

FABIO MARETTO

GENETICS OF DRY CURED HAMS



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GENETICS OF DRY CURED HAMS

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Il rugby è uno sport da gentlemen. Prima di tirare il pallone, indietro, al tuo compagno, tu devi controllare che lui stia bene, che sia ben disposto, aperto, disponibile, ottimista. Non puoi tirargli un pallone vigliacco che gli arriva assieme a due energumeni che gli fanno del male. Però, mentre tu fai tutto questo bel ragionamento etico, ce n'è altri ventinove che ti guardano, di cui quattordici tuoi e quindici no, e di questi tre ti corrono addosso, due grossi e uno piccolo, ma cattivo, e la prima tentazione è di dare il pallone al tuo compagno

— Marco Paolini

Look what Britain have done to Wales. They've taken our coal, our water, our steel. They buy our homes and live in them for a fortnight every year. What have they given us? Absolutely nothing. We've been exploited, raped, controlled and punished by the English -

and that's who you are playing this afternoon

— Phil Bennet 1977

— Pre-game pep talk before facing England —

Il lab non è poi così diverso dal rugby, si vince come squadra ma si perde come singoli...

RIASSUNTO

In questo studio è stata effettuata un'analisi per la ricerca di QTL (Quantitative Trait Locus) per caratteri relativi alla composizione della carcassa e alla qualità del prosciutto crudo stagionato. L'analisi è stata condotta su 369 individui progenie di 15 verri Large White C21 e 82 scrofe ibride Goland. Per questi animali erano disponibili dati fenotipici per un totale di 48 caratteri relativi a crescita e deposizione di grasso (4 caratteri), alla composizione della carcassa (5 caratteri) e alla qualità del prosciutto crudo stagionato (39 caratteri).

Il materiale biologico ed i dati fenotipici utilizzati provengono dal nucleo di selezione Gorzagri (Riese Pio X, TV, Italia) e dal centro genetico di Todi (PG, Italia) dedicato al programma di sib-testing della linea verri C21. Presso questo centro si sviluppa l'attività di produzione di famiglie di suinetti ibridi, originati dall'incrocio di verri C21 con scrofe ibride Goland. Questi suinetti, sui quali viene calcolata la stima del valore genetico dei verri e delle scrofe C21 in selezione, sono di costituzione genetica identica a quella dell'ibrido commerciale Gorzagri.

La linea verri C21 ha come obiettivi di selezione il miglioramento delle performances di allevamento e dell'attitudine alla produzione di prosciutto crudo stagionato DOP. I principali obiettivi di selezione sono il miglioramento dell'attitudine alla trasformazione industriale della carcassa e della coscia, con particolare riferimento alla copertura di grasso della coscia e alla sua qualità (numero di iodio e acido linoleico) e alla presenza di difetti della stessa quali la globosità ed il grado di mazzatura della carne. Inoltre, lo schema selettivo mira alla produzione di animali omogenei in termini di accrescimento per ottenere un'ottimale organizzazione produttiva all'interno degli allevamenti.

Gli animali, sopra descritti, sono stati genotipizzati tramite l'impiego di 269 marcatori molecolari microsatellite (STR) uniformemente distribuiti lungo l'intero genoma suino con particolare attenzione a regioni caratterizzate dalla presenza di QTL implicati in caratteri relativi alla composizione della carcassa e alla qualità della carne e già descritti precedentemente in bibliografia.

Per l'individuazione dei QTL è stato utilizzato un approccio "multi-marker regression for interval mapping" in famiglie di mezzi fratelli. Tramite questa analisi sono stati individuati 52 QTL che superano la soglia di significatività del 5% a livello di linkage group (singolo cromosoma). Di questi, 16 sono risultati significativi all'1% di soglia.

I QTL individuati sono responsabili di caratteri relativi alla qualità dei prosciutti crudi stagionati come la quantità dell'area della noce di grasso in sezioni trasversali delle cosce di prosciutto, la misura della consistenza della massa magra, misurata tramite penetrometro sul muscolo semimebrano, il numero di iodio e altri caratteri importanti per la composizione della carcassa e la qualità del prosciutto crudo stagionato. Lo studio ha dimostrato che i QTL per questi caratteri sono segreganti in questa popolazione commerciale.

I risultati ottenuti saranno utilizzati come punto di partenza per l'individuazione di geni candidati sui quali effettuare analisi di associazione con i caratteri sopra descritti. Il fine ultimo è di implementare l'utilizzo negli schemi di selezione di marcatori molecolari diretti che

permettano di incrementare l'accuratezza e la risposta selettiva in caratteri molto difficili e/o costosi da rilevare come quelli relativi alla qualità del prosciutto crudo stagionato DOP.

ABSTRACT

A quantitative trait loci (QTL) study for carcass composition and dry cured ham quality traits was conducted on 369 individuals progeny of 15 C21 Large White sires and 82 crossbred Goland C40 Large white derived sows of a commercial finishing cross.

Phenotypic records were already available for a total of 48 traits related to growth and fatness (4 traits), carcass composition (5 traits) and dry cured ham quality (39 traits).

The genetic material and phenotypic data used in this study derives from the nucleus and sib-testing program of C21 Large White boar line (Gorzagri, Fonzaso, Italy). In the sib-testing center (Todi, PG Italy) crossbred piglets, deriving from crosses of C21 Large White boars and C21 crossbred Goland sows, are evaluated to obtain genetic breeding values of parents; these animals have the same genetic identity of Gorzagri commercial hybrids.

The main objectives of selection of the C21 boars line are the attitude for production of high quality dry cured hams and commercial performances. Traits involved in the selection of animals are attitude for industrial transformation of raw thighs with emphasis on covering fat and its quality (iodine number and linoleic acid), absence thigh defects such as shape and marbling.

Animals previously described were genotyped for 269 microsatellite markers that covered uniformly the entire porcine genome and with emphasis on regions harbouring QTL affecting carcass composition and meat quality traits already described in literature.

Fifty-two QTL exceeding the 5% chromosome-wise significance level were identified using a multimarker regression approach for interval mapping in half-sib populations. Among these, 16 QTL affecting different traits were significant at 1% chromosome-wise significance level.

Results showed that many QTL affecting dry cured ham quality traits such as cross section ham fat eye area, instrumental firmness on thighs muscles, iodine number and some other important carcass traits segregated within this commercial line.

This study is an important baseline for further investigation of known and unknown candidate genes affecting dry cured ham quality traits with the purpose of gaining further knowledge in the biology of hams' maturing processes and for the opportunity of using direct marker informations for improving ham quality and profitability by within-line selection.

PUBLICATIONS

During the PhD thesis I also worked on other different projects related to molecular genetics, traceability and diversity in different species.

A list of publications can be found here.

Horses:

- **Molecular characterization of Italian Heavy Draught Horse (IHDH) breed using mitochondrial DNA and microsatellite markers.** F. Mareto and R. Mantovani. 2009. Book of Abstract n.15 60th EAAP Annual Meeting, Barcelona. Pag. 219.
- **Genetic variability of Italian Heavy Draught Horse.** F. Mareto and R. Mantovani. 2009. Italian Journal of Animal Science. Vol. 8 (s3) pag. 95-97.
- **Genetic characterization of Italian Heavy Draft Horse (IHDH) breed using microsatellite markers.** Mareto, F.; Ribeca, C. and Mantovani, R. 2008. Proceedings of the 31st International Conference on Animal Genetics, Amsterdam July 20-24 2008. Poster: 2233.

Cattle:

- **Investigation on variability of candidate genes for meat quality traits in Piemontese cattle.** C. Ribeca, G. Bittante, A. Albera, V. Bonfatti, F. Mareto and L. Gallo. 2009. Italian Journal of Animal Science. Vol. 8 (s2) pag. 51-53
- **Effect of Calpain1, Calpastatin and Cathepsin genes and polygene on beef shear force in Piemontese young bulls.** C. Ribeca, F. Mareto, L. Gallo, A. Albera, G. Bittante, and P. Carnier. 2009. Book of Abstract n.15 60th EAAP Annual Meeting, Barcelona. Pag. 145.
- **Caratterizzazione Genetica di razze podoliche con marcatori molecolari microsatellite.** Dalvit, C.; Mareto, F.; Cassandro, M.; De Marchi, M.; Cecchinato, A. and Bittante, G. 2009. Taurus Speciale N.3. pagg. 195-202.
- **Monitoring genetic variability of Bulgarian cattle biodiversity.** C. Dalvit, J. Krastanov, F. Mareto, N. Oblakov, T. Angelova and M. Cassandro. 2009. Italian Journal of Animal Science. Vol. 8 (s3) pag. 89-91.

Other species:

- **Genetic analysis reveals Roe deer (*Capreolus capreolus*) population structure in North-Eastern Italian Alps.** G. Valvo, E. Sturaro, F. Mareto and M. Ramanzin. 2009. Italian Journal of Animal Science. Vol. 8 (s3) pag. 89-91.
- **Survival analysis of piglet pre-weaning mortality.** Cecchinato, F. Mareto, E. Zanetti, P. Carnier. 2007. Ital. J. Anim. Sci.:6 (s1). Pagg; 67-69.

- **Finding 16s rRNA Gene-Based SNPs for the genetic Traceability of Commercial Species belonging to Gadiformes.** F. Maretto, E. Reffo, C. Dalvit, G. Barcaccia, R. Mantovani. 2007. *Ital. J. Anim. Sci.*: 6 (s1). Pagg.161-163.

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ACRONYMS

QTL	Quantitative Trait Locus
SSC	Sus scrofa chromosome
STR	Short Tandem Repeats
DOP	Protected Designation of Origin

Part I

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 MATURED AIR-CURED HAM PRODUCTION

1.1.1 DOP San Daniele Ham

The DOP San Daniele ham is a typical matured air-cured Italian product with a notable economical value.

San Daniele ham is produced in a particular environment in the municipality of San Daniele in the province of Udine (Italy); there are 30 ham-making enterprises that produce more 2,500,000 hams/year generating sales for about 330 million Euro (Consorzio del Prosciutto di San Daniele, data from 2007 [1]). According to GU n. 293/99 [4] and following the EEC reg. no. 2081/92 [3], which established community-level protection for designation of origin-label agricultural and food products, the DPO label for San Daniele ham was finally introduced on the market (Figure 1).

*DOP: protected
designation of origin*

The complex legislation behind the protection and the safeguard of the label can briefly be summarized by the following rules. Pigs used for preparation of San Daniele air-cured ham have to be born, bred and slaughtered in one of these Italian regions: Friuli-Venezia Giulia, Veneto, Lombardia, Emilia Romagna, Piemonte, Toscana, Umbria, Marche, Lazio or Abruzzo (96% of animals are reared in Northern Italy). Pigs must belong to Large White and Landrace breeds and may be cross-bred with Duroc or hybridised following the rules of the National Pedigree Register for "Italian heavy pigs". The pigs must have average weights of no less than 160 kg and an age of at least nine months at slaughter. Many pig breeds are therefore listed as unsuitable and the use of thighs from such boars and sows is explicitly forbidden.

Traceability of pig's thighs is guaranteed by a tattoo reporting the breeder identifier and the animal's date of birth. At slaughtering designated pig thighs should be processed within 120 hours and thighs should weigh a minimum of 12 kg (for satisfactory maturing) with intact trotter; thighs should also own a sufficient quantity of intramuscular and covering (1.5 cm) fat in accordance with defined quality parameters (percentage of linoleic acid and iodine number) and should



(a) Italian PDO Label.



(b) PDO San Daniele Ham label.

Figure 1: DOP labels.

have suitable color of meat and appropriate fat texture. Fresh thighs that meet all the aforementioned requirements are then branded with the DOP label reporting the ham making starting time.

1.1.2 *The Disciplinary of Production*

According to D.Lvo 537/92 [5] and to the disciplinary of production DOP [2] the making process of San Daniele ham is very long and takes at least 13 months (of which a minimum of 8 shall be air-curing). The production stages are the following:

CHILLING. Selected fresh thighs are maintained at 1-3 °C and should have a pH between 5.6 and 6.2.

TRIMMING. Skin and fat are removed to give the ham its typical “chicken drumstick” shape and to facilitate salting.

SALTING. Salt is added to the trimmed ham and is then left for 15-30 days in refrigerated and humidity controlled chambers allowing the initial dehydration steps to start.

PRESSING. Typical of San Daniele ham, this step allows for the typical “guitar-shape” of the ham and to help in dehydration and in compacting of fat and lean tissues.

RESTING. After the removing of the outer layer of salt, hams rest in refrigerated rooms for at least 60 days (depending on size and weight) at a temperature not higher than 6 °C and with less than 80% of humidity. Dehydration started on salting will proceed during this step.

WASHING. Hams are washed with warm water to remove excess salt, microbial layers, etc.

DRYING. Hams rest for a week in a temperature controlled room to allow for the drying of the surface and to let the internal temperature of the ham to reach the optimal curing temperature values.

INITIAL CURING. Ham are hung on frames in well ventilated rooms, due to the high quantity of water still present, during the first 30-60 days, temperature needs to not exceed 15-17 °C. Humidity should also not exceed 50-60% to preserve hams from moulds and from over dehydration.

GREASING. After 6 months of curing, the exposed surface of the hams is softened with a paste of minced lard and salt to preserve hams from over dehydration.

MATURING. Hams are moved to cellar-like rooms and hang on racks until curing is completed.

At the end of the whole process hams are checked for quality. Inspectors pierce each ham in critical points with a porous horse bone needle and perform sensorial analysis to evaluate the quality and possibly branding hams as PDO San Daniele.

1.1.3 *Technological properties of PDO San Daniele ham*

1.2 GENETIC ASPECTS OF PIG SELECTION

In the last 20 years many improvements on productive technologies and plants management were performed by ham's producers to guarantee high quality standards, DOP label and the disciplinary of production [2] were certainly the best driving force to obtain such results. On the other hand, in more recent years, the production of Italian heavy pigs, focused for long times on "traditional genetic types" (Large White and its crosses), has undergone significant changes. Changes were mainly due to global genetic improvements of commercial lines with breeding goals for increasing lean meat, reduced fat deposit and strong muscular growth which are not suited for the production of cured hams (Bosi and Russo [11]).

1.2.1 *Genetic types*

Consortia for the protection of Parma and San Daniele ham admit only some purebred subjects, or hybrids obtained from some breeds. As purebred, only individuals from the Italian Large White and Italian Landrace breeds can be used. In addition, the crosses with the Italian Duroc breed are permitted. Subjects of the same breeds coming from other countries or subjects of other breeds can be used for the production of crossed pigs, provided they are obtained by selection programs with objectives not inconsistent with those of the Italian selection Bosi and Russo [11].

Generally speaking, a greater content of lean meat on the carcass and the different degree of adiposity have adverse effects on color, flavor and firmness of Parma ham (Parolari et al. [59]), moreover, higher content of lean meat on the carcass correspond to higher seasoning loss (Russo and Costa [70]).

The selection schemes used in Large White, Landrace, Duroc breeds (and their crosses) in the Italian breeding programme is different from those used in other countries for how it bears on meat quality traits. For example, maintaining a constant backfat thickness that covers hams, protect the product from seasoning losses and from bad organoleptic characteristics. Intramuscular fat is another selection criterion to reduce, mainly in Duroc breed, in fact, the frequency of excessive presence of inter and intramuscular fat in thighs need to be reduced. Weight and age at slaughtering are also important characteristics and, correlated with fat covering ham, are fundamental to avoid excessive curing loss after salting and maturing due to the fact that covering fat contains less water than muscular tissue (60% less) thus reducing exchanges between muscle and external environment (Carnier et al. [13], Gallo et al. [31]).

1.2.2 *Scheme selection of the C21 boar line*

Data used in this study were collected from May 2001 to March 2005 in a sib testing program of the C21 Large White boar line (Gorzagri, Fonzaso, Italy).

The selection scheme consists in a nucleus farm (Riese Pio X, Italy) where pure C21 boars are produced and mated to pure C21 sows and in a sib testing farm (Todi, Italy), where the same C21 boars

are mated to crossbred sows to produce crossbred piglets. In the sib testing program of the C21 line, crossbred paternal half sib families are produced by mating C21 nucleus boars to a group of crossbred sows which is submitted to minimum intensity replacement policies. Crossbred sows originated from a cross involving boars of a synthetic line, derived from Large White and Pietrain breeds, and sows of a Large White line selected for maternal ability and prolificacy.

Crossbred paternal half sib families provide the genetic evaluation program of C21 purebred breeding candidates with crossbred half sibs phenotypes for quality traits of raw and dry cured hams. Besides growth and residual feed efficiency, the breeding goal of the C21 line includes traits related to the quality of dry-cured ham. Selection is addressed to an intermediate optimum for marbling and for the amount of subcutaneous fat evaluated on the raw ham, to enhance the quality of fat covering, to reduce excessive ham roundness and to reduce curing weight losses at a fixed level of dry-cured ham quality.

1.3 MOLECULAR GENETICS IN PIG

1.3.1 *QTL in the pig*

The current release of the Pig QTLdb (Pig Quantitative Trait Locus database, Release 9) contains 4928 QTLs from 202 publications gathered during the past 15 years (Hu and Reecy [42] <http://www.animalgenome.org/>). Those QTLs represent 499 different traits collected in five different classes:

QTL: quantitative trait locus

- Meat Quality Traits (including anatomy, chemical, conductivity, enzyme activity, fat composition, fatness, flavor, meat color, odor, pH, stiffening and texture traits types)
- Production Traits (including digestive organ, feed intake and growth traits types)
- Health Traits (including blood parameters, disease resistance, immune capacity and pathogen traits types)
- Exterior Traits (including age, behavioral, coat color, conformation and defects traits types)
- Reproduction Traits (including endocrine, litter size, reproductive organ and reproductive traits types)

The first QTL study was carried out in 1994 by Andersson et al. [6] and the QTL that was discovered was a major locus for fat deposition on chromosome 4. Since then, many QTL have been identified for many traits; a frequency distribution of QTL throughout the genome is presented in figure n. 2 with the number of QTL reported for each 10 cM bin across the genome. Descriptive table of number of publications and QTL by years and number of QTL by chromosomes and trait classes are reported in tables n. 1, 2 and 3 respectively.

Only one QTL was discovered in chromosome Y (related to external fat on loin trait) because this sex-chromosome is often not included in QTL analysis. The chromosome SSC1 contains the highest number of QTL (1257). Other chromosomes with a high number of QTL are SSC2, SSC4, SSC7 and SSC8; together with SSC1 they account for more than 60% of the total number of QTL.

Despite the very high number of QTL identified, only few of them are related with ham quality and none, at my current knowledge, with dry cured ham quality traits (Beeckmann et al. [9], Karlskov-Mortensen et al. [47], Harmegnies et al. [40], van Wijk et al. [85], Dragos-Wendrich et al. [24], Cepica et al. [15], Heuven et al. [41], Evans et al. [27]).

Traits like growth and fatness were included in almost all experiments because data are very easily collected, on the other hand traits that are difficult or expensive to measure were investigated in a limited number of studies and with a limited number of animals. Almost 80% (table n.3) of traits analyzed belong to the meat quality trait class followed by growth traits; inside the meat quality trait class fatness and anatomy traits were the most studied.

GROWTH. Average daily gain (ADG) and body weight (BW) measured in different ways and/or at different times are the most reported growth traits. QTL affecting these traits have been reported in all autosomes; SSC6 harbors most of the QTL affecting body weight related traits, while SSC4 followed by SSC1, SSC7 and SSC6 harbor most of the QTL affecting growth related traits.

FATNESS. Among meat quality traits anatomy, fatness and fat composition are the most studied. Backfat measurements at different times and rib number is the most represented trait and numerous QTL were found mainly on SSC2, SSC3, SSC5, SSC7 and SSC8. Fatty acid composition have also been analysed including lipid content (LC), monounsaturated (MUFA) and polyunsaturated (PUFA) content percentages and different types of fatty acid (oleic, myristic, palmitic, palmitoleic, etc.) content percentages. QTLs for fatty acid composition were found in almost all chromosomes but SSC4 and SSC7 are the most frequent. Finally 19 and 9 QTL for intramuscular fat content (IMF) were identified on SSC6 and SSC4 respectively.

CARCASS COMPOSITION. SSC2, SSC4, SSC7 and SSCX contribute most to carcass characteristics. The most cited trait was carcass length (CRCL) QTLs for this trait were found on all chromosomes except SSC15 and SSC16 and the centromeric parts of SSC6, SSC7 and SSC8 have the highest number of citations. Carcass weight (CWT) is influenced by SSC4 which is also associated with growth and fatness traits.

REPRODUCTION. 186 QTL were recorded for reproduction traits. The most cited is Teat Number for which QTLs have been found in all chromosomes except for SSC14, SSC18 and SSCY. This trait is very easy to measure and therefore is one of the most cited. Very few other reproduction traits are currently measured, as an example QTLs for total number born (litter size) have been found on SSC8, SSC12 and SSC15 the same chromosomes harbor QTLs for total number of born alive (prenatal survival) together with SSC16 and SSC18.

MUSCULARITY. The third trait in terms of QTLs reported is loin muscle area that was reported 110 times and for which most of QTLs are reported in SSC2, SSC7, SSC8 and SSC9 scattering over the whole chromosome. Intramuscular fat (IMF) and lean meat percentage (LEANMP) have also been widely studied and SSC7, SSC8 and SSC9 are the most reported chromosomes for those traits.

MEAT QUALITY. Typical meat quality traits are pH, color, marbling, firmness, drip loss, taste and flavor. Some other muscle traits already treated such as IMF, or LEANMP could be considered as quality traits as well but they are often classified under muscle traits. QTLs for pH of longissimus dorsi and semimebranosus taken at different times post mortem have been reported mainly for SSC₁, SSC₃ and SSC₇; QTLs for pH 24h post mortem on loin has been reported also on SSC₁₅ and SSC₁₆.

QTLs responsible for marbling have been reported on SSC₂, SSC₅, SSC₆ and SSC₁₃; in the same position in SSC₂ QTLs have been found for firmness and other muscularity traits. The IGF2 gene with a known mutation with effect on meatiness is located the top of the p-arm of SSC₂ (Laere et al. [52]). On SSC₁₅ QTLs are reported for color, tenderness, flavor, average glycogen and average lactate. The RN gene that is one of the few genes known affecting meat quality traits (Milan et al. [57]) maps on SSC₁₅. Drip loss is the most reported trait analysed and account for 936 QTLs found spanning the whole genome with the exclusion of SSC₁₇ and the sexual chromosomes. Taste and flavor traits are scarcely reported and therefore are not treated.

Performance traits like growth and fatness were the most represented due mainly to ease of recording and were subject of most of the published experiments. Traits that are more difficult or expensive to records, like most meat quality traits, were analyzed in a limited number of studies but still increasing in the last few years (Rothschild et al. [69], Rothschild [67], Evans et al. [27], Heuven et al. [41], Liu et al. [53], Harmegnies et al. [40], Karlskov-Mortensen et al. [47], Slawinska et al. [78], van Wijk et al. [85]). Over-representation of most extensively studies should also be taken into account together with studies that were carried out on a limited number of chromosomes or directed to (QTL-rich) genome regions based on previously published work. As a consequence it is very likely that the number of QTL on the most extensively studied chromosome such as SSC₁, SSC₂, SSC₄, SSC₆, SSC₇, SSC₈ and SSC_x is over represented.

Before the beginning of the genome scan in spring 2007 a total of 110 papers were published reporting identification of 1701 QTL in pig (PigQTLdb Release 5). Informations regarding QTL and traits were collected and are shown in table n.4.

One of the biggest problem encountered in such a survey was the lack of consistency in the nomenclature of traits; this subject is of great interest in the scientific community and this deflection is currently being overtaken by the Animal Trait Ontology Project (Hughes et al. 43).¹

With the aim of exploiting QTL for dry cured ham quality traits presented in this thesis, the selection of genome regions targeted with microsatellite markers in the genome scan were based on this QTL/publications survey. Markers were selected in order to uniformly cover the whole genome and to explore known regions of already mapped QTL but also poorly investigated regions.

ATO: Animal Trait
Ontology

¹ Until recently, from the several bio-ontologies the one that includes phenotypic trait information found in livestock species was missing; the ATO community is then developing a standardized trait ontology for farm animals and software tools to overcome this defect (<http://www.animalgenome.org/atoamigo>). Hopefully such effort will help researchers providing a standard of nomenclature for the descriptions of phenotypes associated with livestock species.

Until now, only a few QTL have been characterized at the gene level and implementation of MAS in commercial pig breeding is limited but still rapidly increasing (Dekkers. [21]). There are several reasons for this. Most of the QTL detection experiments were undertaken by using experimental crosses and initial linkage maps to help determine regions underlying traits of importance to the pig industry. These early QTL scans used families developed by generally crossing European Wild Boar with a commercial breed or crossing the exotic Chinese Meishan breed with a commercial breed. Such scans generally used 300 to 700 pigs and usually produced in a F₂ design (Rothschild et al. [69]). It is not clear however to what extent the detected QTL are polymorphic within commercial populations.

*MAS: marker
assisted selection*

It is not simple to detect the presence of segregating QTLs within commercial pig populations because in such populations the power in QTL mapping is reduced due to the fact that only a limited proportion of parents will be heterozygous for any QTL and the heterozygosity has to be deduced using segregation data. On the other hand, when using experimental populations with divergent intercrosses it can be assumed that all F₁ animals are heterozygous at major QTL.

Only recently very few studies exploited such question and performed QTL mapping experiments within commercial populations (Thomsen et al. [83], van Wijk et al. [85], Heuven et al. [41], Evans et al. [27]).

A second drawback that is being overtaken by new SNP chips technologies but was present for microsatellite markers was the low map resolution of the experiments. Dekkers [21] distinguished three types of markers to be used in MAS: 1) direct markers; loci that code for a functional mutation, 2) linkage disequilibrium (LD) markers; loci that are in population-wide linkage disequilibrium with the functional mutation and 3) linkage equilibrium (LE) markers; loci that are in population-wide linkage equilibrium with the functional mutation in outbred populations. Direct markers are preferred for effective implementation of marker-assisted selection, followed by LD and LE markers, the latter requiring within-family analysis and selection. Ease of application and potential for extra-genetic gain is greatest for direct markers, followed by LD markers, but is antagonistic to ease of detection, which is greatest for LE markers. Microsatellite markers used up to now in a genome scan are not likely in population-wide LD with the QTL and therefore analyses needs to be performed within families as well as selection and the use of such markers in other families requires that the linkage phase of the markers and the QTL is established in each family. Therefore, the search for markers in population-wide LD has become of much interest.

1.3.2 Candidate genes in the pig

Candidate genes for dry-cured ham production traits have been recently investigated in many studies (Ramos et al. [63], Dekkers. [21], ?), these genes are mainly selected among those expressed in skeletal muscle and/or are involved in biological process such as proteolysis and lipolysis that contribute to the ham-curing process. Some of the first genes investigated were RN/PRKAG₃ (Milan et al. [57]), CAST (Ciobanu et al. [18]), cathepsin B, F (Russo et al. [73], Russo et al. [74]) and Z (Ramos et al. [62]), RYR (Fujii et al. [29]), ESR (M. F. Rothschild et al. [54]) CKIT (Marklund et al. [56]), MC₁R (Kijas et al. [48]), MC₄R (Kim et al. [49]), F18 (Frydendahl et al. [28]), K88 (Jørgensen et al. [46]), PRLR (Vincent et al. [86]), RBP₄ (Rothschild et al. [68]), A-FABP/FABP₄ (Gerbens et al. [33]), H-FABP/FABP₃ (Gerbens et al. [34]) and IGF₂ (Jeon et al. [45]).

An example of application of molecular genetic informations in pig breeding industry is PICmarqTM. Since 1991 PIC, currently a subsidiary of Genus plc (UK), one of the leader in animal breeding industry, have developed and uses a panel of direct markers associated with meat quality, production, disease resistance, litter size and breed identity traits (PICmarqTM). Genotypes are directly implemented in their CBVTM (Crossbred Breeding Value) to calculate the breeding values of PIC boars allowing to evaluate and to have better estimation of traits that are impossible to measure in the live pig (meat quality traits) or impossible to measure directly in sires (litter size traits) or impossible to measure in young breeding pigs (lifetime reproduction, disease resistance, coat color-breed identity and congenital defects traits) (de Vries et al. [20], PIC [60]).

Candidate gene approaches for dry cured ham quality traits have not been fully investigated. Numerous studies have demonstrated that several genes and chromosomal regions are associated with fresh pork quality (Bidanel and Rothschild [10]) and candidate genes for specific dry-cured ham production traits can be selected among those expressed in skeletal muscle and/or involved in biological processes that contribute to the ham-curing process, such as proteolysis and lipolysis. Stalder et al. [79] investigated the effects of the PRKAG₃ and CAST genes on dry cured hams processing traits; more recently Ramos et al. [63] found associations of cathepsin F and SCD genes with colour, cured weight and yield and other fresh pork quality traits that could be implemented in selection programs to improve american dry-cured ham.

Despite these findings there is still a lack of informations about genes or chromosome regions responsible for dry cured ham quality traits. Gaining more knowledge on this subject is therefore the aim of this thesis.

1.4 AIM OF THE THESIS

The main objective of this thesis is to gain knowledge in the molecular genetic aspects of dry cured ham quality traits. In particular a genome scan using microsatellite markers (STR) will be performed to investigate chromosomal regions harbouring QTL for dry cured ham quality traits and, in detail:

1. the investigation will be performed on 369 individuals belonging to 15 half-sib families for which phenotypic informations on growth, carcass and dry cured ham quality traits were already available;
2. the genome scan will be performed using 269 microsatellite markers to cover the whole genome uniformly with emphasis on regions harbouring QTL affecting carcass composition and meat quality traits;
3. a multimarker regression approach for interval mapping in half-sib populations will be used to locate position of significative QTL on linkage maps.

Table 1: Number of publications and QTL by years reported from PigQTLdb release 9 (www.animalgenome.org)

Year	Number of Papers	Number of QTL
1994	1	5
1995	1	5
1996	2	6
1997	4	11
1998	8	102
1999	7	42
2000	15	102
2001	14	277
2002	16	193
2003	29	607
2004	8	212
2005	24	500
2006	21	492
2007	17	423
2008	23	1736
2009	12	215

Table 2: Number of QTL by chromosome reported from PigQTLdb release 5 and 9 (www.animalgenome.org)

Chromosome	Number of QTL (r.5 - 2007)	Number of QTL (r.9 - 2009)
Y	1	1
X	108	203
1	207	1257
2	159	432
3	71	177
4	123	439
5	60	119
6	221	456
7	207	488
8	80	197
9	58	148
10	43	127
11	36	93
12	42	127
13	69	164
14	66	151
15	69	142
16	18	71
17	34	64
18	29	72
Total	1701	4928

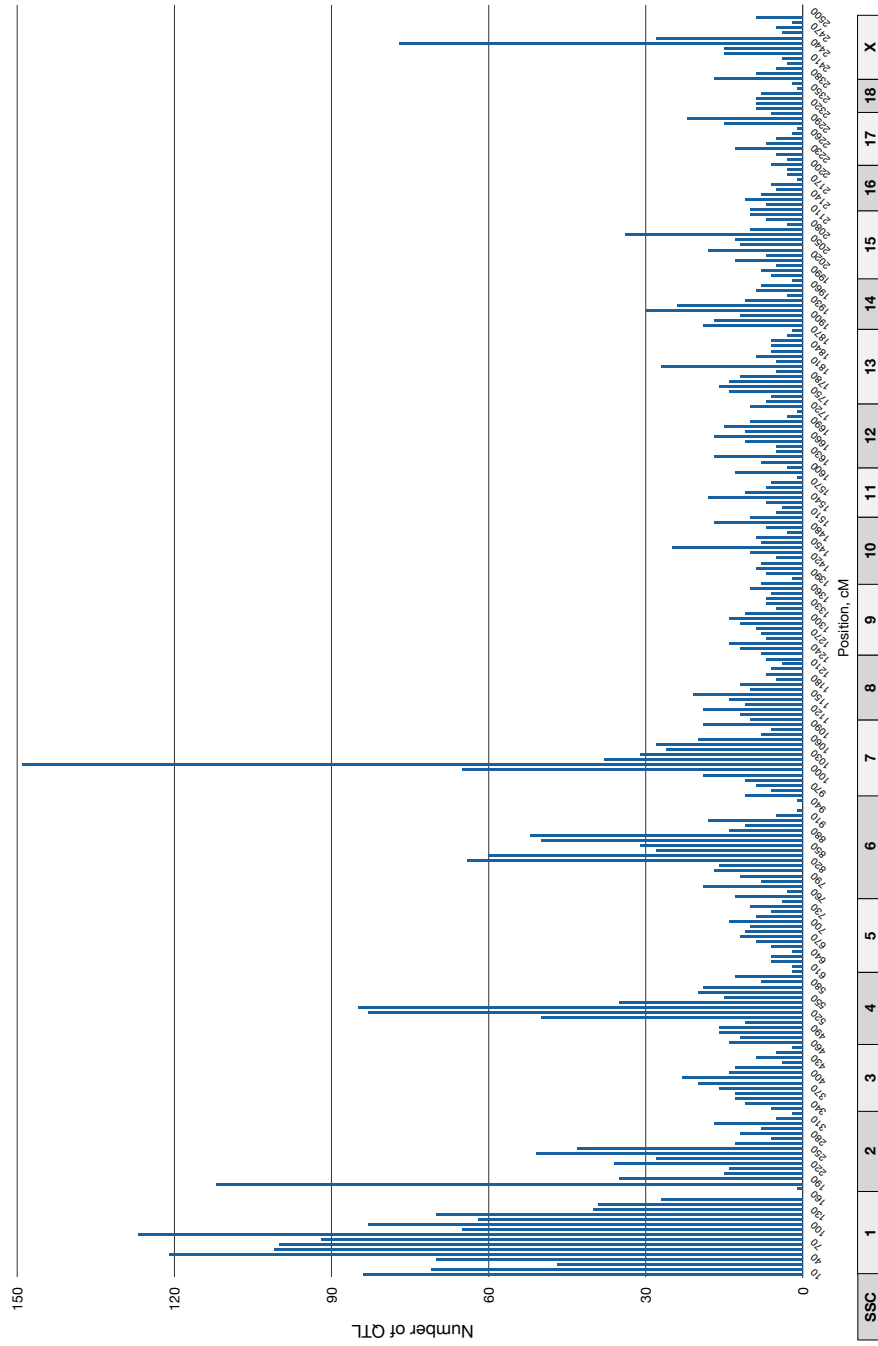
Table 3: Number of QTL by general trait classification reported from PigQTLdb release 5 and 9 (www.animalgenome.org)

Trait classes	Number of QTL (r.5 - 2007)	%	Number of QTL (r.9 - 2009)	%
Exterior	32	1,88	162	3.29
Health	15	0,88	362	7.35
Meat Quality	1327	78,01	3711	75.30
Production	260	15,29	494	10.02
Reproduction	67	3,94	199	4.04
Total	1701	100	4928	100

Table 4: Number of QTL for trait types from pigQTLdb (release 5)

Trait types	QTL found
Anatomy	555
Behavioral	22
Chemical	18
Coat Color	2
Conductivity	25
Conformation	8
Defects	18
Digestive Organ	10
Disease Resistance	7
Endocrine	4
Enzyme Activity	1
Fat Composition	64
Fatness	404
Feed Conversion	8
Feed Intake	18
Flavor	19
Growth	224
Immune Capacity	8
Litter Size	21
Meat Color	69
Odor	5
pH	58
Reproductive Organ	33
Reproductive Traits	9
Stiffening	3
Texture	64

Figure 2: Distribution of QTL throughout the porcine genome with the number of QTL counted per 10 cM bins. The numbers in the grey bar indicates the different chromosomes.



Part II

GENETIC PARAMETERS OF DRY CURED
HAM QUALITY TRAITS

GENETIC PARAMETERS OF DRY CURED HAM QUALITY TRAITS

Analysis and results presented in this chapter were already available at the beginning of this thesis and they were previously published as a PhD thesis [80]. They provided an exceptional background for the development of this thesis and the author is grateful to Dr. Enrico Sturaro for this excellent work.

A brief description of traits and results is presented as introduction and for completeness of analysis of results of the genome scan presented in part 3 and part 4.

2.1 INTRODUCTION

Genetic improvement of dry cured ham quality by traditional breeding is difficult, and hampered by the need of extensive and very expensive measurements of traits. It is expected that for these type of traits and for meat quality traits in general, knowledge of the underlying genes will greatly contribute to the efficiency of selection. Many studies reported the identification of QTL in pigs for a variety of traits (for review see: Bidanel and Rothschild [10], Rothschild et al. [69]).

Dry - cured ham production has a notable economical value and, in Italy, is the most valuable product of the pig industry (see 1.1.1); raw hams account for more than 50% of carcass market value (Bosi et al. [12]). Due to the high value of the product and the long time required for its production the ham represents a conspicuous capital investment, to protect such an investment obtaining very high quality final product is necessary and could be achieved only if the raw ham quality is very satisfactory. Quality characteristics of raw ham could be divided in four categories (Russo et al. [72]):

- sanitary (absence of pathogenic micro-organisms, chemicals residuals and contaminations);
- nutritional (it refers to the chemical composition, nutritional and dietary characteristics of the product);
- organoleptic (i.e. mainly subjective characteristics of the product that make and/or keep a client a faithful consumer such as color, tenderness, taste, etc.);
- technologic (meat attitude of being transformed, packaged and conserved).

Industries that work on the transformation of the dry cured ham are focused on the technological properties of meat that are often correlated with organoleptic features of the final product. Factors that play an important role in the technological properties of meat for its transformation in dry cured hams are:

UMIDITY. High water content could lead to degradation phenomena and would increase drying, curing and maturing steps.

WHC: *Water Holding Capacity.*

WHC AND PH. Water holding capacity and pH are very important physical parameters that directly influence salting and curing steps. Measuring pH at 45 minutes (pH₁) and at 24 hours (pH_u) after slaughter is very important for monitoring PSE and DFD meat respectively (Gallo and Bondesan [30]).

PSE: *Pale, Soft and exudative meat.*

NaCl ABSORPTION. Together with dehydration, NaCl absorption is one of the fundamental process contributing to the stability of dry cured hams (Gou et al. [36]). It depends mainly on size and type of the raw meat, on salting techniques, quantity and duration of salting.

DFD: *Dark, firm and dry meat.*

IODINE VALUE. Determination of iodine value is important for the determination of the amount of unsaturation contained in fatty acids, the latter indicating tendency of fat going rancid.

High quality raw hams are necessary for the transformation industry because the only conservation methodology is salting. Therefore defects on raw thighs could not be corrected thus producing high risks of depreciation or discarding of products with great economic losses.

Lean meat firmness is one of the most important traits for the quality of dry cured hams. Inconsistent firmness generally lead to flaccid meat with problems during slicing and unpleasant sensation of doughy consistency thus producing, again, depreciation or discarding of products. This problem is not only due to an high ratio of humidity/proteins or to a low ratio of salt/humidity but it also depends on the quality of raw meat (Schivazappa et al. [75]).

Weight loss during curing is also one of the most important parameters describing the technological properties of raw hams. This trait records the quantity of dehydration that takes place during transformation processes and it is generally measured as the ratio of dry cured ham weight at the end of curing and as the raw trimmed ham weight (Russo et al. [71]). The loss of water and salting processes during curing inhibit microbial growth and allow for correct maturing and formation of peculiar flavour of products. On the other hand, excess of loss of weight, means, from a pure quantitative point of view, economical losses that the industry try to keep at low levels. Loss of weight during maturing ranges between 20% and 30% of the initial weight (Diaferia and Baldini [22]).

Another fundamental characteristic of raw thighs used in the production of dry cured hams is represented by the sub-cutaneous covering fat. Optimal fat covering prevents from excess of humidity losses allowing for regular and optimal curing processes.

Firmness of fat covering is another fundamental qualitative parameter. Good firmness of covering fat prevent from "fat melting" and percolation in lean tissues during curing processes that could lead to the formation of empty spaces inside the thighs with again high risks of depreciation or discarding of products (Chizzolini et al. [16]).

Fat firmness is normally measured by iodine number values (or more recently by NIR spectroscopy) and by determination of linoleic acid values. Measurements are generally taken on the inner and outer layer of fresh ham subcutaneous covering fat. The iodine number, in particular, measures the amount of unsaturation contained in fatty acids.

NIR: *Near Infrared Spectroscopy.*

The San Daniele DOP provides specific rules for iodine number and for linoleic acid content, the first should not be greater of a value of 70 while the latter cannot exceed the 15% (San Daniele DOP [2]).

2.2 MATERIALS AND METHODS

2.2.1 Genetic material

Phenotypic records analyzed in this study were recorded from animal progeny of C21 Large White boars (Gorzagri, Italy) that were mated to crossbred Goland C40 Large White derived sows (Gorzagri). Animals were reared at the same farm under standard feeding conditions. In particular, piglets were weaned 4 weeks after birth and fed ad libitum up to 75 kg of Body Weight (BW) using two diets with different levels of metabolizable energy (ME) and crude protein content: diet A (17.6% crude protein and 13.2 MJ ME/kg) was provided from 25 to 40 kg BW and diet B (16.2% crude protein and 12.9 MJ ME/kg) was fed to 75 kg BW. From 75 kg onwards, restricted feeding was implemented. From 75 to 110 kg, pigs were fed a diet containing 15.5% crude protein and 12.5 MJ ME/kg whereas crude protein content was reduced to 14% from 110 kg onwards. Animals (9 month of age, with an average BW of 169 ± 17 kg) were slaughtered at the same abattoir on a single day each month. Pigs were slaughtered after CO₂ stunning. (Sturaro et al. [81]).

2.2.2 Carcass and growth traits

A Fat-O-Meter instrument was used to record backfat thickness at 10th (**10RIBBFT**) and at last rib (**LRIBF**) and *longissimus dorsi* depth (**LD**) (ASPA [8]).

After slaughtering carcasses (**CCW**) and lean cuts were weighted. Traits were recorded as percentage on CCW for the different cuts (**HAMP**, **LEANP**, **LOINP**, **SHOUP** and **SPAREP**). Initial pH (**PHI**) was measured 45 min after slaughtering on the *semimembranosus* muscle on left thighs. After 24 h of refrigeration at 4 °C final pH was measured at dressing on the *semimembranosus* muscle (**PHU**).

HAMP: Ham weight (% on CCW)

LEANP: Lean weight (% on CCW)

LOINP: Loin weight (% on CCW)

SHOUP: Shoulder weight (% on CCW)

SPAREP: Sparerib weight (% on CCW)

2.2.3 Meat quality traits

Subjective evaluation regarding quality of raw thighs were performed by trained experts on ham shape (**HAMSS**), amount of blood vessels (**VHS**), amount of haematomas (**HAEHS**), marbling (**MSHS**), color (**CSHS**), covering fat layer (depth) (**CFLHS**), fat firmness (**FFHS**), fat color (**CFHS**) and fat greasyness (**FSHS**). A subjective score was assigned to each trait with values ranging from 0 to 4 for **HAMSS**, **VHS**, **HAEHS**, **MSHS**, **CSHS**, **CFLHS**, **CFHS**, **FFHS**, **FGHS** and **FSHS** and from -3 to 3 for **CSHS** and **CFLHS**. In general smaller values were attributed to undesired characteristics and higher values were attributed to favourable characteristics.

Depth of covering fat on ham was directly measured in cm (**FD**).

Instrumental firmness of covering fat on ham was measured using Hardness Meter MK2 penetrometer. The instrument records values from 0 to 1,000 points corresponding to resistance force recorded by the instrument on air and on a 5 mm aluminium foil respectively.

Measurements were taken in the inner (**FFI**) and outer fat layer (**FFO**); as a derived trait the mean of the two measures was also used (**FFM**).

Finally, Ham Minolta L*, a*, b* (**HAML**, **HAMA** and **HAMB**) values were taken on fresh surface of hams using a Minolta CHR 300 colorimeter (Minolta camera, Osaka, Japan).

2.2.4 Loss of weight after processing steps

WLS: Weight loss after salting (% on trimmed ham weight)

WLR: Weight loss after resting (% on trimmed ham weight)

WLC: Weight loss after curing (% on trimmed ham weight)

After slaughtering thighs and primal cuts were weighted (**HAMP**, **LEANP**, **LOINP**, **SHOUP** and **SPAREP**) and left thighs were refrigerated for 24 h at 0°C and subsequently trimmed. After trimming weights were recorded again and raw hams were transferred for curing according to the San Daniele ham disciplinary of production (DOP [2]). Weight of hams were then recorded after salting, resting and curing processes (**WLS**, **WLR** and **WLC**) together with days of curing (**DC**).

2.2.5 Instrumental and subjective firmness

FSHS: Subjective firmness score of lean in ham

FBF: Instrumental firmness on biceps femoris muscle (FBFM = mean value between point 1 (FBF1) and point 2 (FBF2))

FST: Instrumental firmness on semitendinosus muscle (FSTM = mean value between point 1 (FST1) and point 2 (FST2))

FSM: Instrumental firmness on semimembranosus muscle (FSMM = mean value between point 1 (FSM1) and point 2 (FSM2))

Instrumental and subjective evaluation of lean muscle firmness have been performed after curing. At the end of the transformation process hams were boned and perpendicularly sectioned between one third and half of the total longitudinal length (3). On this section subjective evaluation of lean meat firmness have been performed by trained experts (**FSHS**). Five classes were used for describing the phenotypes with 0 indicating the less firm to 4 indicating the most.

Instrumental firmness of lean on ham was recorded in two spots for each muscle: *biceps femoris*, *semimembranosus* and *semitendinosus* using Hardness Meter MK2 penetrometer (see fig. 3). Traits were recorded as: **FBF1**, **FBF2**, **FBFM**, **FSM1**, **FSM2**, **FSFM**, **FST1**, **FST2** and **FSTM**.

2.2.6 Iodine number

Iodine number (**IN**) measures the amount of unsaturation contained in fatty acids in the form of double bonds which react with iodine compounds. The higher the iodine number, the more unsaturated fatty acid bonds are present in fat. This analysis allows for the evaluation of suitability of hams' fat for curing process. The determination of iodine number is not a quantitative measurement of total insaturation of lipids

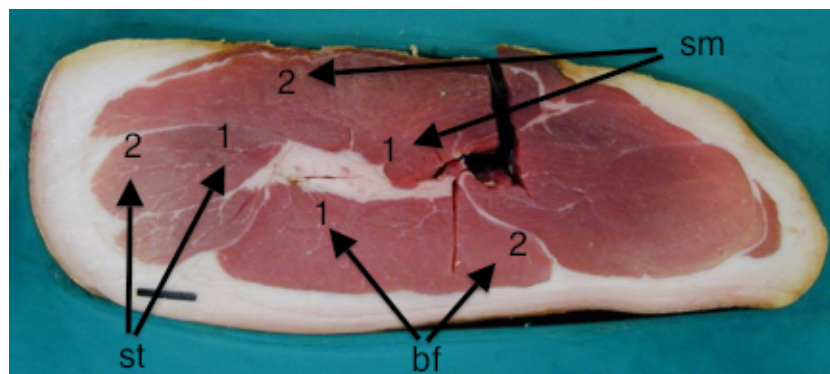


Figure 3: Cross-section of dry cured ham. st: *semitendinosus*, sm: *semimembranosus*, bf: *biceps femoris*



Figure 4: Cross-section of dry cured hams: computer image analysis.

but an empirical number that gives an indicative value. It can predict the tendency for oxydation and therefore the probability of fat going rancid. Iodine number was determined using Wijs method (AOAC [7]) according to the San Daniele procedure DOP [2]. For detailed description of the methodology see Sturaro [80].

2.2.7 Computer image analysis of cross sectioned dry-cured hams

Before image capture, bones were removed from dry cured hams with no fat removal, hams were then cross-sectioned by a cut between one third and half of the total longitudinal length. Images were captured using a digital color camera (model Coolpix 950; Nikon Corp., Tokyo, Japan) mounted on a tripod (4). Digital images were analyzed using Image Pro Plus 4.1 (Media Cybernetics, Silver Spring, MD) (for details see: Carnier et al. [14]).

Area of interest were: the total area of the cross section (**CSA**); the fat eye area (i.e. a visible fatty area approximately centered on the cross section and surrounded by *biceps femoris*, *semimembranosus*, *semitendinosus*, and *quadriceps femoris* muscles, **FA**); the lean, or muscles, area (i.e. the area of the cross section that excluded the area of *subcutaneous* fat, fat eye, and skin, **LA**); *biceps femoris* (**BFA**) and *semitendinosus* area (**STA**) and ratio of the FA to the cross-sectional area (**FESR**).

2.3 DISCUSSION

Sturaro [80] investigated the sources of variation and genetic parameters of some qualitative and technological aspects of dry-cured hams.

On 3370 raw hams for dry-curing (from 61 slaughter groups) he recorded traits for weight losses after salting (14 days), resting (130 days) and at the end of curing (307 days). Average of seasoning weight loss was 27.5%, after resting and after salting weight loss had an average

value of 19.3 and 3.7%, respectively. Data were analyzed according to a linear model accounting for slaughter group, sex and carcass weight as sources of variation. Heritability and genetic correlations between weight losses were estimated using a multivariate REML (Restricted Maximum Likelihood) analysis. Results showed that estimates of heritability were moderate ranging from 0.15 for weight loss after salting to 0.25 for seasoning weight loss. Genetics correlations among weight losses traits were higher than 0.75.

Basing on these results, traits are very likely to be included in a genomic scan for QTL with the aim of finding candidate markers to be used in marker assisted selection for increasing response on selection.

Instrumental and subjective firmness traits were recorded on 2058 and 3275 dry cured hams respectively. Instrumental measures of firmness were strongly correlated and panelist evaluations showed coefficients of genetic correlation nearly to the unit with the instrumental measures collected on *semitendinosus* and *biceps femoris*. Analysis of variance evidenced that the higher was the carcass weight the lower was the firmness of the product.

Relationships between firmness of dry-cured hams and qualitative and quantitative traits of weight losses were analyzed with a logistic regression performed on 2294 linear evaluations of dry-cured hams firmness. Breeding values (BV) of weight loss parameters, estimated for the sires of pigs evaluated for firmness, were included in the model as possible risk factors. Results showed that low weight losses during seasoning and slow instantaneous velocities represented risk factors for the development of insufficient firmness of the final product. Moreover a strong relationship exist between weight loss and firmness of dry-cured hams. Hams with genetic predisposition to have high weight losses favoured the production of firm dry-cured hams with respect to those with low weight losses and instantaneous velocities.

The genetic parameters of iodine number were evaluated on 527 hams. Analysis of variance was performed according to a linear model which considered slaughter group, sex, weight loss and fat covering thickness of raw ham as sources of variation. Results showed that the higher the carcass weight and the thickness of fat covering, the lower the iodine number. Moreover genetic correlations between iodine number and poliunsaturated fatty acid profile were high ($h^2 = 0.42$).

Among the computer image analysis traits, fat eye area was found highly correlated only with its related traits. A negative genetic correlation was found with the lean area ($r = -52\%$) and non significant correlations were found for total cross section area. Fat covering ham depth was highly correlated to total section area ($r = +57\%$) and negatively correlated with percentage of lean area ($r = -69\%$). Fat covering ham area was correlated with total fat area at 99% indicating that the two traits could be consider as one single trait and negatively correlated to percentage of lean area ($r = -77\%$).

Fat eye area, a very important economical trait of dry cured hams, is very variable and genetics effects have not been determined up to now. This trait is another very likely candidate to be further studied in a genome scan analysis, it is a very expensive trait to measure but very important economically. The source of variation could indicate that it is still segregating in the population and genetic improvement using marker assisted selection (if direct markers will be available) could lead to a significative genetic improvement and response on selection.

Part III

GENOME SCAN USING STR MARKERS

GENOME SCAN USING STR MARKERS

Starting from spring 2006, a survey on public available databases for pig microsatellite markers was performed as first step for the whole genome scan object of this thesis.

3.1 INTRODUCTION

3.1.1 *Pig sequencing project*

In 2003 the Swine Genome Sequencing Consortium (SGSC), formed by academic government and industry representatives, was set up to provide coordination for sequencing the pig genome. Currently, pig genome sequencing is underway at the Wellcome Trust Sanger Institute and the first release of the high coverage assembly for chromosome 1 to 18 and the X chromosome has been recently published (Ensembl Assembly and Genebuild release Sscrofa9 - April 2009, http://www.ensembl.org/Sus_scrofa/Info/Index). The assembly used an integrated highly continuous physical map of the pig genome as a template for sequencing (Humphray et al. [44]) and the database version 56.9 accounts for a total $2.39 \cdot 10^9$ bp of which 2798 known protein-coding genes, 9733 projected protein-coding genes, 4962 novel protein-coding genes and 45937 genescan gene predictions.

3.1.2 *Pig genetic linkage map*

One of the most important milestones in pig molecular genetic of the last two decades was the developing of the MARC genetic linkage map by Rohrer et al. [65]. A comprehensive map of the pig genome was firstly available as a starting point for positioning markers and genes on the whole pig genome (18 autosomes and 2 sexual chromosomes). Since then other maps have also been published (for a public repository for genome mapping data see: <http://www.thearkdb.org/arkdb/>), the same MARC map, was released as v2 and it is currently the single largest pig map and its markers are used by most QTL studies for genome / chromosome scans.

Available informations on map position and microsatellite markers details have been a valuable tool for the setup of the whole genome scan presented here.

3.2 MICROSATELLITE SELECTION

A survey on the available public databases (NCBI's UniSTS <http://www.ncbi.nlm.nih.gov/unists>, the ARKdb at Roslin Institute <http://www.thearkdb.org/> and the pigQTLdb www.animalgenome.org/QTLdb/pig.html) was performed as a first step for the selection of microsatellite markers used in the genome scan.

Informations regarding UniSTS available in spring 2006 were collected for a total of 1274 markers. The database was populated with

*UniSTS: Unified
Sequence Tagged
Sites*

data consisting of: name of the marker, type of marker, sequences of forward and reverse primers, temperature of melting, expected PCR size, number of alleles detected in previous studies, UnSTS ID, chromosome, map position along chromosomes and references to published studies for association mapping of markers with common traits. Table n.5 summarizes data collected in the database.

Table 5: Summary of markers data collected in the database

No. of markers	1274
Average Tm	58.95 ± 2.87
Average No. of Alleles	7.08 ± 3.96
No. of references	974

From this dataset, 269 microsatellite markers were then selected for genotyping to cover the whole genome uniformly; selection was made upon the following criteria:

MAP POSITION. Microsatellite markers were firstly selected to span uniformly the whole genome. By using 269 microsatellite markers covering the whole genome there is, on average, one marker every 9 cM (Total length of the MARC Map is 2422 cM).

NUMBER OF ALLELES. Markers that mapped to the same position on a chromosome were then sorted by the number of alleles found in previous studies to avoid the selection of uninformative markers.

ASSOCIATION. Microsatellite markers were then selected with emphasis for already proven association with meat and carcass traits in previous studies.

TECHNICAL PROPERTIES. Tm and PCR size were also taken into account for the selection of markers to facilitate amplification and pooling of amplicons.

A list of selected microsatellite markers together with sequence of primers, map position and other informations is shown in table n. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25. Position of markers along chromosomes in the MARC Map is also shown in figure n.5, 6, 7 and 8.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW1824	AGTAAATATGGAATTAATAATCGCTG	TCAAGCTGGAAACACAGGTGTG	58	1	3	252553	A	6FAM
SW1332	GCATAATGCTGCAGGTACGG	CAGCCTAAGCCAAAGTATGTGG	62	1	29.2	252426	X	6FAM
SWR2300	TTAGTCTCCACTGTGCGGC	TCAAGGGATACACAGAAAGGATG	60	1	33.4	252754	X	PET
S0008	GAGGCAGTGTGTTCTATTCA	GCCATGTGTAAGTGTITGCT	55	1	43.5	252008	N	VIC
APR15	ATTGCCCAATGGAAAATAAATG	TCATTCCAGTGACATTGCTTG	55	1	47	253157	O	VIC
SW2130	TCCAAGTACAATGTGGTTTCTG	AAGCTCTTCCACTCTTCCAGG	58	1	49.4	252736	Q	PET
SW1616	AGAAAGGCCACCTACGTGC	CCCCTATAGATATCCAGTCCCC	62	1	69.3	252496	D	PET
S0313	TGGGTCAAAGTCAACATGTG	GATCTTAGTGGCCCAATTGTG	60	1	78.7	252446	T	VIC
S0113	AGCCTCCGTGTAATATAATCCTTG	AGGACATCTCTCATTTCTTGGCAG	60	1	80.5	252441	C	NED
SWR982	TTCCAATTCGACCCCTAGC	GCAITCTCCATGCAGATAATCC	60	1	86.2	252316	O	6FAM
SW1092	CCTGCTATGTCTTATTGGGAGG	GATCCTGCATTGCCAAAGG	62	1	95.8	252227	O	6FAM
SW974	GGTGAAGTTTTTGTCTTTGAACC	GAAAGAAATCCAAATCCAAACC	58	1	102.9	252064	P	NED
S0302	GCCTACACTATTCAAGACTC	TGCAGCCCTAAAAAGACAAA	52	1	102.9	253041	T	PET
SW1828	AATGCAITGTCTTCAITCAACC	TTAAACCGGGGCACCTTGTG	55	1	118.5	252694	F	6FAM
S0112	AATCCTGAGTATCCTTAATCAGGC	TTGACATGATGCAGAGAAGGAGTC	58	1	121.3	252440	I	6FAM
S0056	AAGCCACAICTCTCTTCT	ATAAGTTGCCCCCTACAC	65	1	127.1	253059	A	VIC
SW1301	TGGATAAGCAATGAGGTCCC	TAGTGGATTTATAATGTGCTAACCC	55	1	140.5	252355	I	6FAM
SW2512	TCTGTCCATCCCTCCATCTC	AGAAAGTGAAAATTGAGCTGGG	58	1	144	252811	F	6FAM

Table 6: Microsatellite marker list for SSC1. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW2443	GAGCACAGAAGATTTTAGGGC	TTAGTTTCTCCTGGGCTGTG	58	2	0	252672	I	VIC
SWC9	GGCTCAGGATCCACAG	AAGCACCTGTACCACACAGG	65	2	0.6	252302	A	PET
SW2623	TCGGAGAATGAGGTAGCTGC	GATTCCACTCTGCTCGAGATG	58	2	9.8	252924	B	6FAM
SW256	ACAAAAGCTTTTGAGAACTCG	TAGCATAGGAACAGGTGCAGC	62	2	19.2	251881	E	6FAM
SWR783	CATACCTGCACATCTCTTCAGC	GCAGCTATAGCTCCGATTTGG	62	2	23.7	252201	I	PET
SWR1910	GGACCCTACTGTAAAGCACAGGG	CATTAACTCATTGAGCGAGGC	62	2	24.7	252968	C	PET
So141	GATCTGCTCTGTCTTGTTCCT	AGACCCCAACTCTTGGTCTCAT	65	2	31.2	252684	E	NED
SW2445	TCCCTTATCCAGAGCAC	GAAGATGGGAAGTTTGTCTTGG	58	2	31.2	252610	U	VIC
SWR1445	CTGGGAACCTCCAAAATCAAATG	AGGAGTGGCCCTAAACACACAC	65	2	32.2	252399	U	VIC
SW240	AGAAATTAGTGCCTCAAATTGG	AAACCATTAAAGTCCCTAGCAAAA	60	2	42	251849	E	VIC
SW1564	ATCAGAACATAGAACGTGTGTG	GTTATATACCCTGTTGGGAGACG	58	2	55	252494	G	6FAM
SB45	GGTGTGGCCCTAAAGAGAAA	CTCCCCCAAGAGAGTTGTG	65	2	63.2	253307	O	NED
So091	TCTACTCCAGGAGATAAGCCAGAT	CAGTGACTCCATGCACAGTTATGA	60	2	64.3	252032	D	VIC
SW354	TGGCTTCTACGCCCTCCAC	GGTTCTCCAACAACATAGCCC	60	2	64.8	251891	U	NED
SCAMP1-4	CAGAACTGAGGCTAAAGTAC	CAGAGTTGTAGGACTGTAGAG	60	2	72.6	253257	U	6FAM
So010	TTAACATGGCTGTCTTGACC	GTCCCTGTCCAACCATAAGA	50	2	77.9	252010	H	PET
SW1408	CAGCCCTGTCACTTGAGTAGC	TTCTGCTCTACAGCAAAAGCG	62	2	88.5	252385	O	PET
SW1879	AGACACATGCACATGTGTTTTAC	AGCATTGTTTCTGTTACTTTTAG	58	2	102.1	252560	I	NED
SW2514	CATGTGCTGTCAAGCAG	AAGGAGGTGACCCTGTGCG	62	2	104.3	252902	I	NED
SWR345	AACAGCTCCGATTCAAACCC	TACTCAGCCCTTAAAAGGAAGGG	62	2	114.4	251978	H	NED
So036	AGTGACCGTGAGGGTCTCTCCCTC	ATGGACCGTGGATTCACACAGCC	62	2	132.1	253055	L	PET

Table 7: Microsatellite marker list for SSC2. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So213	GACCTCAAGCCTGGGAC	GATAGGACGCCCTTCTGGGT	58	3	7.8	252791	X	6FAM
SE47351	GCTGGTCCCTGCTCTTG	AGGCTCAGAGGGAAGGAGAG	58	3	10	253468	P	NED
SW2021	GCGACACATGAGATAAACTGC	AATCCACAGGCTTACTCAGATG	60	3	12.4	252661	K	6FAM
SW2429	TCTTTTAGGTGGAGGATGG	CAITGCCCTATGAACTCTGTG	58	3	17.3	252605	F	NED
SWR1637	CACTGTGCCACAACAGGAAC	ATGAGGACCGCAGTTCAATC	60	3	27.6	252507	N	VIC
SE47329	GCGACCTCTGACTTATTCTGC	AAAACCAGGCTAGGATCGTG	58	3	33	253470	P	6FAM
So206	TGGGTGTGGTCAACAACCAA	ACGTGCCCTGCCCTTACCATC	63	3	42.3	253062	C	VIC
SW139	CGACACCCCTTGGGTTTTG	ATCTAAAATGGGCCCTTTGGG	58	3	52.4	251910	A	6FAM
SWR756	CAGATTTGTTTTCTGCTGAGCC	GATCCTGAATTTTTTTTGAAATT	58	3	61	252296	F	6FAM
SW828	AGAATACAGCACAGTGCCTGG	ATAITCCCAGTGTCTGCCAGC	64	3	64.8	252204	O	PET
SW2047	AGACAAGACCCAGGTGGGTG	TTGGGTTCTGAAAAGCCG	58	3	75.3	252664	K	VIC
So167	AAACTCCAATTTCCATAACATAGG	CTTCATAIATGTGCTAAGACTTCT	60	3	84.7	252641	I	PET
SW2408	AGACACTTGTAGTCGCTCCTCC	AGACAAAAGGGGATGCCAC	55	3	94.2	252595	D	6FAM
SW349	CCTGTTGTAGGCTCCATGAG	CTAGGAGTCGGCCCTGAAC	60	3	112.6	251884	T	NED
SW2532	TTCGACACACAGGTTTTAGGG	GTGGAGGCTTCTGAAAATGTACC	55	3	129.3	252788	G	NED

Table 8: Microsatellite marker list for SSC3. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So227	GATCCATTATAATTTAGCACAAAGT	GCATGGTGTGATGCTATGTCAAGC	58	4	4.1	252796	B	PET
So301	CCGCTTACTTAGGATGTTT	TGATGTGTTTATGTGTTGA	60	4	27.1	252794	B	6FAM
SW835	TGGCTCAGAGTTTTCACCTCG	CAGAGGTTTACCAGAAGTTTGGC	62	4	27.1	252205	J	PET
SW2547	AGATGCCATTAGTGGATGTGC	GACCTGGCTACTCCACTTCC	58	4	29.8	252911	U	6FAM
So001	TGGATGGTCTCATTTCTCAG	TGATTCCTAGCCCTGAGAAGC	58	4	41.8	252001	A	PET
So145	AGAGACATAGAGTCGAGAGG	CACAITTCTCATGGATACGAG	55	4	49	252622	J	VIC
So175	ATATAAGCAAGATGGGTCCGT	CAGGCATAGTCTACTGTGA	58	4	55.9	252643	I	PET
AFABPMS	GGGAACCTTGAAGTCTTTCTC	GGTACTTTCTGATCTAATGGTG	60	4	56	253496	U	PET
So217	TGTGATGCAGGCTGGCAG	GCCTCCTCATCTGGGGTC	58	4	69.6	253028	S	VIC
SW1364	TGGTGCCCTCAATTTCTGTATCC	ACAACCCTTTCATTTGCTGAGGG	60	4	72	252374	E	PET
So214	CCCTGCAAGCGTTTCATCTCA	GGCTGTGCCAAGTCCCAITTAG	58	4	79.3	253064	E	PET
SW512	TATAGTGCAGTTATATCTCAATACAAATGG	TCTGACATTAATACAAACCACCCCC	58	4	80.5	252174	C	NED
SW524	ACCAGGTTGAGTACACATCTGC	AGGTCTGGTACCCGCTCCTG	58	4	99.3	251874	H	VIC
SW445	CCTCCCTGGCACTCAATTG	CACACACACAAGCAGGTGC	58	4	105.8	251923	A	6FAM
SW856	AGGGGGTGGGTGATTGTG	AACTTCCCCATGCTGCTG	60	4	130.1	252156	G	VIC

Table 9: Microsatellite marker list for SSC4. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SJ024	TTCCTTGCTTCAGACTTATAATGCTTGC	CCAGGGGCTCAGTGGATTAAAGG	60	5	0	253274	K	NED
SW413	CAGACACACACCCACAGTGC	AGGTCCAACCCCTCCTGTATG	58	5	8.4	251872	F	VIC
SW491	TTTAAAGCCACTGCACCAGG	CAGGGAACCTCCTCATAGTCCC	58	5	31.5	251953	B	VIC
SW1482	ATTGCAGACTACAGTTCCTTGCC	ACTTACGGGCTGATGCTGTC	62	5	39.9	252475	B	PET
SWR453	TTGAAATTTTTTCATGGAAACC	TCTGGACTTGTGTGACTGTG	58	5	57.9	251985	N	NED
SW2425	ATCTCCATAGGTCAGAGGCTC	ACTCTGTGAGACATTCCTGTATTCC	58	5	72.3	252680	X	VIC
SW332	TTTTCAATATCACATTCACCTCATGC	TTTACAAGTGGGTAGATTAAITA	60	5	73.6	252138	V	VIC
SW1633	AGCAAGGACCAGCAACTTG	ACTCCCTCTTCCTTCCCTTCC	58	5	79.4	252505	O	VIC
SW2003	CATGGAAAAAATGTAATTGTGG	CAGGAAACAGGGATAAAGACAC	60	5	82.4	252719	P	PET
S0005	TCCTTCCCCTCCTGGTAACTA	GCACCTTCTGATTCCTGGGTA	58	5	88.2	252005	D	PET
SW904	CCCCTTTCAGAAAGAATGAAAA	CCTAGTGGCCAAACACCAAAGT	58	5	109.4	252079	I	VIC
SW986	AGGAAGCAAAAATCTTAAGAGGC	GGTGAGCCAGGAACAAGTATG	60	5	115.4	252244	J	NED
IGF1	GCTTGGATGGACCATGTTG	CACCTTGAGGGGCAAAATGATT	58	5	118.7	250858	A	VIC
SWR1112	CTGGGTTTTGTTTCTGTTTTTG	TGGCTTGGGAACCTCCATAC	58	5	130	252088	V	PET
SW1954	GATCGAACCCACACCACAG	TCATTTGGAATAAAGGGATTTC	62	5	130.3	252863	N	PET
SW378	ATTATGCACCCCTACTCCCC	GATTTCTCTTTTGTGTTGCCCC	62	5	134.4	251981	O	VIC
SW967	AGCAGACTGTTTCATCTGTTTCAG	GGGGCAGCTGAAAAGTCC	60	5	145.9	252218	F	VIC
SY12	CAGCAAAGTAACCCAGTCTCTC	ATGAAAATGTTTCCCATCCGG	58	5	152.5	253171	P	VIC

Table 10: Microsatellite marker list for SSC5. STR: Short Tandem Repeats; Chr: chromosome; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So035	GGCCGCTTATACTCTCAGCATTA	CCAAATTAACACAGCAGGCAGCCCT	55	6	7.3	252619	F	NED
SW2406	AATGTCACCCCTTAAAGACCGTGGG	AATGGCAAAACTCCTGAAATTAGC	68	6	21.4	252593	A	6FAM
SW1353	TACTTGTTACCCCCCTGCCCC	AAGTAGCGCAGGTCAGTCTGAG	58	6	29.2	252372	V	VIC
SW1841	TTCTCGAATCTGACCATGACAC	AGCTTCACTGATAAGGAAGTCACTG	65	6	41.5	252556	E	6FAM
SW1057	TCCCCTGTTGTACAGATTGATG	TCCAATTCCAAGTTCACACTAGC	56	6	47.1	252223	U	NED
So087	GACAAGCTCCAGGAAGCTTTCTCTG	ATTGCCCTTGATFCCCAAGGGCA	58	6	62.8	252028	G	PET
SWR1130	ACCTCAACGAACTTGC A AAG	ATATGCCATGGGTGTGGC	55	6	65	252267	L	VIC
SW133	GGCCTGAATTACATATGTTCCC	AATGTGGCAACAAAAACA A AAG	58	6	77.2	251958	A	NED
SW316	TTCTCCAGCCATCATGAGTGTG	AATGACCATTCCTGAGGCTG	65	6	89.3	251948	N	NED
SW1473	TAAGGCTGAATCCACCGCTG	ATGCAAAAGATGCCCAGATTTC	60	6	93.9	252405	E	VIC
So003	G AAGTGTAAAGGA A AAGCCTT	AGCCTCAGTTTCTCTACTTA	50	6	102	252003	P	PET
So228	GGCATAAGGCTGGCAGCAACA	AGCCACCTCAITCTTACTACT	55	6	105.2	252792	J	NED
SW917	AATCTTGGAACTATGGCCC	CCAACA A AATTCAATCAAGTTG	60	6	107	252212	M	PET
So299	TTCTGTGCTTGACTATTGG	AGCATGGCTGACCTCATCTA	60	6	108.7	253040	J	6FAM
So121	TTGTACAATCCACAGTGAATCC	AATAGGGCATGAGGCTGTTTGA	58	6	116	253023	A	VIC
SW322	CATTCAACCTGGAATCTGGG	TCCCTGGAAAAGGCTACACCC	58	6	149.8	252041	H	NED
SW1069	GGCTGTTTTGGTTGTTGTAGC	AAAAAACCACAATGCTGGAGG	58	6	155.2	252224	E	NED
SW2419	AGGGCGTGCTCTTCTA A CTG	TGACTCAGCATCTCCTGCC	58	6	161.4	252602	M	NED

Table 11: Microsatellite marker list for SSC6. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So025	TCTCCCTTCCCTCCATCTCT	CTCCATCAGCCAAAACAT	60	7	3.7	252331	I	6FAM
SWR1343	GATCTGGCAATCCATGTGC	CTCCTAGCCTGGAAACCC	62	7	12.2	252370	N	PET
SW1354	GAGCCAGATTAATGCAGTTGC	CCTAGTCCCGAGCGGTAATC	55	7	22.3	252456	I	NED
So064	TGAGCTGGAGGTTAGCTACC	TGTCAGAAAAGACTGCTTGCG	62	7	30.2	252015	E	NED
SW2155	AGGGTGACAGACCAGAATGG	TCTGGGTCACAGGGAATTC	58	7	32.9	252740	R	6FAM
SW1369	AGCCTTCTCTGGCTCATGG	TCAAATGGAATCATCTTCCC	55	7	48.2	252458	C	VIC
SW1409	GTTGTGCCAAATTTTGCTAATC	CCTGGATAGACCAATGATGG	60	7	57.1	252464	L	NED
TNF	CTGGTCAGCCACCAAGATTT	GGAAATGAGAAATGTGGAGACC	60	7	58.1	251845	Q	6FAM
SW1856	TCATTCCAAACACACAGAGTCC	TTGTATGGTATCCTGTGATGCC	60	7	61.5	252800	C	PET
So102	GTCAAAGCAAACITCCACGCT	ATTTTGTGCCAAAATGCATTGTG	55	7	70.1	252631	O	PET
SWR1928	TAGGGTCAGTGCAATCCTTCC	ACGAGAACTCCGAACCCCTG	60	7	79.3	252972	B	VIC
SWR1806	TGAAATAACCAGGAGTTTCCATC	TGGAACATGATGGAGGATAGTG	60	7	80.8	252690	Q	VIC
SWR1121	TCTGTTGTAGGCTGGCAG	TTGTATGAAATATATTTGCCATTG	62	7	82.8	252228	M	PET
SW147	TTGCCTTTCTCCATGTGACT	ACAACCTAACCAATTTGTCACAGG	58	7	90.1	252165	P	PET
SW252	CTCTGGGTCCATCCATTTTG	TTATGATGCAAAAACATGGAAGC	60	7	99.4	251967	T	PET
So115	TGATGCACTGTGTGGCCACACCA	ACCATGGCTTGAGCTTGAGCCAGC	62	7	102.2	252443	D	VIC
SWR773	GTGGCTGGGGTATAGGCC	TGCTGAAGCATCCACTTCAC	60	7	117.3	252345	F	VIC
SW581	CCCCAGATTGACTCTAGACTCG	CATGATGGAGGATAATGTGGG	60	7	123.8	252114	H	6FAM
So101	GAATGCCAAAAGAGTTCAGTGTAGG	GTCTCCCTCACACITACCCGCAG	58	7	134.9	252630	D	NED
So212	CCACGACTCAAACCTTAG	TCTTTCTTAGAATAATCTCACAT	60	7	141.2	252795	C	6FAM
SW764	TAGCAGATTGTTAGCCTCTGTG	AAGCATCTTTTCTAAGCACAAACA	62	7	156	252051	L	6FAM

Table 12: Microsatellite marker list for SSC7. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So353	TCTGTGGTTTGTAGTTTCCATC	ATGTAACATTAGAGTCTCCAACAAGG	62	8	11.1	252777	G	PET
SW905	AITCCAACCTTCTTCAAAAGG	TCCAGTGGCAGAACCAACATG	58	8	20.8	252240	C	6FAM
KS101	AAGTCTTATTCTGAGTGTGAATCC	GTTAAGGATCCAACATTGCC	58	8	27	253211	Q	NED
KS195	CAGGACATTGTCAAAATAGTGG	AGCCATGTAGATTGACTCCATG	58	8	32.6	253294	K	PET
SWR1101	AACTTCCATATGCCACAGGTG	GGTCTCCTCAGAAAAGTCCC	58	8	38.3	252132	O	6FAM
SW7	TAACCATGCTTTTCCTAGGTGG	CCAGAGCTGAGTAAAAAAGTCA	55	8	55.4	251831	X	PET
KS112	CTTGTTAAGTGCCTTCCTTGGC	CACATGCTGCAGGTGTGAC	58	8	58.2	253220	K	PET
So017	CTAGGAGAAAATCTGAGGTT	GTTTGAATGGAGGTGCTGTA	58	8	60.4	252323	N	6FAM
So225	GCTAATGCCAGAGAAATGCAGA	CAGGTGAAAAGAATGGAATGAA	58	8	82.8	252774	B	NED
SW763	GGGTGCATTGTTCTCATATGG	TGCTCTIAGCAACACACACACC	58	8	92.4	252050	L	VIC
SW1551	TTTACTTGGGAAAACCCCTCC	GATCAACCCAAAATTCTTGGC	58	8	105.9	252414	U	PET
SW61	GAGAGGGATGAGCACTCTGG	AGAGCATTCAGAGCCTTCTCA	55	8	112.3	251835	B	NED
KS141	CAAGCCATTGATGCTTCATG	GGGTTTGATCCCTGGTCTG	62	8	120	253238	K	VIC
So178	TAGCCTGGGAACCTCCACACCGCTG	GGCACCCAGGAATCTGCAATCCAGT	58	8	127.7	252770	J	NED

Table 13: Microsatellite marker list for SSC8. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW983	GCAGTCCCACCTTTAGGTATATATCC	ATAATGCTGCTATGAACACTGTAGTG	60	9	4	252123	F	NED
So024	AAAGAAGGAAAAGAACTGATA	ATGGAGGATAATGTGAAAAA	55	9	27.4	252330	J	PET
SW911	CTCAGTTCITTTGGGACTGAACC	CATCTGTGAAAAAAGCC	60	9	36.8	252211	B	PET
SW2401	TGAACAAGTCCAACCAAGAGC	CCCAACTAACGGGCITGTG	60	9	57.1	252591	T	VIC
SW2074	ATGTGATTATCAITTTGTCTGTAGCC	ATCCAGATTATGAGACACTCTACCC	60	9	65.4	252589	U	6FAM
SWR250	CACTCAAATGCTCGAATCAAGC	CTGGGGCTGTGGTGTAGG	55	9	73.3	251966	D	NED
SWR1939	CTGGACTTAAACACATTGAATGC	TGCTGCAGGTATGACTCTAAAC	58	9	85	252570	C	NED
So019	TTCTTAITTTCTCTGTGTCTT	ATTGTTTCCCTTTCTTCTGA	60	9	86.4	252325	L	NED
So295	GCCTAAAAAGACCCAAAGAA	TACTGCTGAGGCCAAAGGA	60	9	100.5	253038	K	6FAM
SW2093	ATGCACCTGCTCAGAGGC	GTCGTGTGAGGCCCATGGG	60	9	103.6	252729	I	VIC
SW174	GCCAAAATAGCTAATGGACAGC	TCATGCTAATTTTGTCCAGATG	65	9	122.9	251939	O	NED
SW1349	ATTTAATGTTTTTCATTTGTGCCG	CTTACATGATGCCCAAACTGG	62	9	142.5	252455	A	PET

Table 14: Microsatellite marker list for SSC9. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW830	AAGTACCATGGAGAGGGAATG	ACATGGTCCAAAGACCTGTG	58	10	0	252099	K	NED
SWR136	TTCTCTGCCCTCAGCTCACTG	CTGGGACCCCTCCATATGATG	55	10	7.6	252164	D	6FAM
SW443	ACAAAAGGCCAAGCCACATAC	TCACCAGGTTTCTGGGTTTC	55	10	20.4	251921	K	VIC
SW1894	CTCAGCTGCAAAAACAGAGTCTTG	CCTAGGTTCTAGGCTTCTAGGTTG	58	10	23.2	252648	Q	PET
SW2491	GTGTTTGAAGGAAGTGGTAGC	GTAATGCACAGGAAGTGAACAGC	62	10	43	252760	J	6FAM
SW2195	TCCTGAGAGGCTTAGGATGG	TCCCTTCTAATGGGGTGTGTG	58	10	44	252753	V	NED
S0366	TGGATTGGTCTCCCTTCTG	CTCCAAGGTCATGTTTCCCTACTT	65	10	56	253128	L	NED
S0070	GGCGAGGCATTTCAATTCACAG	GAGCAAAACAGCATCCGTGAGC	65	10	62.3	252020	A	VIC
SW1041	ATCAGAAAATGTCACACAGTTCA	GGAGAATTCCCAAAAGTTAATAGG	58	10	67.5	252105	E	PET
SW2000	TTCCCTCGTGAAAAACCCCTC	CACCTCAGCCCCCAGACACC	65	10	86.3	252802	M	6FAM
SW305	AGCTTTCATTTTTTTTAACCCATC	TCACCTTTCAACCCATCACC	62	10	94.5	252112	I	PET
SW951	TTTCACAACCTCTGGCACCAG	GATCGTGCCCAAAATGGAC	60	10	101	252241	B	VIC
SW2067	GAAGAAATTAATGCACCGTCCC	TTGCTGCTTGTGCCCTTTG	58	10	128	252667	J	PET

Table 15: Microsatellite marker list for SSC10. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So385	CTATTAGGCTGGAGGGTIG	AGTTCAGAAAGCTGTGCT	62	11	0	253081	L	PET
So392	TAGCAGATCGTCTAGCACT	CGCTCACCTCCTACTCCC	58	11	1.9	253085	L	6FAM
So391	CTTCCATTCTTTTTCATGGC	TGCGGTGTTATTTGCAGCA	58	11	5	253084	R	6FAM
SWR2071	TGGGGATGAGGGAAACTTC	GAGGATAAGACCCGCCTACC	58	11	11.5	252668	G	VIC
SW1632	GTTTGACAGATAAGGCTCCTGC	ACACGCTCCCTAATCCCC	58	11	16.6	252504	A	NED
So182	CTCCATATGCCACGGAAAT	CAGTCTTCTCCAGACATAAA	60	11	33	253095	B	NED
SW151	TTCCCCATATGATGAGATGGC	GGTGTGGCCCTCAAAGG	60	11	44.3	251934	R	VIC
So394	AGGAACTTTGAGAGGTATG	TATTTTGGTAGCAGACAGG	60	11	48.6	253086	L	VIC
So395	GCAGTTGGACTAAGGTTTAIG	TCACACTTTTGCTCCTCTCC	62	11	52.9	253087	V	6FAM
SW435	ATCATGTGAGAAAAAGAACATATGTG	TGCAAGAGAACTTCCCGGC	50	11	53.3	252170	L	NED
SW1377	TTCAAAGGTTGGAAAAGACAGTCC	ATGAGGAGTTTGAACATAITGGG	58	11	68.5	252377	E	VIC
SW13	TCTTAGCCAGTGCAGGCAC	GATCAATCTCTAAACTGAAGGTG	60	11	80.5	252161	S	6FAM
SW1844	TTTTATGGCTGAGTAGCATTCC	CAACCTACATTCCATTGAGAGG	58	11	107.9	252557	G	6FAM

Table 16: Microsatellite marker list for SSC11. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
S0143	ACTCAGAGCTTGTCCTGGGTGT	CAGTCAGCAGGCTGACAAAAAC	58	12	6.6	252766	C	PET
S0229	TTGGCATTACTGTCTTTAGTGACGA	GGCCATATCTGGTATTTGGGTGCT	62	12	19.3	252775	E	6FAM
SW957	AGGAAGTGAGCTCAGAAAAGTGC	ATGGACAAGCTTGGTTTCC	60	12	33.4	251996	K	NED
SW874	AAAAAGAACCACAACACTACAGCAGC	TTTATGAGGGTATCCGTGACACC	55	12	64.7	252209	B	VIC
SW37	CTTTGTACACCGCTGGTCCT	GAAAGCCACCCTACAAAATCA	55	12	70.5	251833	V	VIC
S0090	CCAAGACTGCCTTGTAGGTGAATA	GCTATCAAGTATTTGTACCATTAGG	58	12	80.2	252031	L	PET
SW1956	AGTCACCCCTCCTCCAGGG	CAGCATCGGTCTTAAAAACTG	58	12	82	252864	L	6FAM
S0147	AGCTGCAGGCTCCAGATCATCT	GCTGTAAGCAGAGATTAAACAC	62	12	89.9	252637	S	NED
SW1936	TGAAAAATAGGATGAAGAAGGGG	TTATGTGAGCACATGTGACACC	55	12	99.6	252569	A	NED
SWR1021	CGCCACAAGTGAACCTCC	CCGCGGGTCCAGCTATAG	62	12	113.1	252000	D	NED

Table 17: Microsatellite marker list for SSC12. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So282	AAC TTCATAATGCCACAGGTGC	AGTGAACACAGAATGGAGAGCCC	58	13	0	253056	G	VIC
SWR1941	AGAAAGCAAATTTGATTTGCATAATC	ACAAGGACCTACTGTATAGCACAGG	62	13	14.1	252676	B	6FAM
SW935	GTGGTGGTTTTGCCCTCTTATAGC	ATATAAGGGAAAATAAATCTGAAAAGAGTATG	58	13	25.4	252213	R	NED
SW344	AGCTTCGTGTGTCAGGAG	GTAGTGTCCAAAGAGAGTGCC	55	13	35.4	251977	U	VIC
SWR1008	ACAGCCACCAACAGTGTTTG	GAAC TTCATATGCTGCAAAGTG	51	13	53	252067	F	NED
SW2448	CTCAGGGACTTATCCCTCAGTGG	GAGGTGGGATTTGGTCCAG	60	13	56.4	252611	V	PET
So068	AGTGTCTCTCTCCCTCTTGCT	CCITCAACCCTTTGAGCAAGAAC	58	13	62.2	252018	M	6FAM
SW1550	GCAGCATAATGTTAGCCACC	TGCATCCAACACAGAAAGCAC	58	13	71.7	252490	M	VIC
SW398	AAGTGCCAAATGCTTTGTTC	CGGAGGAGAAATAAGGGTAGC	58	13	79.3	251848	K	6FAM
SW1056	GGTGGTTGGTTCTCAAAAACA	TTTCTGGGTACAGCAAAGTGA	58	13	96.1	252262	S	PET
So291	GGAGGGACCCCATCTGACAGGA	TTTTGGTGGGACCGCTCCTGAC	60	13	126.2	253057	F	6FAM

Table 18: Microsatellite marker list for SSC13. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW857	TGAGAGGTCAGTTACAGAAGACC	GATCCTCCTCCAATCCCAT	60	14	7.4	252180	L	6FAM
So356	TAGACATGTAACCTCTGGCTGG	AAGAAACCCATCTCTGGGG	60	14	8.6	252779	M	NED
SW1027	AGCAACCTGAGCCACAGTG	GGAACTTCCACACGCCAC	58	14	21.5	252126	H	6FAM
ESTMS18	GCCTAIGTAGAGGACATAAGGGC	TGGTGGTTAGTGCCACATTC	60	14	32.8	253451	D	NED
SW1709	CATTCTCAAGGAAAAGAGCACC	CAGTAGTGCATGAACCTGCTTCC	55	14	41.5	252532	R	VIC
SW1556	TCCCAGCACCTTGATTTTAG	AGGTTGCTGGAGATAGTGAAGC	58	14	51	252493	M	PET
SW2057	CAAATGGTTAGGATGCATTTG	ATTGCTTCCATCCGGTTGG	62	14	62.4	252586	R	PET
SW1081	AAACTGTAGAACCACGCTGAGC	GACCCCTGTAGCATTAGGACTGG	58	14	72.1	252084	P	6FAM
SW1557	TGCTCTAATCTAACCCGGGTC	CCACCCACACTCCCTTCTG	58	14	87.9	252544	B	NED
SW2515	CCATCTCATCCAGAAAACATCC	AGGATGCTGAGGTGTTAGGC	58	14	108.7	252787	D	6FAM
SWC27	CTGAGACTGTGCTGCTCACTG	CCAATTTCCAAAAAACATGGG	58	14	111.5	252280	P	VIC

Table 19: Microsatellite marker list for SSC14. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So355	TCTGGCTCCTACACTCCTCTTGATG	TTGGGTGGGTGCTGAAAAATAGGA	60	15	13.8	253051	A	NED
SW184	CTCCCTGCATATAATTTCAATCC	ATCCCTAGCCTGGAAATGTC	58	15	21.6	251962	N	6FAM
SW1111	AGGTCCTACTGTCCATCACAGG	GAAAGCAGAGTTGGCTTACAGTG	65	15	39.8	252087	B	6FAM
KS111	TCTTGCTGTGGTAGGTGCTG	GGATGTGCCAGCCCTGTATG	60	15	41	253219	S	6FAM
SW964	GTGGTTCCTCTATGCAGAGTCC	ATGTGATGAAACATGATGGAGG	65	15	50.7	252217	N	VIC
So369	GAGAGGAAGGAGAAAGGAAACAAG	AGGTCTATGTGTCAGCATAAAAGAA	58	15	56	253092	G	6FAM
SW2129	CCAATGCCAGACCACTGAC	GGGTGTGGCCCTAAAATGAC	62	15	67.1	252735	J	6FAM
SWR1002	CAAGGAGTATCTTTCTCACAGCA	CTGGGAACCTCCATAAGCCA	58	15	76	252104	M	VIC
SW2053	AAGCAAGGTGCCACTGTTG	CAGTCTCCTGTAGCCCAAGC	62	15	81.1	252724	X	6FAM
SW936	TCTGGAGCTAGCATAAGTGCC	GTGCAAGTACACATGCAGGG	55	15	88.5	251991	D	PET
SW2608	GCTTGAGGAATTGGCAAATG	GAGCTTGTGAGACAGGAATGG	58	15	95	252922	X	NED
SW1262	TTGGGGCTCACAAAAGTCAC	TTGGTAATTTCCGTATGCTGC	60	15	113.1	252251	Q	6FAM
SW1119	CAACCTCAAAAATGGAGAAAAGG	GTTCTTGGCGGTGTTTGGC	58	15	119.9	252135	M	VIC

Table 20: Microsatellite marker list for SSC15. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
So111	TCAGTATTTCTGGCTAATCATCTC	TTGATGTAGACCACCAGCTAGTG	58	16	0	252439	O	NED
SW2411	CCTGGACTCAITTCCTTGCTTG	TTCCATTTCTGTCCCTGCCCTTG	60	16	16.7	252598	X	VIC
So006	TCTGTCTGGCTTATTTCACTT	CAACCTAAGTGTCTGTCCATC	58	16	22.1	252006	N	NED
SWR340	CATTGGTGAITTCGATCCC	ATGGGCTGGCAGCTACAG	55	16	29	251976	E	VIC
SW1305	TATGTGGGAAGAGAATCTGAAGG	CCCCTAGGTAACCTGTTCTGTCTG	58	16	36.5	252356	N	PET
So363	TAACTTGGATGCTGATAGCAC	CATGGTTAAAAATGTTAAACTGC	60	16	37.4	253127	S	VIC
SW557	TGTCGACTGTAAGATGAATGG	CTTTTGAATGTTCTTTTCCCC	58	16	38	251887	V	NED
SW5	TTCAAGTTCCAITCCTTGTGC	AGTGTCCACAGATGGAITGAATG	60	16	44.2	251900	G	NED
SW262	TACTTGGCTTTTTGTGACCAG	TCAGCCAAAAGGGCTCTTTG	62	16	46.9	251882	S	NED
SW2517	ATACTAATGTGCTTGCGTGG	AAGGAACCCTATGAGAGTACTGG	60	16	55.7	252762	T	6FAM
SW1897	GTGCCGTGGCAGGAACCTC	ACTGCCATTTGTTTCAAAAGTG	58	16	86.2	252703	M	6FAM
So105	ACCATCGTCCAGGTGACCATG	CGCGACCATCTTCCCTGTCAAA	60	16	92.6	252438	Q	VIC

Table 21: Microsatellite marker list for SSC16. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW335	GAGTATGGGGAAGCCACG	CCATCAACAACAACTGTATGCACC	62	17	0	251975	G	NED
SW1891	CTAGGTCTTTTCAACGTAAAGCC	CTGCAGAAAGGAAGAGATGG	60	17	17.3	252561	U	NED
SWR1004	TGGGAACACCTGCTTCATTC	TCCATATGCCCCCAAGTGTG	60	17	17.8	252125	H	PET
SWR1120	CAAAATGGAAACCCATTACAGTCC	ACTCCTAGCCCCCAGGAGCTTC	58	17	26.9	252089	X	NED
SWR1133	TGGGATTTGTTACCACTGAGC	TCCATGGGTGAAAAAAGATG	58	17	30.4	252342	Q	NED
SW2441	TCCAACCTAAATGTCCATCATC	CACAAATGGCATTATTTCATCC	58	17	40.6	252681	R	NED
So359	CAACTTCTGGCTGCAGAGC	CAACTTCTGGCTGCAGAGC	60	17	68	253125	C	VIC
So332	TGGTTCCCTCACCAAGACAAGTAC	CCCAAGGAGCTACAGCAAGGCAAG	58	17	88.7	253073	K	PET
SW2427	GCATGTTATTGAGTTGATGTAGG	TCCGAAATTCAGAAAAATTGG	58	17	97	252603	N	6FAM

Table 22: Microsatellite marker list for SSC17. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SY2	TCCCCCATCTTCTCTCC	AGGAGGAAATACCACAGCC	58	18	-4.4	253161	U	PET
SW1808	CCAAAAAAGTGGACTGTAAGCC	TACGGATGATGGAGACAGG	65	18	0	25251	J	VIC
SW1023	AACCTGCTGAGCCACAGTG	GCAAGTACCCAATCTTTTCC	58	18	5	252258	D	VIC
SW1984	TTTTTAGTGTCCAAGGAGGTCC	GGAGCACTAATAGACCACCACC	58	18	29.4	252678	F	PET
SW787	CTGGAGCAGGAGAAAGTAAAGTTC	GGACAGTTACAGACAGAAGAAGG	60	18	31.6	252202	A	6FAM
So120	GCCTAAGTAGAATTAAGCACAAGG	GTGCTCTCACTGCCCTTCATATACC	58	18	45.2	253071	V	PET
Sj061	GCAGAGGCACCTCGGATGTTAG	ACCCGGTGACAAAGCAGAGA	60	18	46	253279	X	NED
So177	TTCACCTGGGATGGTGTGACAT	ATCCACAGAGTTTACTCAGAC	55	18	55.3	252645	R	PET
SWR169	AATCCATTTTGAATTGATTTGTG	TACAGCTCAGATTTGACCCC	60	18	57.6	252097	V	6FAM

Table 23: Microsatellite marker list for SSC18. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

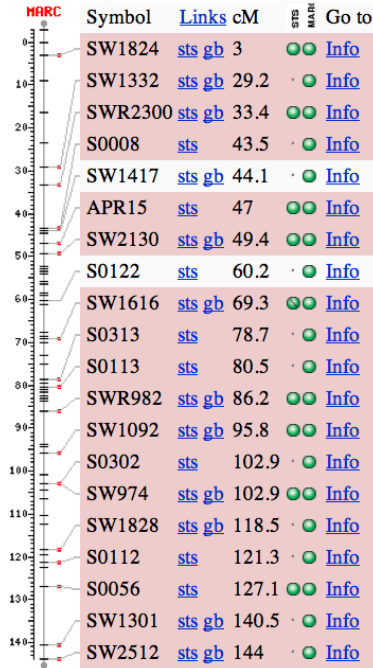
STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW ₂₄₇₀	TAGTACCTAGGCTTCCCCAGG	CTTTGTCTCTCCCTCTCCATAC	50	X	45.8	252615	V	6FAM
SW ₂₄₅₆	GAGCAACCTTGAGCTGGAAC	AATGTGATTGATGCTGTGAAGC	58	X	55.4	252614	F	PET
SWR ₁₈₆₁	TACAGCTCCGGTTGGTCC	TAGCTCTTGAGTGTACTTGCCG	58	X	57.8	252801	G	6FAM
SW ₁₄₂₆	TGGTTGTCACAGTTTATTGGG	TCCCTATCTTCTAAATGCTAGTAGG	62	X	71.7	252393	C	PET
SY ₆	CAITAGGGAAACTCTTGTGGC	TTTTCCAACCTTGGCTGG	60	X	73	253164	T	6FAM
SW ₁₉₉₄	TCTCCAGGTCCCATCCATATTG	TGAACCTCGATGTTAATTGTGG	50	X	74.4	252580	F	6FAM
SY ₁₆	TGTGGTGGAAACCGTGAIG	TAGGTTGACCATCCACATTC	58	X	74.4	253194	T	NED
So ₅₁₁	AAAAACACGGAGCAATAGAAATGTC	GTCCATCCATGTTGCTCCCAAATGG	58	X	97	253998	D	PET
ISU-AR	TGTTTTCCCCCTCTTCCTT	TCCTTTTTCCAGCATAGACC	62	X	200	253191	E	PET

Table 24: Microsatellite marker list for SSCX. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

STR Name	Forward Primer	Reverse Primer	Tm	Chr.	MARC Map	UniSTS	Pool	Dye
SW ₉₄₉	TGAGCAATGAGTTCAATGCC	TCGTTGGTGAAGGCATCC	58	Y	0	251994	J	VIC
SW ₂₅₃₄	TGAGTGAAGGCCCTTACCCAG	TAACGTATAGACCCCAAGTCGCC	58	Y	57.8	252764	H	VIC
SW ₉₈₀	CTTCAGTGTAGTCCAAGTGGC	GATGTTTTGCTGATAGGAAGGG	60	Y	57.8	252243	V	NED
SW ₂₄₇₆	GAGAGGGACAGAGCTGAGAGC	CTTGAGGTTTGATGGCACC	50	Y	77.6	252759	C	6FAM
SW ₁₉₄₃	ATCCCCCTTGACACATTAATGG	TATGGCTGAGTAGTATTCATTTTG	58	Y	87.4	252710	H	6FAM
So ₂₁₈	GTGTAGGCTGGCGGTTGT	CCCTGAAAACCTAAAAAGCAAG	55	Y	114.4	253029	M	NED
SW ₂₅₈₈	TGTCCTTCTCCCCCTCCC	AAAAGCCTGGTGAGGACCC	58	Y	128.4	252917	H	VIC

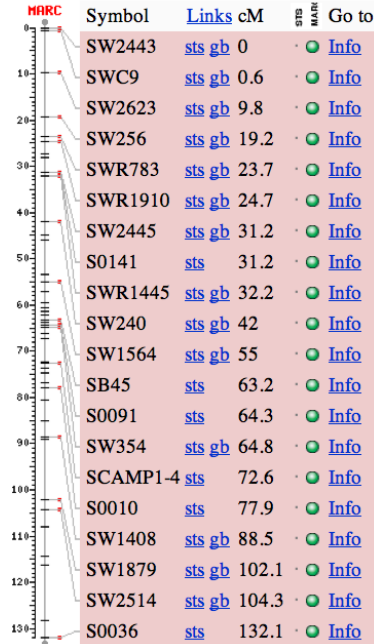
Table 25: Microsatellite marker list for SSCY. STR: Short Tandem Repeats; Tm: temperature of melting; Chr: chromosome; MARC Map: position in cM in the MARC genetic map v2; UniSTS: Unified Sequence Tagged Sites ID number; Dye: dye attached to the 5'-end of the forward primer.

Region Displayed: -3.00-144.00 cM



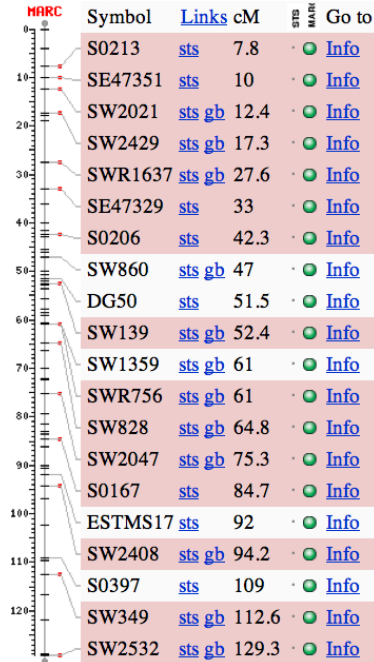
(a) SSC1

Region Displayed: 0.00-132.10 cM



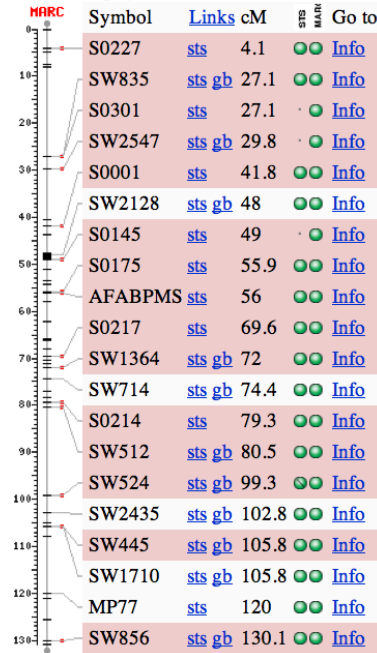
(b) SSC2

Region Displayed: 0.00-129.31 cM



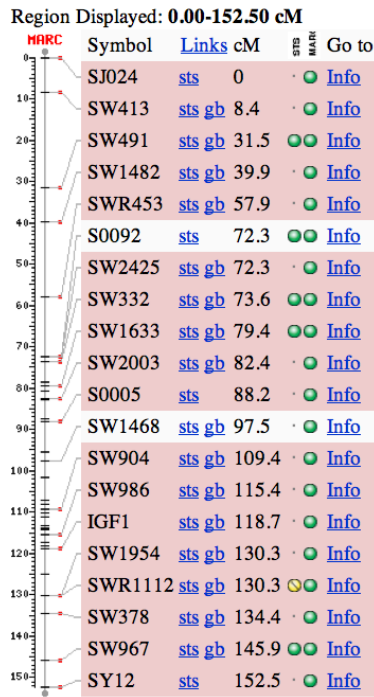
(c) SSC3

Region Displayed: 0.00-131.00 cM

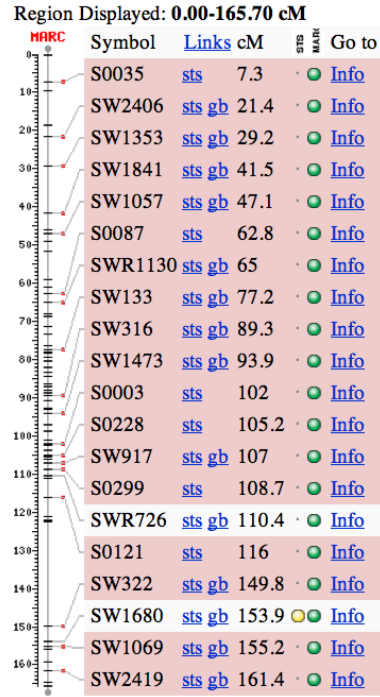


(d) SSC4

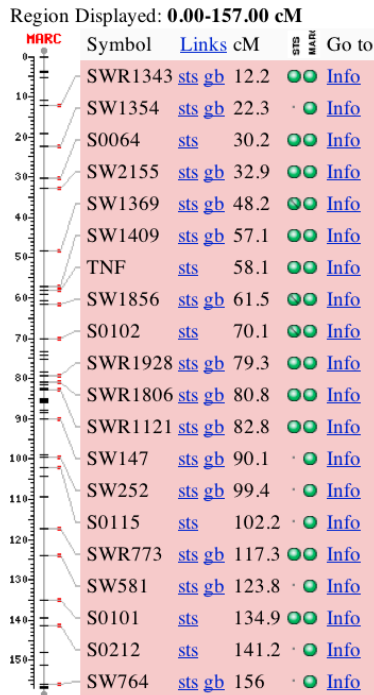
Figure 5: Marker position along chromosomes (source: NCBI Map Viewer <http://www.ncbi.nlm.nih.gov/projects/mapview/>). Selected markers used in this study are highlighted. Sus scrofa chromosomes SSC1, SSC2, SSC3 and SSC4.



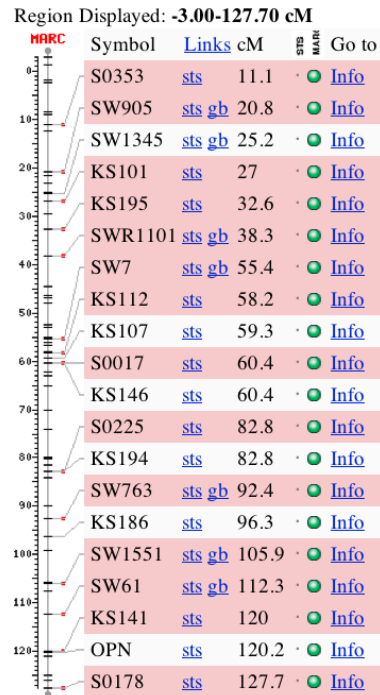
(a) SSC5



(b) SSC6



(c) SSC7



(d) SSC8

Figure 6: Marker position along chromosomes (source: NCBI Map Viewer <http://www.ncbi.nlm.nih.gov/projects/mapview/>). Selected markers used in this study are highlighted. Sus scrofa chromosomes SSC5, SSC6, SSC7 and SSC8.

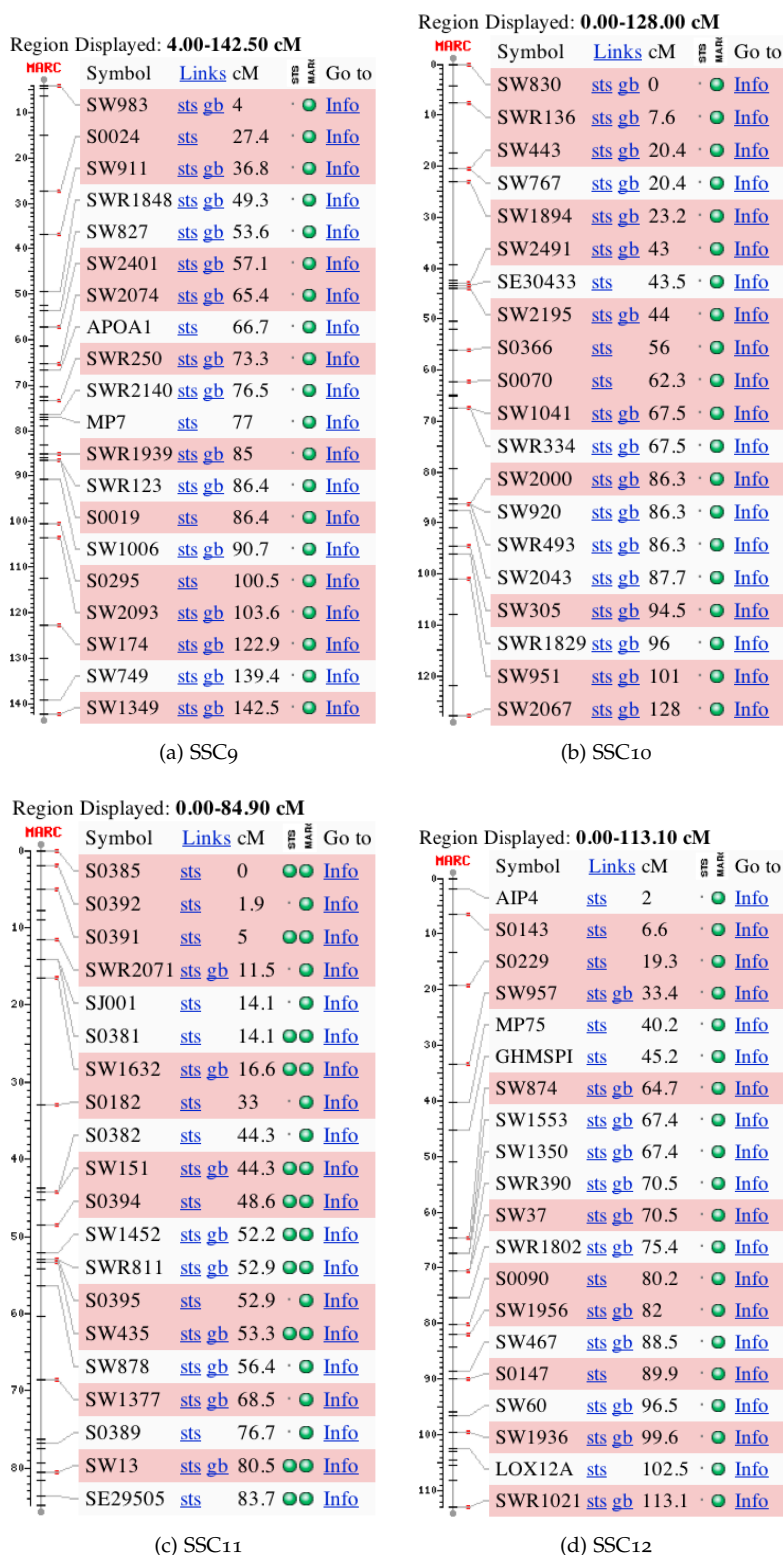


Figure 7: Marker position along chromosomes (source: NCBI Map Viewer <http://www.ncbi.nlm.nih.gov/projects/mapview/>). Selected markers used in this study are highlighted. Sus scrofa chromosomes SSC₉, SSC₁₀, SSC₁₁ and SSC₁₂.

Region Displayed: **0.00-126.20 cM**

MARC	Symbol	Links	cM	STS MARK	Go to
	S0282	sts	0	●	Info
	SN219	sts	1.7	●	Info
	SWR1941	sts gb	14.1	●	Info
	SW935	sts gb	25.4	●	Info
	S0288	sts	35.4	●	Info
	SW344	sts gb	35.4	●	Info
	SWR1008	sts gb	53	●●	Info
	SW882	sts gb	53	●	Info
	SW2459	sts gb	56.4	●	Info
	SW2448	sts gb	56.4	●	Info
	SW1105	sts gb	59	●	Info
	S0084	sts	61.1	●	Info
	S0068	sts	62.2	●	Info
	SW1550	sts gb	71.7	●	Info
	SW1386	sts gb	77.1	●	Info
	SW398	sts gb	79.3	●	Info
	SW1056	sts gb	96.1	●	Info
	SW38	sts gb	101.6	●●	Info
	KS604	sts gb	114	●	Info
	S0291	sts	126.2	●	Info

(a) SSC13

Region Displayed: **7.40-111.50 cM**

MARC	Symbol	Links	cM	STS MARK	Go to
	SW857	sts gb	7.4	●●	Info
	S0356	sts	8.6	●	Info
	SW1027	sts gb	21.5	●	Info
	S0063	sts	31.5	●●	Info
	ESTMS18	sts	32.8	●●	Info
	CATP3	sts	32.8	●●	Info
	S0211	sts	33.6	●●	Info
	SW2439	sts gb	41.5	●●	Info
	SW1709	sts gb	41.5	●●	Info
	SW1536	sts gb	47.1	●●	Info
	SW2519	sts gb	51	●●	Info
	SW1556	sts gb	51	●●	Info
	SW1552	sts gb	53.7	●●	Info
	SW2504	sts gb	60	●	Info
	SWR1042	sts gb	62.4	●	Info
	SW2057	sts gb	62.4	●●	Info
	SW1081	sts gb	72.1	●●	Info
	SW1557	sts gb	87.9	●●	Info
	SW2515	sts gb	108.7	●●	Info
	SWC27	sts gb	111.5	●	Info

(b) SSC14

Region Displayed: **-4.00-124.30 cM**

MARC	Symbol	Links	cM	STS MARK	Go to
	S0355	sts	13.8	●	Info
	SW184	sts gb	21.6	●	Info
	KS197	sts	26	●	Info
	SW1111	sts gb	39.8	●	Info
	KS111	sts	41	●	Info
	SW964	sts gb	50.7	●●	Info
	S0369	sts	56	●	Info
	S0118	sts	60	●	Info
	KS108	sts	67	●	Info
	GLS	sts	67	●●	Info
	SW2129	sts gb	67.1	●●	Info
	SW1263	sts gb	67.5	●	Info
	SWR1002	sts gb	76	●●	Info
	SW2053	sts gb	81.1	●	Info
	SW2083	sts gb	81.1	●	Info
	KS158	sts	81.1	●●	Info
	SW936	sts gb	88.5	●	Info
	SW2608	sts gb	95	●	Info
	SW1262	sts gb	113.1	●●	Info
	SW1119	sts gb	119.9	●●	Info

(c) SSC15

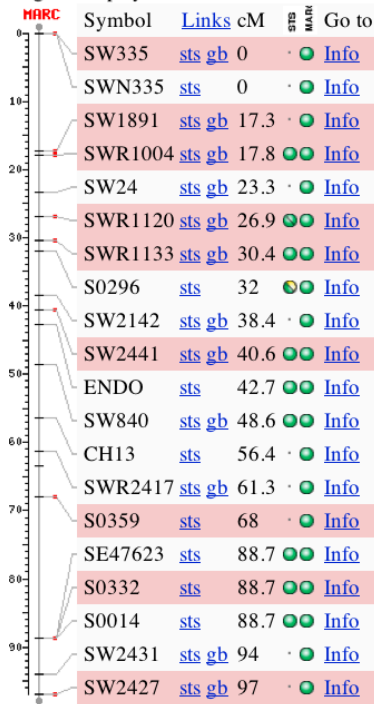
Region Displayed: **0.00-93.20 cM**

MARC	Symbol	Links	cM	STS MARK	Go to
	S0111	sts	0	●	Info
	SW469	sts gb	9.3	●	Info
	SW2411	sts gb	16.7	●	Info
	S0006	sts	22.1	●●	Info
	KS601	sts gb	24	●	Info
	SW1645	sts gb	27.6	●	Info
	SWR340	sts gb	29	●	Info
	SW1305	sts gb	36.5	●	Info
	S0363	sts	37.4	●	Info
	SW557	sts gb	38	●	Info
	SW1341	sts gb	40.1	●	Info
	SW81	sts gb	40.1	●	Info
	SW977	sts gb	44.2	●	Info
	SW5	sts gb	44.2	●	Info
	SW1454	sts gb	44.8	●	Info
	SW262	sts gb	46.9	●	Info
	S0026	sts	46.9	●	Info
	SW2517	sts gb	55.7	●	Info
	SW1897	sts gb	86.2	●	Info
	S0105	sts	92.6	●	Info

(d) SSC16

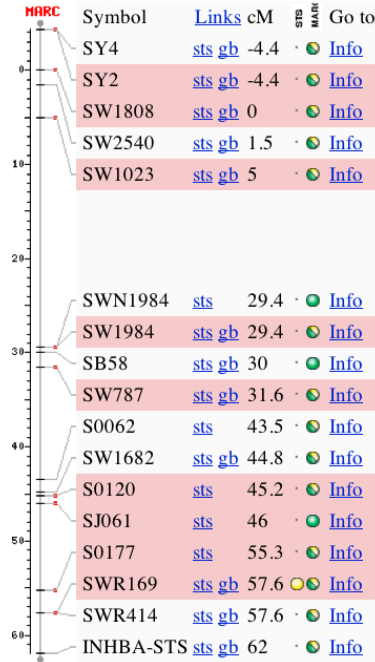
Figure 8: Marker position along chromosomes (source: NCBI Map Viewer <http://www.ncbi.nlm.nih.gov/projects/mapview/>). Selected markers used in this study are highlighted. Sus scrofa chromosomes SSC13, SSC14, SSC15 and SSC16.

Region Displayed: 0.00-97.00 cM



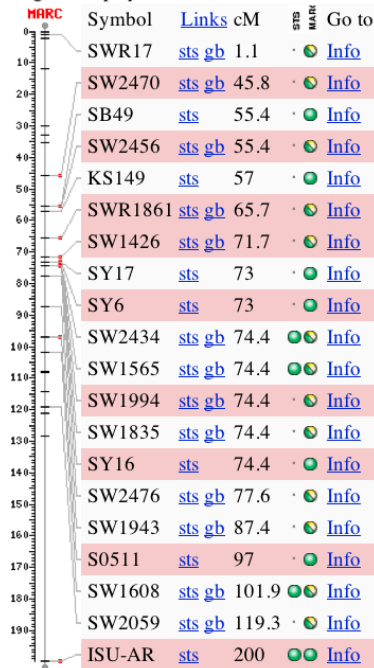
(a) SSC17

Region Displayed: -4.41-62.00 cM



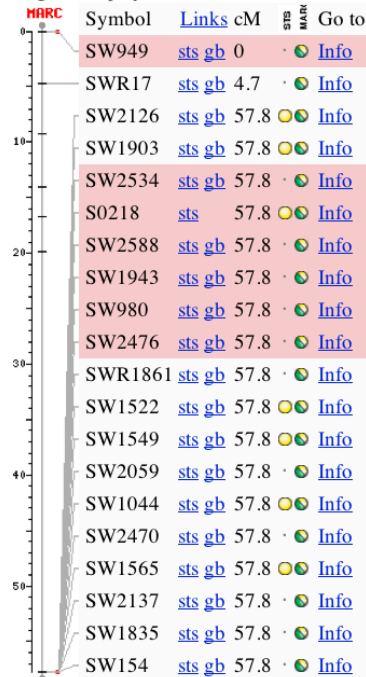
(b) SSC18

Region Displayed: 0.00-200.00 cM



(c) SSCX

Region Displayed: 0.00-57.80 cM



(d) SSCY

Figure 9: Marker position along chromosomes (source: NCBI Map Viewer <http://www.ncbi.nlm.nih.gov/projects/mapview/>). Selected markers used in this study are highlighted. Sus scrofa chromosomes SSC17, SSC18, SSCX and SSCY.

3.3 GENOTYPING

3.3.1 *Selection of animals*

The initial dataset consisted of 5180 animals, progeny of C21 Large white boar line (Gorzagri, Italy) sires and crossbred Goland C40 Large white derived (Gorzagri) sows (see:1.2.2). Animals were born between 17-10-2000 and 28-01-2005 and slaughtered monthly between 31-07-2001 and 8-11-2005 at an average weight of 165 kg. Pigs were reared in one farm and raised under commercial finishing conditions with standard constant controlled diet.

Phenotypical records were routinely collected for growth and carcass traits while dry cured ham quality traits were available only for a limited number of subjects. After editing, with the aim of discarding uninformative individuals for dry cured ham quality traits, 596 animals were available for this study. Further editing of the dataset was necessary because blood samples of sires and/or tissue samples of the progeny were no more available; biological samples of sows (hairs) were also not available due to improper sampling and storing conditions.

Biological samples for a total of 369 individuals sired by 15 C21 boars mated to 82 Goland C40 sows were available (together with the 15 sires) for genotyping.

Actually 15 half-sib families with an average of 26.35 ± 10.34 litter/sire and 6.86 ± 2.41 dam/sire were analyzed.

3.3.2 *DNA Purification*

The DNA of sires was extracted from blood using standard salting out procedures while DNA of offspring was purified from fat tissue using DNeasy 96 Blood & Tissue Purification kit (QIAGEN, Germany) with minor modifications.

100 mg of fat tissue were treated with 180 μ l of ATL buffer (QIAGEN) and 20 μ l of proteinase K (QIAGEN) for 1 to 3 hours at 56°C until the tissue was completely lysed. DNA was then purified following manufacturer's protocol using 96 well plates and Allegra 25R centrifuge (BeckmanCoulter, USA). DNA was finally eluted twice with 200 μ l of AE buffer (QIAGEN).

Quantity of purified DNA was calculated using Qubit fluorometer and Quant-iTTM ds DNA BR assay (Invitrogen, USA) and quality was checked on 1% agarose gel.

DNA was then normalized in ddH₂O at 5 ng/ μ l in 96 well plates using a Biomek3000 robotic liquid handling station (BeckmanCoulter).

3.3.3 *Amplification*

A subset of 8 individuals was initially used to setup the best PCR conditions for the individual amplification of the 269 markers.

For the amplification 15 ng of DNA were added to a reaction mix containing 0.3 μ M of both forward dye-labeled and reverse primers, 1x Taq Gold Buffer (Applied Biosystem, USA), 2.5 mM MgCl₂, 0.16 mM of each dNTPs and 0.2 U of AmpliTaq Gold (Applied Biosystem) in a final volume of 15 μ l.

Amplifications were performed in 384-well PCR plates on an AB9700 (Applied Biosystem) thermal cycler with the following conditions: initial denaturation step of 10 min at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at the primers annealing temperature (see tables n. 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25) and 45 s at 72°C, followed by a final extension of 10 min at 72°C. Before pooling and genotyping, 10 µl of a randomly chosen subset of 16 samples from each 384-well plate were checked and quantified on 2% agarose gel.

3.3.4 Pooling and sequencing

After amplification, compatible amplicons were pooled before capillary electrophoresis. According to expected amplicons sizes and the availability of four different dyes (6FAM, NED, PET and VIC) a total of 23 pools were necessary to analyse the 269 microsatellite markers for the 384 animals.

5 ng of 6FAM-labeled amplicons were pooled with 2x, 3x and 4x VIC, NED and PET-labeled amplicons respectively using a Biomek3000 robotic liquid handling station (BeckmanCoulter). Pooled samples were then dried on a thermal cycler at 50°C, washed twice in cold 70% EtOH and dried again until sequencing.

Before capillary electrophoresis on an automated sequencer (ABI Prism 3100, Applied Biosystem), 20 µl of formamide and 0.5 µl of Liz500 size standard were added to each sample.

3.3.5 Analysis of data and statistical analysis

Sizing of microsatellite markers was performed using Peak Scanner v1.0 (Applied Biosystem) (figure n.10).

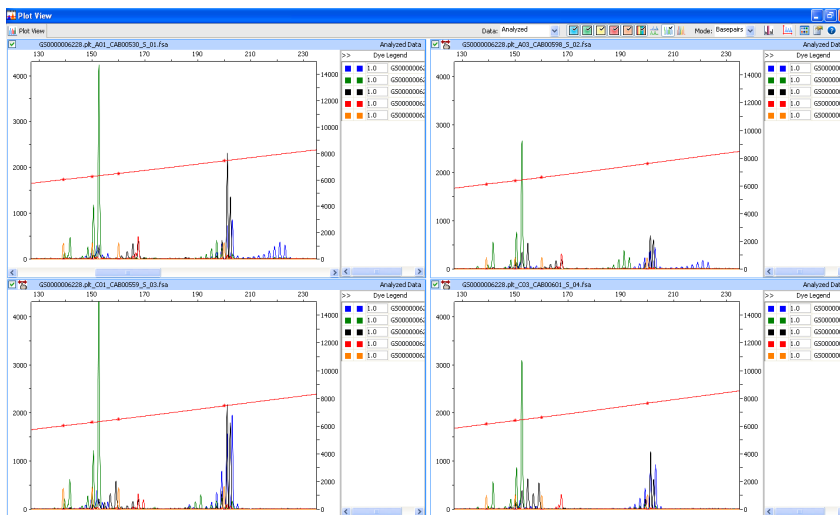


Figure 10: Sizing of STR with PeakScanner v1.0

Genotypes were then checked for errors using MSA Microsatellite Analyzer v. 4.05 [23] and checked against pedigree information using Genoprob v. 2.0 [82]. Observed and expected heterozygosity and number of alleles were calculated using MSA v. 4.05; F_{IS} , PIC and molecular kinship f_{ij} were calculated using Molkin v. 3.0 [39].

PIC: Polymorphic Information Content

3.4 RESULTS

214 microsatellite markers out of 269 were polymorphic in the population analysed. Despite the accurate selection for putative polymorphic microsatellite markers based on previous published studies, 55 markers were found uninformative in our commercial population. This could be due to the fact that the vast majority of studies used experimental population with crosses of exotic breeds; genetic diversity at any random locus is higher in not related breeds than in commercial population therefore decreasing the chance of finding fixed alleles at microsatellite loci.

Observed and expected heterozygosity, number of alleles, Wright's F_{IS} , mean molecular coancestry f_{ij} , PIC and size range for each marker is showed in table n. 26, 27, 28, 29, 30 and 31.

The most polymorphic markers found were SW1482, SW974 and SW2130 with 15 alleles found in the population, while 11 markers had only two alleles. On average, there were 6.60 ± 1.95 alleles per locus. Average observed heterozygosity was 0.51 ± 0.21 and did not differ from expected 0.58 ± 0.18 one. Mean Wright's F_{IS} value for the population was 0.088 and 0.096 when weighted by PIC showing a very small excess of homozygosity among loci. Mean molecular coancestry f_{ij} was 0.43 and 0.37 when weighted by PIC.

PIC values ranged from 0.02 (loci SW262 and S0143) to 0.84 (locus SW2021) with an average of 0.53 indicating a discrete informativeness of selected loci.

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
IGF1	0.61	0.73	0.17	0.68	223-239	8
KS101	0.82	0.82	-0.01	0.79	153-199	13
KS111	0.39	0.37	-0.06	0.35	188-226	9
KS112	0.64	0.62	-0.04	0.56	210-234	9
KS141	0.42	0.50	0.17	0.38	202-208	3
S0001	0.33	0.48	0.32	0.44	181-193	7
S0003	0.63	0.68	0.08	0.61	152-166	4
S0006	0.36	0.79	0.55	0.75	215-245	8
S0008	0.49	0.51	0.05	0.40	183-187	3
S0010	0.67	0.68	0.02	0.62	101-129	4
S0017	0.70	0.63	-0.12	0.55	157-171	5
S0024	0.74	0.69	-0.08	0.63	170-186	4
S0025	0.58	0.55	-0.05	0.52	103-115	5
S0036	0.75	0.79	0.05	0.76	121-133	7
S0064	0.86	0.68	-0.26	0.64	90-112	8
S0068	0.14	0.13	-0.05	0.13	229-257	4
S0070	0.58	0.65	0.11	0.61	263-293	9
S0090	0.72	0.72	0.00	0.67	239-251	6
S0091	0.65	0.61	-0.07	0.54	150-168	8
S0101	0.71	0.71	-0.01	0.65	194-214	6
S0102	0.73	0.72	0.00	0.67	121-139	7
S0105	0.40	0.35	-0.13	0.31	125-147	3
S0111	0.69	0.72	0.04	0.67	149-159	5
S0112	0.51	0.79	0.35	0.75	200-216	6
S0113	0.55	0.52	-0.05	0.47	174-204	9
S0115	0.90	0.75	-0.19	0.71	193-209	6
S0120	0.63	0.67	0.06	0.62	154-172	7
S0121	0.56	0.78	0.28	0.74	163-181	10
S0141	0.81	0.65	-0.25	0.59	206-226	9
S0143	0.01	0.02	0.57	0.02	156-164	4
S0147	0.64	0.65	0.01	0.62	153-183	8
S0167	0.66	0.71	0.06	0.65	212-222	5
S0175	0.16	0.17	0.04	0.17	134-165	6
S0177	0.79	0.72	-0.10	0.68	143-175	9
S0178	0.52	0.56	0.06	0.51	110-128	6
S0182	0.65	0.71	0.08	0.66	119-129	5
S0206	0.39	0.68	0.43	0.62	174-206	9
S0212	0.64	0.71	0.10	0.65	223-241	10
S0213	0.10	0.12	0.15	0.11	142-158	4
S0214	0.27	0.25	-0.07	0.22	125-139	4

Table 26: Ho: observed heterozygosity; He: expected heterozygosity; FIS: Wright's FIS; PIC: Polymorphic information content; allele size and number of alleles per locus

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
S0217	0.28	0.24	-0.16	0.21	142-152	2
S0218	0.15	0.28	0.49	0.24	158-166	2
S0228	0.80	0.76	-0.05	0.72	222-246	7
S0229	0.40	0.42	0.06	0.38	133-151	4
S0282	0.36	0.56	0.35	0.51	123-129	4
S0295	0.51	0.50	-0.02	0.46	227-235	5
S0299	0.61	0.66	0.07	0.59	201-223	6
S0301	0.64	0.64	0.01	0.60	245-265	9
S0302	0.32	0.40	0.20	0.37	208-212	3
S0313	0.70	0.70	0.00	0.65	198-218	7
S0353	0.31	0.70	0.55	0.64	113-125	7
S0355	0.28	0.82	0.66	0.79	233-281	11
S0363	0.40	0.41	0.02	0.39	190-214	8
S0366	0.21	0.21	0.02	0.19	278-282	2
S0385	0.52	0.54	0.04	0.48	168-176	4
S0391	0.74	0.73	-0.01	0.69	170-196	6
S0392	0.69	0.77	0.11	0.74	231-245	8
S0395	0.60	0.73	0.17	0.68	190-228	8
SB45	0.70	0.51	-0.37	0.39	199-209	4
SCAMP1_4	0.43	0.63	0.32	0.56	191-203	5
SE47329	0.60	0.55	-0.10	0.52	184-208	7
SE47351	0.70	0.62	-0.14	0.57	202-210	4
SJ024	0.19	0.38	0.49	0.36	245-283	9
SJ061	0.52	0.61	0.14	0.53	241-257	5
SW1023	0.60	0.56	-0.07	0.52	89-115	5
SW1027	0.36	0.54	0.33	0.43	137-161	6
SW1041	0.47	0.41	-0.13	0.33	94-104	5
SW1056	0.21	0.19	-0.12	0.17	167-169	2
SW1057	0.26	0.41	0.38	0.34	150-186	4
SW1081	0.69	0.65	-0.06	0.58	136-156	3
SW1092	0.57	0.60	0.04	0.54	234-244	5
SW1111	0.69	0.65	-0.07	0.60	161-175	5
SW1119	0.77	0.74	-0.04	0.69	147-175	8
SW1262	0.44	0.45	0.04	0.41	113-147	5
SW13	0.71	0.75	0.05	0.71	142-158	7
SW1301	0.74	0.77	0.05	0.75	160-176	7
SW1305	0.18	0.45	0.60	0.42	214-238	5
SW133	0.52	0.54	0.04	0.50	134-146	7
SW1332	0.49	0.67	0.26	0.64	78-110	14
SW1343	0.61	0.64	0.05	0.57	122-146	4

Table 27: Ho: observed heterozygosity; He: expected heterozygosity; F_{IS} : Wright's F_{IS} ; PIC: Polymorphic information content; allele size and number of alleles per locus

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
SW1349	0.53	0.78	0.32	0.74	143-151	5
SW1353	0.37	0.36	-0.03	0.33	156-168	6
SW1354	0.44	0.63	0.30	0.58	117-141	5
SW1364	0.53	0.51	-0.05	0.41	161-177	7
SW1369	0.35	0.71	0.50	0.66	125-147	8
SW1377	0.57	0.58	0.01	0.50	200-230	8
SW1408	0.73	0.79	0.07	0.76	180-192	6
SW1426	1.00	0.56	-0.78	0.46	87-105	7
SW147	0.72	0.74	0.02	0.69	214-226	6
SW1473	0.50	0.54	0.08	0.44	161-179	5
SW1482	0.44	0.72	0.39	0.68	86-140	15
SW151	0.54	0.50	-0.08	0.37	196-206	2
SW1550	0.55	0.55	0.00	0.50	190-216	6
SW1551	0.36	0.74	0.51	0.70	148-194	13
SW1556	0.33	0.56	0.41	0.46	218-228	5
SW1557	0.49	0.59	0.18	0.54	75-105	11
SW1564	0.54	0.49	-0.09	0.41	112-122	3
SW1616	0.26	0.35	0.26	0.29	130-138	3
SW1633	0.49	0.50	0.01	0.37	146-152	3
SW1709	0.88	0.77	-0.14	0.73	156-172	7
SW174	0.71	0.72	0.01	0.67	119-131	6
SW1808	0.22	0.27	0.21	0.25	106-150	3
SW1824	0.70	0.72	0.02	0.66	85-103	6
SW1844	0.64	0.63	-0.03	0.55	95-103	4
SW1856	0.46	0.59	0.22	0.54	177-201	9
SW1891	0.74	0.83	0.10	0.80	97-123	11
SW1897	0.37	0.36	-0.01	0.31	154-160	3
SW1943	0.25	0.64	0.62	0.57	99-105	3
SW1954	0.54	0.58	0.07	0.53	184-198	6
SW1956	0.07	0.07	0.04	0.07	173-181	2
SW2000	0.20	0.42	0.51	0.39	109-141	5
SW2003	0.64	0.68	0.05	0.62	94-108	6
SW2021	0.89	0.85	-0.04	0.84	102-128	10
SW2047	0.37	0.64	0.42	0.60	167-191	9
SW2067	0.43	0.78	0.45	0.74	110-136	9
SW2093	0.74	0.73	-0.01	0.69	100-138	7
SW2129	0.56	0.50	-0.13	0.38	103-113	3
SW2130	0.51	0.71	0.28	0.70	141-181	15
SW2155	0.37	0.38	0.01	0.33	133-141	3
SW2195	0.12	0.29	0.60	0.27	152-172	6

Table 28: Ho: observed heterozygosity; He: expected heterozygosity; FIS: Wright's FIS; PIC: Polymorphic information content; allele size and number of alleles per locus

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
SW240	0.33	0.29	-0.15	0.25	94-98	3
SW2401	0.70	0.78	0.09	0.74	148-172	8
SW2406	0.41	0.68	0.39	0.63	220-256	9
SW2408	0.80	0.75	-0.07	0.70	152-170	7
SW2411	0.75	0.71	-0.05	0.67	177-203	6
SW2419	0.74	0.70	-0.06	0.66	116-132	7
SW2425	0.65	0.71	0.08	0.66	84-110	11
SW2441	0.72	0.71	-0.01	0.68	140-170	7
SW2443	0.60	0.60	0.01	0.53	200-214	7
SW2445	0.69	0.76	0.10	0.73	210-234	9
SW2448	0.55	0.59	0.06	0.51	204-214	5
SW2470	0.30	0.49	0.39	0.39	155-169	3
SW2476	0.25	0.54	0.53	0.48	88-100	7
SW2491	0.46	0.46	0.00	0.42	132-158	6
SW2515	0.61	0.55	-0.11	0.48	90-106	6
SW2517	0.84	0.72	-0.17	0.67	171-189	5
SW252	0.74	0.81	0.08	0.78	172-188	7
SW2532	0.68	0.80	0.14	0.77	175-195	10
SW2534	0.60	0.51	-0.19	0.46	145-159	6
SW254	0.48	0.48	0.00	0.44	178-204	6
SW2547	0.06	0.08	0.16	0.07	95-113	4
SW256	0.37	0.40	0.07	0.33	95-103	4
SW2588	0.09	0.58	0.85	0.54	104-124	7
SW2608	0.56	0.50	-0.13	0.44	91-121	10
SW262	0.02	0.02	-0.01	0.02	197-201	3
SW2623	0.50	0.51	0.01	0.47	131-139	5
SW305	0.07	0.23	0.70	0.20	119-123	3
SW316	0.79	0.81	0.02	0.78	127-159	8
SW322	0.39	0.60	0.35	0.52	103-115	7
SW322bis	0.61	0.80	0.23	0.77	103-119	8
SW344	0.68	0.68	0.00	0.62	154-186	7
SW349	0.68	0.73	0.06	0.68	154-182	6
SW378	0.34	0.37	0.08	0.31	117-123	4
SW398	0.62	0.61	-0.02	0.53	165-187	4
SW413	0.16	0.66	0.76	0.61	161-175	7
SW435	0.45	0.45	0.01	0.41	161-169	5
SW443	0.31	0.71	0.56	0.67	104-148	11
SW445	0.64	0.67	0.05	0.61	187-205	7
SW491	0.30	0.50	0.41	0.48	148-176	9
SW512	0.41	0.55	0.25	0.48	132-186	12

Table 29: Ho: observed heterozygosity; He: expected heterozygosity; F_{IS} : Wright's F_{IS} ; PIC: Polymorphic information content; allele size and number of alleles per locus

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
SW557	0.42	0.59	0.28	0.52	240-252	5
SW581	0.36	0.37	0.02	0.30	198-204	2
SW61	0.66	0.73	0.10	0.69	237-261	11
SW7	0.38	0.47	0.21	0.43	90-112	5
SW737	0.59	0.61	0.03	0.56	209-227	6
SW763	0.05	0.05	-0.02	0.04	160-174	2
SW764	0.47	0.56	0.15	0.51	114-120	4
SW787	0.79	0.75	-0.05	0.71	148-160	6
SW828	0.02	0.13	0.81	0.12	217-229	5
SW830	0.79	0.77	-0.02	0.73	174-190	8
SW856	0.42	0.82	0.48	0.80	165-203	12
SW857	0.75	0.70	-0.07	0.65	140-156	6
SW874	0.72	0.70	-0.04	0.65	198-218	10
SW904	0.44	0.53	0.16	0.49	157-181	6
SW905	0.42	0.81	0.48	0.79	125-153	13
SW911	0.73	0.75	0.03	0.71	136-170	11
SW917	0.63	0.60	-0.04	0.53	124-132	3
SW935	0.18	0.17	-0.07	0.15	198-200	2
SW936	0.67	0.63	-0.07	0.56	91-113	8
SW949	0.48	0.46	-0.04	0.42	186-208	8
SW951	0.61	0.67	0.09	0.63	111-139	12
SW957	0.48	0.44	-0.09	0.40	111-135	5
SW964	0.81	0.77	-0.05	0.73	205-243	10
SW967	0.56	0.48	-0.17	0.44	90-110	6
SW974	0.47	0.83	0.43	0.80	107-169	15
SW980	0.40	0.76	0.48	0.72	112-130	8
SW983	0.70	0.72	0.03	0.67	89-117	6
SW986	0.73	0.72	-0.02	0.67	146-160	5
SWC27	0.79	0.76	-0.04	0.72	137-167	10
SWR1002	0.72	0.72	-0.01	0.67	114-124	4
SWR1004	0.67	0.76	0.11	0.72	144-162	7
SWR1021	0.51	0.44	-0.16	0.37	89-115	7
SWR1101	0.52	0.61	0.15	0.55	118-160	7
SWR1120	0.64	0.66	0.02	0.60	154-176	6
SWR1121	0.52	0.72	0.27	0.67	156-172	8
SWR1130	0.56	0.56	-0.01	0.51	121-133	5
SWR1133	0.53	0.47	-0.13	0.36	132-134	2
SWR136	0.52	0.65	0.20	0.62	192-232	8
SWR1445	0.51	0.55	0.07	0.49	87-101	4
SWR1806	0.33	0.35	0.05	0.31	208-212	3

Table 30: Ho: observed heterozygosity; He: expected heterozygosity; FIS: Wright's FIS; PIC: Polymorphic information content; allele size and number of alleles per locus

Locus name	Ho	He	FIS	PIC	Allele size	N. alleles
SWR1910	0.42	0.70	0.41	0.66	211-253	10
SWR1928	0.67	0.64	-0.05	0.57	87-105	7
SWR1939	0.52	0.53	0.02	0.50	87-101	7
SWR1941	0.42	0.48	0.12	0.42	200-218	7
SWR2300	0.30	0.36	0.17	0.30	138-156	4
SWR250	0.05	0.05	-0.03	0.05	169-173	2
SWR340	0.66	0.68	0.03	0.62	129-149	7
SWR345	0.31	0.38	0.17	0.36	136-162	9
SWR756	0.27	0.42	0.36	0.39	138-156	5
SWR982	0.63	0.67	0.06	0.62	200-208	5
SY16	0.38	0.65	0.42	0.58	211-219	5
SY2	0.31	0.46	0.32	0.36	77-99	4
SY6	0.23	0.60	0.61	0.52	231-235	3
TNF	0.86	0.82	-0.05	0.80	172-208	13
Mean	0.51 ± 0.21	0.58 ± 0.18	0.088	0.53		6.6

Table 31: Ho: observed heterozygosity; He: expected heterozygosity; FIS: Wright's FIS; PIC: Polymorphic information content; allele size and number of alleles per locus

Part IV

IDENTIFICATION OF QTL FOR DRY
CURED HAM QUALITY TRAITS

IDENTIFICATION OF QTL FOR DRY CURED HAM QUALITY TRAITS

4.1 INTRODUCTION

The production of the Italian heavy pig aims mainly to provide thighs for the production of dry cured hams. Raw hams account for more than 50% of carcass market value (Gigli et al. [35]). Industry of transformation and, in primis, protected origin of designation such as Parma and San Daniele requires high quality raw material for the production of dry cured hams (Bosi and Russo [11]). Many traits involved in the determination of the quality of cured products are difficult to measure, expensive or not standardized. Difficulties in monitoring important economical traits such as wide, visible fatty areas in the cross section of dry cured hams, firmness and marbling depends on the high experimental costs due to the loss of product value. Moreover traits recorded at the end of the maturing process (such as loss of weight due to dehydration) are available too late for an efficient and rapid genetic improvement. Understanding the genetic background of these traits is therefore very important in the prospective of applying marker assisted selection for increasing the response to selection for dry cured ham quality traits.

Aim of this thesis was to scan the whole pig genome for QTL affecting dry cured ham quality traits. Many QTL scans have been conducted in pigs for several traits (reviewed in chapter: 1) but due to the unique features of the Italian market, and the peculiarity of dry cured hams, not many studies have investigated QTL related to traits involved in the quality of cured products. Moreover the vast majority of the genome scan have been performed on segregating experimental crosses between divergent breeds. QTLs identified in these crosses do not necessarily segregate within commercial lines or the allelic effects might be different.

The objective of this study was to identify QTL for body composition and dry cured ham quality traits segregating within a commercial Large White derived line.

4.2 MATERIAL AND METHODS

4.2.1 *Genetic material*

The QTL analysis was performed on 369 individuals progeny of 15 sires of C21 Large White boar line (Gorzagri, Italy) that were mated to 82 crossbred Goland C40 Large White derived sows (Gorzagri).

Animals were reared at the same farm under standard feeding conditions (for details see section: 2.2.1).

Animals (9 month of age, with an average BW of 169 ± 17 kg) were slaughtered at the same abattoir on a single day each month. Pigs were slaughtered after CO₂ stunning.

For the QTL analysis individuals were selected for presence of biological samples and phenotypic records for growth, carcass and dry cured ham quality traits from a dataset of more than 11,000 individuals.

This study was performed on fifteen half-sib families with an average of 26.35 ± 10.34 litter/sire and 6.86 ± 2.41 dam/sire. DNA was purified from blood or fat tissue for sires and offspring respectively; tissue samples from dams were not available.

4.2.2 Phenotypic records

Phenotypic records were available for 48 traits including growth and fatness traits (4 traits), carcass (5 traits) and 39 meat and dry cured ham quality traits (see tables: 32, 33, 34, 35 and 36).

A detailed description of the phenotypic measurements has been described in chapter 2.

Briefly, cold carcass weight (**CCW**) was recorded during the first hour after slaughtering and carcasses were sectioned into typical commercial cuts that were also weighted (**LEANP**, **HAMP**, **LOINP**, **SHOUP** and **SPAREP**).

Backfat measurements were taken in two places (at 10th rib and at last rib) using a Fat-O-Meter instrument (**10RIBBFT** and **LRIBF**); loin depth was also recorded (**LD**).

Meat and ham quality traits for each animal were recorded on the left thigh.

Initial pH (**PHI**) was measured 45 min after slaughtering on the *semimembranosus* muscle. Hams were dressed after 24 h of refrigeration at 4 °C and final pH was measured at dressing on the *semimembranosus* muscle (**PHU**). After dressing hams were weighted and cured according to the San Daniele procedure (DOP [2]).

Minolta L*, a*, b* (**HAML**, **HAMA** and **HAMB**) values were taken on fresh surface of hams using a Minolta CHR 300 colorimeter (Minolta camera, Osaka, Japan). Weight losses of dry cured hams were recorded after salting (**WLS**), resting (**WLR**) and curing (**WLC**) together with the number of days of curing (**DC**).

Subjective evaluation were performed after dressing on traits like: ham shape (**HAMSS**), presence of blood vessels (**VHS**), presence of haematomas (**HAEHS**), marbling (**MSHS**), color (**CSHS**), depth of fat covering ham layer (**CFLHS**), fat color (**CFHS**), fat firmness (**FFHS**), fat greasiness (**FGHS**) and meat firmness (**FFHS**). A linear scoring system was used for the different traits and details can be found in section: 2.2.3.

After dressing, fat sample for the evaluation of iodine number were sampled. Iodine number (**IN**) measures the amount of unsaturation contained in fatty acids in the form of double bonds which react with iodine compounds. The higher the iodine number, the more unsaturated fatty acid bonds are present in fat. This analysis allows for the evaluation of suitability of hams' fat for curing process. It can predict the tendency for oxydation and therefore the probability of fat going rancid. Iodine number was determined using Wijs method (AOAC [7]) according to the San Daniele procedure DOP [2].

Instrumental firmness of dry cured ham was measured by Hardness Meter MK2 at two sites (inner and outer) of *biceps femoris* (**FBF1**, **FSBF2**, **FBFM**), *semitendinosus* (**FST1**, **FST2** and **FSTM**) and *semimembranosus* (**FSM1**, **FSM2** and **FSMM**) muscles as described in Noventa et al. [58].

Instrumental firmness was recorded also for the inner (**FFI**) and the outer (**FFO**) layer of hams' fat; average firmness was also calculated (**FFM**). Fat covering ham (**FD**) in cm was also directly measured.

Computer image analysis of cross-sectioned dry cured hams were performed as described by Carnier et al. [14]. In particular, area of interest included the total area of the cross section (**CSA**); the fat eye area (i.e. a visible fatty area approximately centered on the cross section and surrounded by *biceps femoris*, *semimembranosus*, *semitendinosus*, and *quadriceps femoris* muscles, **FA**); the lean, or muscles, area (i.e. the area of the cross section that excluded the area of subcutaneous fat, fat eye, and skin, **LA**); *biceps femoris* (**BFA**) and *semitendinosus* area (**STA**) and ratio of the FA to the cross-sectional area (**FESR**).

4.2.3 DNA purification and genotyping

Details on DNA isolation and purification have been described in section: 3.3.2.

Two hundred and sixty-nine microsatellite markers were selected to cover the whole genome uniformly (see chapter: 3); markers were amplified with standard PCR protocols and amplicons were pooled before capillary electrophoresis using automated sequencer (see section: 3.3.4).

Sizing was performed using Peak Scanner v1.0 software (Applied Biosystem, USA) and genotypes were checked against pedigree information using Genoprob v2.0 software (Thallman et al. [82]); genotypes that could not be scored unambiguously were treated as missing data.

The linkage map was constructed using CriMap, version 2.4 (Green et al. [37]) and using the Kosamby mapping function. The sex-average linkage map was compared to the USDA Meat Animal Research Centre (MARC) map (Rohrer et al. [65]) used as reference.

4.2.4 Statistical analysis

Phenotypic data of genotyped animals were adjusted for systematic errors before the QTL analysis on whole population data ($n = 11,064$). Effects were estimated using glm procedure in R v.2.10 (R Development Core Team [61]) and the following model was used to describe all phenotypic traits:

$$Y_{ijk} = \text{SEX}_i + \text{SSG}_j + \text{CCW}_k + e_{ijk} \quad (4.1)$$

where,

Y_{ijk} = trait under study;

SEX_i = fixed effect of i^{th} sex (two classes, barrow or gilt);

SSG_j = fixed effect of j^{th} sample stage (156 classes);

CCW_k = fixed effect of carcass weight (kg) (28 classes);

e_{ijk} = the residual effect, $e_{ijk} \sim N(0, I \sigma^2)$.

The adjusted trait score Y^* used in the QTL analysis represents the residual effect (i.e. the phenotypic data adjusted for the non-genetic and litter effects estimated under model [4.1]). The model accounted for the effect of sex (male or female), the effect of sample stage (day of slaughtering, 156 classes) and the effect of carcass weight (class1: less than 108kg, class2: from 108kg to 112kg, class3: from 113kg to 116kg, class4 to class26 different by 2kg increments, class27: from 162kg to 166kg and class28: from 167kg to 170kg). The litter effect included genetic effects of the dams along with common environmental effects.

Table 32: Summary statistics for measured growth, fatness and carcass traits: abbreviations used in text, number of animals per trait (n), means, standard deviation (SD), minimum (Min.) and maximum (Max.) values and coefficient of variation (C.V.).

Trait	Abbreviation	n	Mean	SD	Min.	Max.	C.V. (%)
<i>Growth and fatness traits</i>							
Backfat at tenth rib (mm)	10RIBBFT	282	30.97	5.91	19.00	52.00	19.10
Backfat at last rib (mm)	LRIBF	282	25.91	5.03	15.00	42.00	19.42
Loin depth (mm)	LD	282	56.20	7.51	29.00	81.00	13.36
Lean meat (% on CCW)	LEANP	282	50.17	3.17	41.12	57.03	6.33
<i>Carcass traits</i>							
Cold carcass weight (kg)	CCW	368	136.06	12.38	99.50	178.70	9.10
Ham weight (% on CCW)	HAMP	283	19.65	0.92	16.25	23.23	4.70
Loin weight (% on CCW)	LOINP	283	15.69	0.86	13.32	18.41	5.49
Shoulder weight (% on CCW)	SHOUP	279	13.89	1.20	10.35	18.35	8.63
Spare rib weight (% on CCW)	SPAREP	281	7.90	0.65	6.18	9.82	8.20

Table 33: Summary statistics for measured growth, fatness and carcass traits: abbreviations used in text, number of animals per trait (n), means, standard deviation (SD), minimum (Min.) and maximum (Max.) values and coefficient of variation (C.V.).

Trait	Abbreviation	n	Mean	SD	Min.	Max.	C.V. (%)
<i>Meat quality traits</i>							
Minolta a* ham ^a	HAMA	368	7.27	2.22	-1.40	14.75	30.54
Minolta b* ham ^a	HAMB	368	4.15	2.11	-4.23	9.33	51.00
Minolta L* ham ^a	HAML	368	48.22	5.83	12.81	61.14	12.09
pH initial (45 min)	PHI	366	6.36	0.16	5.85	6.88	2.59
pH ultimate (24 h)	PHU	263	5.80	0.13	5.56	6.29	2.21
<i>Dry cured ham quality traits</i>							
Weight loss after salting (% on ham)	WLS	367	4.11	0.72	1.52	6.96	17.39
Weight loss after resting (% on ham)	WLR	368	20.15	1.67	12.50	26.39	8.28
Weight loss after curing (% on ham)	WLC	368	28.96	2.31	23.27	39.87	7.98
Days of curing	DC	368	310.72	4.07	305.00	327.00	1.31

^aMinolta L* measured lightness of mean, Minolta a* measured redness, Minolta b* measured yellowness.

Table 34: Summary statistics for measured growth, fatness and carcass traits: abbreviations used in text, number of animals per trait (n), means, standard deviation (SD), minimum (Min.) and maximum (Max.) values and coefficient of variation (C.V.).

Trait	Abbreviation	n	Mean	SD	Min.	Max.	C.V. (%)
<i>Dry cured ham quality traits - instrumental firmness</i>							
Instrumental firmness <i>biceps femoris</i> inner	FBF1	363	476.86	81.75	257.00	723.00	17.14
Instrumental firmness <i>biceps femoris</i> outer	FBF2	363	548.21	91.99	270.00	843.00	16.78
Instrumental firmness <i>biceps femoris</i> average	FBFM	363	512.79	81.63	264.00	765.00	15.92
Instrumental firmness <i>seminebranosus</i> inner	FSM1	363	703.43	75.62	458.00	922.00	10.75
Instrumental firmness <i>seminebranosus</i> outer	FSM2	363	901.13	91.19	413.00	1000.00	10.12
Instrumental firmness <i>seminebranosus</i> average	FSMM	363	802.52	68.42	486.00	951.00	8.53
Instrumental firmness <i>semitendinosus</i> inner	FST1	363	536.77	93.23	295.00	790.00	17.37
Instrumental firmness <i>semitendinosus</i> outer	FST2	363	516.69	90.62	247.00	730.00	17.54
Instrumental firmness <i>semitendinosus</i> average	FSTM	363	526.98	87.32	294.00	760.00	16.57
Instrumental firmness fat inner layer	FHI	363	605.13	83.90	345.00	963.00	13.86
Instrumental firmness fat outer layer	FHO	363	578.85	81.45	388.00	962.00	14.07
Instrumental firmness fat average	FFM	363	592.26	75.94	399.00	851.00	12.82
Fat covering ham (cm)	FD	364	1.70	0.22	1.15	2.40	13.17

Table 35: Summary statistics for measured growth, fatness and carcass traits: abbreviations used in text, number of animals per trait (n), means, standard deviation (SD), minimum (Min.) and maximum (Max.) values and coefficient of variation (C.V.).

Trait	Abbreviation	n	Mean	SD	Min.	Max.
<i>Dry cyred ham quality traits - Subjective evaluations^a</i>						
Ham shape value	HAMSS	283	1.78	0.67	1.00	4.00
Blood Vessels ham	VHS	283	1.16	0.76	0.00	3.00
Haematomas ham	HAEHS	283	0.23	0.51	0.00	2.00
Marbling score ham	MSHS	283	1.65	0.61	1.00	4.00
Color score ham	CSHS	283	0.00	0.81	-3.00	2.00
Depth of ham covering fat layer	CFLHS	283	-0.17	0.81	-3.00	3.00
Color fat ham	CFHS	283	1.59	0.89	0.00	4.00
Fat firmness ham	FFHS	283	1.59	0.91	0.00	4.00
Fat greasiness ham	FGHS	283	1.58	0.89	0.00	4.00
Firmness score ham	FSHS	368	1.88	0.54	0.00	4.00

^aDetails on subjective scores are defined in the text.

Table 36: Summary statistics for measured growth, fatness and carcass traits: abbreviations used in text, number of animals per trait (n), means, standard deviation (SD), minimum (Min.) and maximum (Max.) values and coefficient of variation (C.V.).

Trait	Abbreviation	n	Mean	SD	Min.	Max.	C.V. (%)
<i>Dry cured ham quality traits - Computer image analysis</i>							
Ham total cross section area (cm ²)	CSA	364	330.45	32.41	260.24	476.16	9.81
Ham lean area (cm ²)	LA	364	239.95	23.76	181.86	325.72	9.90
Ham fat eye area (cm ²)	FA	364	13.01	5.84	3.79	69.85	44.91
Ratio of FA to CSA (%)	FESR	364	59.23	7.50	19.95	79.49	12.66
Biceps femoris area (cm ²)	BICA	364	20.05	3.14	10.20	32.06	15.68
Semitendinosus area (cm ²)	SEMMA	364	0.04	0.02	0.01	0.19	45.06
Iodine number	IN	364	67.67	2.91	51.40	77.70	4.29

The QTL analysis was performed using the multimarker regression approach for interval mapping in half-sib populations (Knott et al. [50]) that have been widely used and was recently applied by Heuven et al. [41], Uemoto et al. [84], Yang et al. [88], Slawinska et al. [78], Liu et al. [53] in half-sib pig populations.

This method estimates the difference between alternative alleles transmitted by the sire; briefly, marker data on progeny and their common parent are combined in a multi-point approach to calculate probabilities of individuals inheriting allele 1 or allele 2 from the common parent. These probabilities are combined into “coefficients” (with values between 0.0 and 1.0) that can be used to calculate marker information content and marker segregation distortion. The adjusted phenotypic data on progeny are regressed onto these coefficients in a within-common-parent regression analysis (Knott et al. [50]).

For each half-sib offspring, the probability of inheriting one of the sire’s haplotypes was calculated at 1-cM intervals along the genome, conditional upon the flanking marker genotypes.

An *F*-test statistics is then calculated along the chromosome at every 1 cM interval across the half-sib families with the number of degrees of freedom in the numerator equal to the number of common parents that are informative at a given chromosome location.

Analysis were performed on GridQTL platform (<http://www.gridqtl.org.uk/index.htm>) (Seaton et al. [76] and Seaton et al. [77]).

Chromosome-wise significance thresholds (P_{chr}) were determined empirically for each trait by chromosome combination using permutation as described by Churchill and Doerge [17]. Thresholds were obtained based on 1,000 permutations. Chromosome-wise threshold take into account of multiple tests on a specific chromosome but does not correct for testing on the entire genome.

Genome-wise significance thresholds were calculated by applying the Bonferroni correction following the formula:

$$P_{gen} = 1 - (1 - P_{chr})^{1/r} \quad (4.2)$$

where *r* is the chromosome length divided by the total length of the regions covered (Koning et al. [51]). These significance levels do not take into account testing of multiple traits but they allow for comparison between different studies because significance levels are based on total genome length and are not affected by the variable number of independent traits analyzed in different studies.

QTL exceeding the 5% chromosome-wise significance level will be discussed.

4.3 RESULTS

Significance thresholds of the *F*-statistics were estimated based on 1,000 permuted dataset. Threshold differed between trait by chromosome combinations and were, on average, 2.30 and 2.88 for the 5% and 1% significance level respectively. The QTL mapping results are summarized in table n. 37 and 38. Fifty-two QTL affecting 24 of the 41 traits analyzed were identified, among these, 16 QTL were significant at 1% chromosome-wise level and 36 at 5% chromosome wise level. The average genome-wide significance level was $P = 0.04$ among all significant QTLs.

The QTL were found on all autosomal chromosomes excluding SSC18 and ranged from 1 (SSC4, SSC7 and SSC14) to 5 (SSC5, SSC8, SSC9 and SSC12) with an average of 3 QTL per linkage group. Five QTL were identified for the traits ratio of ham fat eye area to cross section area (FESR) and ham fat eye area (FA) and nine for HAEHS.

Profiles of the test statistics for all chromosomes carrying QTL significant at the 5% and 1% chromosome-wise level are presented in figures: SSC1: 11, SSC2: 12, SSC3: 13, SSC4: 14, SSC5: 15, SSC6: 16, SSC7: 17, SSC8: 18, SSC9: 19, SSC10: 20, SSC11: 21, SSC12: 22, SSC13: 23, SSC14: 24, SSC15: 25, SSC16: 26 and SSC17: 27).

For the growth and fatness traits 4 QTL were detected; a QTL for loin depth (LD) on SSC3 with the highest *F*-value at 44 cM between markers So206 and SwR756; a QTL for backfat at last rib (LRIBF) was also found on SSC3 at 54 cM in correspondence to marker Sw828 and with a second significant peak close to marker Sw2408; a QTL for backfat at tenth rib (10RIBBFT) was found on SSC5 at 126 cM in correspondence to marker Sw1954 significant at the 1% genome-wise level; finally a QTL for percentage of lean meat on CCW (LEANP) was found on SSC9 at 138 cM near Sw1349.

For the carcass composition traits two QTLs were found for percentage of loin weight on CCW (LOINP), the first on SSC6 at 111 cM between markers So121 and Sw322 and the second on SSC13 at 23 cM in correspondence to marker Sw935 with the latter significant at the 1% genome-wise level.

No QTLs were detected for meat quality traits such as HAMA, HAMB, HAML, PHI and PHU.

Forty-six QTLs were found for dry cured ham quality traits.

One QTL for percentage of weight loss after resting was found on SSC13 at 20cM between markers SwR1941 and Sw935 significant at the 1% genome-wide level. Six QTLs were found for lean instrumental firmness traits; among the three different muscles analyzed two QTLs were found for instrumental firmness measured on *semimembranosus* muscle (FSM2) at 47 cM in SSC1 (in correspondence to marker Sw2130) and at 44 cM in SSC16 (between markers Sw557 and Sw262) and 4 QTLs for instrumental firmness measured on *semitendinosus* muscle (FST1, FST2 and FSTM). Similar *F*-value profiles for the three aforementioned traits were found on SSC8 with two peaks for each trait at 43 cM (marker Sw7) and 78 cM (marker Sw763); other two QTLs for FST2 were found on SSC10 at 5 cM (between Sw830 and SwR136) and at 74 cM on SSC12 (close to markers So090 and Sw1956). Four QTLs were found for instrumental firmness measured on hams' covering fat (FFI, FFO and FFM); in particular, one QTL for FFO was found on SSC10 at 88 cM (between markers Sw2000 and Sw305) significant at the 1% genome-wide level and four QTLs for FFI were found on SSC3, SSC5, SSC14 and SSC15 respectively. The first QTL on SSC3 has two peaks one in correspondence to marker S2047 and the other in correspondence to marker Sw2408 in the region between 70 and 90 cM; the second QTL for FFI was found on SSC5 at 87 cM between markers Sw2003 and Sw904, the third QTL for FFI on SSC14 was the only one found on this linkage group in correspondence to marker Sw857 and the last was found on SSC15 between markers Sw2129 and SwR1002.

For the fat covering ham trait one QTL located on SSC5 significant at the 1% genome-wide level was found; the highest QTL peak mapped between Sw2003 and Sw904.

QTL for traits recorded in subjective evaluations accounted for one third of the total number of QTLs detected in this study. In particular one QTL for ham shape value was found on SSC2 on a broad chromosomal region of 40 cM mapping between S0010 and SwR345. Seven QTLs were found for HAEHS of which two were significant at the 1% genome-wide level (SSC2 and SSC6); QTLs for this trait were found on SSC2 (in a 30 cM genome region from marker Sw2443 to marker S0141), on SSC5 in correspondence to marker Sj024, on SSC6 in a genome region comprised between Sw133 and S0003 with the highest peak at 73 cM, on SSC7 in correspondance to marker S0025 being the only QTL found on this linkage group, on SSC9 close to marker Sw174 at 119 cM, on SSC12 between markers S0143 and S0229 and on SSC15 close to marker Sw936 significant at the 1% genome-wide level. Two QTLs were found for the ham marbling score (MSHS) on SSC8 with one peak close to marker Sw7 and a broad region with the highest *F*-value at 73 cM between markers S0017 and Sw763 and the second on SSC5 significant at the 1% genome-wide level close to marker Sw332. One QTL was found for the ham color score trait (CSHS) on SSC12 on a wide region before marker Sw874. Three QTLs were found for the color score of fat: the first on SSC1, the second on SSC6 significant at the 1% genome-wide level and with the same profile of FGHS trait and the third on SSC16 also similar in profile to the FGHS trait. Four QTL for the greasiness of fat on ham were found on SSC1, SSC6, SSC9 and SSC16, with the last almost reaching the 1% genome-wide level ($P = 0.0103$).

Among the most interesting traits, those recorded with computer image analysis on cross section of dry cured hams, thirteen QTL were successfully mapped. In particular two QTLs for the total cross section area (CSA) were mapped: the first on SSC4 in correspondance with marker Sw1364 was the only QTL found on this chromosome and the second on SSC8 (in correspondance with marker Sw1551). One QTL for ham lean area (LA) was found on SSC16 in correspondance of S0105 while five QTLs were found for the fat eye area trait. QTLs for fat eye area were mapped on SSC9 (significant at the 1% genome-wide level) with two peaks between markers Sw911 and Sw2401 and markers SwR1939 and S0295, on SSC10, also significant at the 1% genome-wide level, in correspondance of marker Sw2000, on SSC11 (significant at the 1% genome-wide level), in a narrow region between markers S0392 and S0391, on SSC12 on a broader region between markers S0143 and Sw957 and on SSC17 close to marker SwR1120. Ratio of FA to CSA (FESR) followed exactly the same profile of FA trait.

To conclude one QTL was found on SSC13 for iodine number trait in correspondance of marker Sw2448.

Table 37: Summary of QTL mapping results by chromosome (SSC₁ to SSC₈)

SSC	Trait ¹	Pos, cM ²	P _{chr} ³	P _{gen} ⁴	R ²
1	CFHS	59cM	0.0025	0.0374	0.100
1	FGHS	59cM	0.0024	0.0356	0.101
1	FSM ₂	47cM	0.0029	0.0426	0.099
2	HAEHS	1cM	0.0000	0.0000	0.158
2	HAMSS	83cM	0.0010	0.0149	0.108
3	FFI	81cM	0.0008	0.0137	0.110
3	LD	44cM	0.0008	0.0131	0.110
3	LRIBF	54cM	0.0023	0.0379	0.101
4	CSA	45cM	0.0057	0.1084	0.093
5	HAEHS	0cM	0.0003	0.0041	0.118
5	FFI	87cM	0.0013	0.0176	0.106
5	MSHS	68cM	0.0007	0.0092	0.111
5	10RIBBFT	126cM	0.0003	0.0048	0.117
5	FD	91cM	0.0002	0.0027	0.121
6	HAEHS	73cM	0.0000	0.0007	0.132
6	CFHS	0cM	0.0005	0.0078	0.113
6	FGHS	0cM	0.0009	0.0137	0.108
6	LOINP	111cM	0.0012	0.0173	0.106
7	HAEHS	5cM	0.0021	0.0268	0.102
8	CSA	96cM	0.0026	0.0454	0.100
8	MSHS	73cM	0.0024	0.0425	0.100
8	FST ₁	78cM	0.0030	0.0514	0.099
8	FST ₂	43cM	0.0033	0.0569	0.098
8	FSTM	79cM	0.0026	0.0449	0.100

¹See tables 32, 33, 34, 35 and 36 for trait abbreviations.

²Position with greatest *F*-statistics.

³Chromosome-wise *P* value

⁴Genome-wide *P* value calculated using 4.2

Table 38: Summary of QTL mapping results by chromosome (SSC9 to SSC17)

SSC	Trait ¹	Pos, cM ²	P _{chr} ³	P _{gen} ⁴	R ²
9	FESR	46cM	0.0001	0.0009	0.130
9	LEANP	138cM	0.0039	0.0558	0.097
9	HAEHS	119cM	0.0036	0.0529	0.097
9	FGHS	13cM	0.0040	0.0574	0.096
9	FA	46cM	0.0003	0.0045	0.117
10	FESR	83cM	0.0000	0.0001	0.148
10	FFO	88cM	0.0006	0.0092	0.112
10	FA	83cM	0.0000	0.0001	0.149
10	FST2	5cM	0.0051	0.0794	0.094
11	FESR	4cM	0.0000	0.0004	0.138
11	FA	4cM	0.0000	0.0001	0.151
12	FESR	13cM	0.0049	0.0909	0.094
12	CSHS	49cM	0.0027	0.0506	0.100
12	HAEHS	3cM	0.0008	0.0158	0.109
12	FA	4cM	0.0018	0.0344	0.103
12	FST2	74cM	0.0030	0.0557	0.099
13	WLR	20cM	0.0003	0.0065	0.117
13	LOINP	23cM	0.0001	0.0011	0.131
13	IN	56cM	0.0051	0.1036	0.094
14	FFI	0cM	0.0028	0.0533	0.099
15	HAEHS	70cM	0.0006	0.0112	0.112
15	FFI	56cM	0.0038	0.0715	0.097
16	CFHS	56cM	0.0026	0.0555	0.100
16	FGHS	56cM	0.0005	0.0103	0.114
16	LA	92cM	0.0034	0.0730	0.098
16	FSM2	44cM	0.0010	0.0210	0.108
17	FA	9cM	0.0055	0.1475	0.093
17	FESR	9cM	0.0063	0.1680	0.092

¹See tables 32, 33, 34, 35 and 36 for trait abbreviations.

²Position with greatest F -statistics.

³Chromosome-wise P value

⁴Genome-wide P value calculated using 4.2

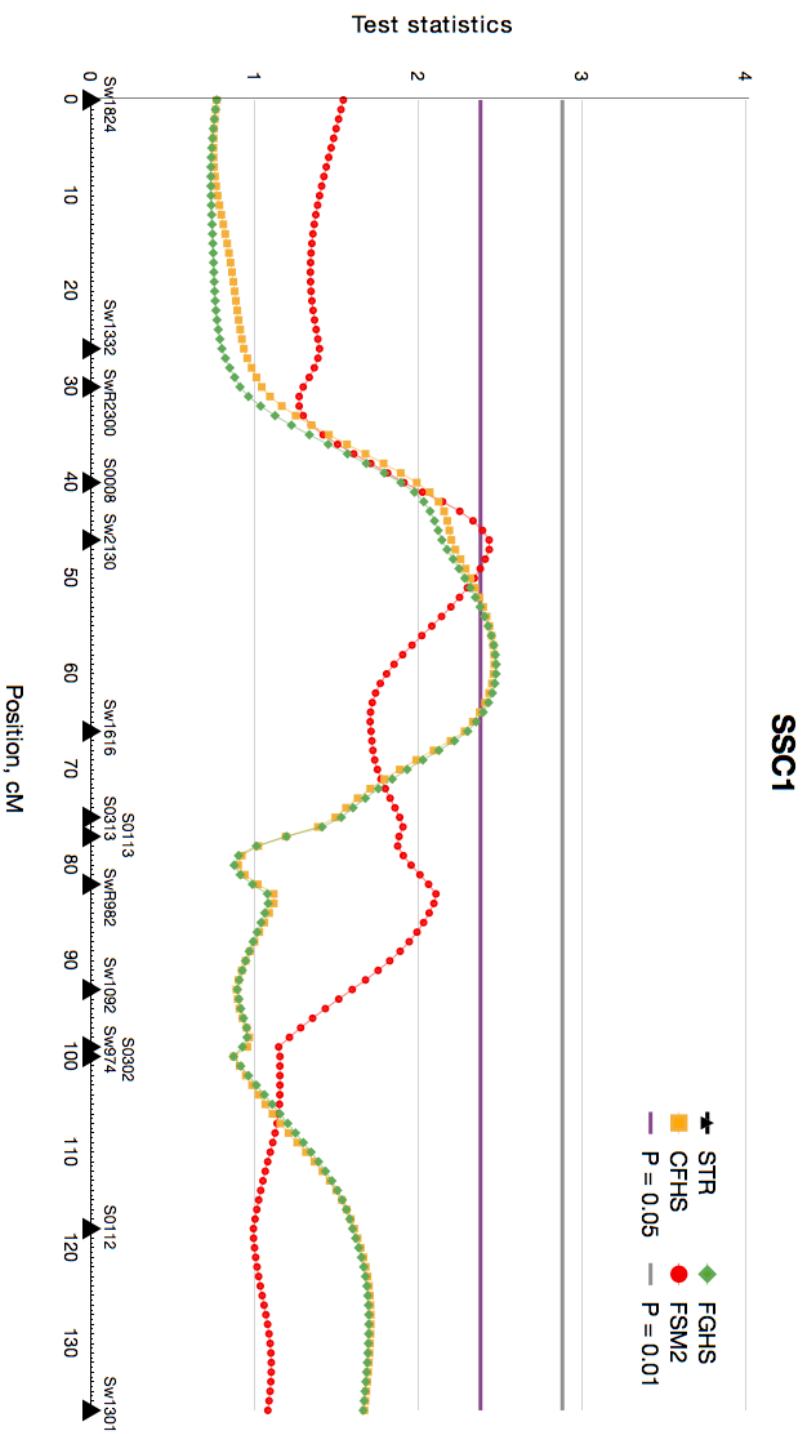


Figure 11: *F*-statistics profiles for SSC1 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

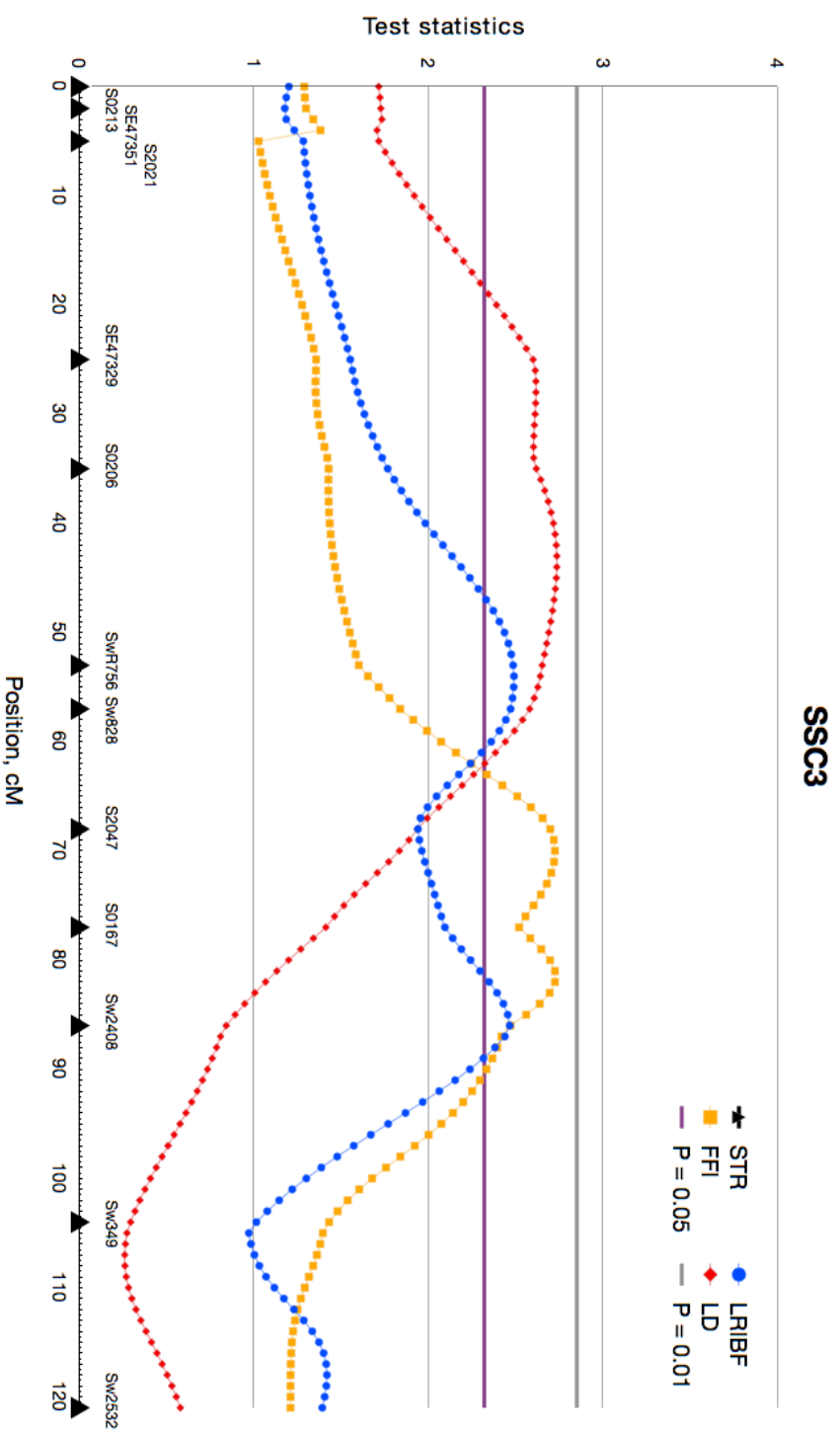


Figure 13: *F*-statistics profiles for SSC3 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

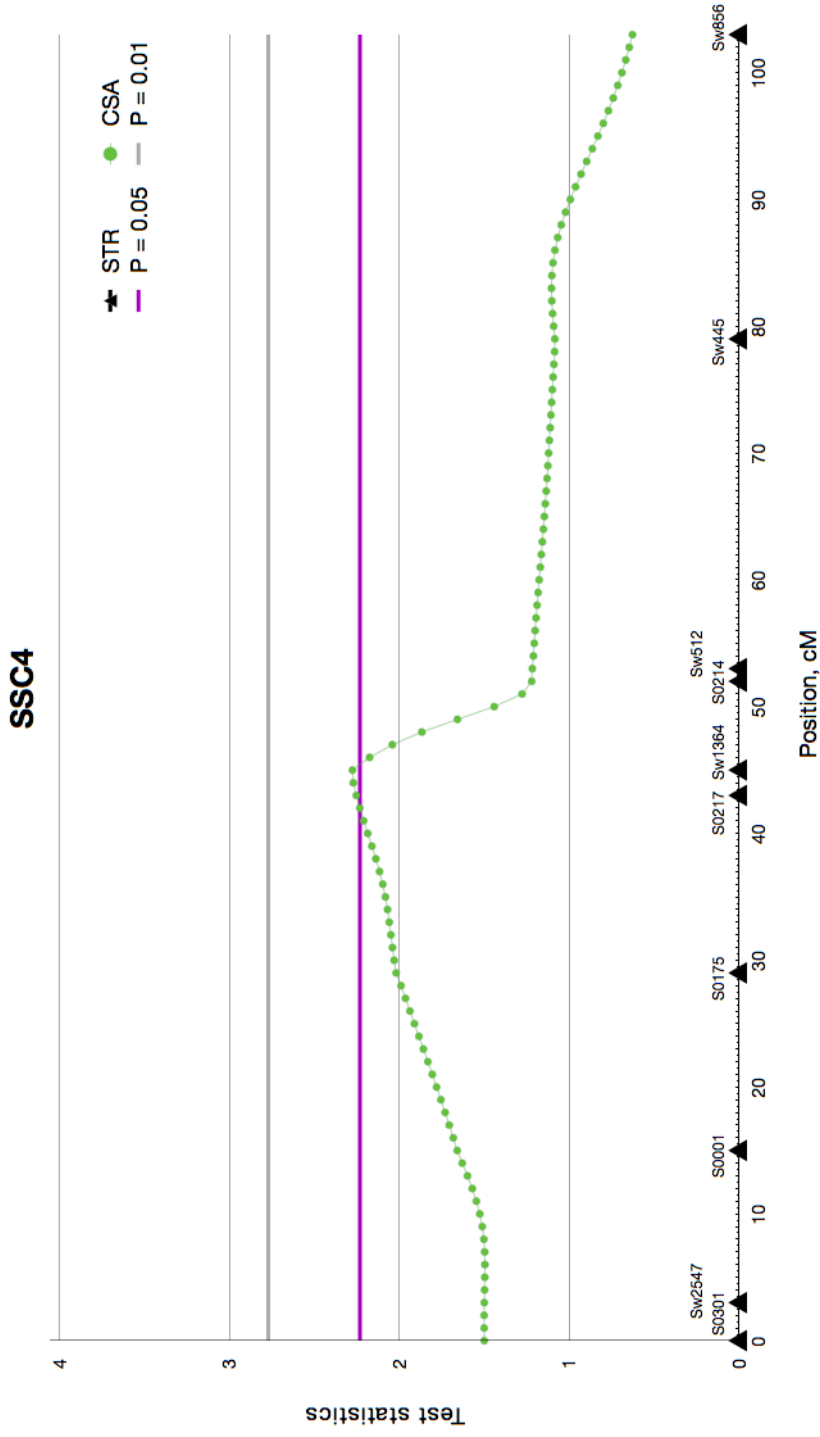


Figure 14: F-statistics profiles for SSC4 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

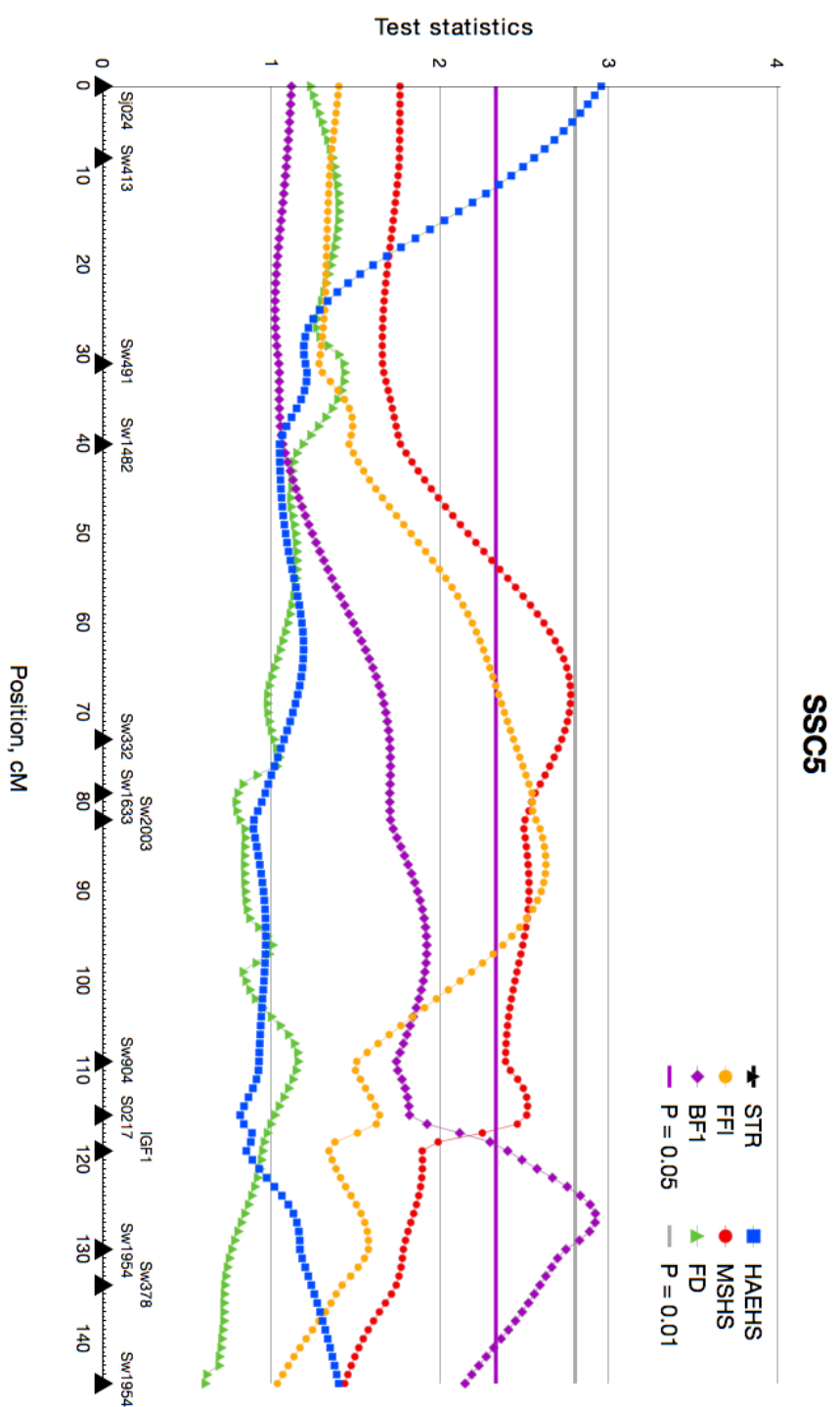


Figure 15: *F*-statistics profiles for SSC5 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

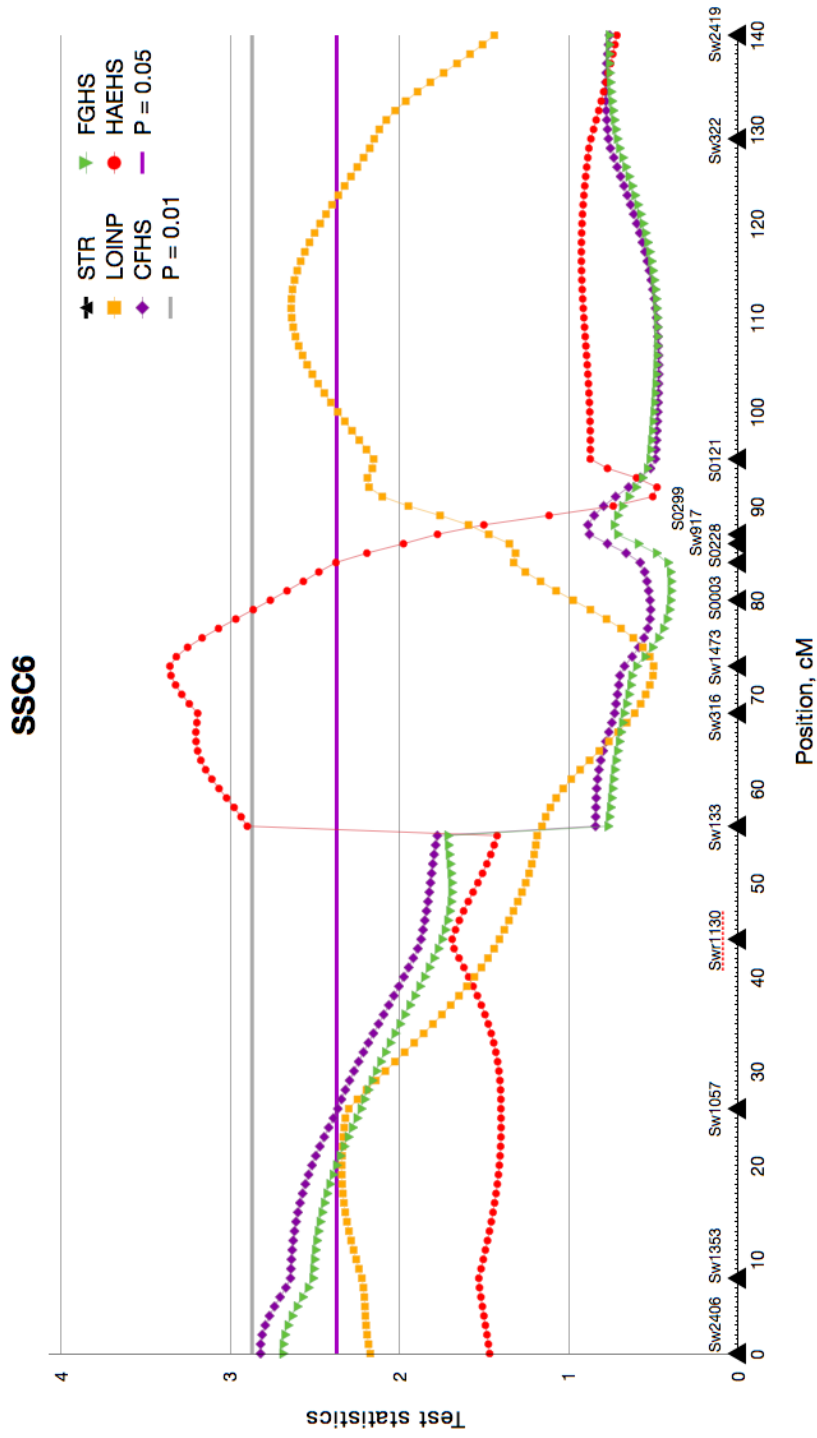


Figure 16: F-statistics profiles for SSC6 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

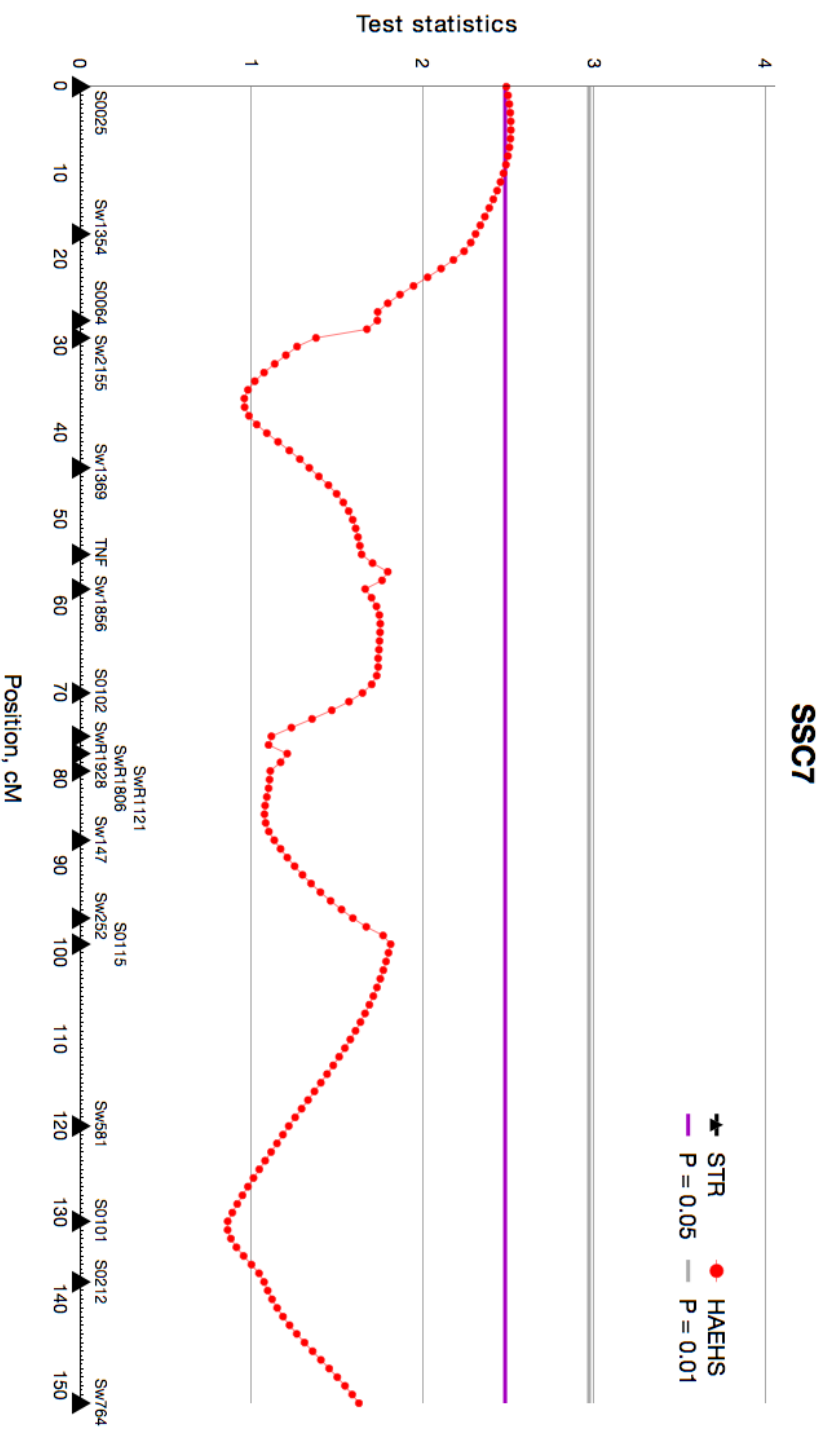


Figure 17: F -statistics profiles for SSC7 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

SSC8

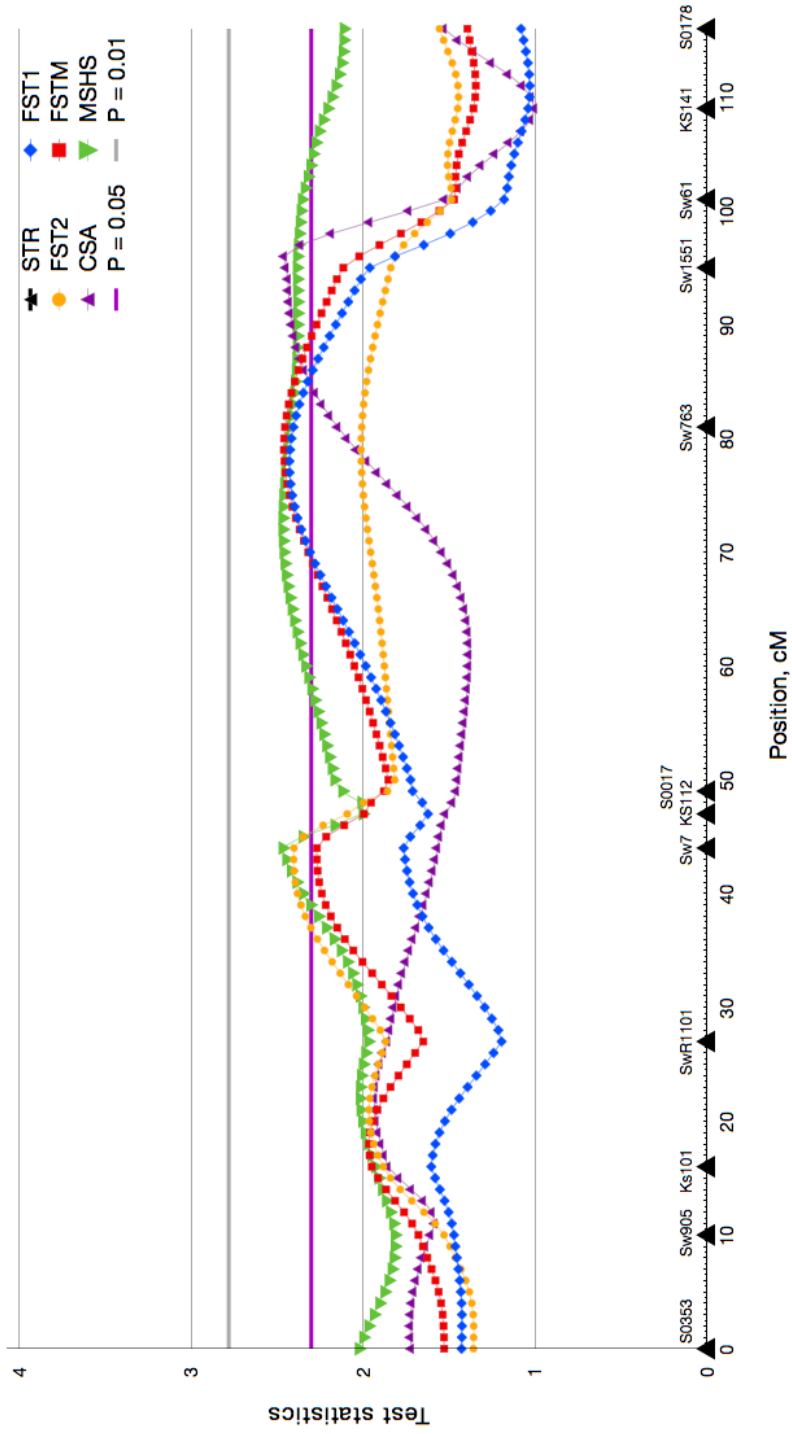


Figure 18: F-statistics profiles for SSC8 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

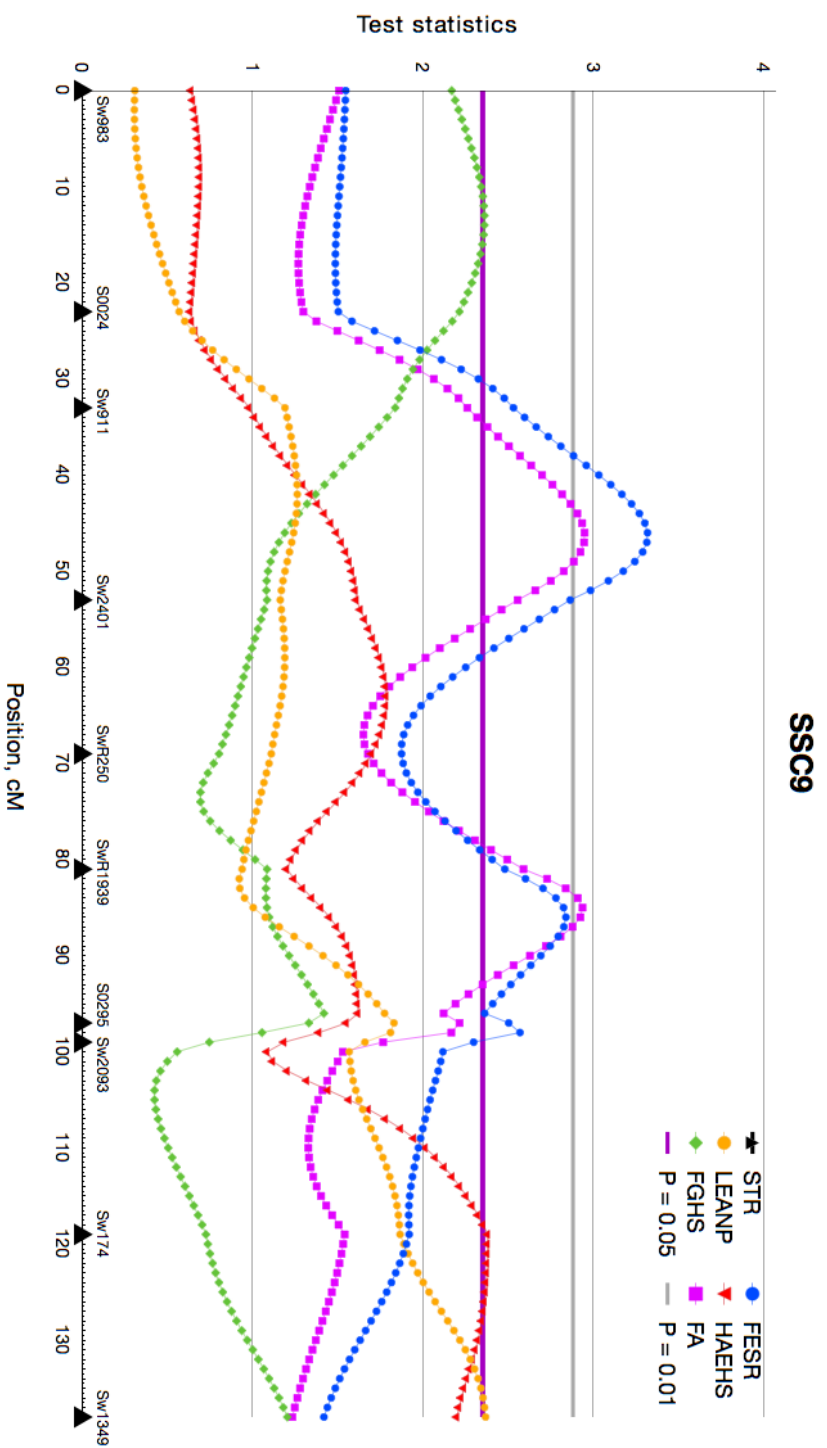


Figure 19: F-statistics profiles for SSC9 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

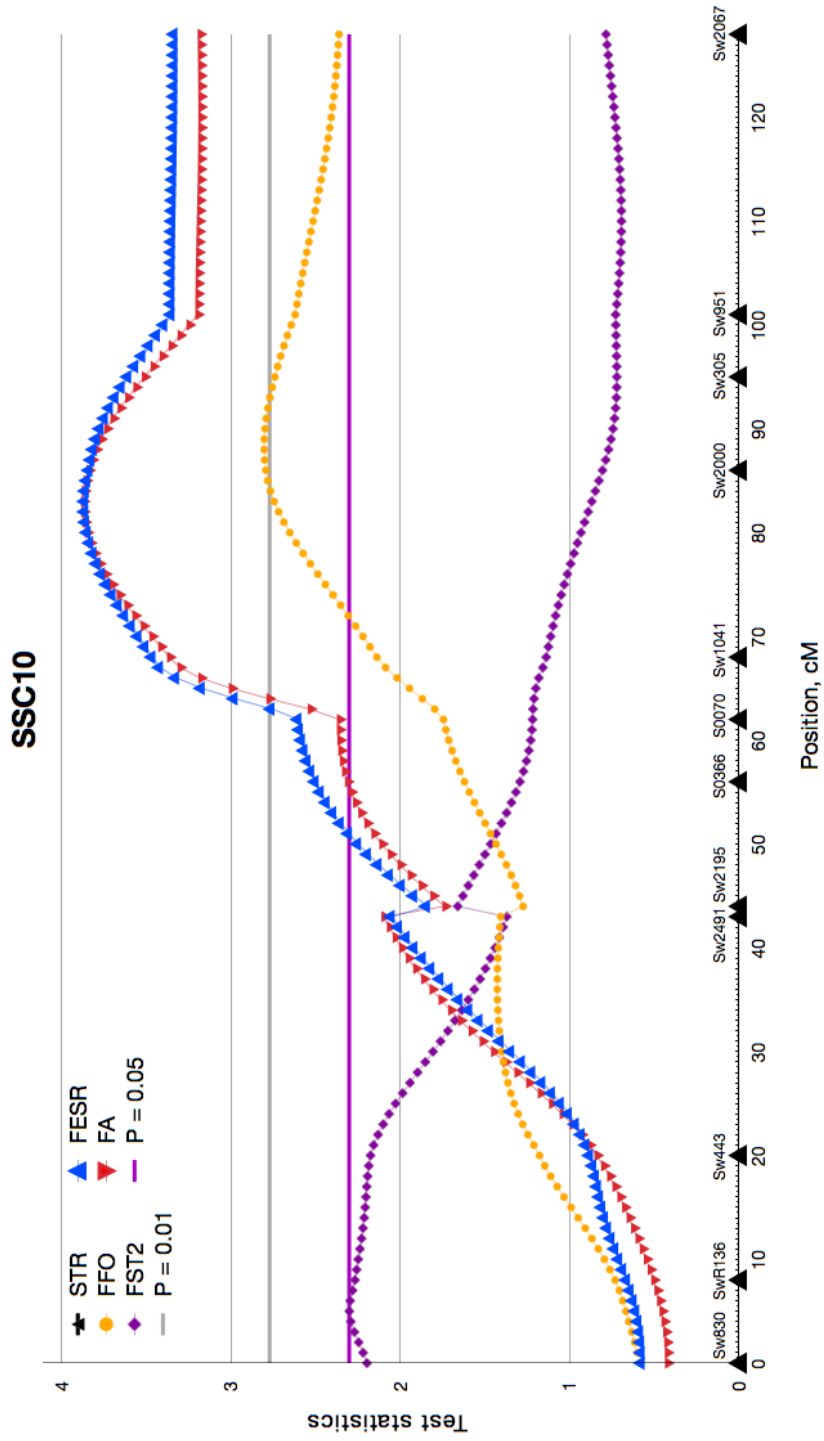


Figure 20: F -statistics profiles for SSC10 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

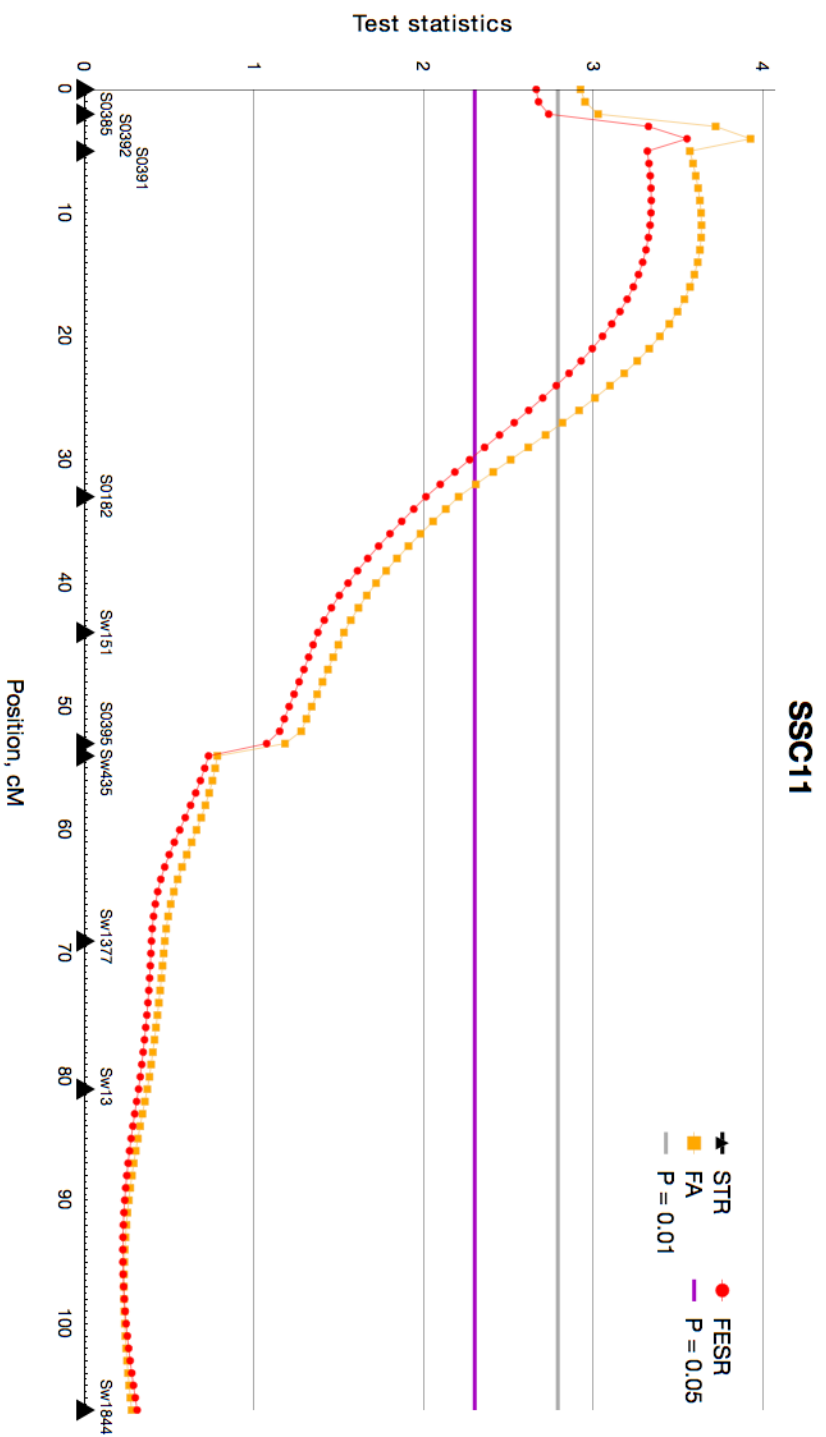


Figure 21: *F*-statistics profiles for SSC11 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

SSC12

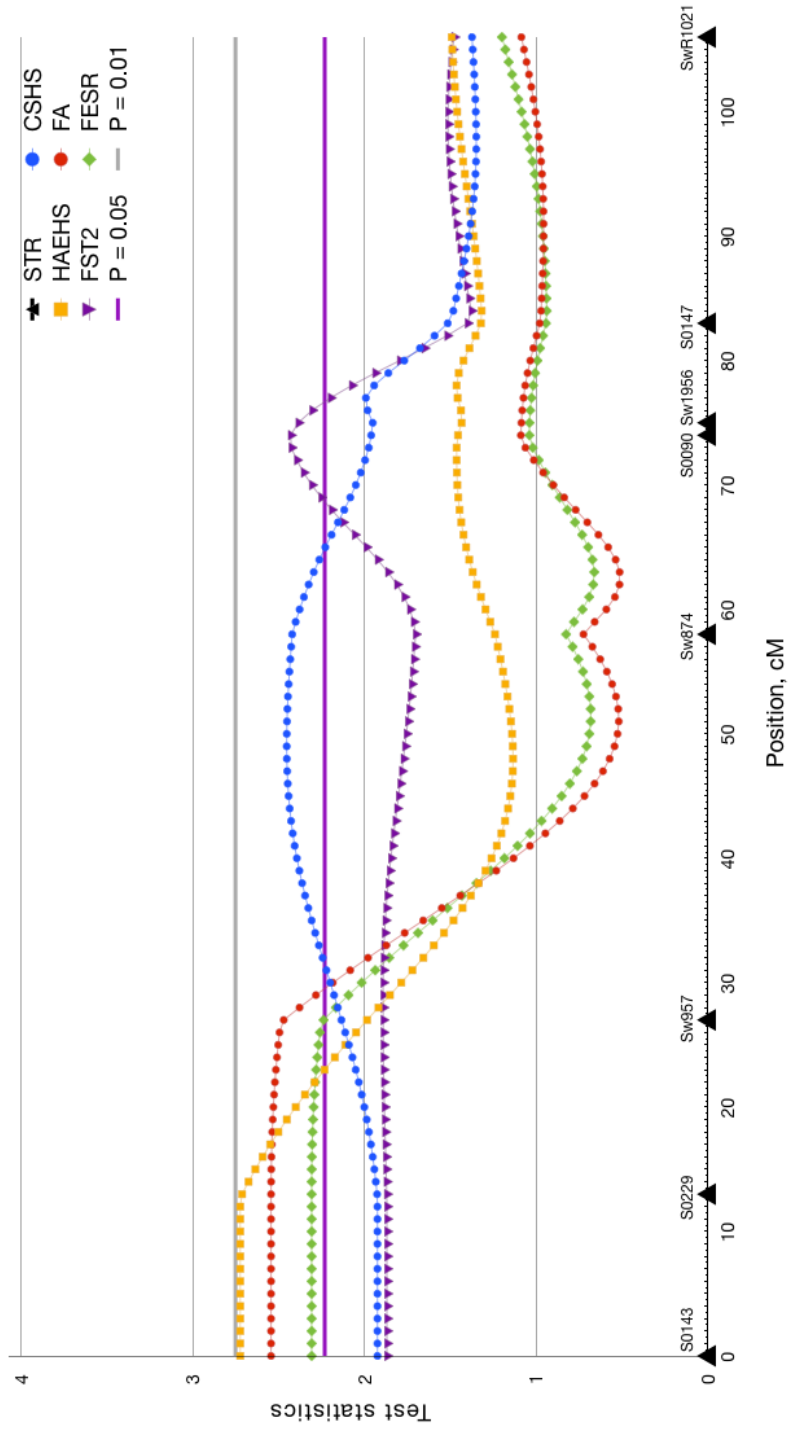


Figure 22: *F*-statistics profiles for SSC12 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36

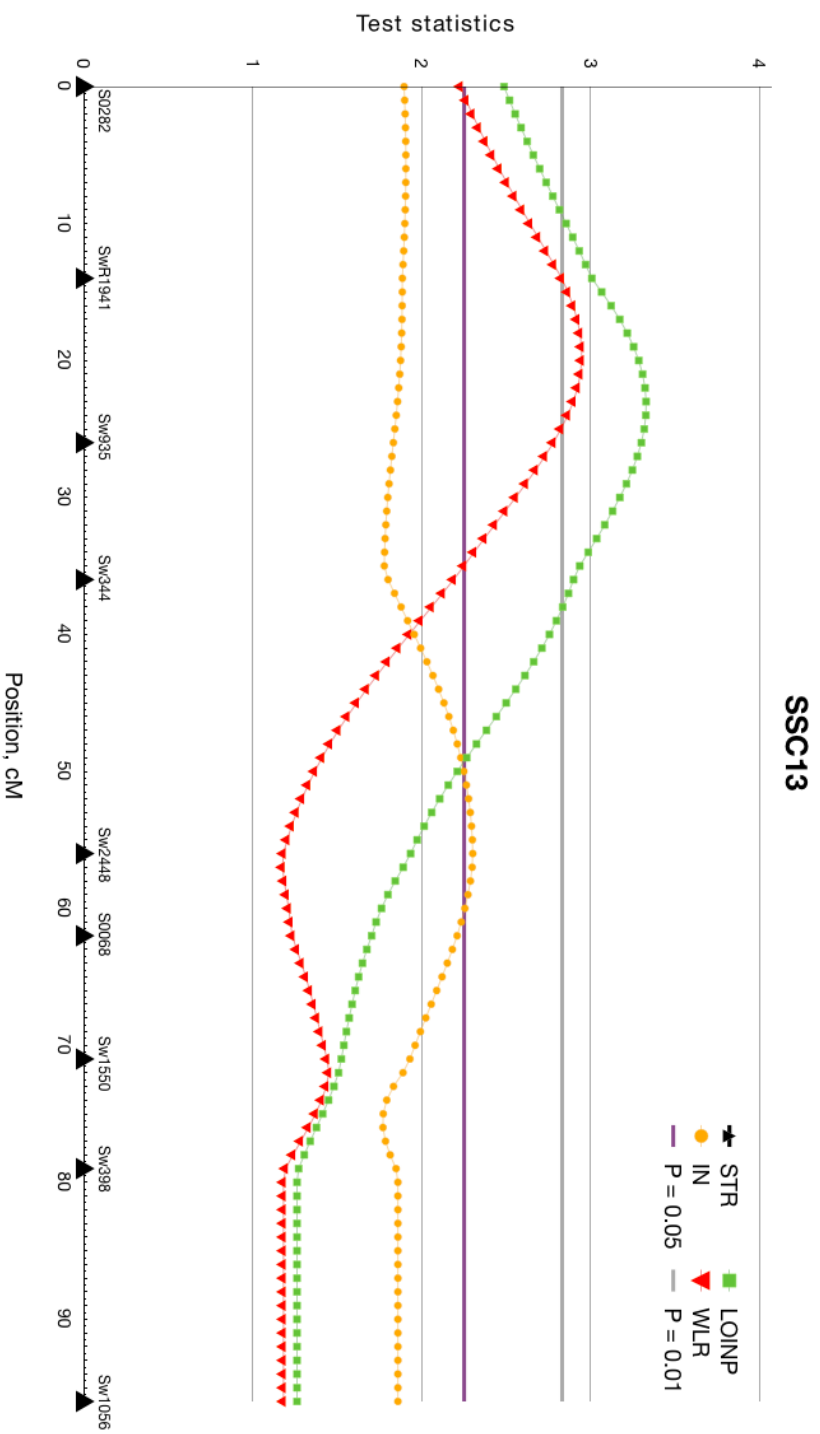


Figure 23: *F*-statistics profiles for SSC13 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

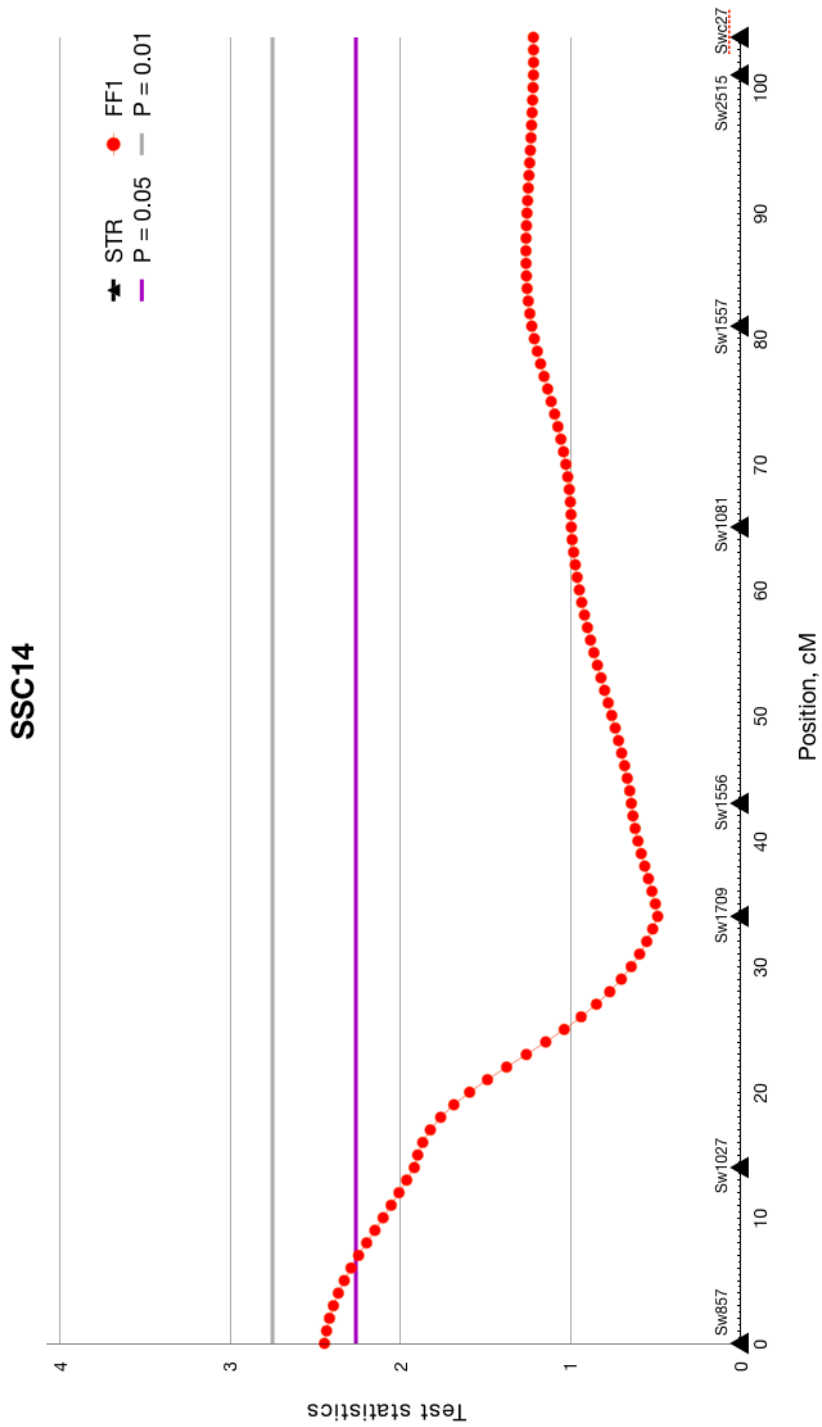


Figure 24: *F*-statistics profiles for SSC14 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36

SSC15

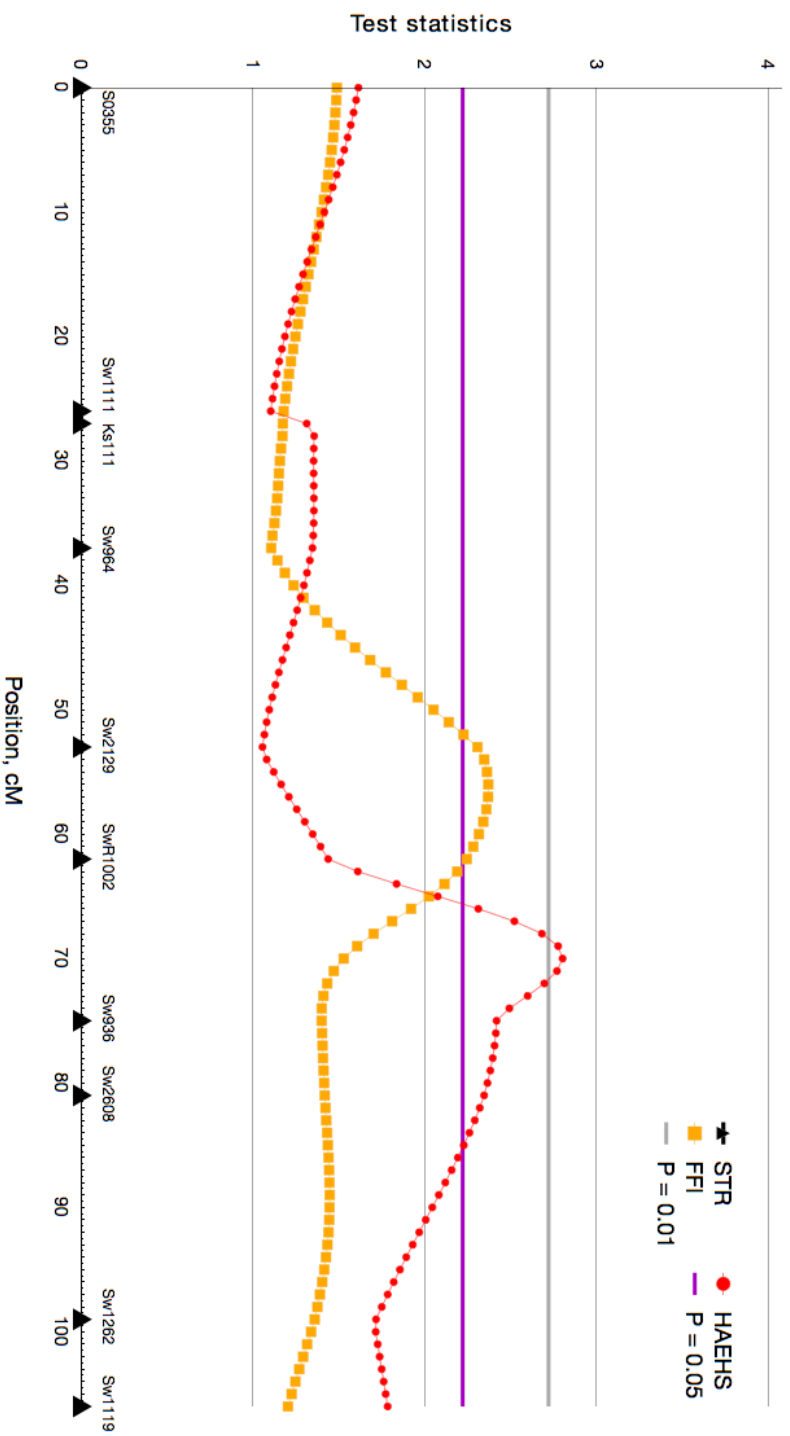


Figure 25: *F*-statistics profiles for SSC15 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

SSC16

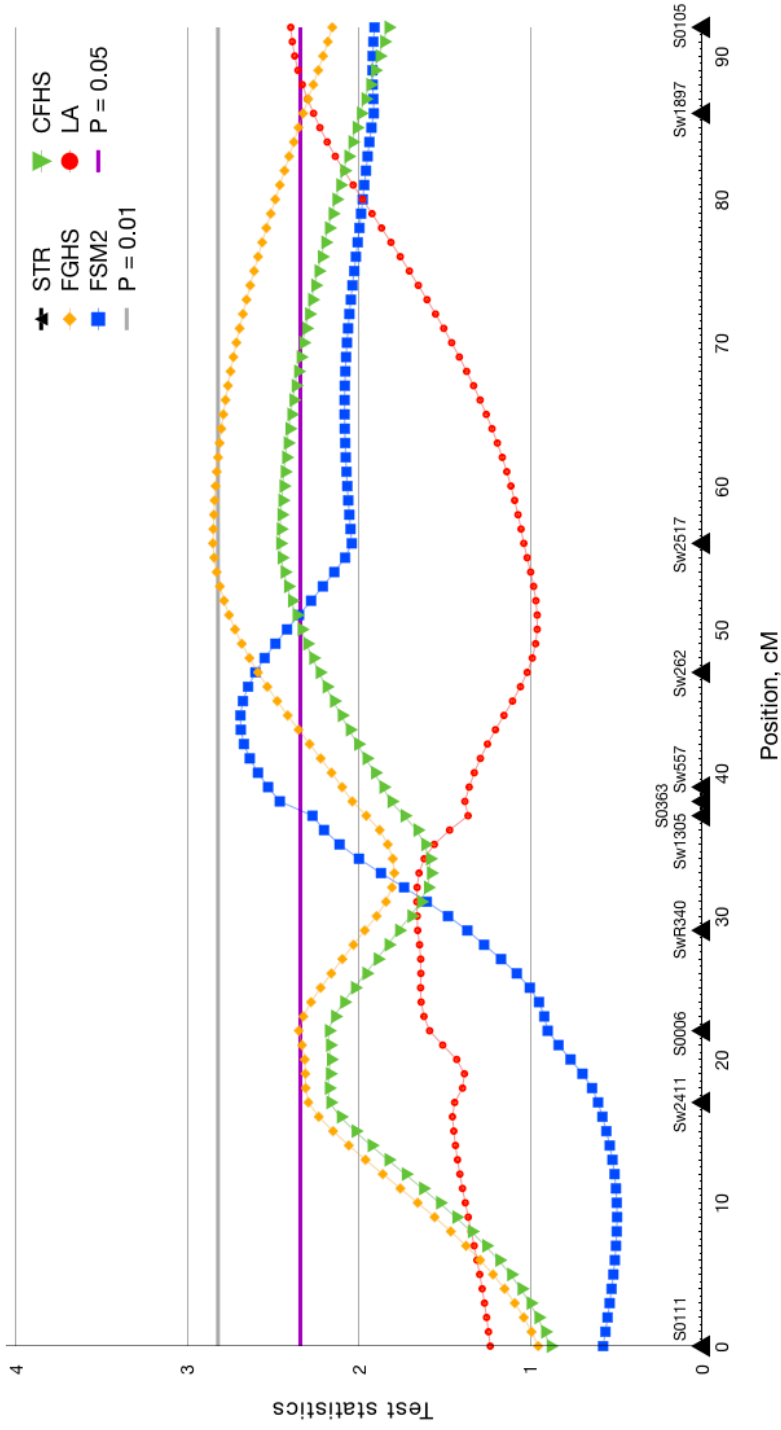


Figure 26: F -statistics profiles for SSC16 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36

SSC17

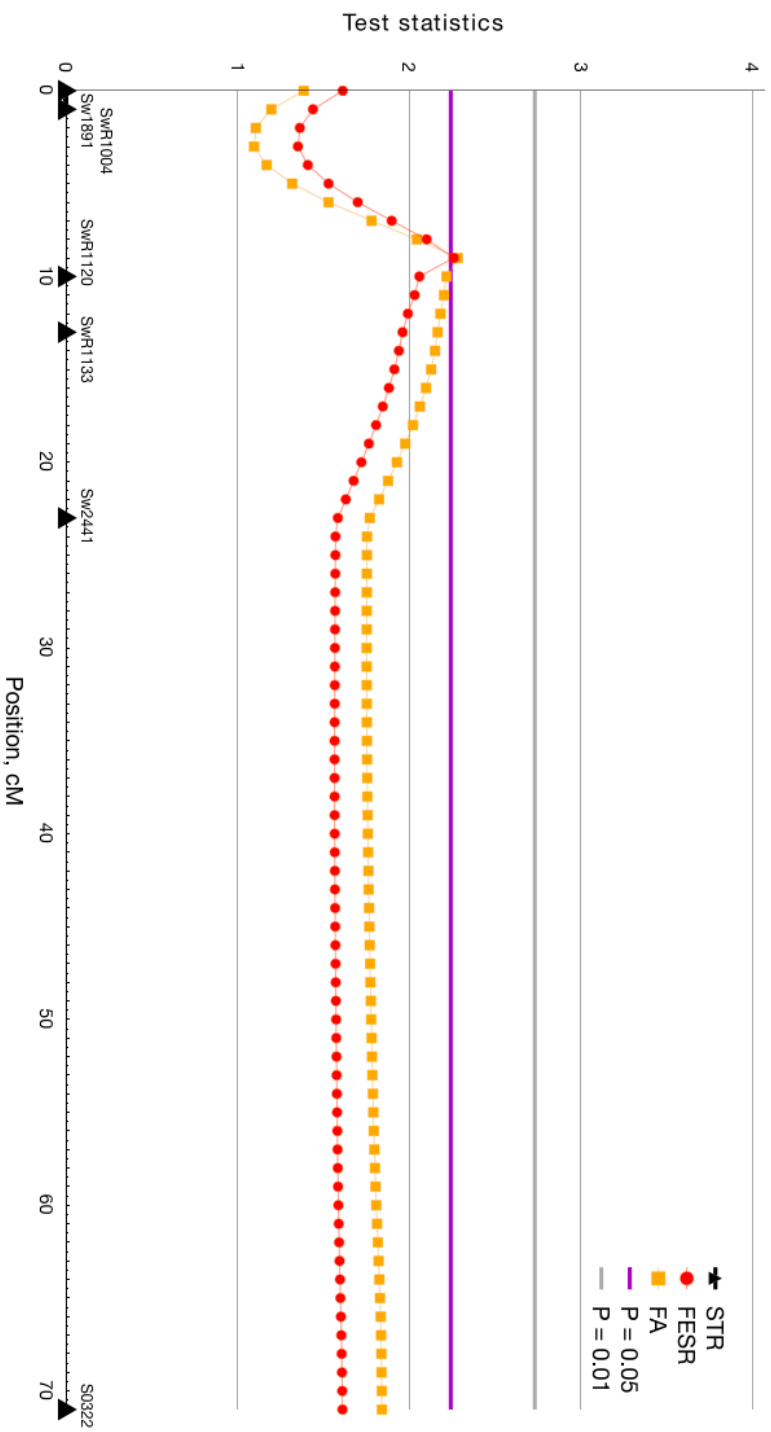


Figure 27: F-statistics profiles for SSC17 carrying QTL with $P < 0.05$ chromosome-wise significance. Traits abbreviations are given in tables 32, 33, 34, 35 and 36.

4.4 DISCUSSION

In this thesis, 52 QTLs exceeding the 5% chromosome-wise significance level were reported which is above the 43 that could be expected by chance only (864 tests performed: 48 traits and 18 linkage groups). Among these, sixteen QTLs exceeded the 1% genome-wide level (almost double of the number of QTLs expected by chance).

According to these results it is very likely that some QTLs, having a medium to large effect, are still segregating within this commercial line although selection for dry cured ham quality traits was already included in the breeding selection scheme (see: 1.2.2). Recent studies searching for QTLs in commercial line crosses (Heuven et al. [41], Slawinska et al. [78], Uemoto et al. [84], Yang et al. [88], Evans et al. [27], de Koning et al. [19], Y. Nagaminea and Visscher [87]) also detected the majority of QTL significant at the 5% chromosome-wise level mainly because presence of boars segregating for QTL is less likely in commercial lines than in experimental segregating population (Geldermann et al. [32]).

4.4.1 *Growth and carcass traits*

For the growth and fatness traits 4 QTL were identified in this study. No similar QTL for loin depth (LD) were previously reported in literature in the same region of SSC₃ but QTL for backfat were detected in the same location of SSC₃ by Edwards et al. [26] in a F₂ Duroc × Pietrain resource population. Different candidate genes mapping in the same chromosomal region of SSC₅ were found significantly associated with 10RIBBFT trait and other fat deposition traits by Ramos et al. [64].

For the carcass composition traits a similar trait (LEANP) was mapped close to the QTL for LOINP, that was found in this study on SSC₆, by de Koning et al. [19] in another commercial population. Many other carcass traits such as average daily gain (ADG), pH for *semimembranosus* (PH), calcium level (CACL), hot carcass weight (HCW), etc. map in the same position close to the major genes RYR₁ (responsible for the malignant hyperthermia, Fujii et al. [29]) and H-FAB known for their association with fatness and intramuscular fat traits. Loin muscle depth (LMDEP), a correlated trait to LOINP, was also found mapping on SSC₁₃ in correspondence to marker Sw935 by Zhang et al. [90]

4.4.2 *Meat quality traits*

Among the meat quality traits no QTL was identified in this study. Reasons for this are not clear; C. V. values for pH were very limited confirming absence of PSE and DFD meat: a low source of variation for this two traits together with the limited number of phenotypes recorded for PHU could be a reason. For Minolta color traits (HAMA, HAMB and HAML) although a discrete variability in phenotypes was recorded were not in linkage with any of the markers genotyped in this study.

4.4.3 *Dry cured ham quality traits*

Loss of weight

Loss of weight during curing is a very interesting trait. For the transformation industry this is one of the most interesting traits to deal with. In this study a QTL for weight loss after resting was indentified on SSC₁₃ with a similar peak conformation to a QTL for LOINP also mapping on this chromosome. Sturaro [80] reported a negative genetic correlation of WLC with CCW on the same population: the lower the carcass weight the higher the water loss after curing. Presence of two QTLs on the same chromosomal location for percentage of loin (LOINP) and for WLC could implicate a common genetic background underlying the two traits.

Instrumental firmness traits

Significative QTL for firmness traits were already mapped on SSC₂ (IGF2 region) by Thomsen et al. [83] using a three generation resource family and by Ciobanu et al. [18] investigating the role of calpastatin (CAST) gene haplotypes. In this study significative QTL for different muscles firmness on dry cured ham were mapped on SSC₁, SSC₈, SSC₁₂ and SSC₁₆ where no previous QTL were detected. The QTL for FSM₂ should be carefully analyzed because the measurement, taken on the outer part of the muscle, could be biased by the excessive dehydration of the muscle due to the absence of covering fat. Instrumental fat firmness here investigated together with muscle firmness on cross sectioned dry cured ham are not common traits analyzed in genome scans and therefore no results have been published, at my knowledge, by now.

External fat on ham trait (EFATHAM) was mapped by different authors in different studies on SSC₁, SSC₂, SSC₃, SSC₄, SSC₆, SSC₉, SSC₁₃, SSC₁₄ and SSC₁₆; in the commercial cross analyzed in this study a QTL significant at 1% genome-wide level was found segregating on SSC₅ in a 'QTL reach' region for carcass and meat quality traits.

Subjective evaluation traits

Many QTLs for subjective evaluation traits have been found in the present study and HAEHS was the most detected. Nevertheless care should be taken in drawing results from this trait because phenotypic variations was very limited and because presence of haemorrhages is mainly due to the stress of animal during CO₂ stunning and therefore a clear genetic background is difficult to prove.

More interesting for dry cured ham quality are traits like CFHS (subjective color of fat on ham) that is highly correlated with fat greasyness (FGHS) and more in general with the quality of fat. Three QTL for each trait were identified in this study mapping on SSC₁, SSC₆ and SSC₁₆, their *F*-statistics profiles were identical as well phenotypic source of variation.

HAMMS (ham shape value) is an important trait mainly for the San Daniele DOP cured ham as its disciplinary of production requires well defined shapes of products. For this trait one QTL was found on SSC₂ but due to its peculiarity in this particular product no other informations are available in literature at the moment.

Marbling (MARB) is a very well studied trait for which many QTL have been identified in literature mainly in SSC₂ in the region of the

IGF2 gene but also, very recently, two QTLs were found by Ramos et al. [64] and by Ma et al. [55] in the same region of SSC5 for which a QTL for MSHS was identified in this study. Another QTL for MARB identified by [?] on SSC8 on a F2 Duroc-Landrace population was confirmed by this study mapping in correspondance of marker Sw763.

Computer image analysis

Among all the traits here analyzed, computer image analysis of cross sectioned dry cured hams are the most important because they are very expensive. Although the analysis is nondestructive, the cross-sectioning of cured hams causes high depreciation of the product therefore markers or candidate genes for traits like ham fat eye area (FA) ham lean area (LA) and other correlated traits such as CSA, BICA, SEMA and FESR would be very useful for the selection of high quality products.

In this genome scan 13 QTL were successfully mapped for these traits. FESR and FA profiles mapped to exactly the same linkage groups with identical profiles and very similar *F*-statistics with the exception of SSC9 (only FESR) indicating the strong correlation between the two traits.

In literature QTLs for fat area (FATAREA) were found on SSC1, SSC2, SSC6, SSC7, SSC12, SSC13 and SSC16 by Yue et al. [89] and Liu et al. [53]. The QTL on SSC12 found in this study maps in the same position of the QTL reported by Yue et al. [89] thus becoming a good candidate for fine mapping. The only drawback is the broad significative chromosomal region that extend for almost 20 cM.

Computer image analysis on cross-sectional areas on *longissimus* were measured by Rohrer et al. [66] in a F2 Duroc-Landrace population; a QTL for MLD (meat area) was found in the same position of FESR and FA on SSC17 found in this study.

All the QTLs for FA and FESR located were highly significative and mostly exceeding the 1% genome-wide significance level thus indicating strong evidence of presence of major genes controlling these traits.

Iodine number

This trait is very important for this commercial line because measurements were routinely taken until recently for sires' evaluation. Iodine number measures the amount of unsaturation of fatty acids; the same trait (FA-UI) was recently analyzed by Guo et al. [38] which identified two QTL on SSC7 and on SSC8. In this thesis a novel QTL for IN was mapped on SSC13.

4.5 IMPLICATIONS

Informations obtained on commercial lines are very important because they give an overview of traits under selection and, if direct markers are available, and are still segregating in the population, they could be directly applied in marker assisted selection (MAS) schemes for rapid genetic gain.

Results of the linkage analysis on half-sib commercial population like the one presented in this thesis could not be directly implemented in MAS because the extent of LD between markers and traits is not maintained over wide map distances and across families. In particular Du et al. [25] calculated, using 4,500 SNP markers, an average $r^2 = 0.1$

LD: linkage disequilibrium

SNP: single nucleotide polymorphisms

for all pairs of SNP 3 cM apart, thus recommending a spacing of 0.1 to 1 cM for a whole genome association study in pig commercial lines using this kind of markers. Nevertheless locating chromosomal regions harbouring QTL for important economical traits such those identified in this study is an important baseline for detecting candidate genes and subsequent identifications of QTN that could be used in MAS.

*QTN: quantitative
trait nucleotides*

The first release of the high coverage assembly of the pig genome has been recently published (see section: 3.1.1) and gene annotation is already started allowing for the selection of candidate genes mapping on QTL regions detected. Comparative mapping for chromosome regions harbouring interesting QTL could also be implemented allowing researchers to use and validate candidate genes from other species such as humans or mouse. Moreover the new sequencing technologies and high-throughput SNP genotyping platform already allow for cost effective SNP and CNV detection on the whole genome or in selected regions (such those found in this study) harbouring interesting QTLs.

*CNV: copy number
variation*

Part V
APPENDIX

BIBLIOGRAPHY

- [1] URL www.prosciuttosandaniele.it. (Cited on page 3.)
- [2] D.o.p. prosciutto di san daniele. 1996. disciplinare della denominazione di origine protetta prosciutto di san daniele. regolamento cee no. 1107. (Cited on pages 4, 5, 21, 22, and 66.)
- [3] Council regulation (eec) no 2081/92 of 14 july 1992 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs. (Cited on page 3.)
- [4] Gazzetta ufficiale n.293 del 15/15/1999. approvazione del protocollo di accordo relativo alla denominazione di origine protetta del "prosciutto di san daniele". (Cited on page 3.)
- [5] D.Lvo 537/92. Decreto legislativo 30 dicembre 1992, n. 537. attuazione della direttiva 92/5/cee relativa a problemi sanitari in materia di scambi intracomunitari di prodotti a base di carne. (Cited on page 4.)
- [6] L. Andersson, C. S. Haley, H. Ellegren, S. A. Knott, M. Johansson, K. Andersson, L. Andersson-Eklund, I. Edfors-Lilja, M. Fredholm, I. Hansson, and al. et. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science*, 263(5154):1771-1774, Mar 1994. (Cited on page 6.)
- [7] AOAC. *Official method of analysis*. ED. Association of Official Analytica Chemists, Washington DC., 13 edition, 1980. (Cited on page 66.)
- [8] ASPA. *Metodologie relative alla macellazione degli animali di interesse zootecnico e alla valutazione e dissezione della loro carcassa*. ISMEA Ed., 1991. (Cited on page 21.)
- [9] P. Beeckmann, J. Schröffel Jr, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. Linkage and qtl mapping for sus scrofa chromosome 3. *Journal of Animal Breeding and Genetics*, 120(s1): 20-27, 2003. (Cited on page 7.)
- [10] J. Bidanel and M. F. Rothschild. Current status of quantitative trait locus mapping in pigs. *Pig News Info*, 23:39-54, 2002. (Cited on pages 10 and 19.)
- [11] P. Bosi and V. Russo. The production of the heavy pig for high quality processed products. *Ital. J. Anim. Science*, 3:309-321, Oct 2004. (Cited on pages 5 and 65.)
- [12] P. Bosi, J. A. Cacciavillani, L. Casini, D. P. Lo Fiego, M. Marchetti, and S. Mattuzzi. Effects of dietary high-oleic acid sunflower oil, copper and vitamin e levels on the fatty acid composition and the quality of dry cured parma ham. *Meat Science*, 54(2):119-126, 2 2000. (Cited on page 19.)

- [13] P. Carnier, M. Cassandro, E. Knol, and D. Padoan. *Genetic parameters for some carcass and fresh ham traits of crossbred Golland pigs.*, pages 221–223. G. Piva, G. Bertoni, F. Masoero, P. Bani, L. Calamari (eds.) *Recent Progress in Animal Science*.1., 1999. (Cited on page 5.)
- [14] P. Carnier, L. Gallo, C. Romani, E. Sturaro, and V. Bondesan. Computer image analysis for measuring lean and fatty areas in cross-sectioned dry-cured hams. *J. Anim. Sci.*, 82(3):808–15, Mar 2004. (Cited on pages 23 and 67.)
- [15] S. Cepica, G. Reiner, H. Bartenschlager, G. Moser, and H. Geldermann. Linkage and qtl mapping for sus scrofa chromosome x. *Journal of Animal Breeding and Genetics*, 120(s1):144–151, 2003. (Cited on page 7.)
- [16] R. Chizzolini, P. ROsa, E. Novelli, E. Zanardi, M. T. Pacchili, E. Goriani, L. Boni, A. Rossi, and L. Rotteglia. La valutazione della consistenza del grasso suino: misura strumentale, valutazione sensoriale e parametri chimici a confronto. *Rivista di suinicoltura*, 36(6):41–49, 1995. (Cited on page 20.)
- [17] G. Churchill and R. Doerge. Empirical threshold values for quantitative trait mapping. *Genetics*, 138(3):963–971, Nov 1994. (Cited on page 73.)
- [18] D. C. Ciobanu, J. W. M. Bastiaansen, S. M. Lonergan, H. Thomsen, J. C. M. Dekkers, G. S. Plastow, and M. F. Rothschild F. New alleles in calpastatin gene are associated with meat quality traits in pigs. *Journal of Animal Science*, 82(10):2829–2839, 10 2004. (Cited on pages 10 and 96.)
- [19] D. J. de Koning, R. Pong-Wong, L. Varona, G. J. Evans, E. Giuffra, A. Sanchez, G. Plastow, J. L. Noguera, L. Andersson, and C. S. Haley. Full pedigree quantitative trait locus analysis in commercial pigs using variance components. *Journal of Animal Science*, 81(9):2155–2163, 9 2003. (Cited on page 95.)
- [20] A. de Vries, L. Faucitano, A. Sosnicki, and G. Plastow. The use of gene technology for optimal development of pork meat quality. *Food Chemistry*, 69(4):397–405, 2000. doi:doi:DOI:10.1016/S0308-8146(00)00049-2. (Cited on page 10.)
- [21] J. C. M. Dekkers. Commercial application of marker and gene assisted selection in livestock: Strategies and lessons. *Journal of Animal Science*, 82(13):313–328, 1 2004. (Cited on pages 9 and 10.)
- [22] C. Diaferia and P. Baldini. Influenza della temperatura di stagionatura e del tempo di stagionatura sulle caratteristiche chimico-fisiche e sensoriali di prosciutti crudi tipo veneto. *Industria conserve*, 69:91–95, 1994. (Cited on page 20.)
- [23] D. Dieringer and C. Schlötterer. Microsatellite analyser (msa): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes*, 3(1):167–169, 2003. (Cited on page 55.)
- [24] M. Dragos-Wendrich, I. Sternstein, C. Brunsch, G. Moser, H. Bartenschlager, G. Reiner, and H. Geldermann. Linkage and qtl mapping for sus scrofa chromosome 14. *Journal of Animal Breeding and Genetics*, 120(s1):111–118, 2003. (Cited on page 7.)

- [25] F. X. Du, A. C. Clutter, and M. M. Lohuis. Characterizing linkage disequilibrium in pig populations. *Int. J. Biol. Sci.*, 3:166–178, 2007. (Cited on page 97.)
- [26] D. B. Edwards, C. W. Ernst, R. J. Tempelman, G. J. M. Rosa, N. E. Raney, M. D. Hoge, and R. O. Bates. Quantitative trait loci mapping in an f₂ duroc x pietrain resource population: I. growth traits. *Journal of Animal Science*, 86(2):241–253, 2 2008. (Cited on page 95.)
- [27] G. Evans, E. Giuffra, A. Sanchez, S. Kerje, G. Davalos, O. Vidal, S. Illan, J. Noguera, L. Varona, I. Velander, O. Southwood, D. J. de Koning, C. Haley, G. Plastow, and L. Andersson. Identification of quantitative trait loci for production traits in commercial pig populations. *Genetics*, 164(2):621–627, Jun 2003. (Cited on pages 7, 8, 9, and 95.)
- [28] K. Frydendahl, T. K. Jensen, J. S. Andersen, M. Fredholm, and G. Evans. Association between the porcine escherichia coli f18 receptor genotype and phenotype and susceptibility to colonisation and postweaning diarrhoea caused by e. coli o138:f18. *Veterinary Microbiology*, 93(1):39–51, 5 2003. (Cited on page 10.)
- [29] J. Fujii, K. Otsu, F. Zorzato, S. de Leon, V. K. Khanna, J. E. Weiler, P. J. O'Brien, and D. H. MacLennan. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*, 253(5018):448–451, 7 1991. (Cited on pages 10 and 95.)
- [30] L. Gallo and V. Bondesan. La qualità della carne suina tra carne fresca e prodotti trasformati. *Eurocarni*, 7:99–107, 2000. (Cited on page 20.)
- [31] L. Gallo, D. Padoan, V. Bondesan, and N. Penzo. *Effect of some carcass and fresh ham traits on curing loss of hams from crossbred Goland pigs.*, pages 689–691. G. Piva, G. Bertoni, F. Masoero, P. Bani, L. Calamari (eds.) *Recent Progress in Animal Science.1.*, 1999. (Cited on page 5.)
- [32] H. Geldermann, E. Müller, G. Moser, G. Reiner, H. Bartenschlager, S. Cepica, A. Stratil, J. Kuryl, C. Moran, R. Davoli, and C. Brunsch. Genome-wide linkage and qtl mapping in porcine f₂ families generated from pietrain, meishan and wild boar crosses. *Journal of Animal Breeding and Genetics*, 120(6):363–393, 2003. (Cited on page 95.)
- [33] F. Gerbens, A. Jansen, A. J. M. van Erp, F. Harders, T. H. E. Meuwissen, G. Rettenberger, J. H. Veerkamp, and F. W. M. te Pas. The adipocyte fatty acid-binding protein locus: characterization and association with intramuscular fat content in pigs. *Mammalian Genome*, 9(12):1022–1026, 12 1998. (Cited on page 10.)
- [34] F. Gerbens, A. J. van Erp, F. L. Harders, F. J. Verburg, T. H. Meuwissen, J. H. Veerkamp, and M. F. te Pas. Effect of genetic variants of the heart fatty acid-binding protein gene on intramuscular fat and performance traits in pigs. *Journal of Animal Science*, 77(4):846–852, 1999. (Cited on page 10.)
- [35] S. Gigli, M. T. Pacchioli, and D. Barchi. Valutazione della coscia per il prosciutto. *Riv. Suinicul.*, 37:31–39, 1993. (Cited on page 65.)

- [36] P. Gou, J. Comaposada, and J. Arnau. Nacl content and temperature effects on moisture diffusivity in the gluteus medius muscle of pork ham. *Meat Science*, 63(1):29 – 34, 2003. (Cited on page 20.)
- [37] P. Green, K. Falls, and S. Crook. *Documentation for CriMap, Version 2.4.*, whashington university school of medicine, st. louis, mo. edition, 1990. (Cited on page 67.)
- [38] T. Guo, J. Ren, K. Yang, J. Ma, Z. Zhang, and L. Huang. Quantitative trait loci for fatty acid composition in longissimus dorsi and abdominal fat: results from a white duroc x erhualian intercross f2 population. *Anim Genet*, 40(2):185–191, Apr 2009. (Cited on page 97.)
- [39] J. Gutierrez, L. Royo, I. Alvarez, and F. Goyache. Molkin v2.0: A computer program for genetic analysis of populations using molecular coancestry information. *J. Hered.*, 96(6):718–721, Nov 2005. (Cited on page 55.)
- [40] N. Harmegnies, F. Davin, S. De Smet, N. Buys, M. Georges, and W. Coppeters. Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. *Anim Genet*, 37(6):543–553, 2006. (Cited on pages 7 and 8.)
- [41] H. C. M. Heuven, R. H. J. van Wijk, B. Dibbits, T. A. van Kampen, E. F. Knol, and H. Bovenhuis. Mapping carcass and meat quality qtl on sus scrofa chromosome 2 in commercial finishing pigs. *Genet Sel Evol*, 41(1):4, Jan 2009. (Cited on pages 7, 8, 9, 73, and 95.)
- [42] Z. Hu and J. Reecy. Animal qtldb: beyond a repository. *Mammalian Genome*, 18(1):1–4, Jan 2007. (Cited on page 6.)
- [43] L. Hughes, J. Bao, Z. L. Hu, V. Honavar, and J. Reecy. Animal trait ontology: The importance and usefulness of a unified trait vocabulary for animal species. *J. Anim Sci.*, 86(6):1485–1491, 2008. (Cited on page 8.)
- [44] S. Humphray, C. Scott, R. Clark, B. Marron, C. Bender, N. Camm, J. Davis, A. Jenks, A. Noon, M. Patel, H. Sehra, F. Yang, M. Rogatcheva, D. Milan, P. Chardon, G. Rohrer, D. Nonneman, P. de Jong, S. Meyers, A. Archibald, J. Beever, L. Schook, and J. Rogers. A high utility integrated map of the pig genome. *Genome Biology*, 8(7):R139, 2007. (Cited on page 27.)
- [45] J. T. Jeon, O. Carlborg, A. Tornsten, E. Giuffra, V. Amarger, P. Chardon, L. Andersson-Eklund, K. Andersson, I. Hansson, K. Lundstrom, and L. Andersson. A paternally expressed qtl affecting skeletal and cardiac muscle mass in pigs maps to the igf2 locus. *Nat Genet*, 21(2):157–158, 02 1999. (Cited on page 10.)
- [46] C. B. Jørgensen, S. Cirera, S. I. Anderson, A. L. Archibald, T. Raudsepp, B. Chowdhary, I. Edfors-Lilja, L. Andersson, and M. Fredholm. Linkage and comparative mapping of the locus controlling susceptibility towards e. coli f4ab/ac diarrhoea in pigs. *Cytogenetic and Genome Research*, 102(1):157–162, 2003. (Cited on page 10.)

- [47] P. Karlskov-Mortensen, C. S. Bruun, M. H. Braunschweig, M. Sawera, E. Markljung, A. C. Enfält, I. Hedebro-Velander, Å. Josell, G. Lindahl, K. Lundström, G. Seth, C. B. Jørgensen, L. Andersson, and M. Fredholm. Genome-wide identification of quantitative trait loci in a cross between hampshire and landrace i: carcass traits. *Anim Genet*, 37(2):156–162, 2006. (Cited on pages 7 and 8.)
- [48] J. M. H. Kijas, R. Wales, A. Tornsten, P. Chardon, M. Moller, and L. Andersson. Melanocortin receptor 1 (*mc1r*) mutations and coat color in pigs. *Genetics*, 150(3):1177–1185, 11 1998. (Cited on page 10.)
- [49] K. S. Kim, N. Larsen, T. Short, G. Plastow, and M. F. Rothschild. A missense variant of the porcine melanocortin-4 receptor (*mc4r*) gene is associated with fatness, growth, and feed intake traits. *Mammalian Genome*, 11(2):131–135, 02 2000. (Cited on page 10.)
- [50] S. Knott, J. Elsen, and C. Haley. Methods for multiple-marker mapping of quantitative trait loci in half-sib populations. *TAG Theoretical and Applied Genetics*, 93(1):71–80, Jul 1996. (Cited on page 73.)
- [51] D. J. De Koning, L. Janss, A. Rattink, P. van Oers, B. de Vries, M. Groenen, Jan van der Poel, Piet de Groot, E Brascamp, and Johan van Arendonk. Detection of quantitative trait loci for back-fat thickness and intramuscular fat content in pigs (*sus scrofa*). *Genetics*, 152(4):1679–1690, Aug 1999. (Cited on page 73.)
- [52] A. Van Laere, M. Nguyen, M. Braunschweig, C. Nezer, C. Collette, L. Moreau, A. Archibald, C. S. Haley, N. Buys, M. Tally, G. Andersson, M. Georges, and L. Andersson. A regulatory mutation in *igf2* causes a major qtl effect on muscle growth in the pig. *Nature*, 425(6960):832, Oct 2003. (Cited on page 8.)
- [53] G. Liu, J. Kim, E. Jonas, K. Wimmers, S. Ponsuksili, E. Murani, C. Phatsara, E. Tholen, H. Juengst, D. Tesfaye, J. Chen, and K. Schellander. Combined line-cross and half-sib qtl analysis in duroc-pietrain population. *Mammalian Genome*, 19(6):429–438, 06 2008. (Cited on pages 8, 73, and 97.)
- [54] D. Vaske M. F. Rothschild, C Jacobson, C. Tuggle, L. Wang, T. Short, G. Eckardt, S. Sasaki, A. Vincent, D. McLaren, O. Southwood, H. van der Steen A. Mileham, and Plastow G. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. *Proceedings of the National Academy of Sciences of the United States of America*, 93(1):201–205, 01 1996. (Cited on page 10.)
- [55] J. Ma, J. Ren, Y. Guo, Y. Duan, N. Ding, L. Zhou, L. Li, X. Yan, K. Yang, L. Huang, Y. Song, J. Xie, D. Milan, and L. Huang. Genome-wide identification of quantitative trait loci for carcass composition and meat quality in a large-scale white duroc x chinese erhualian resource population. *Anim Genet*, 40(5):637–647, Oct 2009. (Cited on page 97.)
- [56] S. Marklund, J. Kijas, H. Rodriguez-Martinez, L. Rönstrand, K. Funä, M. Moller, D. Lange, I. Edfors-Lilja, and L. Andersson. Molecular basis for the dominant white phenotype in the domestic pig. *Genome Research*, 8(8):826–833, 1998. (Cited on page 10.)

- [57] D. Milan, J. T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, C. Rogel-Gaillard, S. Paul, N. Iannuccelli, L. Rask, H. Ronne, K. Lundström, N. Reinsch, J. Gellin, E. Kalm, P. L. Roy, P. Chardon, and L. Andersson. A mutation in *prkag3* associated with excess glycogen content in pig skeletal muscle. *Science*, 288(5469):1248–1251, 2000. (Cited on pages 8 and 10.)
- [58] M. Noventa, L. Gallo, E. Sturaro, O. Bonetti, M. De Marchi, and P. Carnier. Relationship between raw ham cathepsin b activity and firmness of dry-cured ham. *Italian Journal of Animal Science*, 4(s3): 82–84, 2005. (Cited on page 66.)
- [59] G. Parolari, P. Rivaldi, C. Leonelli, M. Bellati, and N. Bovis. Colore e consistenza del prosciutto crudo in rapporto alla materia prima e alla tecnica di stagionatura. *Ind. Conserve.*, 63:45–49, 1988. (Cited on page 5.)
- [60] PIC. Picmarq technology. *PIC Genus plc. Genetics*, 3(1), 2003. (Cited on page 10.)
- [61] R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2009. URL <http://www.R-project.org>. ISBN 3-900051-07-0. (Cited on page 67.)
- [62] A. M. Ramos, K. Stalder, N. T. Nguyen, and M. F. Rothschild. Effect of three cathepsin genes on processing quality traits of fresh and dry-cured hams. In *Proc. Mid-west Regional Meet. Am. Soc. Anim. Sci., Des Moines, IA, USA.*, pages 21–23, 2005. (Cited on page 10.)
- [63] A. M. Ramos, K. L. Glenn, T. V. Serenius, K. J. Stalder, and M. F. Rothschild. Genetic markers for the production of us country hams. *J. Anim. Breed. Genet.*, 125(4):248–57, Aug 2008. (Cited on page 10.)
- [64] A.M. Ramos, R.H. Pita, M. Malek, P.S. Lopes, S.E.F. Guimarães, and M.F. Rothschild. Analysis of the mouse high-growth region in pigs. *Journal of Animal Breeding and Genetics*, 126(5):404–412, 2009. (Cited on pages 95 and 97.)
- [65] G. A. Rohrer, L. Alexander, Z. Hu, T. Smith, J. Keele, and C. Beattie. A comprehensive map of the porcine genome. *Genome Research*, 6(5):371–391, 1996. (Cited on pages 27 and 67.)
- [66] G. A. Rohrer, R. M. Thallman, S. Shackelford, T. Wheeler, and M. Koohmaraie. A genome scan for loci affecting pork quality in a duroc-landrace f2 population. *Anim Genet*, 37(1):17–27, 2006. (Cited on page 97.)
- [67] M. F. Rothschild. From a sow’s ear to a silk purse: real progress in porcine genomics. *Cytogenetic and Genome Research*, 102(1-4):95–99, 2003. (Cited on page 8.)
- [68] M. F. Rothschild, L. Messer, A. Day, R. Wales, T. Short, O. Southwood, and G. Plastow. Investigation of the retinol-binding protein 4 (*rbp4*) gene as a candidate gene for increased litter size in pigs. *Mammalian Genome*, 11(1):75–77, 01 2000. (Cited on page 10.)

- [69] M. F. Rothschild, Z. Hu, and Z. Jiang. Advances in qtl mapping in pigs. *Int. J. Biol. Sci.*, Jan 2007. (Cited on pages 8, 9, and 19.)
- [70] V. Russo and L. Nanni Costa. Suitability of pig meat for salting and the production of quality processed products. *Pig News Inf.*, 16:17N–26N, 1995. (Cited on page 5.)
- [71] V. Russo, D. P. Lo Fiego, L. Nanni Costa, D. Bigi, and M. Pignatti. Relazione tra il contenuto di carne magra della carcassa e le rese tecnologiche e commerciali del prosciutto di parma. *Rivista di suinicoltura*, 4:105–109, 1990. (Cited on page 20.)
- [72] V. Russo, L. Buttazzoni, C. Baiocco, M. R. Davoli, N. L. Nanni Costa, O. C. Schivazappa, and P. C. Virgili. Heritability of muscular cathepsin b activity in italian large white pigs. *Journal of Animal Breeding and Genetics*, 117(1):37–42, 2000. (Cited on page 19.)
- [73] V. Russo, L. Fontanesi, R. Davoli, and S. Galli. Linkage mapping of the porcine cathepsin f (ctsf) gene close to the qtl regions for meat and fat deposition traits on pig chromosome 2. *Anim Genet*, 35(2):155–157, 2004. (Cited on page 10.)
- [74] V. Russo, L. Fontanesi, E. Scotti, F. Beretti, R. Davoli, L. Nanni Costa, R. Virgili, and L. Buttazzoni. Single nucleotide polymorphisms in several porcine cathepsin genes are associated with growth, carcass, and production traits in italian large white pigs. *Journal of Animal Science*, 86(12):3300–3314, 12 2008. (Cited on page 10.)
- [75] C. Schivazappa, R. Virgili, M. Degni, and M. Cerati. Effetto della tipologia suina di provenienza su alcune caratteristiche del prosciutto di parma. *Industria Conserve*, 73(413-416), 1998. (Cited on page 20.)
- [76] G. Seaton, C. Haley, S. Knott, M. Kearsey, and P. Visscher. Qtl express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics*, 18(2):339–340, Feb 2002. (Cited on page 73.)
- [77] G. Seaton, J. Hernandez, J. A. Grunchev, I. White, J. Allen, D. J. De Koning, W. Wei, D. Berry, C. Haley, and S. Knott. Gridqtl: A grid portal for qtl mapping of compute intensive datasets. In *Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006. Belo Horizonte, Brazil.*, 2006. (Cited on page 73.)
- [78] A. Slawinska, M. Siwek, E.F. Knol, D.T. Roelofs-Prins, H.J. van Wijk, B. Dibbits, and M. Bednarczyk. Validation of the qtl on ssc4 for meat and carcass quality traits in a commercial crossbred pig population. *Journal of Animal Breeding and Genetics*, 126(1):43–51, 2009. (Cited on pages 8, 73, and 95.)
- [79] K. J. Stalder, M. F. Rothschild, and S. M. Lonergan. Associations between two gene markers and indicator traits affecting fresh and dry-cured ham processing quality. *Meat Sci.*, 69:451–457, 2005. (Cited on page 10.)
- [80] E. Sturaro. *Caratteristiche Tecnologiche e Qualitative di Cosce Suine-Fresche Destinate alla Trasformazione in Prodotti Tipici Stagionati: Aspetti Genetici e Relazioni con la Qualità del Prodotto Finale*. PhD thesis,

- Dottorato di Ricerca in: Conservazione, gestione e miglioramento delle risorse genetiche animali. Ciclo XVII. Dipartimento di Scienze Zootecniche. Università degli Studi di Padova., 2004. (Cited on pages 19, 23, and 96.)
- [81] E. Sturaro, L. Gallo, M. Noventa, and P. Carnier. The genetic relationship between enzymatic activity of cathepsin b and firmness of dry-cured hams. *Meat Science*, 79(2):375–381, 2008. (Cited on page 21.)
- [82] R. Thallman, G. Bennett, J. Keele, and S. Kappes. Efficient computation of genotype probabilities for loci with many alleles: I. allelic peeling. *J. Anim Sci.*, 79(1):26–33, Jan 2001. (Cited on pages 55 and 67.)
- [83] H. Thomsen, H. K. Lee, M. F. Rothschild, M. Malek, and J. C. M. Dekkers. Characterization of quantitative trait loci for growth and meat quality in a cross between commercial breeds of swine. *J. Anim. Sci.*, 82(8):2213–2218, Aug 2004. (Cited on pages 9 and 96.)
- [84] Y. Uemoto, S. Sato, C. Ohnishi, K. Hirose, K. Kamayama, K. Fukawa, O. Kudo, and E. Kobayashi. Quantitative trait loci for leg weakness traits in a landrace purebred population. *Animal Science Journal*, In press, 2010. (Cited on pages 73 and 95.)
- [85] H. J. van Wijk, B. Dibbits, E. E. Baron, A. D. Brings, B. Harlizius, M. A. M. Groenen, E. F. Knol, and H. Bovenhuis. Identification of quantitative trait loci for carcass composition and pork quality traits in a commercial finishing cross. *J. Anim. Sci.*, 84(4):789–99, 2006. (Cited on pages 7, 8, and 9.)
- [86] A. L. Vincent, G. Evans, T. H. Short, O. I. Southwood, G. S. Plastow, C. K. Tuggle, and M. F. Rothschild. The prolactin receptor gene is associated with increased litter size in pigs. In *Proc. 6th World Congr. Genet. Appl. Livest. Prod., Armidale, Australia.*, pages 15–18, 1998. (Cited on page 10.)
- [87] A. Sewalema Y. Nagaminea, C. S. Haleya and P. M. Visscher. Quantitative trait loci variation for growth and obesity between and within lines of pigs (*sus scrofa*). *Genetics*, 164(2):629–635, 6 2003. (Cited on page 95.)
- [88] B. Yang, X. Huang, X. Yan, J. Ren, S. Yang and Z. Zou, W. Zeng, Y. Ou, W. Huang, and L. Huang. Detection of quantitative trait loci for porcine susceptibility to enterotoxigenic *escherichia coli* f41 in a white duroc x chinese erhualian resource population. *Animal*, 3 (7):946–950, 2009. (Cited on pages 73 and 95.)
- [89] G. H. Yue, H. Bartenschlager, G. Moser, and H. Geldermann. Identification of qtl affecting important traits on porcine chromosome 12. *Acta Genetica Sinica*, 27(10):858–865, 2000. (Cited on page 97.)
- [90] J. H. Zhang, Y. Z. Xiong, B. Zuo, M. G. Lei, A. W. Jiang, F. E. Li, R. Zheng, J. L. Li, and D. Q. Xu. Quantitative trait loci for carcass traits on pig chromosomes 4, 6, 7, 8 and 13. *J. Applied Genetics*, 48 (4):363–369, 2007. (Cited on page 95.)

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