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1 **Use of Locally Weighted Scatterplot Smoothing (LOWESS) regression to study selection**  
2 **signatures in Piedmontese and Italian Brown cattle breeds**

3

4 Elia Pintus<sup>1</sup>, Silvia Sorbolini<sup>1</sup>, Andrea Albera<sup>2</sup>, Giustino Gaspa<sup>1</sup>, Corrado Dimauro<sup>1</sup>, Roberto Steri<sup>1</sup>,  
5 Gabriele Marras<sup>1</sup>, Nicolò Pietro Paolo Macciotta\*<sup>1</sup>

6

7 Address: <sup>1</sup>Dipartimento di Agraria, Sezione di Scienze Zootecniche Università degli Studi di  
8 Sassari, 07100 Sassari, Italy, <sup>2</sup>Associazione Nazionale Allevatori Bovini di Razza Piemontese  
9 (ANABORAPI), 12061 Carrù (CN), Italy.

10

11 Email: Elia Pintus – [epintus@uniss.it](mailto:epintus@uniss.it); Silvia Sorbolini - [ssorbolini@uniss.it](mailto:ssorbolini@uniss.it); Andrea Albera -  
12 [info@anaborapi.it](mailto:info@anaborapi.it); Giustino Gaspa – [gigaspa@uniss.it](mailto:gigaspa@uniss.it); Corrado Dimauro - [dimauro@uniss.it](mailto:dimauro@uniss.it);  
13 Roberto Steri - [rsteri@uniss.it](mailto:rsteri@uniss.it); Gabriele Marras - [gbmarras@uniss.it](mailto:gbmarras@uniss.it); Nicolò Pietro Paolo  
14 Macciotta\* - [macciott@uniss.it](mailto:macciott@uniss.it);

15 \*Corresponding author

16

17 **Summary**

18 Selection is the major force affecting local levels of genetic variation in species. The  
19 availability of dense marker maps offers new opportunities for a detailed understanding of genetic  
20 diversity distribution across the animal genome. Over the last fifty years, cattle breeds have been  
21 subjected to intense artificial selection. Consequently, regions controlling traits of economic  
22 importance are expected to exhibit selection signatures. The fixation index ( $F_{st}$ ) is an estimate of  
23 population differentiation, based on genetic polymorphism data and it is calculated using the  
24 relationship between inbreeding and heterozygosity. In the present study, the locally weighted

25 scatterplot smoothing regression (LOWESS) and a Control Chart approach were used to investigate  
26 selection signatures in two cattle breeds with different production aptitude (dairy and beef).  
27  $F_{st}$  was calculated for 42,514 SNPs marker loci distributed across the genome in 749 Italian Brown  
28 and 364 Piedmontese bulls respectively. The statistical significance of  $F_{st}$  values was assessed using  
29 a Control Chart. The LOWESS technique was efficient in removing noise from the raw data and  
30 was able to highlight selection signatures in chromosomes known to harbour genes affecting dairy  
31 and beef traits. Examples are, the peaks detected for BTA2 in the region where the *myostatin* gene is  
32 located and for BTA6 in the region harbouring the *ABCG2* locus. Moreover, several loci not  
33 previously reported in cattle studies were detected.

34 **Key words:** SNPs,  $F_{st}$ , LOWESS, cattle breeds

35

## 36 **Introduction**

37 The study of the genetic basis of differences among animal populations is a hot topic of  
38 animal genetics. The quantification of allelic richness and the evaluation of their association with  
39 phenotypes represent tools for the safeguard and the management of local populations. **Moreover,**  
40 identification of genomic regions involved in phenotypic differences between individuals provide  
41 useful knowledge for gene assisted selection programmes.

42 Specialized breeds have been subjected to intense selection. A main consequence has been  
43 the progressive erosion of local levels of genetic variation that may have compromised the ability to  
44 challenge environmental factors (Mäki-Tanila et al. 2010). Thus a genetic comparison between  
45 selected and autochthonous populations may allow for the identification of genomic regions  
46 involved in the control of fitness traits. On the other hand, studies involving highly selected breeds  
47 with different production aptitudes, as the case of dairy and beef cattle, can provide an exciting  
48 opportunity for studying signatures of selective breeding (Hayes et al. 2008a; Qanbari et al. 2010).

49 Actually, little is known about the effects of intensive, directional and prolonged selection on  
50 genome sub-structure of domestic species.

51 In population genetics, the identification of a locus target of selection is based on the  
52 existence of a reduction in nucleotidic diversity, or on an increase linkage disequilibrium (LD)  
53 and/or a changed allele frequency (Doebley et al. 2006). Currently, different statistical methods are  
54 used for the detection of selection signatures. Many of them are based on the comparison of allele  
55 frequencies or haplotype structure (for a review see Biswas and Akey 2006). The most commonly  
56 used metrics are the  $r^2$  for measuring LD and the fixation index  $F_{st}$  (Weir and Cockerham, 1984). A  
57 quite recent approach, extensively studied in human populations, is based on the detection of runs  
58 of homozygosity (ROH), defined as uninterrupted stretch of homozygous genotypes (Gibson et al.,  
59 2006). The extent and frequency of ROHs can be used as an indication of past or recent inbreeding  
60 (Khatkar et al., 2010; Purfield et al., 2012; Ferencakovic et al., 2012). However, for many of these  
61 methods it is difficult to develop a proper statistical test. This is particularly true when searching for  
62 selective signatures within a single population.

63 High throughput platforms able to simultaneously genotype for many thousands of SNP  
64 offer a powerful tool for the assessment of the genetic diversity across the genome (Andersson and  
65 Georges 2004). Genome Wide Analysis (GWA) have been performed to clarify the role of selection  
66 and drift in the evolutionary processes (Biswas and Akey 2006). Several recent studies have  
67 proposed the hitch-hiking mapping approach for identification of target of positive selection. The  
68 basic assumption is that the substitution of favourable allele at one site results in a reduction of  
69 variability at closely linked sites and lead to the allele fixation in a population (Przeworski et al.  
70 2005). Actually, the abundance of SNP throughout the genome makes them particularly suitable in  
71 the detection of such selective sweeps (Andersson and Georges 2004).

72 However, such a huge amount of information has become rather problematic to interpret. A

73 major issue is represented by the great variability of the signal pattern (for example heterozygosity  
74 or other related statistics as  $F_{st}$ ) along the chromosome. An usual empirical practice to smooth data  
75 is to work on average values of sliding windows of predetermined size (Weir et al. 2005; Barendse  
76 et al. 2009). A common problem is represented by the development of a suitable statistical test able  
77 to assess whether an index of the genetic difference between two populations can be considered  
78 significant. Different approaches have been proposed to assess  $F_{st}$  statistical significance, as the  
79 calculation of q-values for Kernel-smoothed values (Flori et al., 2009), permutation test based on  
80 binomial distribution of the SNP allelic frequencies (Stella et al., 2010) or the setting of a threshold  
81 of one standard deviation from the mean for smoothed  $F_{st}$  values (Kijas et al., 2012).

82 In the present work, an approach for studying selection signatures in two Italian cattle breeds  
83 with different production aptitude, Italian Brown and Piedmontese (dairy and beef, respectively), is  
84 proposed. In particular, a local regression is used to smooth raw  $F_{st}$  data and a Control Chart is  
85 applied to predicted data for identifying significant values. The method is challenged to identify  
86 genes that have been reported to be involved in the genetic determinism of dairy and beef traits in  
87 cattle.

88

## 89 **Materials and Methods**

90 A sample of 749 Italian Brown and 364 Piedmontese bulls was considered in the study.  
91 Animals were genotyped with the Illumina Bovine bead-chip containing 54,001 SNP  
92 (<http://www.illumina.com>). Only SNP located on the 29 autosomes in the Btau4.0 build of the  
93 Bovine Genome assembly were considered. Quality control was performed on the genotypes. SNP  
94 were **removed** if: monomorphic in both breeds; **had** a percentage of missing data higher than 2.5%  
95 **had** a minor allele frequency lower than 1%. After data editing, 42,514 markers were retained for  
96 the study. Missing data were replaced with the most frequent allele at that specific locus for each

97 breed.

98 Allele frequencies, observed and expected heterozygosity were calculated for each breed.

99 Total allelic frequencies for each locus,  $f_p$  and  $f_q$ , considering all animals as a single population were  
100 calculated as:

$$101 f_p = [f_B \cdot (2 \cdot n_B) + f_P \cdot (2 \cdot n_P)] / (n_B + n_P);$$

102 Where  $f_B$  and  $n_B$  are frequencies of alleles and number of individuals in Brown, and  $f_P$  and  
103  $n_P$  are frequencies of alleles and number of individuals in Piedmontese.

$$104 f_q = 1 - f_p$$

105 Then, expected heterozygosity in populations ( $H_s$ ) and overall ( $H_t$ ) were calculated. Finally,  
106  $F_{st}$  was calculated according to Weir and Cockerham (1984) as:

$$107 F_{st} = H_t - H_s / H_t$$

108 In order to smooth  $F_{st}$  pattern, data were fitted with a Locally Weighted Scatterplot  
109 Smoothing (LOWESS) regression using the PROC LOWESS of SAS/STAT software version 9.2  
110 (SAS Institute, Inc; Cary NC) (Cleveland 1979). The LOWESS has been used in genetics for  
111 smoothing model  $R^2$  in the statistical analysis of molecular marker data (Questa-Marcos et al.,  
112 2010). In the LOWESS, the space of the independent variable is fragmented into different intervals  
113 for which separate regressions are fitted. The aim of the method is to remove noise from raw data  
114 and to clarify graphical presentations. A critical point in fitting LOWESS is the identification of a  
115 suitable dimension of the data interval to be included in the analysis. In other words, if  $x$  is the  
116 number of adjacent points to be used in the estimation procedure of a set of  $n$  data, each region  
117 contains a fraction of points given by  $x/n$ . This ratio is defined as the smoothing parameter  $S$  of the  
118 LOWESS regression. As  $S$  increases, the fitted line will be smoother until  $S = 1$  that corresponds to  
119 a single line (i.e. the standard linear regression). Consequently, the goodness of fit depends strongly  
120 on the smoothing parameter used (Cohen 1999).

121 In general, the number of markers considered in the local regression was different across  
122 chromosomes, being directly related to their length. Therefore the use of the same  $S$  parameter in all  
123 chromosomes could not be feasible. In the present work, a smoothing parameter corresponding to  
124 an interval of 20 SNPs for each separated regression gave the best results. The different smoothing  
125 parameters used for each chromosome are reported in Table 1 (supplemental material).

126 In order to identify  $F_{st}$  values different from the average pattern that could be evidence of  
127 selection signatures, LOWESS smoothed  $F_{st}$  were analysed with a Control Chart approach. This  
128 methodology aims at checking a process and its variability and it can be used to identify sources of  
129 variation. In the specific case of the present study, the goal was to partition  $F_{st}$  variation into a  
130 component due to selection, that causes a drop of heterozygosity, and a remaining random variation  
131 along the chromosome. Control Charts are graphically displayed as stream of data falling within  
132 control limits. Data exceeding these limits are flagged as outlier signals. A similar approach has  
133 been used by Kijas et al. (2012) for identifying selection signatures in sheep breeds. A Control Chart  
134 approach has been recently used to identify sites of preferential location of genetic variation in  
135 *Mycobacterium tuberculosis* (Das et al., 2012). In the present study, smoothed  $F_{st}$  values were  
136 plotted against their position along the chromosome. Limits of the Control Chart were set at 3  
137 standard deviations from the mean.

138 In order to compare the results with an assessed methodology for studying selection  
139 signatures,  $F_{st}$  values were also smoothed with a sliding windows approach. The genome was  
140 divided into windows and average  $F_{st}$  values for each interval were calculated. Sliding windows are  
141 a graphical method widely used for detect genomic regions under positive or balancing selection  
142 (Hayes et al., 2008a; Stella et al., 2010). In the present study, the size of the window was fixed at 20  
143 SNP each (i.e. of the same size of those used in the LOWESS smoothing). The two methods were  
144 compared by examining patterns of smoothed  $F_{st}$  signals.



145 Annotated genes in genomic regions corresponding to peaks exceeding Control Chart limits  
146 were derived from the UCSC Genome Browser Gateway (<http://genome.ucsc.edu/>). Intervals of  
147 500Kbp (0,25 Mbp upstream and downstream the significant region) were considered.

148

## 149 Results and Discussion

150 The comparison of chromosome average heterozygosity (Hobs) between the two breeds  
151 highlights lower values for the Italian Brown (average difference of 0.04) (Figure 1). The largest  
152 difference was found for BTA6 (0.07) the smallest for BTA2 (0.02). Differences in heterozygosity  
153 between cattle breeds have been reported by other authors (Ciampolini et al. 1995; Cañón et al.  
154 2001).

155 In general, non smoothed SNP  $F_{st}$  values were characterized by some well defined peaks that  
156 could be evidence of divergent selection, and by a large background of low to moderate values  
157 indicating random noise. The largest number of high raw  $F_{st}$  values ( $n= 17$ , between 0.4 and 0.9)  
158 was detected on BTA6, the smallest ( $n= 1$  with  $F_{st}$  value= 0.26) on BTA23 (Figures 2a and b,  
159 respectively). Few raw  $F_{st}$  signals (both in frequency and magnitude) were also detected on BTA28  
160 and 29 (supplemental material). The pattern of raw  $F_{st}$  data for BTA6 was more regular compared to  
161 BTA23. This result may be interpreted as a consequence of the hitchhiking effect, because a  
162 reduction of heterozygosity (selective sweep) affect polymorphism of both individual and  
163 associated loci (Stephan et al., 2006).

164 The LOWESS correction resulted in a better definition of highest peaks, even if with an  
165 expected reduction in scale due to the regression (supplemental material). Moreover, other peaks of  
166 moderate height have been disentangled from the background noise of raw  $F_{st}$  data. As an example,  
167 LOWESS corrected  $F_{st}$  values for BTA6 and BTA23 are reported in Figures 2c and d, respectively.  
168 It can be clearly seen that the smoothing procedure enhanced clustered peaks whereas isolated

169 signals were regressed towards lower values. This behaviour was observed for the whole genome  
170 (supplemental material).

171 Compared to other methods currently used for studying selection signatures, such a relative  
172 simplicity could be interpreted as a sign of weakness. Actually, the LOWESS regression is a robust  
173 non parametric method, does not relies on strong assumptions on data distribution and it could be  
174 considered as a sort “of vertical sliding windows” (Jacoby, 2000). Such a property was evident also  
175 in the comparison with the sliding windows approach performed in this study (Figure 3): the  
176 LOWESS was actually able to yield more defined and clear signals. The enhancement of cluster of  
177 peaks and the lowering of isolated signals are evidence of robustness of the method that is not  
178 affected by the variation of a single marker. This feature is particularly useful for fitting the hitch-  
179 hiking effect that occurs in the surroundings of a selectively favourable mutation (Maynard Smith  
180 and Haigh, 1974).

181 The control chart analysis detected a total of 98 outliers on the whole genome. BTA6, 8 and  
182 15 showed the largest number of signals (8, 8 and 7 respectively). On BTA11, 12, 17 and 22 a  
183 single signal was detected, whereas no peaks were found on BTA23, 25 and 29. These figures are  
184 lower than those reported by Stella et al. (2010) that, on a large number of breeds, found 699  
185 different putative selection signatures on the whole genome. However, Flori et al. (2009) using  
186 smoothed  $F_{st}$  across three different dairy or dual purpose breeds identified a total of 13 significant  
187 regions under selection distributed on seven distinct chromosomes. Some of these regions  
188 correspond to those detected in the present work. An example are peaks of smoothed  $F_{st}$  that have  
189 been found on BTA6 regions where *LAP3* (*leucine aminopeptidase 3* at 37,871,423-37,896,860 bp)  
190 and *LCORL* (*ligand dependent nuclear receptor corepressor-like* at 38,137,617-38,288,047 bp) loci  
191 map.

192 Five peaks distributed throughout the chromosome were detected BTA19. A total of 66

193 different annotated loci for the corresponding genomic regions were retrieved from UCSC Genome  
194 Browser Gateway data base. This is the highest number of genes per chromosome found in the  
195 present analysis. This result is in agreement with the study of Band et al. (2000), that reported a  
196 significantly larger number of mapped genes for BTA19 compared to the other autosomes.

197 As far as the use of the Control Chart for testing outliers is concerned, the way confidence  
198 limits are set implies an assumption of normality for data distribution. Actually  $F_{st}$  often shows a  
199 heavily skewed distribution (Deng et al., 2007). However, a way to deal with this problem is to  
200 divide data into subgroups and then use their averages which could be considered approximately  
201 normally distributed (Morrison, 2008). Such an approach is similar to what has been done in the  
202 present work, where actually intervals of  $F_{st}$  data were considered for the LOWESS smoothing.  
203 Recently, to investigate levels of genetic diversity and to characterise the role of domestication and  
204 selection on the sheep genome, Kijas and co-workers (2012) performed a genome-wide analysis  
205 using smoothed SNP-specific  $F_{st}$  plotted for values on excess of one standard deviation from the  
206 mean. In any case, being straightforward to interpret without specific statistical background and  
207 simple to update, the Control Chart approach has been widely used in genetics, medicine and other  
208 fields of applied biology (Westgard et al., 1981; Coskun et al., 2008; Das et al., 2012).

209 The reliability of the proposed method was confirmed by smoothed  $F_{st}$  values that exceeded  
210 Control Chart limits in regions of the genome where genes known to affect productive traits are  
211 located. An evident example was the highest peak detected at about 37 Mbp on BTA6 (Figure 2e).  
212 It was the largest smoothed  $F_{st}$  predicted value (0.30) observed across the whole genome in the  
213 present study. Some genes known to affect milk production traits have been mapped in this region.  
214 Examples are *Family with sequence similarity 13 member A (FAM13A1)* (36,740,247-36,843,133  
215 bp) (Cohen et al. 2004), *ATP-binding Cassette, sub-family G (WHITE), member 2 (ABCG2)*  
216 (37,342,201-37,433,870 bp), *secreted phosphoprotein 1 (SPPI)* (37,511,672-37,511,830 bp) and

217 *peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A)* (44,797,216-  
218 44,935,623 bp) (Cohen-Zinder et al. 2005; Ron and Weller 2007; Sheehy et al. 2009). On the other  
219 hand, no LOWESS predicted Fst peaks were detected on BTA23 (Figure 2f).

220 A further example is represented by a peak exceeding the chart limits that was detected  
221 between 6,5-7,5 Mbp on BTA2. It is well known that *myostatin (MSTN)* locus that controls double  
222 muscling phenotype in cattle is located in position 2q14-q15 between 6,532,697 and 6,539,265 bp.  
223 Actually this gene is reported to be fixed for the p.Cys313Tyr variant in the Piedmontese breed  
224 (Casas et al. 1999). Even though this causative mutation is not present in the SNP chip, the signal  
225 has been detected in the adjacent markers.

226 A rather unexpected result has been obtained on BTA14, where no relevant signals in the  
227 region where the *DGATI* locus (*diacylglycerol O-acyltransferase 1*) maps were found. However, it  
228 should be remembered that some studies have reported the fixation of the p.Lys232Ala variant both  
229 for the Italian Brown and Piedmontese breeds (Kaupe et al., 2004).

230 Other detected genomic regions, in agreement with previous researches on selection  
231 signatures in cattle, were those harbouring genes affecting coat colour. These loci have been under  
232 strong selection considering the importance of this trait in defining cattle breeds (Flori et al., 2009;  
233 Wiener and Wilkinson 2011). In the present study, two selection signatures were observed on  
234 BTA18 (12-13Mbp) and between 72-73 Mbp still on BTA6. In these chromosomic regions are  
235 located the *Melanocortin 1 receptor (MC1R)* and the *Kit (V-kit Hardy-Zuckerman 4 feline sarcoma*  
236 *viral oncogene homolog)*, loci respectively. The Extension locus controls melanine synthesis. The  
237 presence of three different alleles (E, E<sup>1</sup> and e) in both cattle breeds considered in this study has  
238 been reported (Russo et al., 2007). The *Kit* locus is responsible for the “Piebald” spotted coat-colour  
239 pattern in cattle and other species. This is interesting because Brown Swiss and Piedmontese breeds  
240 did not show Piebald phenotype (Stella et al. 2010), confirming the complex genetic architecture of

241 coat colour in mammals.

242 In the present study, strong selection signals have been identified also in genomic regions  
243 not previously associated to traits of economic importance.

244 Several genes related to calcium homeostasis and metabolism were found. *Osteocrin*  
245 (*OSTN*) on BTA1, the *calcitonin receptor (CALCR)* and *calmodulin 2 (CAM2)* on BTA4 encode for  
246 bone specific proteins that appears to act as soluble osteoblast/osteoclast regulators (Thomas et al.,  
247 2003). The analysis of BTA10 outliers has revealed the presence of one interesting gene, the  
248 *GREM1* that encode for the *gremlin 1*, a protein required for the osteoblastic activity and mineral  
249 apposition (Canalis et al. 2012). Moreover, on BTA15 *STIMI (Stromal interaction molecule 1*, was  
250 highlighted. It is expressed in mammary gland and it is essential for the cellular storage of calcium  
251 and the activation of the calcium influx pathway (Li et al., 2012). Actually, milk production is a  
252 complex biological process involving different tissues and governed by many genes (Finucane et  
253 al., 2007; Lemay et al., 2009). Bone and mammary tissues are related via the same signalling  
254 pathways (Cohen et al., 2004). Bone is a dynamic tissue continually modelled through the  
255 coordinate actions of the bone forming osteoblast and resorbing osteoclast (Budayr et al., 1989).  
256 Lactation is considered one of the most important events that determine bone remodelling due to the  
257 relevant calcium mobilization (Qing et al., 2012). During the production of milk, plasmatic  $Ca^{+2}$   
258 entries into the mammary epithelial cells through a phenomenon called calcium influx pathway  
259 (McAndrew et al., 2011).

260 A further set of highlighted genomic regions are those were genes related to epithelial cell  
261 proliferation, skeletal muscle and bone morphogenesis map. As reported in the previous section, a  
262 selection signature in a large region spanning between 7 and 10 Mbp was found on BTA2. Several  
263 genes involved in the biology of muscular apparatus have been mapped in this region. Examples are  
264 the *Bridging integrator 1 (BIN1)*, that plays an important role in muscle cell biology (Sedwick

265 2010), and the *Solute Carrier family 40 (iron regulated transporter), member1 (SLC40A1)* locus,  
266 that codes for the ferroportin 1 (FPN1) a protein with an essential role in the regulation of iron  
267 levels on the body.

268 On BTA11 the *Bone morphogenetic protein 10 (BMP10)* a growth factor belonging to the  
269 TGF- $\beta$  superfamily known for its ability to induce bone and cartilage development (Groenveld and  
270 Burger, 2000) was found. Moreover, *SNAI3 (Snail homolog 3)* and *CDH15 (cadherin 15, type1, M-*  
271 *cadherin (myotubule)* were highlighted on BTA18 (12,908,122-13,260,964 bp). They are involved  
272 in the skeletal morphogenesis and myoblast differentiation (Moran et al., 2002; Zhuge et al. 2005).  
273 An evident peak around 26-27 Mbp was observed on BTA20. In this genomic region is annotated  
274 the *Follistatin (FST)* locus. This protein acts blocking the binding of *Myostatin* to its receptor and  
275 causing an abnormal muscle development (McPherron and Lee, 2001). Table 2 reports other  
276 regions identified by peaks exceeding Control Chart limits and the annotated genes involved in  
277 skeletal muscle development and metabolism. Strong selection signatures observed in regions of  
278 genes related to muscle development, differentiation and metabolism could be interpreted as signs  
279 of selection within the Piedmontese. However, it should be remembered that the Brown Swiss was  
280 originally a dual purpose breed. Thus some of these genes might have also contributed to determine  
281 the Brown's phenotype. A deeper knowledge of the role of these genes in muscular cells could be of  
282 help for selecting markers useful for beef cattle breeding.

283 In this study few putative candidate genes were detected for lipid metabolism (Table 2). This  
284 is probably due to the fact that intramuscular fat deposition not only depends on the genetic  
285 background but also by other factors such as age, sex, nutrition and farm conditions.

286 An interesting result was the identification of numerous putative candidate genes involved  
287 in the reproductive function (Table 2). Among them, the specific ligand (*KITLG*) for the *Kit*  
288 receptor was identified on BTA5. Actually the interaction between *kit* and its ligand is crucial for

289 fertility (Mithraprabhn and Loveland, 2009). Such results suggest a further deepening of the genetic  
290 basis of relationships between production and fertility traits (Bello et al., 2012).

291 Finally, this genome wide analysis highlighted the presence of selection signatures for a  
292 group of similar genes. Six genes belonging to the Calpains gene family were detected in four  
293 different chromosomes: *CAPN 7* on BTA1, *CAPN 13* and *14* on BTA11, *CAPN 5* on BTA15, and  
294 *CAPN 2* and *8* on BTA16, respectively. Several studies indicate calpains as regulators of apoptosis  
295 and suggest an involvement of the calpain system during the muscle postmortem apoptotic pathway  
296 (Mohanty et al., 2010). The interaction among calpains and other proteases is considered a  
297 fundamental step for after slaughtering meat tenderization (Koochmaraie, 1992). A multi gene  
298 family is formed by duplication of a single original gene. In cattle, 3.1% of the genome is  
299 composed of duplicated genes, most of which encoding proteins involved in innate immunity,  
300 sensory receptors and reproduction (Elsik et al., 2009). Generally, the expansion or contraction of  
301 gene families can be due to chance or is the result of natural selection. Gene gain or loss are so  
302 considered to be an incentive for evolutionary change and as a common advantageous response to  
303 selective regimes (Demuth et al., 2006).

304 A detailed list of putative genes for all 29 bovine chromosomes highlighted by the Control  
305 Chart outliers values is summarized in Table 2. All gene content information presented was derived  
306 from the UCSC Genome Browser Gateway (<http://genome.ucsc.edu/>) using the fourth draft of  
307 bovine genome sequence assembly (Btau 4.0) and from NCBI or Swiss ProtK Source consultation.

308 Results obtained in this study on the comparison between two cattle breeds with different  
309 production aptitude, beef and dairy, agree with previous report on milk QTL (Cohen et al., 2004)  
310 and transcriptome analysis (Bionaz and Looor, 2008; Lemay et al., 2009). Moreover it confirmed  
311 what observed in previous comparisons between cattle breeds (Flori et al., 2009; Stella et al., 2010;  
312 Qanbari et al. 2011). Differences have been found with the QTL analysis carried out by Prasad et al.

313 (2008) on BTA19 and 29, where selection signatures in different chromosomal regions were found.  
314 A possible explanation could be represented in the different genomic assemblies used.

315

## 316 **Conclusions**

317 The combined use of a LOWESS regression and a Control Chart approach here proposed  
318 was effective in studying the genetic differences between the Piedmontese and the Italian Brown  
319 cattle breeds. In particular, the local regression was able to yield a smooth  $F_{st}$  pattern, easy to  
320 interpret compared to raw data. The Control Chart allowed for a quite simple detection of  
321 significant  $F_{st}$  values that may indicate selection signatures. The method was validated by  
322 comparing results obtained on several chromosomes with previous reports in cattle (Hayes et al.  
323 2008a and b; Flori et al., 2009; Stella et al 2010; Qanbari et al. 2011). Moreover, some regions  
324 harboring genes not yet associated to traits of economic importance for livestock have been  
325 detected. In particular, genes involved in the calcium metabolism and muscle biology have been  
326 highlighted. The methodology could be proposed as an easy approach for performing a whole  
327 genome scan in studies aimed at identifying selection signatures by using high throughput SNP  
328 maps.

329

## 330 **Authors' contributions**

331 E.P., S.S. and N.P.P. M. planned and performed analysis and drafted the manuscript. A.A., G.G.,  
332 C.D., R.S. and G.M., performed the analysis. All authors reviewed the manuscript.

333

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337 genotypes.

338

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521 Table 2 List of putative candidate genes obtained on the basis of Control Chart outliers

Biological Function	BTA	Position Mbp	Gene name
Immune Response	5	81,763,516-81,779,866	<i>USP18 ubiquitin specific peptidase 18</i>
	16	23,622,572-23,625,154	<i>TLR5 toll-like receptor 5</i>
	17	57,084,217-57,115,368	<i>HVCN1 hydrogen voltage-gated channel 1</i>
	18	1,880,236-12,887,173	<i>CYBA cytochrome b-245, alpha polypeptide</i>
	19	21,395,686-21,409,196	<i>TMIGD1 transmembrane and immunoglobulin domain containing 1</i>
	26	23,471,864-23,478,382	<i>NFKB2 nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)</i>
Reproduction	1	155,943,716-155,956,150	<i>EAF1 ELL-associated factor 1</i>
	3	86,007,282-86,200,728	<i>AK4 Adenylate kinase 4</i>
	5	20,587,724-20,612,963	<i>KITLG Kit ligand</i>
	6	37,961,724-37,987,164; 38,153,046-38,199,153; 38,227,954-38,378,385	<i>LAP3 leucine aminopeptidase 3;</i> <i>NCAPG non-SMC condensing I complex, subunit G;</i> <i>LCORL ligand dependent nuclear receptor corepressor-like</i>
	8	104,876,401-104,908,801	<i>TXNDC8 Thioredoxin domain containing 8 (spermatozoa)</i>
	9	41,225,543-41,246,855	<i>AMD1 adenosylmethionine decarboxylase 1</i>
	10	36,873,000-36,890,219	<i>TYRO3 TYRO3 protein tyrosine kinase</i>
	11	70,119,086-70,174,863	<i>GMCL1 germ cell-less, spermatogenesis associated 1</i>
	14	60,023,782-60,033,403	<i>ODF1 outer dense fiber of sperm tails 1</i>
	15	18,520,179-18,520,292; 24,046,636-24,397,152	<i>FDX1 ferredoxin 1;</i> <i>CADM1 cell adhesion molecule 1</i>
	18	13,648,996-13,652,641	<i>SPATA2L spermatogenesis associated 2-like</i>
	19	24,498,808-24,501,792; 24,628,862-24,646,107;  50,216,969-50,223,538	<i>GSG2 germ cell associated 2 (haspin);</i> <i>P2RX1 purinergic receptor P2X, ligand-gated ion channel, 1 ;</i> <i>DDX5 DEAD (Asp-Glu-Ala-Asp) box helicase 5</i>
	Cell growth, proliferation and differentiation	1	76,216,039-76,832,685
12		70,974,850-71,682,818	<i>HS6ST3 heparan-sulfate 6-O-sulfotransferase 3</i>
13		47,627,052-47,683,993  48,488,115-48,536,904	<i>CDS2 CDP-diacylglycerol synthase (phosphatidate cytidyltransferase) 2 ;</i> <i>FERMT1 fermitin family member 1</i>
14		60,169,396-60,307,900	<i>UBR5 ubiquitin protein ligase E3</i>

			<i>component n-recognin 5</i>
	16	20,839,081-20,931,656 26,878,683-26,905,046	<i>TGFB2 transforming growth factor, beta2;</i> <i>PSEN2 presenilin 2 (Alzheimer disease 4)</i>
	17	57,146,787-57,165,849	<i>PPP1CC protein phosphatase 1, catalytic subunit, gamma isozyme</i>
	19	35,124,710-35,129,750 35,535,495-35,544,295 35,953,771-35,969,817 45,567,703-45,574,688	<i>MAPK7 mitogen- activated protein kinase 7;</i> <i>DRG2 developmentally regulated GTP binding protein 2;</i> <i>FLCN folliculin;</i> <i>GRN granulin</i>
Ions metabolism	1	78,466,667-78,488,928	<i>CLDN16 claudin 16</i>
	2	9,451,265-9,580,452	<i>CALCRL calcitonin receptor- like</i>
	4	11,016,143-11,126,171	<i>CALCR calcitonin receptor</i>
	5	81,136,111-81,146,812	<i>KCTD17 potassium channel tetramerisation domain containing 17</i>
	8	11,700,825-11,763,811	<i>ACO1 aconitase 1, soluble</i>
	17	56,466,582-56,498,348,; 56,790,348-56,488,450	<i>CAMKK2 calcium/calmodulin-dependent protein kinase kinase 2, beta;</i> <i>ATP2A2 ATPase, Ca<sup>++</sup> transporting, cardiac muscle, slow twitch 2</i>
	18	47,597,196-47,605,452	<i>KCNK6 potassium channel, subfamily k, member 6</i>
	19	56,790,348-56,844,450; 24,594,778-24,623,204	<i>ATP2A3 ATPase, Ca<sup>++</sup> transporting, ubiquitous;</i> <i>CAMKK1 calcium/calmodulin-dependent protein kinase kinase 1, alpha</i>
	24	31,254,115-31,532,051	<i>KCTD1 potassium channel tetramerisation domain containing 1</i>
	26	22,854,587-22,857,882; 24,558,695-24,564,440	<i>KCNIP2 Kv channel interacting protein 2;</i> <i>CALHM3 calcium homeostasis modulator 3</i>
Lipid metabolism	2	6,192,072-6,348,621	<i>HIBCH 3 hydroxibutirril o idrolase</i>
	5	53,700,174-53,700,270	<i>ACAT2 AcetylCoA acetyltransferase 2</i>
	10	59,440,432-59,504,627	<i>CYP19A1 cytochrome P450, family 19, subfamily A, polypeptide1</i>
	13	48,423,438-48,446,513	<i>CRLS1 cardiolipin synthase 1</i>
	15	55,827,654-56,160,380	<i>ACER3 alkaline ceramidase 3</i>
	18	13,212,190-13,250,827	<i>ACSF3 Acyl-CoA synthase family member 3</i>
	19	35,671,152-35,687,188	<i>SREFB1 sterol regulatory element</i>

			<i>binding transcription factor 1</i>
mammary gland metabolism	2	10,226,975-10,322,817	<i>ITGA V integrin alpha V</i>
	6	37,351,167-37,421,683 37,431,966-37,490,645 37,511,673-37,518,636 72,298,906-72,346,677 72,741,252-72,828,528	<i>ABCG2 ATP-binding cassette, sub-family G, (WHIT), member 2;</i> <i>PKD2 polycystic kidney disease 2;</i> <i>SPP1 secreted phosphoprotein 1;</i> <i>PDGFRA platelet-derived growth factor receptor, alpha polypeptide;</i> <i>KIT V-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog</i>
	7	62,635,246-62,657,995	<i>SPARC secreted protein, acidic, cysteine-rich (osteonectin)</i>
	10	29,529,387-29,541,874 36,595,794-36,596,071	<i>GREM1 gremlin1, DAN family BMP antagonist;</i> <i>IGFBP3 insulin-like growth factor binding protein 3</i>
	14	12,506,878-12,583,201	<i>MTMR2 myotubularin related protein 2;</i>
	15	20,478,802-20,482,029 50,442,087-50,753,021	<i>CRYAB crystalline alpha B;</i> <i>STIM1 stromal interaction molecule 1</i>
	18	14,699,407-14,998,970	<i>ITGF1 integrin alpha FG-GAP repeat containing 1</i>
	19	35,122,081-35,124,619 35,823,315-35,854,048	<i>MFAP4 microfibrillar associated protein 4;</i> <i>PEMT phosphatidylethanolamine N-methyltransferase</i>
	24	30,845,569-30,860,104	<i>AQP4 aquaporin 4</i>
	27	48,475,540-48,478,931	<i>OXSM 3-oxoacyl-ACP synthase, mitochondrial</i>
bone and muscle metabolism	1	77,682,355-77,718,578 155,717,664-155,777,449	<i>OSTN osteocrin;</i> <i>CAPN7 calpain 7</i>
	2	5,595,799-5,652,801 6,532,697-6,539,265 7,066,569-7,148,685 7,740,061-7,779,695	<i>BINI bridging integrator 1;</i> <i>MSTN myostatin;</i> <i>SLC40A1 solute carrier family 40 (iron regulated transporter) member 1;</i> <i>COL3A1 collagen type (III) alpha 1</i>
	7	5,824,715-5,935,402	<i>MYO9B myosin IXB</i>
	8	11,291,512-11,308,875 105,221,050-105,315,564	<i>CLU clusterin</i> <i>MUSK muscle, skeletal, receptor tyrosine kinase</i>
	10	19,387,377-19,414,041	<i>PKM pyruvate kinase, muscle</i>
	11	69,145,567-69,152,285 70,648,036-70,648,340 71,029,777-71,105,164	<i>BMP10 bone morphogenetic protein 10;</i> <i>CAPN14 calpain 14;</i> <i>CAPN13 calpain 13</i>

	13	48,488,115-48,536,904	<i>FERMT1</i> fermitin family member 1;
	15	11,852,140-11,854,278 56,045,818-56,103,271	<i>PPP1R14C</i> protein phosphatase 1, regulatory (inhibitor) subunit 14C ; <i>CAPN5</i> calpain 5
	16	24,021,217-24,065,788 24,108,032-24,166,355	<i>CAPN8</i> calpain 8; <i>CAPN2</i> calpain 2
	17	56,905,068-56,915,878 57,330,762-57,338,500	<i>ARPC3</i> actin related protein 2/3 complex, subunit3 21kDa; <i>MYL2</i> myosin light chain 2,regulatory, cardiac, slow
	18	12,908,122-12,913,750 13,260,964-13,279,948 47,527,738-47,531,970 47,701,775-47,875,177	<i>SNAI3</i> snail homolog 3; <i>CDH15</i> cadherin 15, type1,M-cadherin (myotubule); <i>PPP1R14A</i> protein phosphatase 1, regulatory (inhibitor) subunit 14A; <i>RYR1</i> ryanodine receptor 1(skeletal)
	20	23,624,160-23,688,918 27,297,146-27,302,564	<i>GPBP1</i> GC-rich promoter binding protein 1; <i>FST</i> follistatin
	21	45,895,690-45,898,343	<i>CFL2</i> cofilin 2 (muscle)
	26	12,908,235-12,917,607 23,540,685-23,557,026	<i>ANKRD1</i> ankyrin repeat domain 1 (cardiac muscle); <i>ACTR1A</i> ARP1 actinn related protein 1 homolog a, centractyn alpha (yeast)
others	10	19,817,179-19,849,769	<i>ADPGK</i> ADP-dependent glukonidase
	11	68,612,764-68,639,385 69,642,777-69,707,857	<i>CNRIP1</i> cannabinoid receptor interacting protein 1; <i>GFTP1</i> glutamine-fructose-6 phosphate transaminase 1
	15	20,576,533-20,611,864	<i>DLAT</i> dihydrolipoamide S-acetyltransferase
	18	13,776,888-13,778,639	<i>MC1R</i> melanocortin 1 receptor (alpha melanocyte stimulating hormone receptor)
	19	45,226,420-45,227,150 45,325,106-45,329,822	<i>PPY</i> pancreatic polypeptide <i>G6PC3</i> glucose 6 phosphatase, catalytic, 3

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528 **Captions to figures:**

529 **Figure 1** Comparison of average heterozygosity (Hobs) per chromosome between the two breeds  
530 (black = Piedmontese, grey = Italian Brown).

531 **Figures 2.** Pattern of raw  $F_{st}$  data calculated for SNP located along the BTA 6 (a) and 23 (b);  
532 predicted  $F_{st}$  values for the SNP located along BTA6 (c) and 23 (d) using the LOWESS regression  
533 with a smoothing parameter of 0.009 and 0,021 respectively; Control Chart of predicted  $F_{st}$  values  
534 for BTA6 (e) and 23 (f). Solid line: Mean, dotted lines are: upper control limit (UCLI) and lower  
535 control limit (LCLI).

536 **Figure 3** Plot of comparison between Sliding Windows versus LOWESS on BTA6. Solid line:  
537 Sliding Windows method, dotted line: LOWESS methodology

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