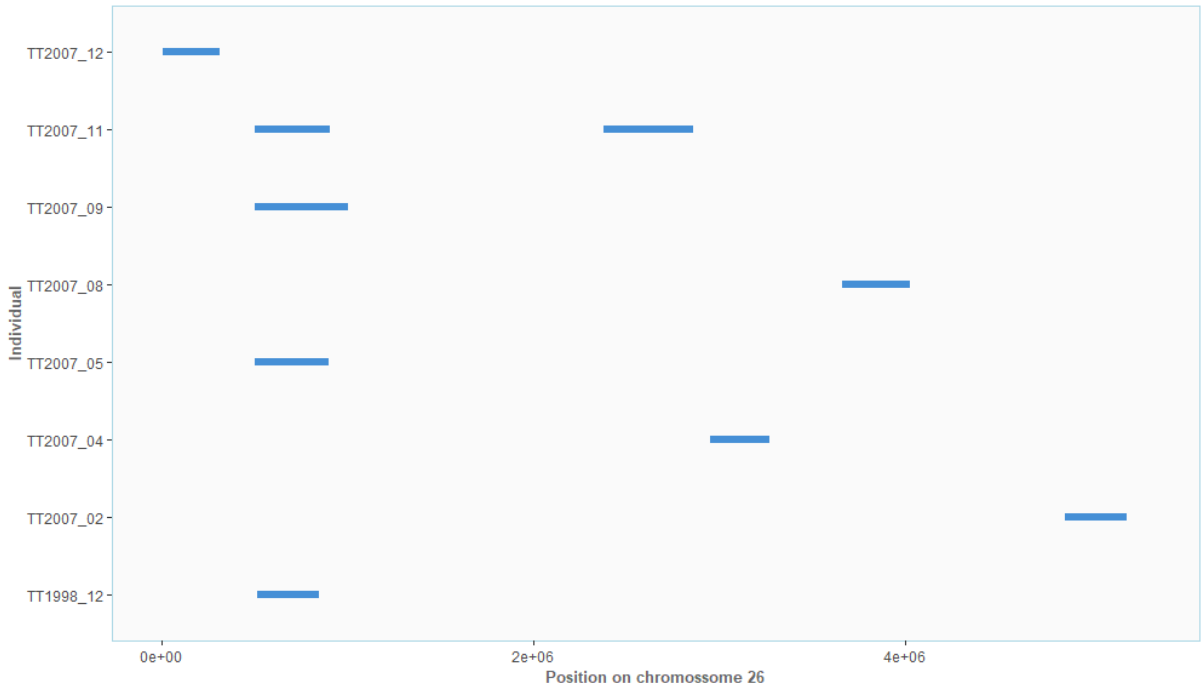

Figure 32 Genome wide distribution of runs of homozygosity on chromosome 26 in TT line.

SUPPLEMENTARY MATERIAL G – FIGURES 33, 34 AND 35.

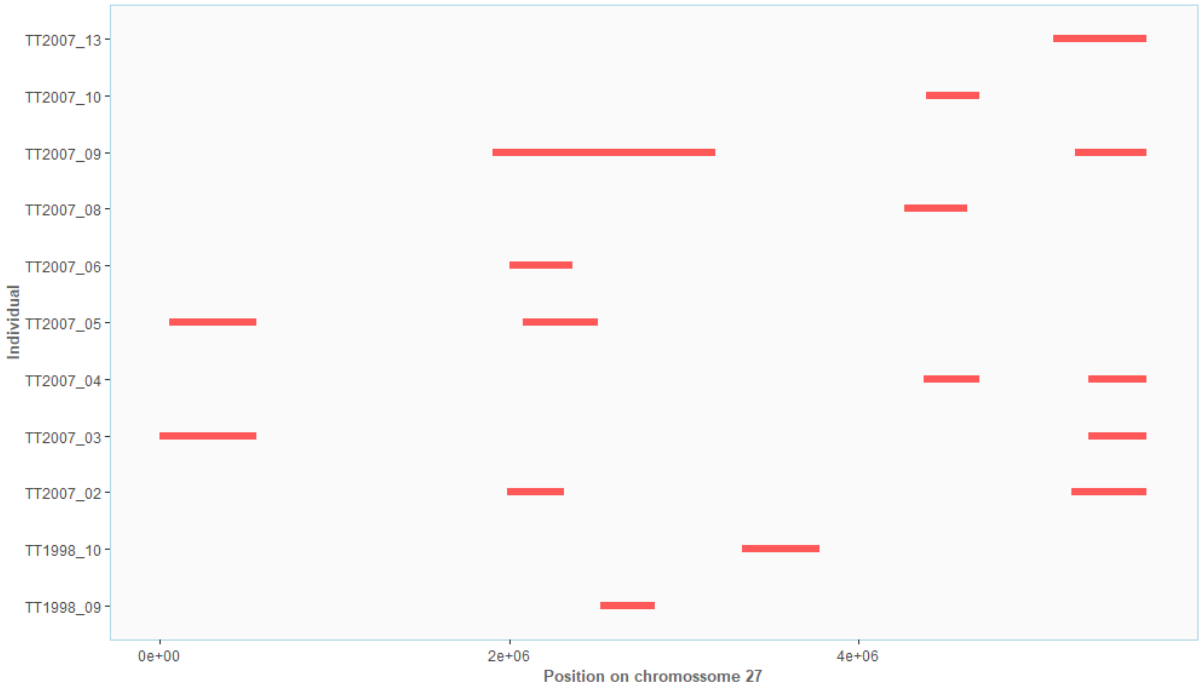

Figure 33 Genome wide distribution of runs of homozygosity on chromosome 27 in TT line.

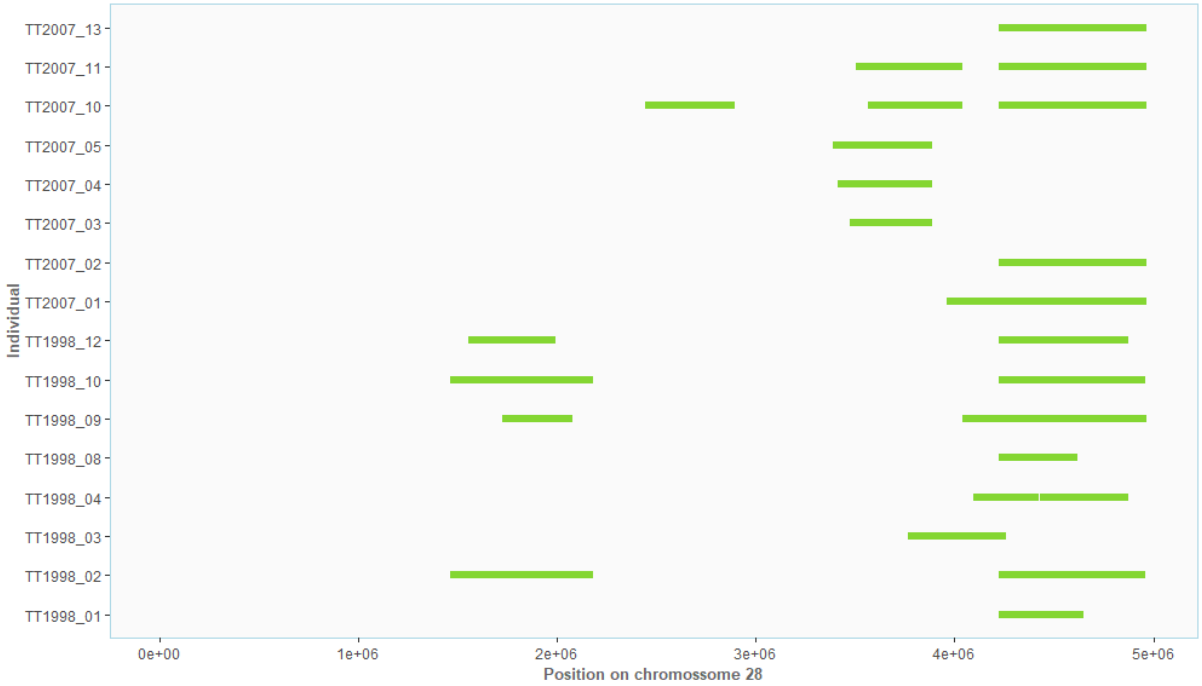

Figure 34 Genome wide distribution of runs of homozygosity on chromosome 28 in TT line.

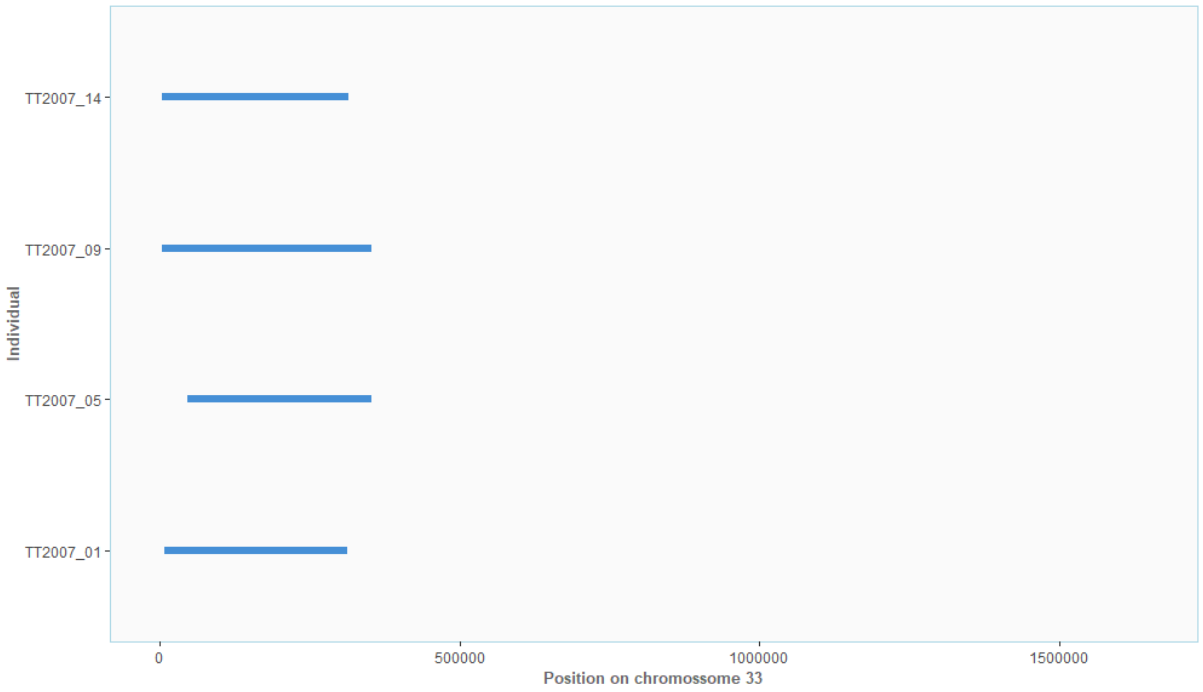

Figure 35 Genome wide distribution of runs of homozygosity on chromosome 33 in TT line.

SUPPLEMENTARY MATERIAL H – SUPPLEMENTARY TABLE 1.

Supplementary Table 1. Genes annotated commonly between selection signatures of two or more datasets: the 1,941 consensus ROH (cROH), the FST SNP windows (>0.3), and the FST INDEL windows (>0.3).

F_{ST} SNP and INDEL windows (≥0.3) datasets		
Gene ID	**Gene name**	**Description**
ENSGALG00000000242	*EBF2*	early B cell factor 2
ENSGALG00000002370	*SH2D4B*	SH2 domain containing 4B
ENSGALG00000002414	*TSPAN14*	tetraspanin 14
ENSGALG00000004045	*AGAP1*	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
ENSGALG00000004116	*TRPM8*	transient receptor potential cation channel subfamily M member 8
ENSGALG00000004129	*SPP2*	secreted phosphoprotein 2
ENSGALG00000007555	*CCND1*	cyclin D1
ENSGALG00000007556	*LTO1*	LTO1, ABCE1 maturation factor
ENSGALG00000012542	*RASD2*	RASD family member 2
ENSGALG00000015402	*C3orf38*	chromosome 3 open reading frame 38
ENSGALG00000015403	*EPHA3*	EPH receptor A3
ENSGALG00000015570	*GPR63*	G protein-coupled receptor 63
ENSGALG00000015573	*FHL5*	four and a half LIM domains 5
ENSGALG00000016518	*PHKA2*	phosphorylase kinase regulatory subunit alpha 2
ENSGALG00000016522	*PPEF1*	protein phosphatase with EF-hand domain 1
ENSGALG00000016529	*CDKL5*	cyclin dependent kinase like 5
ENSGALG00000016541		BEN domain containing 2
ENSGALG00000016543	*NHS*	NHS actin remodeling regulator
ENSGALG00000022866	*ZNF654*	zinc finger protein 654
ENSGALG00000026372		
ENSGALG00000028376	*FGF19*	fibroblast growth factor 19
ENSGALG00000032974	*ADAMTS2*	ADAM metallopeptidase with thrombospondin type 1 motif 2
ENSGALG00000033076		
ENSGALG00000035116	*STAG1*	stromal antigen 1
ENSGALG00000035393	*LRRC14B*	leucine rich repeat containing 14B
ENSGALG00000035906	*YTHDC1*	YTH domain containing 1
ENSGALG00000036204		
ENSGALG00000036327	*NGEF*	neuronal guanine nucleotide exchange
ENSGALG00000036730	*MRPS35*	mitochondrial ribosomal protein S35
ENSGALG00000036938	*RALYL*	RALY RNA binding protein like

ENSGALG00000038154	*YAP1*	Yes associated protein 1
ENSGALG00000038730	*GIGYF2*	GRB10 interacting GYF protein 2
ENSGALG00000039139	*TNS3*	tensin 3
ENSGALG00000039690	*STMN2*	stathmin 2
ENSGALG00000039738	*SLC9A3*	solute carrier family 9 member A3
ENSGALG00000040264	*C9H2ORF82*	chromosome 9 open reading frame, human C2orf82
ENSGALG00000042764	*COG5*	component of oligomeric golgi complex 5

cROH and F_{ST} SNP windows (≥0.3)

Gene ID	**Gene name**	**Description**
ENSGALG00000000242	*EBF2*	early B cell factor 2
ENSGALG00000001153	*AUTS2*	AUTS2, activator of transcription and developmental regulator
ENSGALG00000003705	*VPS13C*	vacuolar protein sorting 13 homolog C
ENSGALG00000004045	*AGAP1*	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
ENSGALG00000004116	*TRPM8*	transient receptor potential cation channel subfamily M member 8
ENSGALG00000006237	*PKN2*	protein kinase N2
ENSGALG00000007555	*CCND1*	cyclin D1
ENSGALG00000007556	*LTO1*	LTO1, ABCE1 maturation factor
ENSGALG00000015402	*C3orf38*	chromosome 3 open reading frame 38
ENSGALG00000015403	*EPHA3*	EPH receptor A3
ENSGALG00000016518	*PHKA2*	phosphorylase kinase regulatory subunit alpha
ENSGALG00000016522	*PPEF1*	protein phosphatase with EF-hand domain 1
ENSGALG00000016529	*CDKL5*	cyclin dependent kinase like 5
ENSGALG00000022866	*ZNF654*	zinc finger protein 654
ENSGALG00000025253	*gga-mir-1694*	gga-mir-1694
ENSGALG00000028376	*FGF19*	fibroblast growth factor 19
ENSGALG00000030580	*RPS6KA5*	ribosomal protein S6 kinase A5
ENSGALG00000032958	*AMPH*	amphiphysin
ENSGALG00000034119		collagen type XV alpha 1 chain
ENSGALG00000035116	*STAG1*	stromal antigen 1
ENSGALG00000035906	*YTHDC1*	YTH domain containing 1
ENSGALG00000036938	*RALYL*	RALY RNA binding protein like
ENSGALG00000038730	*GIGYF2*	GRB10 interacting GYF protein 2
ENSGALG00000040167	*TPD52*	tumor protein D52

cROH and F_{ST} INDEL windows (≥0.3) datasets

Gene ID	**Gene name**	**Description**
ENSGALG00000000242	*EBF2*	early B cell factor 2
ENSGALG00000000667	*EDN2*	endothelin 2

ENSGALG00000004045	*AGAP1*	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
ENSGALG00000004116	*TRPM8*	transient receptor potential cation channel subfamily M member 8
ENSGALG00000007555	*CCND1*	cyclin D1
ENSGALG00000007556	*LTO1*	LTO1, ABCE1 maturation factor
ENSGALG00000008163	*PSME4*	proteasome activator subunit 4
ENSGALG00000015402	*C3orf38*	chromosome 3 open reading frame 38
ENSGALG00000015403	*EPHA3*	EPH receptor A3
ENSGALG00000016518	*PHKA2*	phosphorylase kinase regulatory subunit alpha
ENSGALG00000016522	*PPEF1*	protein phosphatase with EF-hand domain 1
ENSGALG00000016529	*CDKL5*	cyclin dependent kinase like 5
ENSGALG00000022866	*ZNF654*	zinc finger protein 654
ENSGALG00000025789	*gga-mir-6614*	gga-mir-6614
ENSGALG00000027632	*ACYP2*	acylphosphatase 2
ENSGALG00000027960	*GRPR*	gastrin releasing peptide receptor
ENSGALG00000028376	*FGF19*	fibroblast growth factor 19
ENSGALG00000034516	*SHISA6*	shisa family member 6
ENSGALG00000035116	*STAG1*	stromal antigen 1
ENSGALG00000035906	*YTHDC1*	YTH domain containing 1
ENSGALG00000036810		
ENSGALG00000036938	*RALYL*	RALY RNA binding protein like
ENSGALG00000038730	*GIGYF2*	GRB10 interacting GYF protein 2
ENSGALG00000039102	*TOX*	thymocyte selection associated high mobility
ENSGALG00000040322		

cROH and F_{ST} SNP and INDEL windows (≥0.3) datasets

Gene ID	**Gene name**	**Description**
ENSGALG00000000242	*EBF2*	early B cell factor 2
ENSGALG00000004045	*AGAP1*	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
ENSGALG00000004116	*TRPM8*	transient receptor potential cation channel subfamily M member 8
ENSGALG00000007555	*CCND1*	cyclin D1
ENSGALG00000007556	*LTO1*	LTO1, ABCE1 maturation factor
ENSGALG00000015402	*C3orf38*	chromosome 3 open reading frame 38
ENSGALG00000015403	*EPHA3*	EPH receptor A3
ENSGALG00000016518	*PHKA2*	phosphorylase kinase regulatory subunit alpha
ENSGALG00000016522	*PPEF1*	protein phosphatase with EF-hand domain 1
ENSGALG00000016529	*CDKL5*	cyclin dependent kinase like 5
ENSGALG00000022866	*ZNF654*	zinc finger protein 654

ENSGALG00000028376	*FGF19*	fibroblast growth factor 19
ENSGALG00000035116	*STAG1*	stromal antigen 1
ENSGALG00000035906	*YTHDC1*	YTH domain containing 1
ENSGALG00000036938	*RALYL*	RALY RNA binding protein like
ENSGALG00000038730	*GIGYF2*	GRB10 interacting GYF protein 2

SUPPLEMENTARY MATERIAL I – SUPPLEMENTARY TABLE 2.

Supplementary Table 2. Genes previously associated with traits of interest located in consensus sequences of runs of homozygosity (cROH) with different frequencies of sharing between animals of TT98 and TT2007.

Chr	Gene	Gene position	cROH position	n animais TT98/TT2007[a]
1	IGF-I	55,335,204 – 55,383,631	55,149,208 – 55,359,089	7/13
1	CACNA1C	61,471,636 – 61,671,542	61,493,137 – 61,582,897	0/3
1	ITPR2	67,751,004 – 67,983,940	67,475,528 – 67,977,445	2/7
1	MYO1C	138,120,025 – 138,390,526	137,959,482 – 138,138,743	2/4
			138,355,619 – 138,459,726	4/5
1	MYO7A	193,221,562 – 193,279,711	193,062,724 – 193,346,675	2/1
2	VIPR1	1,742,703 – 1,846,900	1,745,421 – 1,863,999	1/4
2	MYO10	75,715,508 – 75,869,282	75,651,510 – 75,752,520	5/14
2	ADCY2	79,496,841 – 79,702,592	79,332,597 – 79,659,423	2/5
2	NSUN2	80,034,291 – 80,050,765	79,940,076 – 80,187,400	2/4
2	MOCOS	85,073,411 – 85,199,421	84,971,233 – 85,451,910	3/1
2	BAG1	85,906,463 – 85,915,694	85,485,648 – 85,943,053	2/2
2	ELP2	88,712,300 – 88,744,632	88,409,172 – 88,718,424	2/1
			88,718,448 – 88,734,342	2/1
3	MYO6	80,736,607 – 80,807,004	80,805,954 – 80,905,165	2/3
3	MATN3	101,853,568 – 101,868,845	101,659,552 – 101,995,693	1/2
3	APOB	102,659,050 – 102,693,303	102,641,927 – 102,729,608	1/5
3	POMC	105,818,021 – 105,834,013	105,658,152 – 106,099,077	1/2
5	ACTC1	32,283,463 – 32,288,278	32,232,519 – 32,293,369	5/8
5	AKAP6	34,843,920 – 35,050,252	34,751,946 – 34,917,590	2/7
6	KCNMA1	13,362,771 – 13,800,472	13,160,512 – 13,373,205	2/3
			13,532,853 – 13,536,574	2/3
			13,537,312 – 13,573,098	2/3
			13,573,470 – 14,246,233	2/3
6	VCL	8,014,556 – 8,105,794	8,136,748 – 8,477,976	1/3
			8,783,567 – 9,165,313	0/3
7	MYO1B	8,014,556 – 8,105,794	7,849,666 – 8,160,956	8/5
7	HOXD9	16,362,036 – 16,363,342	15,889,809 – 16,664,514	0/2
7	HOXD10	16,367,140 – 16,369,673	15,889,809 – 16,664,514	0/2
7	PLA2R1	21,941,750 – 21,977,517	21,726,831 – 21,964,077	0/5
7	IGFBP2	23,382,780 – 23,435,025	23,428,863 – 23,483,121	4/3
10	MYO1E	6,672,861 – 6,750,075	6,643,521 – 6,674,804	1/4
12	PPARG	4,859,854 – 4,880,521	4,774,152 – 4,978,240	1/4
15	SCARB1	4,544,980 – 4,558,143	4,423,619 – 4,565,327	5/5

15	ATP2A2	5,344,147 – 5,385,167	5,346,615 – 5,480,296	3/7
19	MYO1C	5,189,500 – 5,240,189	5,054,141 – 5,419,934	1/5
21	MRTO4	4,666,392 – 4,668,550	4,532,882 – 4,841,708	1/1
21	PQLC2	4,672,913 – 4,677,272	4,532,882 – 4,841,708	1/1
24	HSPA8	3,073,404 – 3,080,079	2,904,030 – 3,095,369	1/3
27	ACE	2,830,975 – 2,847,223	2,523,474 – 2,834,249	1/1
28	MYO1F	1,741,275 – 1,754,399	1,723,513 – 1,789,434	0/4
28	TPM4	4,446,782 – 4,454,984	4,428,153 – 4,621,310	7/5

Chr = chromosome

[a]Number of animals of TT98 and TT2007 with ROH overlapping the same region.