Title:

Detection of QTL affecting flesh quality traits (body lipid percentage and flesh colour) using molecular markers (microsatellites and AFLP markers) in Atlantic salmon (*Salmo salar* L.)

By

Derayat A.

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Institute of Aquaculture

University of Stirling

Stirling, Scotland, UK.

Declaration

This thesis has been produced by me and the work described in it was carried out by me. The specific contributions of others have been acknowledged. This thesis has not been accepted in any previous application for a degree.

Amid Derayat

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Abstract

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Flesh colour and fillet fat percentage are the two most important attributes to salmon fillet quality. A medium genetic component to body lipid percentage within commercial lines has previously been shown (h^2 = 0.17-0.24). A low level of heritability (h^2 = 0.16) has also been reported for flesh colour in Atlantic salmon. To investigate whether this genetic component includes loci of major effect, a genome-wide QTL scan was performed with commercially bred Atlantic salmon (Landcatch Natural Selection). Five large full-sib families (10 parents with 153 offspring) were genotyped using microsatellite markers. To utilize the large difference between sire and dam recombination rate, a two-stage genotyping was employed. Initially, the parents and offspring were genotyped for two microsatellite markers per linkage group, and sire based QTL analysis was used to detect linkage groups with significant effects on those flesh quality traits. A linear-regression based interval as analytical method was applied for QTL detection. The results revealed evidence of QTLs affecting percentage fat percentage and flesh colour on linkage groups LNS16 and LNS1 respectively.

To confirm the QTL and to provide an improved estimate of position, a dam-based analysis was then employed. One major QTL was located on the genome-wide significance level for percentage fat percentage. Microsatellite marker *Ssa*0016NVH (at position of 1.3 cM) was found to be tightly linked to QTL affecting percentage fat percentage. In addition, a QTL affecting flesh colour was found to be flanked by microsatellite markers *Ssa*9.44NUIG at position of 68.7 cM and *Ssa*0021NVH at

position of 50.6 on linkage group LNS16. The evidence for suggestive QTL affecting flesh colour on linkage group LNS1 was also revealed.

In order to increase marker density within these and other linkage groups, AFLP markers were employed, 24 primer combinations resulted in a total of 489 polymorphic fragments. Among 11 fragments that were found to be linked to the microsatellite markers on linkage group LNS16, four fragments (AAG-CAC328, AGG-CAG447, AGG-CTA237 and AGG-CTC237) were tightly linked to microsatellite marker *Ssa*9.44NUIG, but none were found to be linked to microsatellite *Ssa*0021NVH. Moreover, none of the AFLP markers were found to be linked to microsatellites residing on linkage group LNS1. Using a constructed map of microsatellite and AFLP markers for linkage group LNS16, the dam based analysis revealed a significant QTL for flesh colour at the location of 189 cM, while the sire based analysis detected a significant QTL for fat percentage at the location of 80 cM.

Considering the dominant nature and clustering character of AFLP markers, it was concluded that a certain primer combination in AFLP markers could be of limited use for fine mapping and QTL detection in Atlantic salmon.

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List of Abbreviation

Actual inbreeding level	F
Amplified fragment length polymorphism	AFLP
Base pair	bp
Biotechnology and biological sciences research council	BBSRC
centiMorgan	cM
Deoxyribonucleic acid	DNA
Ethylenediaminetetraacetic acid	EDTA
Expressed sequence tags	EST
Genomic research on Atlantic salmon project	GRASP
Heritability	h^2
Infection pancreatic necrosis virus	IPN
Infectious hematopoietic necrosis	IHN
Landcatch Natural Selection	LNS
Log of odds ratio	LOD
Marker assisted selection	MAS
Mitochondrial DNA	mtDNA
N,N,N',N'- Tetramethylethylenediamine	TEMED
Passive Integrated Transponder	PIT
Polymerase chain reaction	PCR
Polymorphic information content	PIC
Quantitative trait loci	QTL
Random fragment length polymorphism	RFLP
Random amplified polymorphism DNA	RAPD
Rate of accumulation of inbreeding	ΔF
Ribonucleic acid	RNA
Salmon genome project	SGP
Simple sequence length polymorphisms	SSLPs
Simple sequence repeats	SSR
Single nucleotide polymorphism	SNP
The best linear unbiased prediction	BLUP
The United Nation Food and Agriculture Organization	FAO
Tris borate	TE
Tris borate EDTA	TBE
Variable number of tandem repeats	VNTRs

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Chapter 1 - General Introduction

1.1 The State of World Aquaculture

The world's fast growing human population is consuming the earth's natural resources at an increasing rate. Tropical forests, fertile ranches and aquatic animals are among the list of these diminishing and limited natural resources. World-wide demand for food is growing rapidly and there is every sign that it will continue to grow in the future too. Historically, turning forests and fertile ranches into agricultural fields or catching more fish from the seas have been a natural response to supply food demands. Advances in science and agricultural techniques have increased productivity per surface area, noticeably in the last century. Aquaculture, probably the fastest growing segment of the world food production, is defined as the cultivation of aquatic animals and plants such as fish, shellfish and seaweed in natural or controlled marine or freshwater environments.

The practice of aquaculture is ancient and dates as far back as 2500 BC in China. But modern intensive aquaculture started only in the 1970s when the first group of salmon was introduced and reared in sea cages in Norway. Ever since the world-wide production of aquatic animals has been increasing at a high rate and will continue to do so for the coming years.

According to The United Nation Food and Agriculture Organization (FAO 2005) today's aquaculture production accounts for almost 50 percent of the total world fish supply for human consumption and it predicts that by 2030 aquaculture should provide at least an additional 40 million tonnes to meet the fish food demand. This is a realistic prediction, when we look at the increase in aquaculture production from 3.9

percent of total fish production in 1973 to 27.3 percent in the year 2000 (FAO 2002). There is now sufficient evidence that suggests that capture fisheries from open seas are dangerously reaching their carrying capacity. The damaging effects of over-fishing have raised serious concerns about diminishing fish stocks in many places in the world. With no prospects for further increase, the total catches from the sea have now become stable at around 100 million tons per year. On the other hand, due to inadequate fish supply and the world's growing population, global per capita fish supply has decreased from 14.6 kg in 1987 to 13.1 kg in the year 2000. This situation has led to constant and continuous human pressure on ocean resources in general. It is currently a matter of debate whether aquaculture could and should bridge the gap between the high demand for sea food and the limited resources of marine fisheries. Since 1970, aquaculture has been growing at rate of 8.8 % with its production increasing from below one million tons in 1950s to more than 59 million tons in 2004 (FAO 2007) and it is assumed that it will continue to increase even further as human population continues to grow. It is predicted that world supply for sea food must reach to 183 million tons by 2030 to sustain world-wild fish demand (De Silva 2001). Therefore, it is expected that the total aquaculture production should reach to 85 million tons of fish per year by 2030 to maintain the current per capita consumption rate (Bilio 2008). By volume, however, the contributions of various carp production from freshwater ponds constitute the vast bulk of total aquaculture production. Among today's diverse aquacultural products, Atlantic salmon is the focal point of this study. Therefore, I shall only discuss ways to further improve performance of this species by genetic means and specifically concentrate on genetic prospects of this fish for future aquaculture.

1.2 Biology of Atlantic salmon

The ancestrally tetraploid Atlantic salmon (*Salmo salar* Linnaeus) is from the family Salmonidae, subfamily Salmoninae and order Salmoniformes. The Salmonidae family is divided into three subfamilies; Salmoninae, Coregoninae and Thymallinae. Subfamily Salmoninae includes three genera; *Oncorhynchus* (e.g. rainbow trout (*O. mykiss)*), *Salvelinus* (e.g. Arctic charr (*S. alpinus*)) and *Salmo*, which includes the Atlantic salmon.

According to Phillips and Ráb (2001), the majority of salmonids can be divided into two groups based on their karyotype. Group A karyotypes contain approximately 80 chromosomes (2N = 80) and chromosome arm number of 100 (NF =100) and they tend to be more subtelocentric than metacentric chromosomes. Group B karyotypes (2N = 60, NF 104) tend to have more metacentric than subtelocentric chromosomes. Atlantic salmon having a diploid chromosome number of 54 – 58 (across North American and European populations) and chromosome arm numbers of 72 – 74 do not conform to either of these categories. Differences in diploid number, chromosome arm number and allele frequencies at minisatellites and microsatellites between North American salmon populations and European populations have also been reported (reviewed in Verspoor 2005). There is considerable evidence that the ancestor of the existing salmonids underwent an autotetraploid (intraspecific genome duplication) event 25 – 100 million years ago (Allendorf and Thorgaard 1984). The genome size is 2.5×10^9 base pairs and the number of chromosome arms varies from 74 to 170. Both are almost twice that of related fishes (Phillips and Ráb 2001). The autoteraploid ancestry of salmonids causes some difficulties in interpreting the genetic basis of quantitative traits (Allendorf and Thorgaard 1984).

Since the tetraploid event the salmonid genome has undergone re-diploidization process through the differentiation of duplicated chromosome sets into distinct pairs of homeologs. Disomic inheritance (independent segregation of homeologs) has been re-establish across most of the genome, but some chromosomes still form multivalents and exchange chromatid segments with their ancestral counterpart during meiosis.

Another consequence of the residual tetrasomy in salmonid fish that seems to retard the diploidization process is due to a false linkage observed in males termed pseudolinkage (Allendorf and Thorgaard 1984; Allendorf Danzmann 1997). In the phenomenon of pseudolinkage, ancestrally duplicated (homeologus) regions allow pairing of chromosomes in multivalent formations in males. As a result of pseudolinkage recombinant progeny types are produced in excess of parental ones, causing physically unlinked loci to appear in linkage disequilibrium (Allendorf and Thorgaard 1984).

In nature, female salmon can reach 150 cm in length with weight up to 45 kilos. This fish inhabits cooler waters with strong to moderate flow. Atlantic salmon is an anadromous species that spends normally 2 - 4 years (but ranges 1 - 6 years) of its early life in fresh water and then migrate to the ocean and remains there for 1 - 4 years before returning to freshwater for spawning. In the freshwater stage, Atlantic salmon has a slow growth rate while they grow more rapidly in seawater. Adult salmon go back to the river of their origin to spawn (natal stream homing) and cease eating altogether prior to spawning. As a result a lot of adults die after spawning while returning to sea. At the fresh water stage salmon feed mainly on aquatic insects, crustaceans some molluscs and other fish. Adult salmon in the sea consume squids, shrimps and other fish. Atlantic salmon are marketable fresh, dried or salted, hot and

cold smoked, frozen or even more recently processed in the shape of a sausage. Atlantic salmon are considered a luxury food and are eaten in many different ways including: fried, broiled, steamed, microwaved and baked.

1.3 Distribution of Atlantic salmon

The Salmonidae are found throughout the northern hemisphere and are resident in both fresh and sea water as well as in temperate and Arctic waters. In nature, Atlantic salmon live in very diverse habitats during their life span. Atlantic salmon has a distribution throughout North America in streams along the Atlantic coast from northern Quebec in Canada to Connecticut in the USA. In Europe, salmon occur from the south of Greenland toward the Icelandic coast, extend along the Atlantic coastal drainage to northern Portugal and the Bay of Biscay (MacCrimmon et al. 1979). In the ocean, Atlantic salmon are found over large areas in the North Atlantic. Many rivers in Iceland, Scotland, Ireland and Norway create substantial spawning grounds for salmon. Salmon appear along the German North Sea coast, northern coast of France, and many rivers in Spain. The overall picture of population structure of salmon in Europe has been also shown by Verspoor et al. (2005).

1.4 Farming of Atlantic salmon

The global catch of wild salmon has shown a steady decrease during the last three decades while during the same period of time production of farmed Atlantic salmon has been rapidly increasing. Today, world-wide production of farmed Atlantic salmon has reached well over one million tonnes, according to statistics from an online source (Intrafish). The capture of wild salmon has stabilized around 5000 tonnes per year which is equal to 0.5% of total farmed salmon production.

Today, Atlantic salmon has become the most important domesticated animal in terms of biomass as well as economy in Europe and many other countries in the world. As a result of excellent environmental conditions, Norway is the top producer of farmed Atlantic salmon with yearly production of 420,000 tonnes. Chile is the second most important salmon producer and other countries such as Scotland, Ireland, Canada, USA and Japan are major salmon producers as well. Table 1 shows world farmed salmon production in years1988, 1995, 2002 and 2006 that demonstrates the significant production increases that have occurred in this period, particularly in Norway and Chile.

Table 1: The world production of farmed salmon from 1988 to 2006 (source: FAO, Fishstat Plus Aquaculture Production 1950-2006).

	Production 1988		**Production 1995**		**Production 2002**		**Production 2006**	
	1000 Tons	**Share %**	**1000 Tons**	**Share %**	**1000 Tons**	**Share %**	**1000 Tons**	**Share %**
Norway	78.7	70.5	261.5	56.2	462.4	42.6	626.4	47.9
Chile	0.2	0.2	55.2	11.9	265.7	24.5	386.3	29.5
UK	18.8	16.8	70.3	15.1	145.6	13.4	131.9	10.1
Canada	3.3	3.0	33.6	7.2	113.7	10.5	101.6	7.8
Ireland	4.1	3.7	11.8	2.5	23.2	2.1	11.1	0.8
United State	1.0	0.9	14.1	3.0	12.7	1.2	9.4	0.7
Faeroe Islands	3.4	3.0	8.5	1.8	44.9	4.1	13.1	1.0
Other Countries	2.2	2.0	10.1	2.2	16.3	1.5	27.7	2.1
Total	109.5	100	455.0	100	1068.3	100	1279.8	100

Europe, Japan and North America have been the major marketplace for farmed Atlantic salmon, but more recently other countries such as Russia and China are becoming suitable markets for Atlantic salmon products as well. In turn, this can probably explain the current highest ever demand for farmed salmon.

1.5 Genetic selection in Atlantic salmon

Traditionally, selective breeding has played an important role in the domestication of terrestrial farm animals for many decades by increasing the survival rate and improvement in product quality. As a result, economic gains from genetic improvement have been well realized by livestock farmers. Several breeding programs have been developed and utilized in farm animals resulting in numerous increases in production. This progress has largely been achieved through selection based on phenotype characteristics of farmed animals (Harris 1998).

Atlantic salmon was introduced into sea cages for the first time in 1969 in Norway and the earliest attempt at genetic improvements commenced in 1971. In the last three decades, selection for increased growth rate together with improvements in nutrition and management have reduced the production cycle by approximately 1.5 years, leading to massive savings. So far, at least eight generations of Atlantic salmon have undergone these improvements. Although many techniques have been suggested and applied for genetic improvements in fish and other animals in the past, selective breeding has proved to be paramount for genetic improvements, especially in the case of Atlantic salmon. As a result, selective breeding has significantly improved the growth rate in farmed Atlantic salmon (Refstie 1990). The earliest breeding program for Atlantic salmon primarily aimed to increase the growth rate and decrease the incidence of early maturation (known as grilse in salmon). The founding population originated from 41 stocks taken from various rivers and localities in Norway (Gjedrem et al. 1991a; Gjedrem 2000). The progeny of this founding population produce most of the eggs used in Atlantic salmon farming in Norway as well as in many other countries.

AKVAFORSK (Norwegian research institution for breeding and genetics of aquatic animals) developed the base populations of the Norwegian breeding program for Atlantic salmon in 1971 to 1974 (four sub-populations) and implemented this breeding program until 1986. Thereafter, the Norwegian Fish Farmer's Breeding Centre took control of the breeding program. Apart from a breeding centre at Sunndalsora, an additional breeding station was also established at Kyrksaeterora because of safety reasons. In 1992 the Aqua Gen Company took over and became the owner of these two breeding stations (Gjedrem 2000). The earliest selection programs for Atlantic salmon were carried out at both of these stations. The first breeding goal in 1975 was body weight at slaughter followed by age at sexual maturation in 1981, disease resistance in 1993, flesh colour in 1994 and the trait of fat content in 1995. It is estimated that 80 percent of the farmed Atlantic salmon in Norway originate from the selection program that was initiated at Sunndalsora in 1971 (Gjedrem et al. 1991a).

Today, there are several large-scale breeding companies implementing breeding programs and are supplying salmon seed world-wide. Aqua Gen is the major breeding company and egg producer for Atlantic salmon in Norway with a considerable export to Chile and Scotland. Other large-scale breeding companies are also well established in countries such as: Canada (Atlantic Salmon Federation), Iceland (Stofnfiskur EHF), Scotland (Landcatch), Ireland (Fanad) and Chile (Aquachile Gentec SA and Landcatch). In addition to these breeding programs, many other small-scale hatcheries are thought to practice selection programs to produce improved stocks of salmonids. Yet, it is worth bearing in mind that in terms of total aquaculture production only 1 to 2 percent of farmed fish and shellfish in the world have originated from breeding programs (Gjedrem 1997), indicating that there is much to be gained by using this

powerful tool in world aquaculture. From my own experience it seems that the lack of knowledge and resources is the main reason for the situation that only a few fish farmers are committed to the concept of fish breeding programs. Furthermore, modern genetic improvements and application of up-to-date technologies such as sex and chromosome-set manipulations, transgenesis, genome mapping and use of DNA markers into the cultured fish and shellfish, hold even greater impact for future industries. Although, there is a long way to go in applying the existing scientific information and modern genetic technologies into the diverse aquaculture industries, some of these techniques have begun finding their application in species like Atlantic salmon. Farming of Atlantic salmon has been developing so rapidly that despite being a relatively new enterprise, it has inspired other sections in aquaculture such as shrimp, carp and tilapia farming.

1.6 Selective breeding in Atlantic salmon

The knowledge and methodology that can be used to select the best individuals for animal breeding is called a breeding program. Selective Breeding is comprised of two principal components: selecting the parents for the next generation (selection) and determining how the selected parents will be mated (mating system). In order to conduct such a program it is essential that the entire reproductive cycle for a species to be controlled in captivity. Breeding objectives need to be defined and traits included must show genetic variation.

Apart from salmonids, aquaculture species have not benefited much from modern developments in animal breeding, despite the fact that fishes have typically high reproductive capacity and occurrence of a higher genetic variation in fish. In Atlantic salmon, selective breeding has been practised for traits such as growth rate, late

maturation, disease resistance, and more recently quality traits such as fillet colour and fat content. The extent to which genetic improvement can be achieved in any trait is dependent on the amount of genetic variation that exists for the trait. Genetic variation among individuals can be divided into additive and non-additive components (Falconer and Mackay 1996). A prerequisite for continual selection response is the presence of additive genetic variation (Knibb 2000). As long as inbreeding can be avoided, selection over many generations is not expected to reduce the additive genetic variability (Gjoen and Bentsen 1997). In other words, the improvement in one generation is preserved and the genetic gain in the following generation is added.

The selected traits in any breeding program must be sufficiently heritable and easy to measure. Effectively a large and genetically diverse breeding population is fundamental when establishing a breeding program. In a discussion on suitable breeding programs for sustainable aquaculture, Olesen et al. (2003) recommended that the definition of breeding goals for sustainable fish production must be based on long term biological, ecological and sociological principles rather than on short term market values. In order to create a breeding program that contributes to a sustainable production, they suggest that the breeding operators must communicate and cooperate with farmers as well as the consumers and governmental representatives. Others have only expressed economic views for definition of breeding objectives. For instance, Davis et al. (2000) suggested that traits in breeding objectives are the traits that make money or cost money. By specifying sources of income and expenditure in aquaculture enterprises, they recommended that biological traits that are related to these sources of income and expense should be the components of the breeding

objective. Nonetheless, most of the breeding goals in current aquacultural breeding programs are based on economic values.

After specifying the breeding objective, the selection strategy must be described. The most important factors that need to be considered in genetic selection strategies are accuracy of selection, selection intensity, effective population size and mating system (Muir 1997). Optimum response to selection can be achieved by maximizing the first three factors and using a mating system that allows for optimization of reproductive characteristics in dam lines and production characteristics in sire line. Today, several methods of the selection are practised for the genetic improvement of aquaculture species, among them mass selection, between family selection and the best linear unbiased prediction (BLUP) are more commonly used. BLUP is a generalized procedure for combining information on the individual and all relatives in a selection program and greatly increases the accuracy of selection, especially in traits with low heritabilities (Muir 1997). Marker assisted selection (also known as MAS) utilizes recent developments in the field of molecular genetics to assist in the selection of individuals to become parents in the next generation and its application is currently under debate (Davis et al. 2000). Furthermore, Gomez-Raya & Klemetsdal (1999), using a stochastic simulation of a closed nucleus herd for beef production, predicted that genetic gain may increase by 11 percent when the MAS program is combined with the conventional BLUP method.

The genetic gain for growth rate in salmonids is primarily because of high fertility and large phenotypic variance (Gjedrem 1975). In addition, the large genetic variation in fish (coefficient of variation = 20 - 35%) in comparison with farm animals (coefficient of variation = 7 - 10%) has been suggested for the higher genetic gain

(Gjedrem 1997). In the Norwegian breeding program, genetic gain for growth rate of Atlantic salmon is reported to be 14% per generation (Fjalestad et al. 2003). This means that the growth rate of Atlantic salmon could be doubled after eight generations of selection. Growth rate can vary among salmonids fishes at different stages of life. For example, rainbow trout may grow better in both fresh and sea water to a smaller harvestable size whereas Atlantic salmon is more suitable for production to a larger marketing size (Gjedrem and Knut 1978).

In rainbow trout traits such as growth rate, feed efficiency, age at sexual maturation, disease resistance and flesh quality are the important traits for improvement (Gjedrem 1992). Apart from progresses made in breeding programs currently underway in countries such as Finland and the UK these traits have not been considerably improved on a commercial scale in other countries. With Atlantic salmon the selection goals may vary between the freshwater and seawater environment. For instance, early survival and smolting rates are more important during freshwater stages while growth rate and age at maturity becomes the main target for selection in seawater. The response to selection in different environments such as freshwater and seawater may vary for the same group of fish. Application of a selection index (multiple objective selection) for improvement of several traits in freshwater and seawater have led to substantial genetic gain in Atlantic salmon (O'Flynn et al. 1999). An earlier study conducted by Friars et al. (1995) also reported higher responses to multiple-trait-index selection than mass selection for the reason that heritabilities are higher for family means than for individual performance.

1.7 Heritability estimations in Atlantic salmon

In general, improvements in phenotypic traits through selection are dependent on the heritability and selection intensity. Heritability (h^2) is the proportion of phenotypic variation due to additive genetic variation of the trait (Winter 1998). $h^2 = V_G/V_{ph}$

Heritability is usually estimated with individuals of known relatedness generated using a controlled breeding program or through response to selection. Heritability and genetic correlations are the parameters that define the extent of genetic variation for the trait of interest. For example, a significant difference in resistance to vibrio disease (*Vibrio anguillarum*) between river strains of salmon parr was reported by Gjedrem and Aulstad (1974). Heritability estimation based on the sire and dam component ranged from 0.12 to 0.07.

Heritability estimates can vary considerably among traits in salmonids fishes. In Atlantic salmon, heritability estimations have been subjected in various studies and many reports have been published. In Table 2, some estimates of heritabilities are given for traits of economic importance in Atlantic salmon.

Table 2: Heritability estimates for economical important traits in Atlantic salmon.

Trait	Estimated heritability and standard error	Authors
Body weight	0.35 ±0.10	Rye & Refstie (1995)
Age at sexual maturation	0.15	Gjerde (1986)
Fat percentage	0.30 ± 0.09	Rye & Gjerde (1996)
Flesh colour score	0.09 ± 0.05	Rye & Gjerde (1996)
Survival	0.0 ± 0.02	Rye & et al (1990)
Survival in the sea	0.0 to 0.21	Standal &Gjerde (1987)
Furunculosis	0.4 ± 0.17	Gjedrem et al. (1991b)
BKD	0.2 ± 0.10	Gjedrem &Gjon (1995)
Vertebral deformity [a]	0.00 ± 0.0 to 0.36 ± 0.14	Gjerde et al. (2005)
Fillet fat percentage	0.28 ± 0.05	Powell et al. (2008)
Flesh colour (Hue)	0.16 ± 0.043	Powell et al. (2008)
Harvest weight	0.15 ± 0.05	Quinton et al. (2005)

[a] The difference in the heritability estimation (on the liability scale) is due to differences in incidence of deformity in 4 year-class in Atlantic salmon.

Early mortality of eggs and fry can be a great cause for concerns in fish breeding programs. Differences in the heritabilities for mortality of eggs, alevins and fry was reported by Kanis et al. (1976) where heritability based on sire component was highest at eyed egg stage ($h^2 = 0.08$), followed by the alevin stage ($h^2 = 0.05$) and zero heritability for fry.

External parasites such as the sea lice poses a major dilemma for the European salmon industry and it takes its toll, especially in fish cages in Ireland and Scotland. Atlantic salmon infected with large numbers of lice need to be deloused several times during the marine phase of the rearing cycle, stressing the fish and costing effort and money. The degree to which Atlantic salmon families may differ in susceptibility to infection to the sea lice was studied by Glover et al. (2005) where they found significant differences in abundance of lice among the families. Despite the occurrence of a genetic component for this trait, it was suggested that a strong environmental component influenced fish susceptibility to sea lice. Low heritability (0.074 ± 0.22) was reported for susceptibility to sea lice.

Vertebral deformities are also considered a problem that causes financial losses to salmon farmers. According to Gjerde et al. (2005), vertebral malformation in Atlantic salmon is determined by a substantial additive genetic component. The reported estimation of heritability for deformity in Atlantic salmon is quite high (0.36 ± 0.14). It has been recommended not to select the breeders from families with high incidence of deformed fish and certainly not at all the breeders showing the deformity themselves.

1.8 Growth rate improvement through selective breeding

Growth rate has been the trait of highest economic importance in farmed Atlantic salmon. An early study of Gunnes et al. (1978) showed that there is a significant genetic variation in body weight and length of Atlantic salmon after a growth period of 2 years in the sea. Their results were in agreement with that obtained for growth in the freshwater period and indicated that most of the growth variation has a genetic basis which can be exploited by a selection program. As a result of selection for increased growth rate, feed consumption and feed utilizations have altered in selected fish. A 4.6% increase in feed efficiency ratio per generation of selection has been reported (Thodesen et al. 1999).

It is worth bearing in the mind that length can probably be a more useful measure of growth in selection programs for fish since the reported heritability for length are higher than those estimated for weight, for instance Refstie et al. (1978). Other studies have similarly reported moderate to high levels of heritability for body weights in Atlantic salmon. For example, Gjerde and Gjedrem (1984) reported heritability of 0.38 - 0.44 for body weight in Atlantic salmon, Gjerde et al. (1994) estimated heritability of 0.04 – 0.26 for specific growth rate (% day^{-1}). The observed genetic

gains in body weight in breeding programs for rainbow trout ranged from 4.8 to 12.5% per generation in fresh and sea water (Kause et al. 2005).

For proper implementation of selective breeding, growth rate between different families in separate tanks must regularly be measured. Therefore, fish density plays an important role and must be taken into account as early as possible. There is enough evidence that high density can considerably influence growth rate at different stages of rearing Atlantic salmon (Refstie et al. 1976). This is an important consideration especially for newly hatched salmon where they must be kept in separate tanks until they have reached the appropriate size for external tagging. After the early stages of life, fingerling fish are to be pooled to provide the same environmental condition (such as tank density, water quality, food availability competition and etc.). With regard to the tagging or marking of fish, many different methods have been developed in which each method depends on the purpose and need for tagging. In the past, a combination of fin-clipping and freeze-branding has been commonly used for tagging small Atlantic salmon. Freeze branding requires trained personnel and good light conditions (preferably sunlight) to obtain a high percentage of correct identification (Gunnes and Refstie 1980). Spaghetti tags (also called arrow tags) and PIT (Passive Integrated Transponder) tags are also commonly used in salmon of larger size. Recent innovations in molecular genetics (including DNA fingerprinting) have shown to be promising to assist traditional selection programs and will be discussed in the coming pages.

1.9 Difference between cultured and wild Atlantic salmon

The differences between cultured and wild Atlantic salmon have been the subject of various studies. From an aquacultural point of view, the domesticated salmon differ

from wild salmon in fitness related traits such as growth and aggression. Unlike nature, culture facilities provide predator-free, high density, rapid growth environments that can affect the morphological and behavioural development of the fish.

It has been reported that growth of selected fish at smolting age can be as twice that of wild salmon (Gjedrem 2000). In the wild, salmon smoltify at 2-5 years of age (Refstie et al. 1977) while farmed salmon smoltify at less than one year old. Smoltification is the process by which a juvenile salmon becomes ready for entry into marine water, and involves change of external colouration, change in osmoregulatory structures and processes to maintain physiologically appropriate water and salt concentrations in the tissues, and increase in growth rate. Early smolting is commercially desirable therefore, salmon farmers routinely manipulate temperature and photoperiod in order to induce smoltification in cultured stocks.

Results of a study carried out by Fleming et al. (1997) showed that intentional and unintentional selection during the seven generations of domestication has led to divergence in morphology (i.e. body becoming more robust with smaller rayed fins), behaviour (including aggression, dominance and predator avoidance), growth (higher growth performance in the absence of competition) and even life history (such as higher rate of smolting and lower incidence of male parr maturity) of the farmed salmon from their wild ancestor. This evidence suggests that the farmed salmon have diverged from their wild founder population in several fitness-related traits.

1.10 Age at sexual maturity

Age at sexual maturity is one of the economically important traits in salmon farming and it is a matter of debate whether growth and early maturation are interrelated. In

general, salmonids display a high degree of sexual dimorphism in body size and timing of maturity. Early maturation especially in male Atlantic salmon has deleterious effects on fillet quality and reduces economic benefits. In the past, early maturation has led to disastrous financial consequences for farmers especially in the case of Atlantic salmon. As an alternative, the use of lights to prevent problems related to grilsing are now applied in most salmon farms. In addition, most breeding programs for Atlantic salmon use late maturation as a trait to prevent grilsing. There has also been some attempt to examine whether it is possible to change the degree of the sexual dimorphism in salmonids using selective breeding in many studies. For example, Kause et al. (2003) reported that the heritabilities of male and female maturity in rainbow trout were of different magnitude (estimated heritability for male maturity was 0.34 while for female maturity it was only 0.12). This suggests that strong selection for rapid growth can lead to initiation of maturity. Reversely, strong selection for late maturity can reduce the rate of genetic improvement for growth rate. It is concluded that it would be unlikely to achieve rapid genetic changes in the sexual dimorphism of age at maturity through selective breeding, although selective breeding can delay the timing of maturity in both sexes of rainbow trout.

The trait of age at sexual maturity in the sea is largely influenced by the age of parents at sexual maturity. Gjerde et al. (1994) reported a high level of heritability for the trait of age at sexual maturity in the sea (0.48 ± 0.20) for Atlantic salmon whereas Wild et al. (1994) reported a medium level of heritability (0.10 - 0.17) for this trait. It is concluded that genotype by environmental interaction plays a very important role for the trait of early sexual maturity suggesting that selection programs must be based on records of this trait at different fish rearing locations. In Coho salmon, a relatively

high level of heritability (for male $h^2 = 0.49 – 57$ and for female $h^2 = 0.39 - 0.41$) for the trait of age at sexual maturity has been estimated (Hankin et al. 1993).

As an alternative, the culture of mono-sex all female salmon can be advantageous by eliminating precocious maturation of the male. Sex determination in salmonids appears to be controlled by an X-Y chromosomal system. The production of all female fish can be achieved in different ways, e.g. by feeding offspring with added male hormone producing neo-male stock for the next generation. Sperm of such male stock (XX) can fertilize the normal eggs (XX) and produce all female progeny. Sex identification in salmonids is currently accomplished by observing morphological differences between males and females. Identification of genetic sex can carry some practical application for development of mono-sex stocks in salmonids in the future. As an example of early contribution of molecular genetics towards the practical aquaculture, work of Devlin et al. (1998) is noticeable where they identified the DNA sequence of the Y- chromosome of the Chinook salmon (*Oncorhynchus tshawytscha*). PCR amplification of this sequence yielded a 209-bp fragment that was specific to males. It was suggested that this method can rapidly and reliably be used to determine the genetic sex of precociously mature males.

1.11 Selective breeding for quality traits

Consumer appreciation of Atlantic salmon is mostly influenced by colour, texture and fat content of the flesh. With increasing production of Atlantic salmon, these quality traits have become of increased interest to producers as well. As a result, traits such as flesh colour and fat content are now considered as traits of economic importance.

The pink colour in Atlantic salmon is produced by the addition of carotenoids pigments such as astaxanthin (3,3′dihydroxy-β,β-carotene-4,4′-dione) and

canthaxanthin (β,β-carotene-4,4′-dione) in the diet of the fish. Leading to higher expense to fish farmers, only around 10-18% of these pigments are retained in the flesh (Nickell and Bromage 1998). Flesh colour measurement is not as straightforward as with other traits and is more subjective, therefore prone to error. Using three different methods of colour measurement for assessment of flesh colour in Atlantic salmon Norris et al. (2004) noticed that as a result of carotenoids migration from the muscle into the gonads, all the scores from these three measurements were negatively correlated with the gonadosomatic index. Low to medium heritabilities (0.12 - 0.14) for colour traits was estimated implying that this trait can be improved by BLUP selection. Earlier work of Withler et al. (1994) with progenies of 6 Coho salmon (*Oncorhynchus kisutch*) populations has shown occurrence of genetic variation for intensity of red flesh colour within populations. The positive genetic correlation between flesh colour and weight suggests that flesh colouration in salmonids can be improved indirectly through the selection for increased harvest body weight.

There is also evidence that direct selection for harvest body weight can result in unfavourable consequences for fat content (Quinton et al. 2005). Flesh fat content in excess of 18% can lead to detrimental effect on fillet quality such as texture and flavour (Gjedrem 1997).

1.12 Effects of inbreeding on Atlantic salmon

Inbreeding or mating amongst relatives is a major cause for concern with fish breeders. Inbreeding can reduce response to selection in two ways: it causes the loss of favourable alleles (increasing homozygosity) leading to an increased chance of expression of lethal or undesirable recessive genes and it can also lead to a reduction

in the mean phenotypic value of fitness traits such as growth and reproductive capacity (Falconer and MacKay, 1996). Inbreeding rate can be estimated through pedigree analysis or through direct experimental measures of changes in genetic variability.

The deleterious effects of inbreeding have been well documented in salmonids. The majority of these studies have reported relatively high inbreeding levels (ΔF = 10 -25%). Generally, in selective breeding programs the rate of accumulation of inbreeding (ΔF) is more important than the actual inbreeding level (F) due to its influence on genetic progress. Therefore, careful attention must be given to the rate of inbreeding, which is increased by increasing either accuracy of the selection or the selection intensity. By increasing selection intensity inbreeding rate may become more intense and can lead to phenomenon known as inbreeding depression.

The high level of fecundity in fish permits high selection intensities, which in a way can increase the risk of mating among closely related individuals. It is known that traits related to fitness are most subject to inbreeding depression. A common method for calculating the magnitude of inbreeding depression is by comparing the mean performance of systematically inbred groups with the performance of randomly bred groups. In the earliest report published by Gjerde et al. (1983) the effect of inbreeding on survival and growth in rainbow trout at three levels (F = 0.25, F = 0.38 and F = 0.50) was investigated. Highly significant differences at each level of inbreeding for survival of eyed-eggs, alevins and fry were found. The overall inbreeding depression of 10.0% for survival of eyed –eggs, 5.3% for alevins and 11.1% for fry was reported as a result of inbreeding. Although, growth of adults was significantly depressed by

inbreeding effects at each level of inbreeding no significant inbreeding effects on growth of fingerlings (160 days after first feeding) was detected.

The magnitude of the inbreeding depression as may normally occur within selected strains of rainbow trout was studied by Su et al. (1996) whereas no significant inbreeding depression in body weight at early stages suggested that inbreeding depression of body weight could increase with advancing age. Highly significant depression for spawning age and egg number in females was reported (10% increase of the inbreeding coefficient resulted in delay on spawning age of females by 0.53% and reduction in egg number by 6.1% of the mean).

Inbreeding coefficient is the probability that an individual has both alleles of a gene identical by descent from the same allele in a common ancestor (Winter et al. 1998). Pante et al. (2001) examined the effect of inbreeding on body weight in rainbow trout at harvest by fitting the individual inbreeding coefficients as a linear covariate and reported a considerably lower estimate of inbreeding depression (max 3.3% decrease in mean body weight per 10% unit increase in inbreeding coefficient) than that reported by Gjerde et al. (1983). Estimation of inbreeding depression in populations of rainbow trout after one generation of brother-sister mating was calculated by Kincaid (1976) where 37.6% increase in fry deformities, 5.6% decrease in feed conversion efficiency, 19% reduction in fry survival and 23.2% decrease in fish body weight at 364 days of age was reported. The deleterious effects of inbreeding were even greater after two generations of brother and sister mating. A thorough review on the effect of inbreeding in fish populations was also done by Kincaid (1983) where two approaches of large random mating and systematic line crossing for reducing the rate of inbreeding accumulation was discussed.

In Atlantic salmon, Rye et al. (1998) reported a 0.6% to 2.3% inbreeding depression for growth per 10% increase in inbreeding coefficients. A moderate effective population size of about 25 to 100 per generation with strict mating policy for breeders selected as parents can delay the accumulation of inbreeding and minimize its effect on growth in salmonids. For example, Gallardo et al. (2004) reported that in two Coho salmon populations of 61 and 106 founders respectively no significant inbreeding depression was found on survival of eggs at the eyed stage or traits such as body weight at spawning, weight of gonads and relative fecundity despite the differing rate of inbreeding ($\Delta F = 2.45\%$ per generation) and ($\Delta F = 1.10\%$ per generation) in the respective populations. Optimum design in terms of number of sire and dam can be very helpful in fish breeding programs to achieve maximum genetic gain while restricting the rate of inbreeding.

In order to avoid mating among related individuals, more recently knowledge of parental assignment is beginning to find its application in commercially valuable aquacultural species. Several studies have demonstrated the ability to determine parentage in communally reared fish using molecular markers, in particular microsatellites. The next pages of this introduction will discuss the use of molecular genetics and its integration into genetic improvement programs.

1.13 Genetic markers and their application in salmonids

DNA marker technologies have revolutionized the research studies in aquaculture and fisheries genetics in the last three decades. As a result, many genetic markers including allozymes, mitochondrial DNA (mtDNA, these markers are maternally inherited) random fragment length polymorphism (RFLP), random amplified polymorphism DNA (RAPD), amplified fragment length polymorphism (AFLP),

microsatellites and single nucleotide polymorphism (SNP) have been introduced in the field of fisheries and aquaculture research. In particular, microsatellite markers and AFLP are widely used by researchers interested in solving problems in aquaculture genetics. However, not a single type of marker is appropriate for all applications, and it has been recommended that the choice of a genetic marker must be based on both the characteristics of a particular species and the marker loci themselves. For example, RFLP are not as abundant in the genome as other markers and have a limited amount of variability. RAPD can obtain considerable coverage of the genome but their usefulness depends on the species being studied. RAPD are dominant markers that have shown inconsistencies in amplification (Ferguson and Danzmann 1998).

Genetic markers in general have been categorized into different classes by various authors. For example, Danzmann and Gharbi (2001) classified the genetic markers largely into two groups of sequence specific (such as simple sequence repeat SSR) and sequence-independent markers (e.g. AFLP and RAPD). Sequence-independent markers are characterized as dominant markers leading to less utilization for inheritance traceability across family lines whereas sequence-specific markers (e.g. microsatellites) are the markers of choice especially in aquaculture genetics. Due to the importance of microsatellite markers and AFLP in this study, I will further discuss the advantages and disadvantages, requirements for use, along with their application in parental assignment and QTL detection.

1.14 Microsatellite markers

Microsatellite markers are numerous and widely spread in the genome, providing a massive supply of genetic markers. Microsatellites also known as simple sequence length polymorphisms (SSLPs), simple sequence repeats (SSRs), or short tandem repeats (STR) are regions of DNA that exhibit short repetitive sequence motifs. These repetitive sequence motifs are often composed of 1-6 bp, such as CA, AGA, ATA and the like. The repeat numbers are variable between individuals, which make microsatellites polymorphic. Microsatellites can be found in both protein-encoding (type I) and non-coding (type II) DNA. However, for the reason that microsatellites are commonly located in non-coding region, they are known as type II markers. Di-nucleotide and tetra-nucleotide motifs are mostly spread in non-coding regions. Despite the fact that microsatellite markers generally require more time and effort to develop than markers such as AFLP, they offer the advantages of reliable amplification, extensive genome coverage, very high levels of polymorphism and co-dominant inheritance. Microsatellites can rapidly be amplified by polymerase chain reaction (PCR) using two unique oligonucleotide primers that flank the microsatellite. Moreover, microsatellites have been extensively used, as the marker of choice in the field of genetic mapping, because they are highly informative and require a small amount of DNA. Microsatellite markers are usually neutral and often represent non-functional sequences; therefore they are not directly responsible for phenotypic variation of economical traits. However, some microsatellite loci showing linkage association with the trait of interest may be in strong linkage disequilibrium functional genetic variations that cause the phenotypic variation (Chistiakov et al. 2006).

Microsatellite loci were originally described from eukaryotic and mamlian genome (e.g. Tautz & Renz 1984 and Beckmann & Weber 1992), but the use of microsatellite loci as polymorphic DNA markers has expanded considerably over the past two decades both in the number of studies and in the number of aquatic organisms studied. This rapid application of microsatellites as genetic markers can be explained because of the following advantages; their relative ease of amplification by the polymerase chain reaction (PCR) from a small sample of genomic DNA, co-dominant Mendelian inheritance, high variability, possibility of cross-amplification in related species, potential for automated assay and accuracy of scoring allele types (O'Reilly and Wright 1995).

One of the earliest applications of microsatellites in aquaculture research was to use these markers as a tool in parental assignment, assessing the genetic value of fish reared as separate families in individual tanks with those from the same families raised in a single communal tank. An example of a successful application of microsatellite markers in parental assignment of communally reared Atlantic salmon was shown by Herbinger et al. (1999). Using four polymorphic microsatellite markers (*Ssa*85, *Ssa*171, *Ssa*197 and *Ssa*202) for parental assignment, they concluded that rearing single families in single tanks results in an artificially high level of variation among family means, reflecting environmental differences among tanks rather than genetic differences among families. Casting doubt on the earlier findings, this meant that heritabilities for important traits such as length and weight obtained from the single family tanks were probably over-estimated. The results from studies of this sort have encouraged the application of microsatellite marker techniques especially in the form of paternity assignment and have shown that even more families can be kept in the breeding centre without the need for using separate tanks.

Currently, there is a debate going on about the number of microsatellites and how informative (i.e. number of alleles /locus and allelic frequencies) they need to be for accurate parental identification in different populations of fishes. Three loci consisting of 4 base pair (bp) repeat units (*Ssa*171, *Ssa*197, *Ssa*202), with a di-nucleotide locus (*Ssa*85) reported by O'Reilly et al. (1996) have shown great application with high level of success for parental application in Atlantic salmon. These four loci (which can be amplified in a single reaction and exhibit non-overlapping allele size distributions) also proved to be ideal for assessing genetic variation in wild population of Atlantic salmon. High heterozygosity (80 to 91%), multiplexing in a single reaction and minimal stuttering are reported as the main advantages of this set of microsatellite markers.

Based on exclusion probabilities, Villanueva et al. (2002) investigated the same four informative microsatellites and assigned at least 99% of the offspring to the correct pair of parents (the number of parents involved in the crosses were 100 males and 100 females). They acknowledged that an additional locus is needed for correctly assigning 99% of the offspring when the 100 crosses are produced with 10 males and 10 females. The same set of microsatellite markers has also been used by O'Reilly et al. (1998) to assign parentage of communally reared Atlantic salmon originating from a river in New Brunswick with 99.5% success. It was reported that the di-nucleotide locus (*Ssa*85) was less variable and uninformative than those tetra-nucleotide loci. In a population studied by Letcher et al. (1999), loci *Ssa*171 and *Ssa*202 were more informative for family identification. Recently, Paterson et al. (2004) described seven highly variable tetra-nucleotide microsatellite markers along with conditions for multiplexing and genotyping them in a single run for analysis of genetic studies in Atlantic salmon. In an attempt to asses the mortality in challenged Atlantic salmon

with infection pancreatic necrosis virus (IPN), Guy at el. (2006) performed parental assignment using a multiplex of 10 microsatellite markers and achieved more than 99.8% success. Nonetheless, to save time and effort in parental assignment in Atlantic salmon populations, the best types of microsatellite markers to use are those which have already been isolated and characterized in previous studies (e.g. Slettan et al. 1996; O'Reilly et al. 1998).

In the absence of pedigree information, microsatellite markers can also be used to discriminate related fish reducing the risk of mating closely related individuals and avoiding problems associated with inbreeding. The four most informative microsatellite markers developed for Atlantic salmon in combination with four other variable microsatellites were shown to be capable of discriminating between related and unrelated salmon in a situation where no pedigree information was available (Norris et al. 2000). The precision of assignment to one correct parental pair depends on not only on the number and variability of the microsatellite markers, but also on the number of potential pairings from which to choose. In other words, the more families in the breeding program, the more microsatellites might be required to discriminate between them. On a commercial scale, the wealth behind the salmon industry has sustained the application of microsatellite markers as genetic tags for use in parental assignment. Undoubtedly, the application of microsatellite markers for family identification represents a milestone in selective breeding of Atlantic salmon and all other aquatic species (Gjoen et al. 1997).

Rainbow trout is also a commercially important fish, but due to the complexity and cost of maintaining pedigree information a mainly mass selection approach has been used for its genetic improvements. In countries such as Finland, national breeding

programs have been designed and well developed for genetic improvements in rainbow trout. Application of microsatellite markers to determine paternity in rainbow trout has also been reported in several studies. For example, Herbinger et al. (1995) used four out of five microsatellite loci in communally reared rainbow trout and traced 91% of offspring to one or two parental couples of 100 possible parental pairs (10 sires × ten dams). Morris et al. (1996) reported that amongst 76 microsatellites isolated for rainbow trout two microsatellites showed very high level of polymorphism (at least 8 - 9 alleles) and displayed great potential for use in pedigree analysis in rainbow trout.

It is known that microsatellite markers in marine fishes tend to display significantly higher genetic variation than in freshwater fishes with anadromous fishes showing an intermediate level of genetic variation (DeWoody and Avise 2000). In commercially important marine fish such as the Atlantic cod, microsatellite markers are also finding their application for parental identification. For instance, Wesmajervi et al. (2006) using penta-plex amplification of microsatellite markers assigned 91% of cod juveniles which were collected from different mass spawning tanks.

For paternity and relatedness analysis of hatchery broodstock, the most useful microsatellite loci are only those that exhibit a high level of PIC (Polymorphic Information Content), show robustness in reproducibility, have well distinguishable allele size, and can easily be multiplexed. In addition an appropriate methodology should also be chosen for accurate and precise analysis of genotyping data to determine parentage in the population (Jones et al. 2003). PIC is used for measuring the informativeness of a genetic marker and it has been defined as the probability that

one could identify which homologue of a given parent was transmitted to a given offspring, the other parent being genotyped as well.

A number of studies have applied microsatellites to population genetics of salmonids. For example, McConnell et al. (1995) using two di-nucleotides microsatellite loci (*Ssa*4, *Ssa*14) isolated from Atlantic salmon along with two loci from rainbow trout (*Omy*27 and *Omy*380) found a clear differences in genetic variation between Canadian and European Atlantic salmon stocks. In support of that finding, Koljonen et al. (2002) reported clear difference between European and North American Atlantic salmon populations specifically at microsatellite SSOSL311. McConnell et al. (1997) using genetic variation of microsatellites (*Ssa*4, Ssa14, *Ssa*289, *Ssa*171 and *Ssa*197, *Omy*27, *Omy*38 and *Omy*105) showed that stocks of Atlantic salmon in eastern Canada are highly genetically diverse reflecting their phenotypic and behavioural diversity. In support of earlier studies, where it was claimed that domesticated salmon differ from wild salmon in fitness related traits such as growth, aggression and predator response, Skaala et al. (2004) using variation of 12 microsatellite loci (*Ssa*20, *Ssa*62NVH, *Ssa*71NVH, *Ssa*90NVH, *Ssa*103NVH, *Ssa*105NVH, *Ssa*F43, *Ssa*20.19, *Ssa*13.37, SsOSL85, *Ssa*197, *Ssa*28) showed that are cultured salmon are genetically less variable than wild salmon.

Similar findings have also been reported for other commercially important salmonids such as Arctic charr, whereas Lundrigan et al. (2005) used allelic variation at seven di-nucleotide microsatellite markers to compare the genetic diversity of Arctic charr to that of natural populations and drew the conclusion that hatchery strains and natural populations of Arctic charr are genetically differentiated. In the literature review carried out by Jones and Arden (2003), the appropriate techniques and the choice of

computer programs for solving the most common problems (such as insufficient genetic variation, scoring errors, mutations, null alleles and incomplete sampling) occurring in parental assignment in natural populations have been discussed. With the emerging of new statistical techniques such as likelihood approach for even more accurate assignment the major drawback for parental assignment in natural populations appeared to be obtaining appropriate and complete field samples. Although applications of microsatellite markers for parentage assignment and quantifying genetic variability have found great utility, but there are suggestions that the key component of aquaculture genomics in the near future could be QTL mapping (Liu and Cordes 2004).

1.15 AFLP markers

ALFP is a technique originally developed by Vos et al. (1995) and is known to be highly reproducible. This technique combines the power of restriction fragment length polymorphism (RFLP) with the flexibility of PCR-based technology. AFLP technology can rapidly generate hundreds of highly replicable markers (Liu and Cordes 2004). In addition, ease of use in AFLP technique has led to emergence of these types of marker as a major novel class of genetic marker with broad application, particularly in species with a poorly characterized genome. A major advantage of the AFLP technique is the high marker density that can be obtained without the availability of prior sequence information. As major disadvantage, AFLPs are dominant markers, meaning that without using special software, the homozygous genotypes cannot be distinguished from the heterozygous genotype, and that makes

their use in mapping experiments more difficult. Thus, microsatellite-based genotyping is probably more useful for linkage analysis and parentage testing.

ALFP technique has been applied for DNA fingerprinting of prokaryotes and eukaryotes. In plants, AFLP technique has been used for construction of genetic maps in *Pinus taeda* (Remington et al. 1999) and soybean *Glycine mar* (Keim et al. 1997), as well as in genetic studies for assessment of genetic relationships between wild and cultivated carrots *Daucus carota* (Shim and Jorgensen 2000). AFLP has also important applications for gene mapping and QTL detection in animals. For instance, Otsen et al. (1996) by adding 18 AFLP markers into the linkage map of the rat demonstrated the potential of AFLP markers for detection of QTL. They found suggestive correlation between the blood pressure regulatory gene and two closely linked AFLP markers located on chromosome 20.

In aquatic species, AFLP genetic markers are increasingly gaining attention among fish geneticists. Maldini et al. (2006) demonstrated the power of discrimination and suitability of AFLP for assessment of species identification and authenticity testing in fish and seafood species in processed products. As genetic tags, Miggiano et al. (2005) used 147 AFLPs in conjunction to 4 microsatellite markers for identification of escapee gilthead seabream. Their method proved to be reliable in differentiate two different hatchery broodstock (one of Atlantic and one of Mediterranean origin) from wild fishes of natural population. AFLP markers have also been used to assess genetic variation in fish population. For example, the level of genetic variation among wild channel catfish populations and its genetic similarities to the domestic population were compared by Simmons et al. (2006). The suitability of AFLPs in generating polymorphic markers for gene mapping of catfish has also been evaluated by Liu et al.

(1999). The difficulty in isolating microsatellites from penaeid species in a number of laboratories has led to broad application of AFLP technique for the construction of genetic linkage maps in penaeid shrimp. AFLP based linkage maps in black tiger shrimp *Penaeus monodon* (Wilson et al. 2002) and white shrimp *Penaeus vannamei* (Perez et al. 2004) have been reported.

In salmonids, AFLPs have found a broad application in genetic linkage mapping and QTL detection. For instance, identification and mapping of sex linked markers in rainbow trout (Felip et al. 2004), detection of a QTL for resistance to infectious salmon anemia (ISA) in Atlantic salmon (Moen et al. 2004b), finding the gene involved in dominant albino locus in rainbow trout (Nakamura et al. 2001), detection of two major and a minor QTL influencing hatchability time in rainbow trout (Robinson et al. 2001), detection of chromosomal region responsible for natural killer cell-like activity in rainbow trout (Zimmerman et al. 2004), and association of 15 AFLP markers with three major QTL linked to pyloric caeca number in rainbow trout (Zimmerman et al. 2005). AFLP markers have also been used for maximizing genetic diversity in a base population for an Atlantic salmon breeding program (Hayes et al. 2006).

It should be mentioned that although AFLPs have been broadly used for the construction of linkage maps in a number of aquatic species, but the majority of these linkage maps utilize a combination of different techniques such as microsatellite and AFLP markers. For instance in Arctic charr, Woram (2001) constructed a linkage map using 107 AFLP, 138 microsatellite markers, 7 known genes and one phenotypic marker. In Atlantic salmon Moen et al. (2004a) reported a genetic linkage map containing 473 AFLP and 54 microsatellite markers.

1.16 Genetic Linkage Map

As a first step towards the detection of QTL, the construction of genetic linkage maps is an essential task. For economically important fish species, genetic linkage maps is created by assigning highly polymorphic DNA markers to chromosomal region based on their segregation relationships (Liu and Cordes 2004). The idea behind that is to identify genetic markers which are linked to a group of genes that control the desired characters, for example, genes controlling disease resistance or growth rate. A genetic map is constructed using the recombination rate between selected genetic markers. The recombination rate is determined by the frequency of crossover that occurs between two loci during meiosis of either parent, as observed in the progeny genotype. Two loci that are located physically close to each other on the chromosome will show lower recombination rates between them. Map distances are measured in centiMorgans (cM), whereas one centiMorgan equals one percent recombination between two loci (Hartl 1996).

For the construction of genomic maps, a collection of a large number of genetic markers to cover a high proportion of the genome is required (Poompuang and Hallerman 1997). In aquaculture species, molecular markers such as microsatellite loci randomly amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) have been used for constructing genetic linkage maps. However, microsatellite markers are one of the most employed types of marker used for the construction of genetic maps. Advantages of using microsatellite markers for QTL analyses can be listed as follows:

1) Microsatellites are co-dominant.

2) Microsatellites are highly abundant and well distributed throughout the genome.

3) Microsatellites are highly variable and can be quickly and reliably screened.

4) Many microsatellites amplify well across closely related species.

Disadvantages of using microsatellite markers can be summarized as:

1) High cost of development.

2) High cost of analysis that includes a PCR machine and a genotyping instrument.

3) Potential scoring difficulties.

Linkage maps with a density of markers around 20-30 cM are recommended for detecting the presence of QTL (Lynch and Walsh 1998). However, increasing marker density will allow more precise positioning of the QTL. The earliest salmonid linkage map is a composite derived from several species and consists of 54 allozyme defining 22 chromosomal arms (May and Johnson, 1990). This map was constructed using three genera, *Salvelinus*, *Oncorhynchus* and *Salmo*. Microsatellite based genetic linkage maps have been reported for a number of fish species such as zebrafish (*Danio rerio*) (Knapik et al. 1998; Shimoda et al. 1999), yellowtails (*Seriola quinqueradiata* and *Seriola lalandi*) (Ohara et al. 2005), Japanese flounder (*Paralichthys olivaceus*) (Coimbra et al. 2003), tilapia (*Oreochromis niloticus*) (Kocher et al. 1998), channel catfish (Liu 2003) common carp (*Cyprinus carpio*) (Sun and Liang 2004), rainbow trout (*Oncorhynchus mykiss*) (Sakamoto et al. 2000; Nichols et al. 2003) and Arctic charr (*Salvelinus alpinus*) (Woram et al. 2004).

The first linkage map of Atlantic salmon consisting of 54 microsatellites and 473 polymorphic AFLP markers was reported by Moen et al. (2004a). The length of the male map was reported to be 103 cM with 31 linkage groups, while that of the female

map was 901 cM (with 33 linkage groups) resulting in the highest difference in recombination rate between the sexes reported for vertebrates (the ratio of female vs. male recombination rate = 8.26). A microsatellite based linkage map of the Atlantic salmon was also reported by Gilbey et al. (2004) where the ratio of female vs. male recombination rate at 3.9:1 was reported. Most of vertebrates including fishes show higher recombination rates in females rather than males except in Japanese flounder where Coimbra et al. (2003) reported higher recombination rates in male flounder compared to the female (7.4:1). Table 3 shows the details of genetic linkage map constructed for three commercially important salmonids.

Table 3: Genetic linkage map for three commercially important salmonids (2N refers to chromosome number in each species).

Species	number of markers on linkage map	LG	length cM	2N	reference
Atlantic salmon	Male: 251 (31 microsatellites + 215 AFLPs)	31	Male:103	58-60	Moen et al. (2004a)
	Female: 230 (31 microsatellites +199 AFLPs)	25	Female: 910		
Arctic charr	327 (184 microsatellites, 129 AFLPs, 13 ESTs, 1 phenotypic marker sex)	46	Male: 3900	80	Woram et al. (2004)
			Female: 9920		
Rainbow trout	208 (191 microsatellites, 3 RAPD, 7genes, 7 allozymes)	29	Male: 463.2	60	Sakamoto et al. (2000)
			Female: 1152.8		

AFLP based linkage maps for Zhikong scallop *Chlamys farreri* (Wang et al. 2005; Li et al. 2006), the Eastern oyster *Crasssostrea virginica* (Yu and Guo 2003) have also been published among other shellfish species. AFLP has also been used for linkage map construction of sea urchin using crosses between male of *Strogylocentrotus nudus* and female of *S. intermedius* (Zhou et al. 2006).

The genetic linkage maps are also very useful in population genetic studies for improving the quality of the management of wild stocks of salmonids. In the past, a collaboration project (SALMAP) using informative DNA markers has generated low

resolution genetic maps for Atlantic salmon. Currently, there are two major consortia, GRASP (Genomic Research on Atlantic Salmon Project) and SGP (Salmon Genome Project); working on genetic maps for Atlantic salmon and both using SALMAP derived markers and maps as the basis for their work. As a result of their work, more markers (primarily microsatellites and also AFLP, SNP and structural gene markers) have been introduced into the existing maps. These maps are now published at: http://grasp.mbb.sfu.ca/GRASPlinkage.html and http://www.salmongenome.no/cgi-bin/sgp.cgi. These web pages provide valuable databases, tools and information about genetic markers and genetic maps for salmonids fish and they have been very beneficial towards my study.

1.17 QTL Detection

QTL detection is emerging as a very important part of aquaculture genetics. Most production traits such as growth and disease resistance are controlled by a number of genes and inherited as quantitative traits. A quantitative trait (for example, weight or length) is defined as measurable phenotypic variation under genetic and/or environmental influences. A QTL is a genetic locus that affects phenotypic variation. One or several QTL can influence a particular trait. If many QTLs are involved, then each might have an effect of different magnitude on a trait. When a linkage map has been created, using the phenotypic data in combination with the statistical methods such as linear regression can be used to identify markers that are closely linked to the QTL of interest, thus allowing the QTL to be positioned on the linkage map.

In theory, this information can be utilized to maximize growth, resistance to diseases, or quality trait through means of marker-assisted selection. However, not all the traits are suitable for undergoing this process and priority must be given to certain traits that

can justify effort and expenses. According to Davis and Hetzel (2000) there are five areas where QTL detection can be most effective and deliver significant gains:

1) Traits which are difficult or costly to measure, such as feed conversion efficiency.

2) Traits which are only measurable on one sex, for example fecundity of females.

3) Traits that can only be measured after the selection has taken place, such as reproductive characteristics.

4) Traits that can only be measured after slaughter such as flesh colour and fat percentage.

5) Traits that can only be measured on animals under experimental challenges, such as IPN resistance.

There are two major methods available for QTL detection; candidate locus and genome scan (Cheverud and Routman 1993). The candidate locus approach investigates loci that have a known biochemical and physiological relationships to the phenotype of interest. According to Cheverud and Routman (1993) the advantages of this approach are as below:

1- It only concentrates on relevant genomic regions for the traits of interest.

2- The results can easily be interpreted especially for physiological traits.

3- It presents direct measures of genotypic values.

4- It is easily applicable to variation within natural population.

This method, however, is limited by the number of loci available and genome coverage.

The advantages of the genome scan approach are:

1- It covers the entire genome.

2- It can effectively be applied in phenotypically divergent crosses.

3- Searches for unknown QTL.

Theoretical basis of the QTL mapping based on the interval mapping method with the maximum likelihood approach was initially proposed by Lander and Botstein (1989). In interval mapping, the putative QTL is assumed to occur within a segment of the genome bordered by 2 genetic markers (A and B). The recombination fraction between these 2 markers can be estimated prior to the QTL analysis. The assumption can then be made as to the likelihood that an individual has a particular genotype at a putative QTL, based on the function of the relevant recombination frequencies. The evidence for a QTL can then be summarized through a LOD score which is calculated by the equation $LOD = \log_{10} (L_1/L_0)$, where L_1 is the likelihood where the estimated QTL effects are included, and L_0 is the likelihood where QTL effects are considered to be zero. The position where this likelihood ratio is maximal is the most likely location for a QTL within the marker interval. Appropriate LOD thresholds based on genome size and marker density can be set and if the LOD score exceeds the threshold, there is significant evidence for a QTL in region of the genome under analysis (Lander and Botstein 1989).

QTL analysis in livestock started in the early 1990s, ever since many QTLs have been reported in many livestock species, mostly related to traits of prime economic

importance. QTL for traits such as growth and fatness in swine were reported by Andersson et al. (1994) where they found evidence of QTL on chromosome 4 with large effects on growth, length of the small intestine and fat deposition. This chromosome 4 was the centre of focus in study carried out by Walling et al. (1998) where in agreement with the previous study the occurrence of QTL with major effects on growth rate and fat depths on chromosome 4 was confirmed.

A tight linkage (3% recombination) between the TGLA116 microsatellite marker and the gene causing weaver disease in cattle was reported by Georges et al. (1993). It was claimed that this microsatellite marker can assist breeders in selecting efficiently against weaver disorder without having to rely on lengthy and expensive progeny testing procedures.

In a genome-wide search for QTL in poultry, van Kaam et al. (1998) mapped 368 genetic markers on 24 autosomal linkage groups and found the most likely position for a QTL affecting body weight on chromosome 1 at 240 cM. The within-family linkage disequilibrium was utilized by Ikeobi et al. (2002) for mapping 102 microsatellite markers to 27 linkage groups and led to detection of QTL affecting fatness in the chicken.

QTL detection in aquaculture species is not as advanced as livestock species. This is mainly due to the fact that the science of animal and plant breeding programmes began during the 1930s, whereas the earliest salmon breeding programmes were started in the 1970s (Gjedrem 2000). As the domestication of farmed fish continues, these techniques are emerging as powerful tools for the application of genetic improvement in aquaculture species as well. In particular, a number of QTL have been identified in salmonids fish. The most reports in the area of QTL detection have

been published for rainbow trout. The first report of QTL detection in rainbow trout was carried out by Jackson et al. (1998) where they identified two major and several minor QTL influencing upper temperature tolerance. This was followed by reports of QTL identification for spawning time on linkage group 5, 15, A, B, G, J and N (Sakamoto et al. 1999), Martyniuk (2001) found support for these results and identified QTL for spawning time. QTL for upper thermal tolerance has also been identified by Perry et al. (2001). QTL reports for growth related traits have been reported by Martyniuk et al. (2003) and Perry et al. (2005). Other work has identified QTL for disease resistance in rainbow trout for example Ozaki et al. (2001) reported QTL for resistance to infectious pancreatic necrosis virus (IPN) and Palti et al. (1999) identified the candidate QTL associated with ischaemic haematopoietic necrosis virus (IHNV) resistance.

In Arctic charr, using microsatellite markers two QTL affecting upper temperature tolerance was reported by Somorjai et al. (2003).

Genotyping 91 microsatellite markers located on 16 linkage groups in three full-sib families of Atlantic salmon, Reid et al. (2005) detected QTL affecting body weight and condition factor. In order to identify molecular markers linked to QTL influencing *Gyrodactylus salaris* in Atlantic salmon, Gilbey et al. (2006) utilized 39 microsatellite markers identified 10 genomic regions associated with resistance to this parasite, explaining 27.3% of the total variation in parasite loads. The summery of QTL detection in commercially important salmonids is given in Table 4.

Table 4. Summery of detected QTL in commercially important salmonids.

QTL	Magnitude of the QTL effect	Markers linked to QTL	Species	Publisher
Upper temperature tolerance	9-13%	Omy32UoG; Ssa14DU	Rainbow trout	Jackson et al. (1998)
Susceptibility to ectoparasite	27,30%	-	Atlantic salmon	Gilby et al. (2006)
Body weight	4,0-7,0%	OmyRGT14TUF, One2ASC	Rainbow trout	Martyniuk et al. (2003)
Condition factor	2-3%	One2ASC, One19ASC, OmyRGT36TUF	Rainbow trout	Martyniuk et al. (2003)
Precocious maturation	-	OmyRGT1TUF; One2ASC; OmyRGT14TUF	Rainbow trout	Martyniuk et al. (2003)
Upper temperature tolerance	7,5%	Ssa20.19UNIG	Rainbow trout	Perry et al. (2001)
Spawning time	-	OmyFGT12TUF; Ssa311NCVM; One5ASC; One2ASC; Ssa85DU; Ssa4DU; Ssa289DU and OmyFGT34TUF	Rainbow trout	Sakamoto et al. (1999)
Body weight	11,7-21,1%	Ssa401UoS; Ssa417UoS	Atlantic salmon	Reid et al. (2005)
Condition factor	11,9-24,9%	One102ADFG; BHMS159	Atlantic salmon	Reid et al. (2005)
Upper temperature tolerance	-	Ssa189NVH; SsaF43NUIG	Arctic charr	Somorjai (2003)

The identification of markers linked to the sex-determining region (e.g. Devlin et al. 1998) can lead to the detection of sex-linked genes that may influence fitness and other economically important traits in salmonids. As mentioned previously, autosomal QTL for physiological traits such as body weight or resistance to diseases have been identified in Atlantic salmon, but so far no sex-linked QTL for any trait has been identified in Atlantic salmon. A sex determining locus in Atlantic salmon has already been mapped in linkage group 1 and close association between microsatellite marker *Ssa*202 with the male phenotype has been reported (Reid 2003). Physical location of the sex chromosome has also been identified using fluorescent in situ hybridization (FISH) in Atlantic salmon on chromosome 2 (Artieri et al. 2006). A study of Y-chromosome linkage map of four salmonid species (Artic charr, Atlantic salmon, brown trout and rainbow trout) carried out by Woram et al. (2003) demonstrated that Y-chromosomes are not conserved among salmonid species. As a result of a general

lack of conservation for sex linkage among salmonid fishes, it is concluded that different Y-chromosomes have evolved in each of the species (Woram et al. 2003).

Comparative mapping of QTL among relevant species is becoming an efficient approach to detect QTL in commercially important fish species (Somorjai et al. 2003). In addition, this approach has provided important insight into the evolutionary dynamics of duplicated loci (Small and Wendel 2002). As the first example of comparative QTL mapping, Somorjai et al. (2003) detected two significant QTL for upper temperature tolerance in Arctic charr and then using comparative mapping approach, they localized these two QTL to homologous linkage groups containing the same QTL in rainbow trout. Nonetheless, sex-specific recombination rates in salmonids and differences in the composition of the marker sets were reported as two main obstacles for determining the homologies of chromosomal regions between the salmonid fishes. These studies have opened a new chapter in salmonid genetics with a massive prospect for future exploitation in the field of aquaculture.

Despite several reports for occurrence of genetic component for fat level in salmonids, genetic improvement of this trait has received comparatively little attention. Historically, quantitative genetics have been used to improve production traits such as body weight and delay maturation in salmonids. With advances made in the field of molecular genetics, new opportunities are emerging for enhancement of quality traits (such as fillet pigmentation and fat percentage) based on genotypic data as well as phenotypic records. Several studies have shown that genetic markers (in particular microsatellite markers) can be used to search and localize the genes responsible for health and production traits. A brief overview of past achievements and new trends for genetic improvements of farmed salmonids is given below.

In the salmon industry, there is an urgent need to enhance quality traits such as fat percentage and flesh colour (both traits are difficult to measure and demand slaughter of fish). The purpose of my project was to identify genetic markers associated with QTLs for these commercially important traits, work to identify QTLs affecting fat percentage and flesh colour in Atlantic salmon that could aid in marker-assisted breeding.

Chapter 2 - Genome-wide scan

2.1 Introduction

Alongside growth rate and feed conversion efficiency, quality traits (such as flesh colour, fat percentage and fillet yields) have recently become of considerable importance to the salmonids industry. The lipid content of the salmonid fillet is an important attribute that influences the characteristics of the product and is of major importance to flesh texture and flavour (Johansson et al. 2000).

The standard method for aquacultural improvement is to select for desirable traits based on phenotypic values. In principle, phenotypic variation is the result of genetic components and environmental effects along with interactions and associations of these two sources of variation (Hartl 1996).

Fat content in fish is generally influenced by the availability of dietary energy. However, increasing the lipid supply in the feed leads to increased fat deposition throughout the whole body of the fish (including the fish fillet and the slaughter waste of fish such as the viscera and abdominal belly). On the other hand, insufficient lipid content in the diet can adversely affect the growth rate.

In Atlantic salmon, an estimate of the heritability for fat percentage is fairly high. According to Rye and Gjerde (1996) the heritability for fat percentage is 0.30 indicating the prospect of achieving a rapid genetic gain in the reduction of fat percentage by selection in Atlantic salmon. However, this potential has not been exploited fully because of a number of reasons such as the difficulty in measuring the fat content in a large number of fish; the measurements are very time-consuming and

prone to error, and above all require destruction of fish and possible breeding candidates.

The occurrence of high levels of unsaturated fatty acids in fillet fish is of great interest from a consumer perspective. According to Tobin et al. (2006), lipid traits display higher heritabilities ($h^2 = 0.40$) than protein traits ($h^2 = 0.18$) in rainbow trout. These moderate estimates of heritabilities for percentage fillet fat and flesh colour in salmonid fish confirm that there is a considerable amount of genetic variation available for genetic improvement through traditional breeding programs or in combination with marker assisted selection. It seems that there is no positive genetic correlation between body weight and fillet fat percentage. For example, Gjerde (1997) has reported that there is an adverse genetic correlation between body weight and the fat percentage. Quillet et al. (2005) showed that no difference in growth of two selected lines for muscle lipid content in rainbow trout. Kause et al. (2002) claimed that there are very low genetic correlations (−0.12 to 0.36) between body weight with percentage fillet fat and flesh colour in rainbow trout, suggesting that the quality of fillets is not strongly changed when fish are selected for rapid growth rate.

Flesh colour in farmed salmonids is an important attribute which influences product marketability and consumer perspective. Colouration of flesh is influenced by carotenoids which cannot be synthesized by the fish and must therefore be given in the feed (by adding of astaxanthin or canthaxanthin). The high cost of carotenoids supplementation in the feed and poor retention of it in the flesh can increase the feed price by 10 to 15% of the total production (Norris et al. 2004). Therefore, flesh colour is considered as an economically important trait in Atlantic salmon.

Despite the importance of flesh colour traits to the salmonid industry, few estimates of genetic variation have been reported for flesh colour. In Coho salmon, a medium to high heritability for flesh colour (0.30 to 0.50) has been estimated (Iwamoto and Hershberger 1996). Low to medium heritabilities for flesh colour (0.12 to 0.14) have been reported for Atlantic salmon (Rye and Gjerde 1996; Norris and Cunningham 2004).

Genome-wide scan strategy for the identification of linked markers to a chromosomal region harbouring the QTL for the trait of interest have proven to be a feasible means of detecting QTL in different species. To save time and effort, these genome scans are usually performed with a minimal set of markers spanning the whole genome, leading to a low resolution of mapped QTL locations. For narrowing QTL location, additional polymorphic markers (such as microsatellites and AFLP markers) could also be integrated into the selected genome regions.

Currently, a collaborative group consisting of Landcatch Natural Selection, Roslin Institute, University of Edinburgh and University of Stirling with the UK's principal funder BBSRC (Biotechnology and Biological Sciences Research Council) are investigating the incidence of infectious pancreatic necrosis (IPN) mortality in pedigreed Atlantic salmon. Within their chosen families, my primary objective was to use microsatellite loci to screen the whole genome to identify linkage groups that may harbour QTL for fillet quality traits such as fat percentage and flesh colour. The following chapters will then focus on the saturation of candidate linkage groups with a new set of genetic markers such as microsatellite and AFLPs markers.

2.2 Materials and methods

2.2.1 Mapping family

One hundred and ninety seven full-sib family groups were originally generated by 149 males and 197 females in November 2000 in Landcatch Natural Selection (LNS) to investigate the incidence of infectious pancreatic necrosis (IPN) mortality. The parents of these families were spawned in 1999, and offspring were hatched in March 2000. Approximately 55.000 smolts from these families were used in experimental trial to assess level of mortality caused by IPN in a sea cage in Shetland, UK. After 2 to 3 months of seawater transfer, the collected the dead fish confirmed the incidence of IPN as a cause of mortality. The dead fish were genotyped and assigned to 10 full-sib families. Based on DNA availability these 10 families were chosen for the study of QTL detection affecting resistance to IPN (as outlined in Houston et al. 2008).

Within these 10 families, DNA samples from five full-sib families were given to me for assessing the genetic control on quality traits (fat percentage and flesh colour) in this study. DNA sample of 153 individual fish were derived from fin clips. Families one, two, three, four and five contained 25, 33, 17, 34 and 44 offspring, respectively.

2.2.2 Phenol/chloroform DNA Extraction

For DNA extraction, phenol/chloroform is a common and preferred technique used to purify a DNA sample. Fin clips were used for DNA extraction. Each sample was placed into the 1.5 ml microcentrifuge tubes containing 340 µl of 0.2M EDTA solution (pH 8.0), with 0.5% SDS (sodium lauroylsarcosine, Sigma). Then 10 µl of 20 mg/ml proteinase K (ABgene) was added into each tube. Each tube was briefly mixed and incubated overnight at approximately 55 °C in a hybridization oven (Techne

Hybridizer HB-1). The day after, 10 µl of 20 mg/ml DNAse free RNAse (ABgene) was added into each microcentrifuge tube. The mixture was vigorously mixed, and then incubated for 1 hour at 37 °C in a hybridization oven. Approximately 350 µl of buffered phenol (Fisher Scientific) was added into each tube and vigorously vortexed for 10 seconds, and then centrifuged for 5 minutes at 10.000 g to enact phase separation. The upper aqueous layer carefully was removed to a new tube. Then 900 µl of chilled 92% ethanol was add to the tube and mixed by vigorous inversion of the tubes for 5 to 6 times and was left aside for DNA precipitation. Then ethanol was removed and discarded. Then 1 ml of 70% ethanol was added to the DNA pellet and tubes were paced in a rotator (Stuart Scientific) over night at room temperature. The day after, ethanol was removed and the DNA was kept out for 10 minutes to dry at room temperature. The dried DNA was dissolved in 50 µl TE buffer (10 mM Tris-HCl, pH 7.6-8.0, 0.1 mM EDTA). Aliquots of 100 µg of purified DNA in several 0.5 ml microcentrifuge tubes were frozen at -20 °C.

The DNA was first quantified by measuring absorbance of the DNA solution in a spectrophotometer (Jenway Ltd.) at 260 nm. The quality of the DNA samples were assessed by running them through a 1.2% agarose gel electrophoresis in 1X TAE buffer (50X stock solution containing 242 g Tris base, 57.1 ml glacial acetic acid, 100 ml of 0.5 M EDTA (pH 8.0) in 1 litre solution). 1.2 g of agarose (Sigma, UK) was added to 100 ml of 1X TAE in a 200 ml Pyrex beaker and mixed well. This mixture was heated for 2 minutes at 75% power in an 800 W microwave, with occasional breaks for mixing until the solution became clear. The solution was equilibrated to approximately 55 °C in a fume hood and 2 µl of ethidium bromide (20 mg/ml; Sigma, UK) was added. After mixing, the solution was poured into a proper gel-casting tray and a 12 samples comb was positioned appropriately in the gel. The gel was allowed

to set for approximately 20 minutes. Agarose gel was run for 15 min at 5 V/cm. The gels were visualized using a UV transilluminator (Figure 1). A basic descriptive analysis was carried out to determine the means and standard deviations of each trait, as is shown in Table 5. The harvest trait measurements and estimation of heritabilities for these traits were carried out by LNS and the raw data is given in Appendix 1. A univariate analysis was used to estimate the heritability for harvest traits, and a multivariate analysis was applied for heritability estimation in quality traits. .

Figure 1: DNA quality assessment using 1.2% agarose gel. View of the agarose gel on a UV transillminator. The first lane was loaded with 0.5 µg of 100 bp DNA ladder. One µg of DNA samples of parent fish are shown. A visible smear in the 100-500 bp range of last four fish can be a result of the DNA degradation.

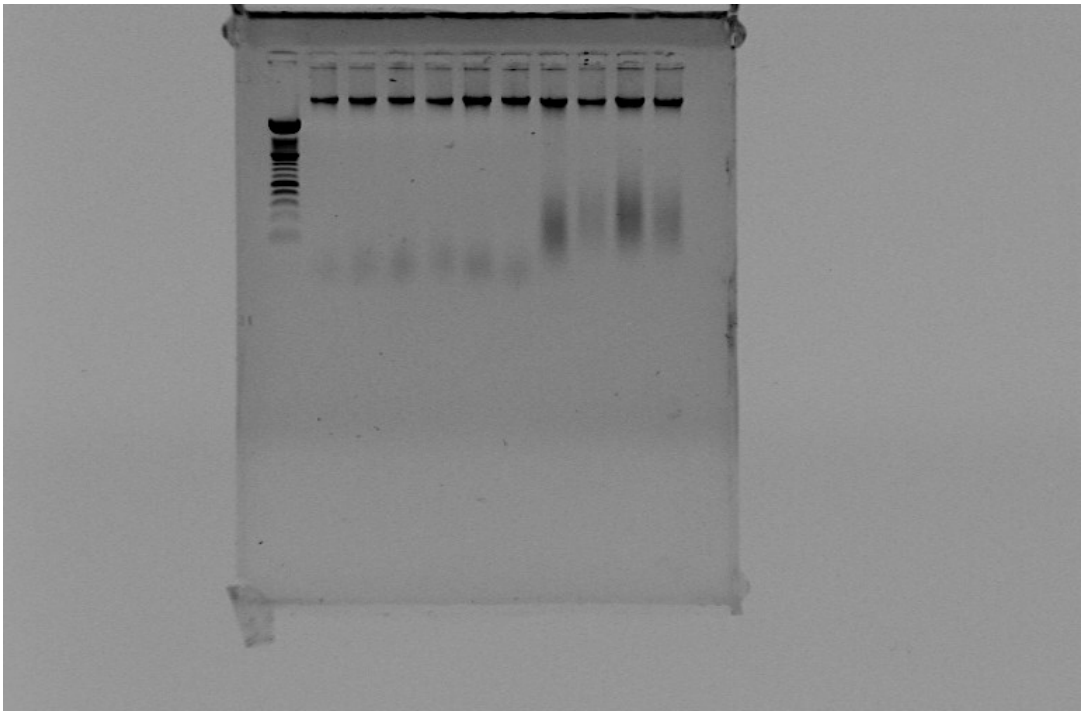


Table 5: The mean and the standard deviation for some of the commercially important traits in whole population of LNS fish.

No.	Traits	Mean	S.D.	Heritability
1	Harvest Weight (kg)	2.69	0.57	0.52
2	Harvest Length (cm)	64.97	4.20	0.42
3	Harvest Condition Factor	0.97	0.10	0.02
4	Gutted Weight (kg)	2.47	0.52	0.02
5	Weight of Guts (g)	214.35	72.70	-
6	Gutted Yield	90.86	9.94	0.12
7	Head Weight (g)	340.36	75.89	0.04
8	Fillet Weight (kg)	1.80	0.42	0.53
9	Waste Weight (g)	337.50	134.78	0.30
10	Fillet Yield	71.41	9.64	0.04
11	Fat % (Torry)	12.62	5.62	0.17
12	Colour % (Minolta L)	47.85	7.53	0.10
13	Colour % (Chroma)	35.78	4.47	0.10
14	Colour % (Hue)	44.03	5.72	0.03
15	Colour % (Roche)	28.57	3.28	0.15

2.2.3 Genetic markers and genotyping

Microsatellites markers were employed for genome-wide scanning across the families. Based on the nomenclature adopted by the SALMAP consortium, microsatellite markers names begin with the first letter (capital) of the genus followed by the first two letters of the species in which the microsatellite sequence was originally isolated. These three letters are generally followed by a number designation and ends with the institution where the marker was developed. For instance, the primer used to amplify *Ssa*416UoS was developed from *Salmo salar* at University of Stirling. Markers that were developed before 1998 are an exception to this nomenclature, for example, SSOSL85 keeps its original designation. Duplicated microsatellite markers detected with a single pair of primers are indicated by a forward slash and a lower-case i or ii to distinguish each separate locus. Table 6 demonstrates abbreviations and scientific names and common names of species for

microsatellites primer sets in salmonids. Table 7 shows Institution abbreviations, official institution names at which microsatellite primer sets were designed.

Table 6: Species name abbreviations for microsatellites primer sets in salmonids (taken from Sakamoto et al. 2000).

Species abbreviation	Scientific name	Common name
Ssa	*Salmo salar*	Atlantic salmon
Sal	*Salvelinus alpinus*	Arctic charr
Sfo	*Salvelinus fontinalis*	Brook charr
Str	*Salmo trutta*	Brown trout
Omy	*Oncorhynchus mykiss*	Rainbow trout
One	*Oncorhynchus nerka*	Sockeye salmon
Ots	*Oncorhynchus tshawytscha*	Chinook salmon
Ocl	*Oncorhynchus clarki*	Cutthroat trout
Ogo	*Oncorhynchus gorbuscha*	Pink salmon

Table 7: Institution abbreviations for institutes where microsatellite primer sets were designed.

Institution abbreviation	Official institution names
UoS	University of Stirling (UK)
INRA	Institute National de la Recherché Agronomique (France)
NVH	Norwegian College of Veterinary Medicine (Norway)
NUIG	National University of Ireland, Galway (Ireland)
UoG	University of Guelph (Canada)
TUF	Tokyo University of Fisheries (Japan)
UW	University of Washington (USA)

For linkage analysis at least one parent must be heterozygous at each microsatellite locus. Microsatellite markers can be informative in three cases.

1- Microsatellite loci are considered as highly informative when each parent is heterozygous at different alleles.

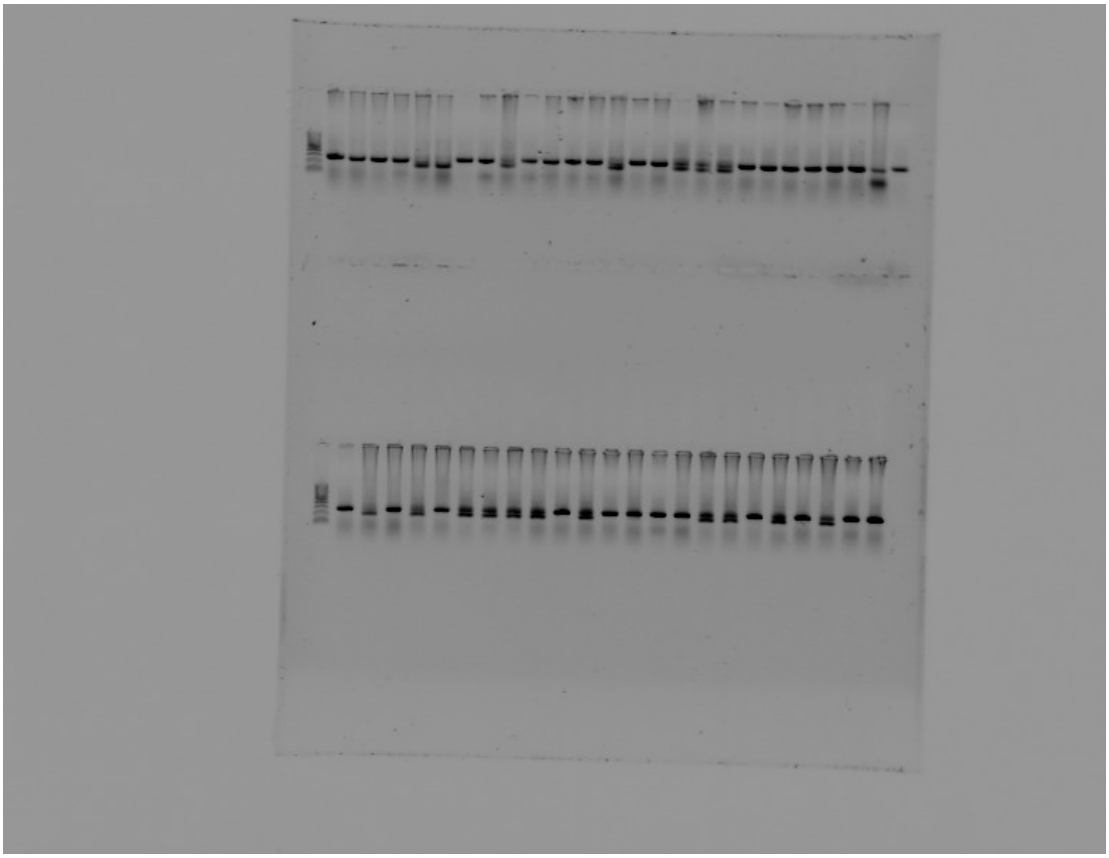
2- Microsatellite loci are considered as informative when one parent is heterozygous and the other is homozygous.

3- Microsatellites loci are considered less informative when both parents are heterozygous for the same alleles, thereby only the homozygous progeny (approximately have the progeny panel) are informative.

Microsatellite markers were amplified by PCR in a thermal cycler (TGRADIENT Biometra) with a heated lid at 99 °C. The routinely used temperature profile for PCR was: 95 °C for 1 min (minutes); 35cycles of 95 °C for 1 min, annealing temperature for 1 min and 72 °C for 1 min with a 5 min final extension at 72 °C.

The standard PCR cocktail included: 1X PCR buffer IV (ABgene Ltd; 75 mM Tris-HCl, pH 8.8, 20 mM (NH4) SO4, 0.01% (v/v) Tween 20®); dNTPs 150 µM each, 1.5 mM MgCl2 (several microsatellite loci required alteration to the MgCl2 concentration during optimization), forward and reverse primers 0.15 pmol/µl each, 0.2 U Taq DNA polymerase ABgene Ltd;, 70 ng DNA and PCR water to make a total volume of 15 µl. Prior to automated detection of DNA fragments on an ABI 377 sequencer, an aliquot of each of PCR product was separated through an agarose gel (1.2%) to confirm successful DNA amplification (Figure 2). The forward primers were dye labeled with one of three different fluorescent dyes; FAM (6-carboxyfluorecein), TET (6-tetrachlorofluorecein), HEX (6-hexachlorofluorescein) for detection via an ABI 377 sequencer.

Figure 2: Visualization of PCR products from microsatellite assays in agarose gel prior to use ABI 377 DNA sequencer. This procedure was routinely done to make sure of existence of PCR products.



The DNA fragments were visualized with the ABI PRISM 377 DNA sequencer. 6% denaturing polyacrylamide gels were prepared by dissolving 18 g urea (Bio-Rad Laboratories) in 5 ml of Long Ranger® gel solution (Acrylamide, 50% stock solution, Cambrex), 5 ml of 10x TBE (108 g Tris, 55 g boric acid, 8.3 g EDTA in 1 litre solution) and 26 ml of distilled water. In order to remove charged particles from the gel solution 0.5 g of resin beads (Sigma) was added and stirred with a magnetic bean for about 20 min. The solution was filtered through a 0.2 µM membrane and degassed for about 10 minutes by vacuum. 35 µl of TEMED (N,N,N',N'-tetramethylethylenediamine, Sigma- Aldrich) and 250 µl of 0.1 APS (Ammoniom persulphate, Amersco) were added into the gel solution to initiate polymerisation and mixed well. The gel solution was injected into 0.2mm space of two 42 cm glass plates

using a 50 ml syringe. The gel was left for 2 hours and then mounted in the sequencing machine.

In order to reduce the intensity of the fluorescence, PCR products were diluted 5 fold with distilled water. From this dilution, 0.5 µl was taken and mixed with 0.5 µl of GeneScan™ -350 TAMRA™ size standard (Applied Biosystems, UK) and 1.5 µl of a loading solution. The loading solution comprised of 5 parts of deionised formamide (pH> 7.0) and 1 part of EDTA (25mM) mixed with blue dextran (50 mg/ml). Samples were denatured for 5 minutes at 95 °C and then transferred onto ice immediately. Samples were loaded onto the gel by a membrane comb after performing a pre-run (to bring the gel to the appropriate temperature). The electrophoresis was performed under the following conditions; voltage 1.00 kV, temperature 51 °C and laser power 40 mW, for duration of 2 hours.

The raw data stored in a gel file was analysed using the Genescan® Analysis software version 2.1 and ABI Prism Genotyper version 2.0. These data together with the optimized primer conditions were passed onto LNS to speed up their large scale genotyping. As part of IPN project funded by BBSRC (Biotechnology and biological sciences research council), LNS was responsible to perform all large scale screening (using ABI 377 technology) for the detection of QTL affecting IPN. In total, 60 microsatellite markers were used in the analysis, 45 of these being optimised by myself.

Table 8 demonstrates the names and conditions in which these microsatellite markers were amplified. In this study linkage groups are identified by a three-letter codes (LNS) referring to the Landcatch Natural Selection breeding stock followed by their numerical code taken from SALMAP linkage code. In order to cover the whole

genome, we initially decided to select three loci from each known linkage groups according to available SALMAP and SGP linkage maps. Polymorphism was the main criteria for selecting the loci. Microsatellites from other salmonids species were also selected, when polymorphisms were of highest possibility. The parents and offspring were genotyped for at least two markers per linkage group and a sire-based QTL analysis was used to detect linkage groups with significant effects on quality traits such as fat percentage and flesh colour.

Optimization of PCR products were essentially carried out by testing different annealing temperature (Ta). Using a Biometra TGradient Thermocycler a gradient of temperature in the range of 10 °C below the estimated melting temperature (Tm) was examined. The annealing temperature that gave the clearest amplification result was chosen for subsequent PCR. Depending upon a success or a failure of PCR amplification some adjustments in the concentrations of the reagents were carried out as follows: MgCl2 between 1.5 to 2 mM, dNTPs between 150 µM, primers between 0.15 and 0.2 pmol/µl and Taq concentration between 0.2 to - 0.50 U.

Table 8: The list of all 60 microsatellite loci used in genome-wide scan showing the dye used, forward and reverse primer sequence the optimised $MgCl_2$ in mM, the optimized annealing temperature and the Genebank Accession number if known.

No	Microsatellite	Dye	Forward primer	Reverse primer	$MgCl_2$ mM	AT °C	Accession No.
1	Ocl2	TET	ATTGACCGGTGAAAC TCGAC	AACATACCCACACAC ACGGA	1.5	63	AF028699
2	Ogo4	FAM	GTCGTCACTGGCATC AGCTA	GAGTGGAGATGCAGC CAAAG	1.0	63	AF009796
3	Ogo8	FAM	TCGCAGAGCGATACC AATG	GAGGAAGACCATTGA GGTGG	1.0	63	AF009780
4	Omy23INRA	TET	CCTGTAGCTGGGGAT TTGG	CTTTGGTATCCATCAC TCAGC	1.0	56	Not known
5	Omy21INRA	TET	GCATTGGCGTAATGA GAAGG	CTGACGGACATATCA GCCC	1.0	55	Not known
6	Omy27/1INRA	HEX	CCAATCACCATCTGCT GGG	GCCCATCGTTTAGCC AGG	1.5	57	Not known
7	Sfo23	FAM	GTGTTCTTTTCTCAGC CC	AATGAGCGTTACGAG AGG	1.5	60	Not known
8	Ssa0011NVH	HEX	TTACACAGCCCTGCTC AC	TCCTGTCACACTCACT ACC	1.5	60	AF256662
9	Ssa0014NVH	TET	TTGTGCCGATTTAGG ACG	GCCTTTAACGTAAGT GGTAG	1.5	56	AF256665
10	Ssa0023NVH	TET	AAAGACACGGAGCAA GGC	AAGACAGGAGTCTGG GTG	1.5	60	Not known
11	Ssa0025NVH	TET	AAGGTCCCTGTAAAA GATAC	AGGAGAAGGCAAAGT CGG	1.5	60	AF256675
12	Ssa0047NVH	FAM	TCTGTCACTGTCACCC TG	CACACGTCTCTATCCG TG	1.5	60	AF256697
13	Ssa0054NVH	FAM	TGTTCTCCCAGGAAG CAC	AGCCTAGCAGCTCAT TGG	1.5	60	AF256704
14	Ssa0058NVH	TET	GAACAACTTCAGAAC TTGAC	CGCCTCATAGCTGAT ATTTAAC	1.5	60	AF256708
15	Ssa0062NVH	TET	CGTTAAAACCCCGTG GAG	GACTAAAAAGCGTCT GGC	1.5	57	AF256712
16	Ssa0083NVH	HEX	GGTAAGTCAAGGTTT CACC	TTACTCCCCAACTCTG AG	1.5	55	AF256729
17	Ssa0084NVH	TET	ACCTCAGCACATGAA CAC	TGACAGAGCCATAGA CCG	1.5	55	AF256730
18	Ssa0086NVH	TET	GATGGGTGCTATTGA CTC	CCACACAATCACCGT TGC	1.5	55	AF256731
19	Ssa0087NVH	FAM	CTGTAAACATCACAG GCG	CTCCACTAATAGTCTG AAGG	1.5	55	AF256732
20	Ssa0089NVH	Hex	CCATTAGCTTCTGTTG GAG	ATTGCGTTCCTCTGGA GC	1.5	57	AF256734
21	Ssa0097NVH	FAM	TTGAGCCATCCTCACC TC	CACTGGTTTGTTGTTG TTG	1.5	57	AF256741
22	Ssa0099NVH	TET	TTCATGTGTGCGAGA GCG	AGAATGCAGTATTAG ACTGG	1.5	57	AF257052
23	Ssa0100NVH	FAM	CTGTCATTCCCTTGGC AC	GATGCTGCTAGGAGA GAG	1.5	60	AF256743
24	Ssa0103NVH	FAM	GCTGTGATTTCTCTCT GC	AAAGGTGGGTCCAAG GAC	1.5	57	AF256746
25	Ssa0105NVH	HEX	CTAGATCACTCACCC AGG	GTGCTTTTGGCTTATG TTAG	1.5	57	AF256748
26	Ssa0106NVH	HEX	ACCTTTTGGCTGAATG AC	TAACCGAATGACTGT GAG	1.5	55	AF256749

No	Microsatellite	Dye	Forward primer	Reverse primer	$MgCl_2$ mM	AT °C	Accession No.
27	Ssa0112NVH	HEX	AGGTCCCATGTAACATTC	ATGGCATTATCTCTCTCC	1.5	55	AF256753
28	Ssa0120NVH	FAM	AGCTCTGTCACCAAAGGG	ATGTGCTGTGTCAGCGTG	1.5	57	AF256760
29	Ssa0128NVH	HEX	TCCAGGATAGTCCTCATAG	CCAGAACATTTAGAACTCTC	1.5	57	Not known
30	Ssa0152NVH	HEX	GCTGTTCATTTCTGAGCAG	GACACACCGAATCAGTGC	1.5	60	AF256786
31	Ssa0168NVH	FAM	GCCTTTTCCCAACAATCC	AAATCGCTACCCTGACTG	1.5	56	Not known
32	Ssa0179NVH	HEX	CGTTCAATTCTCCCATATC	GACAGATTTACCAGGAGC	1.5	56	Not known
33	Ssa0185NVH	HEX	AGAGAAGTATAAACCCTGC	AATATGGTAGGAAGACACAG	1.5	55	AF256816
34	Ssa0216NVH	HEX	GCACTGGGGTTTAATGTC	TGTATAGGGGCAATCAGC	1.5	50	Not known
35	Ssa0217NVH	FAM	AGCGAGCTTTCTTTCCAG	AGCTGTCTATTCACGACTC	1.5	50	Not known
36	Ssa20.19NUIG	FAM	TCAACCTGGTCTGCTTCGAC	CTAGTTTCCCCAGCACAGCC	1.5	57	AJ290344
37	Ssa405UOS	TET	CTGAGTGGGAATGGACCAGACA	ACTCGGGAGGCCCAGACTTGAT	1.0	63	AJ402722
38	Ssa79NUIG	FAM	TGGGACCAAATAGAACAG	ATGGAGTCTCTTGTCACT	1.5	55	Not known
39	SSLEER15	HEX	ACAACAGCGTCACCTGTC	ACTGACTTGAAGGACATTAC	1.5	57	U86708
40	SSOSL438	FAM	TGACAACACACAACCAAGG	GTAAAATGGAAGCATCTGTG	1.5	57	Z49134
41	Ssa0082NVH	FAM	AGAGCGAATACAACAGCC	AGAGCGAATACAACAGCC	1.2	57	AF256728
42	Ssa0059NVH	FAM	GTGTCACTCCATCCTTGC	CAGTCATTTCTCCAAACAG	1.5		AF256709
43	Ssa0028NVH	FAM	CCCCATGATGTGTTCTTC	CACAATGAGGCTTGACAC	1.5	57	AF256678
44	Ssa0071NVH	HEX	CCCCTGTCAAACGTCTTC	AGCACACTGGATTCAAGG	1.2	57	AF256719
45	Ssa0054NVH	FAM	TGTTCTCCCAGGAAGCAC	AGCCTAGCAGCTCATTGG	1.5	55	AF256704
46	Ssa418UoS	FAM	CACACCTCAACCTGGACACT	GACATCAACAACCTCAAGACTG	1.5	55	AJ402735
47	Omy27INRA	HEX	CCAATCACCATCTGCTGGG	GCCCATCGTTTAGCCAGG	1.2	55	Not known
48	Ssa0042NVH	TET	ACTAAGAGTCCACATTTGAG	TTAGGATGGAGAATGGTAG	1.5	57	AF256692
49	Ssa0064NVH	HEX	CCTGCCATCATCCAACTC	TCCACACCCAACATACTC	1.5	57	AF256714
50	Ssa0010NVH	FAM	TTCCCCTCTGATCCCAAG	TGTTCTCTACACAGTTGCC	1.5	57	AF257048
51	Ssa0003NVH	FAM	TTGTGGGTGGGTGTAAGC	CTCTGTCATGGCAGGATG	1.5	55	AF256656
52	Ssa0096NVH	FAM	ACTTCCATTCAGATGACAC	CCTGTATCTCCTCCATTAC	1.0	59	AF257053
53	Ssa0048NVH	FAM	CAGAACCGTGATCTGAAG	TGGACATTCTCTGGCGTC	1.2	57	AF256698

No	Microsatellite	Dye	Forward primer	Reverse primer	$MgCl_2$ mM	AT °C	Accession No.
54	Ssa0149NVH	TET	TGAGTCACACCTGTC ACG	GTGATGATGATTAAA GCCAG	1.5	57	AF257049
55	Omy14INRA	HEX	GTCAGCGATAATCCA CATGG	CCGTTATGGAGATGT GTAGGG	1.5	57	Not known
56	Ssa0046NVH	HEX	TATCACCCAGTGAAC GTG	CAAATGAGCCATCAA CAG	1.5	57	AF256696
57	Ssa0065NVH	TET	GCAACACAAACACAT TTGC	TATGGAGAGGGTTGG TAG	1.5	57	AF256715
58	Ssa0055NVH	HEX	AATAAGAGGGCAGTG GAG	TGCACCAGAGAGAGT AGC	1.2	55	AF256705
59	Ssa0016NVH	TET	TGAAACTAGGATGCC TGG	TCTGACCCACACACA AGC	1.2	57	AF256667
60	Ssa0070NVH	FAM	ATAGACGTATGACTT TGCC	AAGCTGTGTCAATCA GCG	1.5	55	AF256718

2.2.4 Linkage analysis

Based on the published SALMAP linkage groups, 'twopoint' option in Cri-map 2.4 (Green et al., 1990), available at: http://www.animalgenome.org/bioinfo/resources/manuals/Embnetut/Crimap) was utilized to detect the expected linkage between markers. This option performs linkage analysis for each pair of loci and estimates the maximum likelihood of the female and male recombination fractions. Marker pairs with a LOD score of over 3.0 were considered as linked markers. To confirm marker order based on recombination fractions using the Haldane mapping function, the BUILD option in Cri-map 2.4 was used. Where marker positions were not available through the published maps, the markers were entered as "inserted" rather than "ordered" in the parameter (PAR) file to obtain the best estimate of order and position according to the data in the current study.

The detection of QTL is dependent on finding a clear association between trait values and marker genotypes, within families. This association can be detected by various

statistic methods such as the linear regression technique, whereby coefficients are calculated based on the probabilities of offspring inheriting a particular allele from the parent, and the trait data regressed onto those coefficients (Knott et al. 1998). In this study, the linear regression approach was used for interval mapping through the QTL Express Program (Seaton et al. 2002).

The QTL Express Program is web-based software for the analysis of quantitative trait data and is available at http://qtl.cap.ed.ac.uk. Using a general linear model, the QTL Express Program applies various linear models to fit the phenotypic data. This program checks the genotype file for Mendelian errors, by comparing the observed alleles in parents and progeny.

The phenotypic, genotype and map information was converted into a format suitable for analysis with the QTL Express Program. Suggestive and significant thresholds were obtained using a permutation test as described by Churchill and Doerge (1994). The suggestive level is the level at which we expected to obtain one significant result per genome-wide analysis by chance ($p<0.05$), whereas the genome-wide significant level is the level at which we could obtain 0.05 significant results per genome analysis by chance (Lander Kruglyak 1995). One thousand permutations were fulfilled in the QTL Express Program which calculates automatically the F Ratio which is equivalent to the 0.05 level genome and chromosome wide thresholds. This was repeated 5 times and significant and suggestive thresholds were estimated by taking a mean of the 5 values obtained. The 10% and 1% genome wide significance thresholds were also calculated through the same procedure in order to have F Ratios which correspond to nearly significant ($P<0.1$) and highly significant ($P<0.01$) respectively.

2.3 Results

The estimated heritability for fat percentage and flesh colour in the population under study were 0.17 and 0.15, respectively. To investigate whether these genetic components include loci of major effect, a genome-wide QTL scan was performed within commercially bred families that were analysed for a range of commercially important harvest traits.

Using DNA samples from other fish population, the optimisation of amplification condition for the microsatellite loci used in this study was carried out by me. Then this information was passed onto Landcatch Natural Selection where microsatellite genotyping of DNA samples from the collected dead fish (caused by IPN) were carried out. As I was in the early stage of this study, for map construction and QTL detection a great deal of help was given to me by Dr. Ross Houston at the Roslin Institute. At this stage, most part of the map construction and statistical analysis of this study was carried out by Dr. Houston as a part of a collaborative QTL mapping affecting IPN in Atlantic salmon.

The genome-wide search and subsequent interval mapping revealed significant QTLs for fat percentage and flesh colour on linkage groups LNS16 and LNS1, respectively. A significant QTL for harvest length and suggestive QTL for harvest weight and gutted weight were also detected on linkage group LNS1. Suggestive QTL for condition factor were found on linkage groups LNS3, LNS10 and LNS23. In addition, suggestive QTLs for flesh colour (measured by Minlota and Roche) were also detected on linkage groups LNS5, LNS16 and LNS18. In this study, significant and suggestive QTLs affecting the body weight were found on linkage groups LNS1,

LNS4, LNS5, LNS14 and LNS19. Table 9 shows the summary of QTL analysis for commercial trait based on sire analysis.

Table 9: Identification of linkage groups harbouring QTL for harvest traits based on sire analysis (Critical R Ratio values for suggestive and significant linkage calculated through the permutation analysis described in section 2.2.3).

Linkage Group	Trait	F Ratio >2 = suggestive level and >3.5 = genome-wide significant	Position cM
1	Harvest weight	3.20	0
	Harvest length	**3.53**	0
	Gutted weight	3.06	0
	Flesh colour (Measured with Chroma)	**3.55**	0
	Fillet weight	2.96	0
3	Harvest CF	2.86	1
4	Harvest weight	2.34	1
	Harvest length	2.01	0
	Gutted weight	2.24	0
	Head weight	2.09	0
5	Harvest weight	2.00	6
	Harvest length	2.51	6
	Head weight	3.17	6
	Waste weight	3.28	0
	Filled Yield	2.14	0
	Flesh colour (Minlota)	2.29	0
10	Harvest CF	2.77	0
11	Harvest weight	2.10	9
13	Flesh colour (Minlota)	2.14	3
14	Harvest weight	2.03	3
	Harvest length	2.51	3
	Gutted weight	2.90	3
16	Fat % (Torry Fish Fatmeter)	3.32	0
	Flesh colour (Roche)	2.39	1
18	Flesh colour (Roche)	2.09	10
19	Harvest weight	2.45	0
	Harvest Length	2.93	15
	Gutted weight	2.48	15
	Head weight	2.33	15
	Fillet weight	2.67	15
23	Harvest CF	2.15	0

2.4 Discussion

In the near future, the knowledge of QTL and the candidate genes influencing phenotypic character could be integrated into breeding programs for salmonids species to achieve higher response to selection. In the Atlantic salmon, intermediate estimates of heritability for fat content (0.20 to 0.30) and flesh colour (0.12 to 0.14) have previously been shown (Rye and Gjerde 1996; Norris and Cunningham 2004). In Coho salmon, high levels of genetic variability for flesh colour (0.30 - 0.50) has been reported. Furthermore, positive correlation between flesh colour and fish body weight will indirectly help for considerable genetic improvement in flesh colouration via selective breeding (Iwamoto and Hershberger 1990).

So far, QTLs for spawning time, maturation and growth in rainbow trout have been identified using microsatellite linkage maps (Sakamoto et al. 1999; Martyniuk 2001, O'Malley 2001). In Atlantic salmon, Gross and Nilsson (1999) showed that the *GH* gene fragments caused by a polymorphic *Taq*1 restriction enzyme were associated with the growth.

The results from genome scan in the current study showed that linkage groups LNS1, LNS4, LNS5, LNS14 and LNS 19 are carrying QTLs affecting the body weight. In North American population of Atlantic salmon, Reid et al. (2004) using variation among 91 microsatellite loci located on 16-18 linkage groups searched for QTL affecting body weight and condition factor. Their results showed that QTLs affecting body weight are located on different linkage groups (AS-8, AS-10 and AS-11) than the ones found in this study. A minimum of two common markers are generally needed as an indicator of homology among linkage groups from the different studies.

No homology was found between the linkage groups carrying QTLs in this study with those of reported by Reid et al. (2005).

In addition, Reid et al. (2004) reported four significant QTL for condition factor residing on linkage groups AS-2, AS-5, AS-11 and AS-14 while my results showed that suggestive QTLs affecting condition factor are residing on LNS3, LNS10 and LNS23. It should also be mentioned that the genome coverage by Reid et al. (2004) was 1.5 times more than the genome coverage in my experiment (as I only used 60 microsatellite loci). Therefore, this may have had some negative impacts on the reliability of the detected QTLs in this study. Another reason for the discrepancy could be explained by differences in QTL regions in the different stock.

The results from this study showed that QTLs controlling body weight are on different linkage groups (LNS1, LNS4, LNS5, LNS14 and LNS19) than that affecting condition factor. This indicates that the genetic correlation between body weight and condition factor could be low in this population of Atlantic salmon. This may also suggest that the two traits have some degree of independence in genetic control and probably different sets of genes might be involved.

Overall, the results found here show significant evidence of QTL for fat percentage and flesh colour on the linkage groups LNS16 and LNS1. We found that the significant QTL affecting flesh colour (measured with Chroma) is residing on linkage group LNS1. Furthermore, suggestive QTLs for flesh colour (measured with different instruments) were also located within linkage groups of LNS5, LNS13 and LNS18 (Table 7). Results from this study revealed that multiple QTLs on a linkage group LNS1 are controlling body weight and flesh colour in this population of Atlantic salmon. In Atlantic salmon, loci on linkage group one are linked to the sex

determination factor which is closely linked to locus *Ssa*202DU (Artieri et al. 2006). QTL for upper thermal tolerance have been reported on the sex linkage group in rainbow trout (Perry 2001) and Arctic charr (Somorjai 2001). It is also reported that the location of sex determining locus is not conserved among salmonids fish (Woram et al. 2003)

Search for QTL that affect performance traits is emerging as a very important section of modern aquaculture. Relative chromosomal positions of QTL for fat percentage and flesh colour found in this study deserve further attention. A higher marker density is required for fine-mapping and localization of the sire-based detected QTLs on linkage groups LNS16 and LNS1 in this study. Poompuang and Hallerman (1997) suggested that QTL can reliably be identified within high resolution linkage maps with at least 20 cM marker distance.

On the other hand, the detection of QTL based on a sire map often result in the revealing of weaker associations over longer map distances because of largely reduced amounts of recombination in male Atlantic salmon (Moen et al. 2004a). Since recombination rates are more representative of map distance in female salmon the QTL detected in the dam are more representative of the true QTL location in comparison to male based analysis. Another reason for the differences for detected QTLs between male and female salmonids could be explained by the occurrence of false linkage in the male known as pseudolinkage (recombination by means of crossovers between homeologous chromosomes during meiosis), as discussed by Allendorf and Danzmann (1997). Pseudolinkage in male salmonids is a well known event but it has never been reported in female salmonids.

The differences in recombination between male and female of Atlantic salmon is amongst the highest ratio (8.26) reported in vertebrate (Moen et al 2004a). As a result, genome scan approach can benefit greatly from recombination differences observed in male and female Atlantic salmon in detecting chromosomal regions carrying QTLs. Since the data in this study are derived from the limited number of microsatellite markers in sire it is difficult to conclude the actual QTLs location on these linkage groups.

Therefore, we consider that fat percentage and flesh colour QTLs localized in the sire-based linkage groups of LNS16 and LNS1 need further investigation and it must be tested by incorporation of additional markers into these linkage groups. Based on the location of microsatellites on the SALMAP linkage groups, my next step is to incorporate more microsatellites within these linkage groups to provide stronger evidence for these QTLs. In addition, AFLP markers are also to be employed to search the genomic region harbouring these QTLs. In the next two chapters I will discuss the employment of microsatellite and AFLP markers for localization of these putative QTLs identified through the genome scan approach.

Chapter 3 - Microsatellites based search for QTL linked to quality traits

3.1 Introduction

In addition to genetic improvement of growth rate and body weight in salmonids, quality traits (colour and fillet lipid) have also become of considerable importance during the last decade. Flesh colour is an economically important trait in Atlantic salmon. For consumers, the red-pinkish flesh colour is considered as an important characteristic for purchasing the food. The pink colour of salmonid fishes is due to their ability to accumulate relatively high amounts of carotenoids such as astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) in their muscle. Animals, including fishes, are unable to synthesize carotenoids and therefore rely on dietary supply. Deposition of carotenoids in flesh is a result of a process that begins with the absorption of pigments in the gut, followed by transport of the pigment in the blood and then its deposition and retention in the muscle. Carotenoids are expensive to manufacture and are poorly utilized by the fish. The muscle retention of astaxanthin in salmonids is usually around 10% (Nickell and Bromage 1998). On the other hand, the cost of feed pigmentation is approximately 10-15% of the total cost of feed production (Torrissen et al. 1995). Considering that the cost of salmonids feed can reach around 50% of their total cost production (Rasmussen 2001), the extra expense of pigmentation can put huge economical pressure on salmonids producers. Low temperature can affect absorption of astaxanthin in salmonids. The results from a recent study suggest that low temperature may have a negative effect on the utilisation of astaxanthin. A reduction of approximately 10% of the absorption of astaxanthin was observed when the temperature dropped from 12 to 8 °C (Ytrestoyl et al. 2005).

Previous studies have shown that the flesh pigmentation in salmonids is under considerable genetic control, for example Iwamoto et al. (1990). The intermediate estimate of heritability for flesh colour (0.15) in Atlantic salmon population under study has been shown. Therefore, flesh pigmentation could be considerably enhanced through genetic improvement leading to profitability for salmon farmers.

The fillet fat is known as another important quality attribute in farmed Atlantic salmon (Bjerkeng et al. 1997). Fish body composition seems to be strongly influenced by feed composition. For example, the proportion of fat in both the fillet and viscera of Atlantic salmon increase significantly as the concentration of fat in the feed is increased (Refstie et al. 2001). It is known that increasing fillet lipid content alters the taste and texture of the fish fillet. However, the quality criteria can considerably differ with respect to various demands of the fish processing industry. For instance, it is desirable to have smoked fish containing more fat than frozen fish. Moreover, quality demands alter with geographical regions and different cultures (Rasmussen 2001).

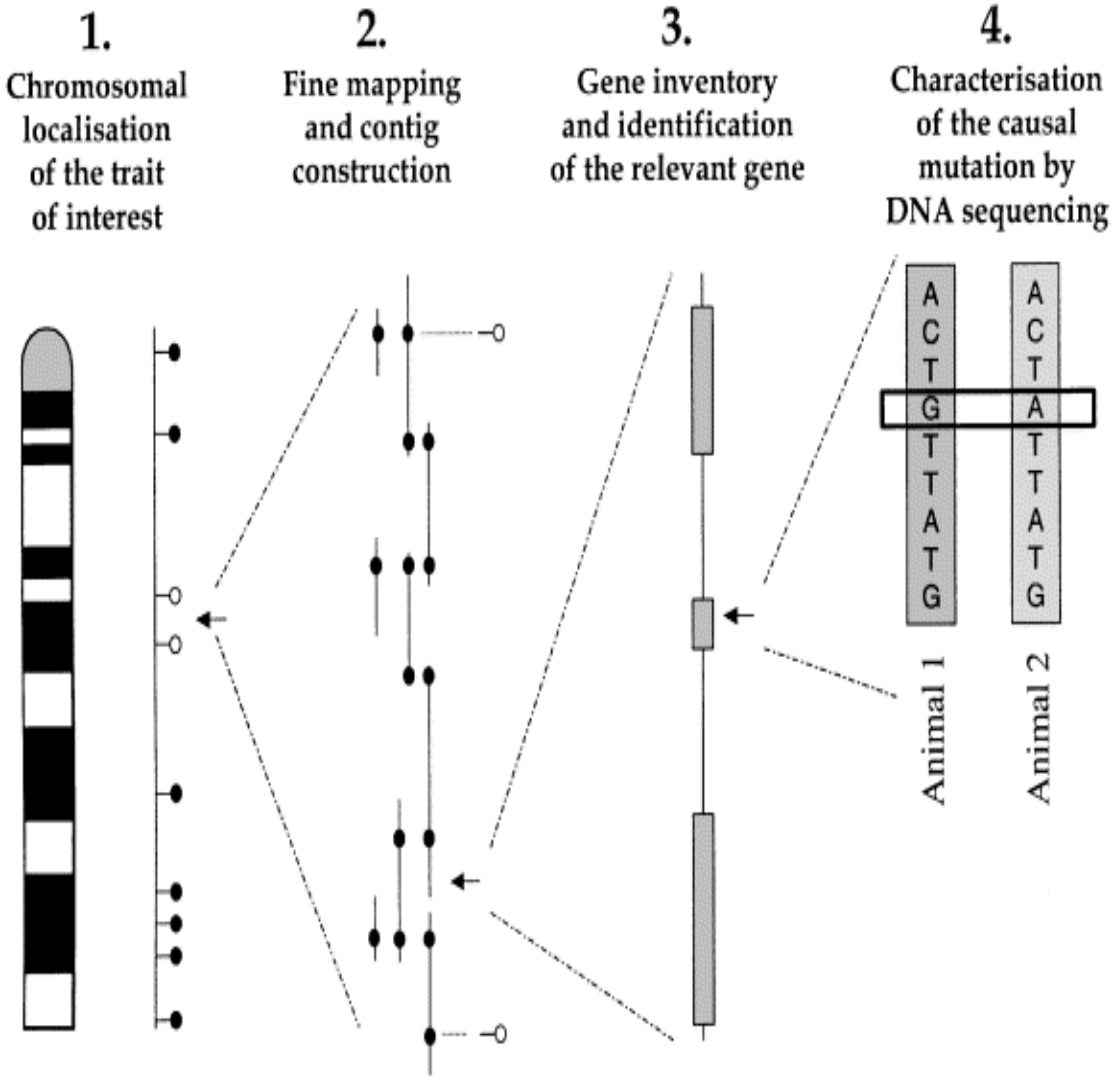
For salmon farmers though, the primary goal is to convert the feed into edible fillet weight with acceptable quality rather than into excess visceral lipid and waste. The quality of salmonids is affected by parameters such as feed type, ration and growth. It seems that the fat percentage in the fillet of salmon tends to increase during the seawater stage and the salmon fillet has the capacity to contain up to 55% of fat reserves as a total of body fat (Jobling and Johansen 2003). However, little is known about changes in the distribution of body fat during seawater growth. While fat percentage is considerably high in the carcass(belly, skeleton, head and skin) of Atlantic salmon smolt, the fillet becomes the major site of fat storage as the fish increase in body size (Jobling et al. 2002).

According to Rye and Gjerde (1996), fat concentration in the carcass is positively correlated with body weight and there is a negative genetic correlation between muscle fat percentage and final body weight, therefore they concluded that fat deposition in the carcass is negatively correlated with growth rate. In another words, the increase in carcass fat with size appears to be less in faster growing fish.

There are several methods for estimating the fat content of fish including: chemical analysis, Torry fat meter, computerized tomography and near-infra-red spectrophotometer, which are mostly laborious and costly.

The results from previous chapter showed suggestive evidence of QTL for fat percentage and flesh colour in farmed Atlantic salmon, but lacks closely linked marker information. After application of genome-wide scanning (a process by which evenly spaced markers covering the entire genome are selected for screening of trait-linked markers), then fine mapping can be applied using polymorphic markers near the chromosomal regions containing the QTL (Liu and Cordes 2004). This process can be followed by focusing on the construction of a set of overlapping clones (known as clone contig) for the region of interest to fulfil a gene inventory leading to the identification of the gene itself and finally of causal mutation. This process can be summarized in four steps as shown in Figure 3 (taken from Eggen and Hocquette 2004).

Figure 3: The four steps involved in positional cloning (taken from Eggen and Hocquette (2004)



The objective of this chapter was to more finely map the chromosomal region (linkage groups LNS16 and LNS1) harbouring potential QTLs for the quality traits previously reported in chapter 2. Using microsatellite markers based on the SALMAP linkage groups (unpublished) my aim was to find possible association between the QTL affecting quality traits (fat percentage and flesh colour) and microsatellite loci residing on these 2 linkage groups.

3.2 Material and methods

3.2.1 Family structure

Extracted DNA from five families of Atlantic salmon as explained in previous chapter (2.2.1) was used for microsatellite genotyping in this study.

3.2.2 Microsatellites Analysis

Based on location of microsatellite markers on the SALMAP linkage groups (unpublished), twenty one microsatellites from linkage group 16 and 1 were tested in a 15 µl reaction volume containing 0.15 pmol of reverse primer, 0.02 pmol of M13 tailed forward primer, 0.15 pmol of M13 fluorescent dye labelled primer, plus 200 mM of each dNTP, 1 µl buffer (20 mM Tris-HCl, $(NH_4)_2SO_4$, Tween 20 at PH 8.8) (ABgene), 1.5 mM $MgCl_2$, 0.5 U of Taq DNA polymerase and 70 ng template DNA. Most of the microsatellites amplified satisfactorily using an annealing temperature of 57 °C. A different annealing temperature was used for a minority of loci (see Table 9). The main reason behind using M13 tailed primer was to minimize the cost of genotyping. I used the M13 tailed primer method to label forward primer for visualization of capillary sequencer. Forward primers of each one of the microsatellites were 5'-tailed with the M13 blue fluorescence (5'-CACGACGTTGTAAAACGAC-3'). As result, the entire forward primer would look like (5'-CACGACGTTGTAAAACGACXXXXXXXXXXXXXXXXXXXX-3') where the Xs denote the microsatellite-specific primer sequence (Table 10).

A Single primer set for each microsatellite was amplified using a thermal cycler (TGRADIENT Biometra) under the following reaction conditions: an initial denaturation at 95 °C for 2 min, followed by 35 cycles consisting of 1 min at 95°C, 1 min at 57 °C, 1 min at 72 °C, and final extension of 3 min at 72 °C. Most primers

amplified at an annealing temperature of 57 °C. Gradient PCR was used to determine optimal annealing temperatures for primers that failed to amplify at 57 °C.

Electrophoresis and data collection were carried out on Beckman Coulter 8800 CEQ genetic analyser. 0.8 µl of undiluted PCR product was added to 96-well plate containing 30 µl of Sample Loading Solution (SLS) and 0.30 µl size standard (Beckman 400 base pair size standard WELLRED™ dye D1). This solution was thoroughly mixed for a minimum of two minutes. Each well was overlaid with a drop of mineral oil before loading onto the capillary based genetic analyser. Table 10 shows the list of microsatellite markers with their known linkage group used in this study.

The CEQ 8800 utilizes the capillary electrophoresis technology to separate the DNA fragments. This genotyping machine has 192 sample capabilities (two 96-well sample microplates). Each row of eight samples, containing labelled DNA fragments is automatically denatured and then separated by capillary electrophoresis. After each separation, the gel is automatically replaced in eight capillaries. Detection takes place by laser-induced fluorescence in four spectral channels. The four-channel raw data sets generated by each of the eight capillaries are automatically processed to produce high quality fragment lists after separation. Information about CEQ™ 8800 can be found on user's guide published by manufacturer at: (http://www.beckmancoulter.com).

CEQ 8800 version 7.0 software was used for data capture and basic data analysis. From six modules available in the main menu of software, Sample Setup, Run Module, Fragment Analysis and Data Manager Module were used for data collection. The Sample Setup Module was used to create, save and modify methods and sample

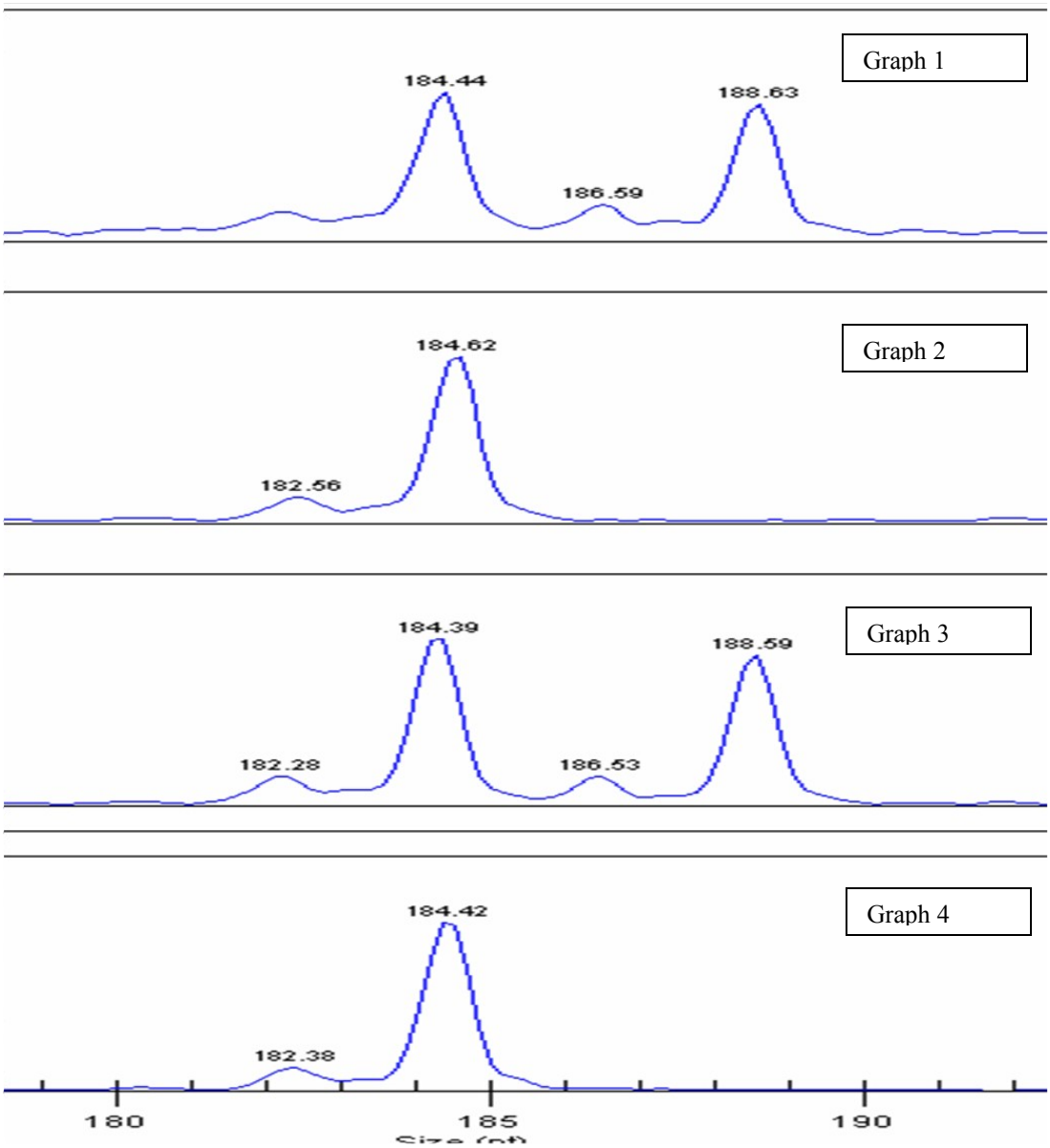
plates. The Run Module provides the capability to carry out the pre-programmed sample plates and controlling individual functions of the instrument. The Fragment Analysis Module was used to view and analyse the raw data. The Data Manager Module was utilized to save databases containing fragment results and fragment analysis parameters.

Table 10: List of microsatellite markers used for QTL detection on linkage group 1 and 16, forward and reverse primer sequence, accession number (if known) and annealing temperature (AT).

No	Locus name	Forward primer	Reverse primer, tailed with M13	Access No.	Linkage group	AT °C
1	Ssa0021NVH	GACTTGGAGACTC TTTGG	GAGAGGGAGAT AGCATCG	AF256672	16	54
2	CL19368	GAGTAACGTAAGG GACAG	GAAAAGCCAAG TAAAATG	Not known	16	54
3	Ssa0042NVH	ACTAAGAGTCCAC ATTTGAG	TTAGGATGGAGA ATGGTAG	AF256692	16	57
4	Ssa0016NVH	TGAAACTAGGATG CCTGG	TCTGACCCACAC ACAAGC	AF256667	16	57
5	Ssa0050NVH	TTTTCTACCTGCCA CTGC	AAAGTGAGGAT GCAACCG	AF256700	16	54
6	AluI387	CTTACTTACTACA CAACCACTG	CCTAAAGAGCAA ATGGAAG	AY543898	16	57
7	Ssa9.44NUIGa	GCATTGGAGTCAT TACAGTTC	GGAATCGAACTC ACAACCC	AJ290333	16	57
8	CL17121	GACAACGCTGACT GTGACTT	TGGTCTACACTG GAATAAAGGA	Not known	16	57
9	Ssa416UOS	TGACCAACAACAA ACGCACAT	CCCACCCATTAA CACAACTAT	AJ402733	16	60
10	RsaI466R	GTCTTCGGCATCT GTAAC	AGTGGGCAGTCT GGAAAC	AY543746	16	54
11	Ssa0037NVH	CACTAATGCACAG TGTCAG	GCATAAATGGCA TGTGTTC	AF256687	16	57
12	CL10695	CAACAAAAAGAG GCAATGGT	GAAGGATTACGA CAAACAGGA	Not known	16	57
13	OMYRGT55TUF	CGTTTTATCCGCTG CCAG	CACGTCCAACAA TATGGTGC	Not known	16	60
14	RsaI485	GGTTAGGGTTAGG AAAATAG	ACACACAAGGG CAGTCAC	AY544058	1	55
15	RsaI458	TTATTCCCCTGGTA TCCG	GAGCAAACAGT GGTCCTG	AY544058	1	57
16	OmyFGT8/1TUF	AAGTGTTGGCCTC AGACCTG	GAGCTCCCTCCT CAGAATACC	Not known	1	60
17	Oneμ18/1	ATGGCTGCATCTA ATGGAGAGTAA	AAACCACACACA CTGTACGCCAA	Not known	1	55
18	Omy1032UW	TCTCATTGCTCTGG CACTGGTTCTAC	CACATGGCGAGT CTTCCAAACG	AY505337	-	57
19	Omi116TUF	CTCGTTCTCTCTCT CTGTGTCA	ATGTCAAGATGC CCGGAG	Not known	-	60
20	OMM1134/ii	GAAGTTCATCTCC AGGTCAAACTG	TGCGTAGGTTGA TGAATCCTC	AY039628	-	57
21	OMY17DIAS	AGCTAAGACTTGC CAAGGTT	GGTCCATTGGAT ATTGTCAG	AF239034	-	57

Allele sizing was undertaken manually and did not rely on automated scoring option. The size given by genotyping is extrapolated from a standard curve and requires to be rounded to a whole number. This was done once all genotyping was completed to determine the range of sizes representing each allele and to ensure consistent rounding. Figure 4 shows example chromatograms of microsatellite genotyping in sire and dam (graph 1 and 2) and its segregating pattern in offspring (graph 3 and 4). The small peaks were ignored.

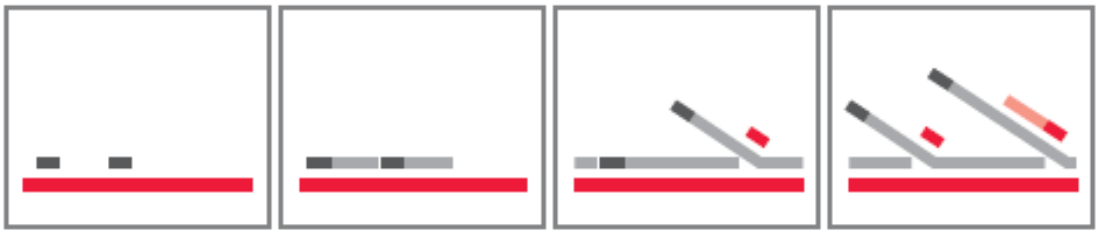
Figure 4: Example chromatograms from the screening of both parents and two offspring with microsatellite marker Alu387 showing the predicted allele size.



3.2.3 Whole Genome Amplification

Due to limited amounts of DNA in the parental fish families, a method of whole genome amplification was applied to compensate for this short-coming. The REPLI-g Midi Kit (QIAGEN) was purchased for whole genome amplification of DNA samples. This method provides uniform amplification across the entire genome. The method is based on multiple displacement amplification (MDA) technology which carries out isothermal genome amplification utilizing a processive DNA polymerase. The DNA polymerase has a 3' to 5' exonuclease proofreading activity during replication. The principle of this method relies on DNA denaturation by adding denaturation buffer (chemical denaturation of DNA), then cession of denaturation by addition of neutralization buffer and finally addition of master mix containing buffer and DNA polymerase. The amplification reaction is carried out for overnight at 30 °C. Figure 5 shows the schematic of REPL-g amplification. In this method the DNA polymerase moves along the DNA template strand displacing the complementary strand. The displaced strand becomes a template for replication allowing high yields of high-molecular-weight DNA to be generated.

Figure 5: A schematic of whole DNA amplification shows that the DNA polymerase moves along the DNA template strand displacing the complementary strand. Then the displaced strand becomes a template for replication, leading to high-molecular-weight DNA to be generated.



For the whole genome amplification reactions the following procedure was applied: sufficient denaturation buffer and neutralization buffer were prepared. 2.5 µl of

template DNA was added into microcentrifuge tube. 2.5 µl of denaturation buffer was added and mixed by vortexing and centrifuged briefly. This solution was incubated at room temperature (20 °C) for 3 min. 5 µl of neutralization buffer was then added, mixed and centrifuged briefly.

A master mix for 10 DNA samples with the following component was prepared: 100 µl nuclease-free water, 290 µl REPLI-g Mini reaction buffer, 10 µl REPLI-g Mini DNA polymerase, then briefly vortexed and centrifuged. This mater mix was kept on ice and used immediately upon addition of the REPLI-g Mini DNA polymerase. 40 µl of the master mix was added into 10 µl of denatured DNA, making a final volume of 50 µl of amplification reaction. The solution was incubated at 30 °C overnight then DNA polymerase was inactivated by heating the samples for 3 min at 65 °C. Amplified DNA was quantified using the NanoDrop® ND-1000 and stored at 4 °C (Table 11).

Table 11: Quantification of amplified DNA using NanoDrop® ND-1000.

	DNA pre-amplification ng/µl	Amplified DNA ng/µl	A260	A280	260/280
Dam family 1	132	458	9.20	4.95	1.85
Sire family 1	70	475	9.50	5.10	1.86
Dam family 2	18	429	8.58	4.55	1.89
Sire family 2	134	483	9.68	5.28	1.83
Dam family 3	128	438	8.75	4.67	1.87
Sire family 3	124	525	10.51	5.66	1.86
Dam family 4	61	670	13.94	7.47	1.87
Sire family 4	81	701	14.03	7.45	1.88
Dam family 5	89	433	8.66	4.64	1.87
Sire family 5	87	432	8.65	4.46	1.94

Quantification of DNA concentrations for all the DNA samples was carried out using the NanoDrop® ND-1000 device. The NanoDrop® ND-1000 (a full-spectrum 220-750 spectrophotometer) was employed for accurately measuring the DNA

concentration. This equipment utilizes a sample retention technology that employs surface tension to hold the sample in place, therefore eliminates the need for cuvettes and other sample containment devices. It also claimed that has the capability to measure 50 times higher concentrations than samples measured by a standard cuvette spectrophotometer (see manufacturer catalogue).

The main steps for DNA measurements were as below: A blank measurement was initially done using 1 µl of mixture of amplification buffer and $TE_{0.1}$ buffer. After wiping the water from both the upper and lower pedestals, 1.5 µl of DNA sample was loaded onto the lower pedestal for DNA concentration measurements.

The ratio of sample absorbance at 260 and 280 nm was checked to assess the purity of the DNA samples. A ratio of approximately 1.8 is generally accepted as pure for DNA and if the ratio is considerably lower than that it may indicate the presence of protein, phenol or other contaminants (Table 10).

3.2.4 Map construction of linkage group LNS16 and linkage group LNS1

A microsatellite based linkage map for commercial Atlantic salmon from Landcatch families was created. The Twopoint option in Crimap 2.4 (Green et al. 1990) followed by Build option was used for map construction. The highest likelihood of marker orders was chosen utilizing the Flipsn option. A linear-regression based interval QTL detection method was used in all the analysis. Due to the large difference in recombination frequency between males and females of Atlantic salmon, sex-specific maps were constructed. An interval mapping method was used to identify significant associations between the markers and phenotypic records, using the web-based software package QTL Express (Seaton et al. 2002). Due to full-sib family groups,

sire and dam based half sib analysis were possible and sex-specific maps were created. It should be mentioned that I personally carried out all microsatellite genotyping for this chapter, and as a beginner a considerable amount of assistance (by Dr. Houston from Roslin Research Institute) was given to me for the construction of microsatellite linkage map.

3.3 Results

All loci showed reliable PCR amplification, stutter free and easy to score, except loci Omy1032UW, OMY17DIAS and OMYRGT55TUF which did not amplify in all the families. Loci OMM1134/ii, *Ssa*0021NVH, CL17121, RsaI458 and CL19368 in family one, loci *Ssa*0050NVH, CL17121 and *Rsa*I485 in family 2, loci *Ssa*416UoS, *Ssa*0021NVH and CL17121 in family 3, loci *Ssa*0050NVH, CL17121 and OMM1134/ii in family 4 and loci CL19368, *Ssa*0016NVH, RsaI458 and OMM1134/ii in family 5 were homozygous at the same alleles in both parents and therefore considered as non-informative. I did not undertake their genotyping in offspring (Table 12). From the genotyping results of offspring Mendelian segregation was examined. There was no evidence of non-random assortment in these families, indicating the loci were showing disomic segregation.

The raw data of microsatellite genotyping is provided in Appendix 2. primarily, two region of genome showed significant evidence for QTL effects on fat percentage and flesh colour.

Table 12: Genotyping results of parents in families under study using microsatellite markers.

Locus	Family	Parent	Genotype	Locus	Family	Parent	Genotype
AluI387	1	M	188/188	OmyFGT8/1TUF	1	M	217/221
		F	184/188			F	213/223
	2	M	184/188		2	M	190/217
		F	184/184			F	215/223
	3	M	188/188		3	M	190/221
		F	184/188			F	198/217
	4	M	184/188		4	M	219/223
		F	184/184			F	215/217
	5	M	184/188		5	M	190/190
		F	184/188			F	215/223
Ssa0050NVH	1	M	175/183	CL10695	1	M	277/297
		F	175/175			F	263/307
	2	M	**175/175**		2	M	261/269
		F	**175/175**			F	265/277
	3	M	175/175		3	M	277/277
		F	175/179			F	247/263
	4	M	**175/175**		4	M	241/269
		F	**175/175**			F	261/263
	5	M	175/179		5	M	273/307
		F	175/179			F	307/307
Ssa9.44UNIGa	1	M	133/133	CL17121	1	M	**345/345**
		F	131/133			F	**377/377**
	2	M	129/131		2	M	**345/345**
		F	131/131			F	**345/345**
	3	M	129/133		3	M	**345/345**
		F	131/131			F	**345/345**
	4	M	131/133		4	M	**345/345**
		F	133/133			F	**345/345**

	5	M	131/133		5	M	335/377
		F	131/131			F	335/377
Omi116TUF	1	M	218/226	CL19368	1	M	**165/165**
		F	178/226			F	**165/165**
	2	M	178/226		2	M	163/165
		F	188/220			F	163/163
	3	M	158/218		3	M	165/165
		F	188/200			F	163/165
	4	M	212/220		4	M	**165/165**
		F	220/246			F	**165/165**
	5	M	226/230		5	M	**165/165**
		F	178/226			F	**165/165**
RsaI485	1	M	182/194	Ssa0021NVH	1	M	**133/133**
		F	182/194			F	**133/133**
	2	M	**194/194**		2	M	133/147
		F	**182/182**			F	133/133
	3	M	182/182		3	M	**147/147**
		F	182/194			F	**133/133**
	4	M	180/180		4	M	133/147
		F	180/192			F	133/133
	5	M	180/180		5	M	133/145
		F	180/192			F	133/145
Locus	Family	Parent	Genotype	Locus	Family	Parent	Genotype
OMM1134/ii	1	M	**231/231**	Ssa0042NVH	1	M	181/195
		F	**231/231**			F	187/199
	2	M	205/231		2	M	185/187
		F	205/205			F	185/193
	3	M	205/231		3	M	187/195
		F	205/231			F	187/193
	4	M	**205/205**		4	M	187/205

		F	**205/205**			F	187/201
	5	M	**205/205**		5	M	187/201
		F	**205/205**			F	195/205
Ssa416UOS	1	M	312/312	Ssa0016NVH	1	M	181/181
		F	227/312			F	167/181
	2	M	312/312		2	M	163/167
		F	227/312			F	167/169
	3	M	**312/312**		3	M	163/181
		F	**312/312**			F	167/169
	4	M	312/312		4	M	167/181
		F	227/312			F	167/167
	5	M	227/398		5	M	**181/181**
		F	227/312			F	**167/167**
RsaI458	1	M	**180/180**	RsaI466	1	M	248/280
		F	**176/176**			F	268/280
	2	M	176/180		2	M	248/248
		F	176/176			F	190/248
	3	M	180/180		3	M	248/248
		F	176/180			F	226/248
	4	M	180/182		4	M	194/248
		F	180/180			F	226/248
	5	M	**180/180**		5	M	190/248
		F	**176/176**			F	268/278
Oneu18/1	1	M	194/278				
		F	278/278				
	2	M	225/294				
		F	225/225				
	3	M	209/279				
		F	303/317				

	4	M	275/275				
		F	275/317				
	5	M	275/295				
		F	225/275				

3.3.1 Fine mapping of linkage group LNS16

Using microsatellite markers for fine mapping of linkage group LNS16, I confirmed a QTL for fat percentage in sire-based analysis at position of 3.0 cM (Figure 6). In the male map of linkage group LNS16, the closest marker to this QTL is *Ssa*0016NVH at the position of 1.3 cM (Table 13).

In dam based analysis of linkage group LNS16, there was evidence for a QTL for flesh colour (measured by Roche) at the location of 63.0 cM (Figure 7). On the female map of linkage group LNS16 this QTL is flanked by microsatellite markers *Ssa*0021NVH at the position of 50.6 cM and *Ssa*9.44NUIG at the position of 68.7 cM (Table14). The positions of the markers on male and female based analysis of are demonstrated in Figure 8 and 9, respectively.

Male based analysis did not detect any QTL with effect on flesh colour. The arbitrary value for total variation of QTL effect for flesh colour in dam based analysis shown in Table 15. The effect of detected QTL for flesh colour trait was significant in family two and family three with 4.96% and 5.0% of total variation, respectively. The arbitrary value for total variation of QTL effect in sire based analysis on the fat percentage is significant in family 4 which 5.6% of total variation (Table 16).

Table 13: Position of microsatellite markers on male and female based map in linkage group LNS16.

Marker Code	Marker Name	Position on male map cM	Position on female map cM
1	*Rsa*I466	-	96.4
2	*Rsa*I485(i)	24.1	93.7
3	*Ssa*0050NVH	18.0	88.1
4	*Ssa*416UOS	18.0	76.1
5	*Ssa*9.44NUIGa	13.5	68.7
6	*Ssa*0021NVH	13.5	50.6
7	*Ssa*0016NVH	1.3	36.1
8	*Ssa*0042NVH	0.0	17.1
9	*Omi*116TUF	-	0

Table 14: Result of QTL analysis of linkage group LNS16.

	QTL on Linkage 16	Position cM	F Ratio
Sire based analysis	Fat % (Torry Fatmeter)	3.0	3.69
	Flesh colour (Roche)	15.2	1.05
Dam based analysis	Fat % (Torry Fatmeter)	93.3	1.06
	Flesh colour (Roche)	63.0	3.52

Table 15: The arbitrary value of QTL effects on flesh colour in dam based analysis.

Source	Estimate	S.E.
Dam Effect family 1	0.92	1.68
Dam Effect family 2	**4.96**	1.44
Dam Effect family 3	**5.00**	2.38
Dam Effect family 4	0.93	1.35
Dam Effect family 5	1.02	1.37

Table 16: The arbitrary value of QTL effects on fat percentage in Sire based analysis.

Source	Estimate	S. E.
Sire effect family 1	3.46	2.61
Sire effect family 2	2.69	2.07
Sire effect family 3	3.64	2.71
Sire effect family 4	**5.60**	1.82
Sire effect family 5	3.48	1.79

Figure 6: QTL analysis of linkage group LNS16, indication of QTL for flesh colour based on dam analysis.

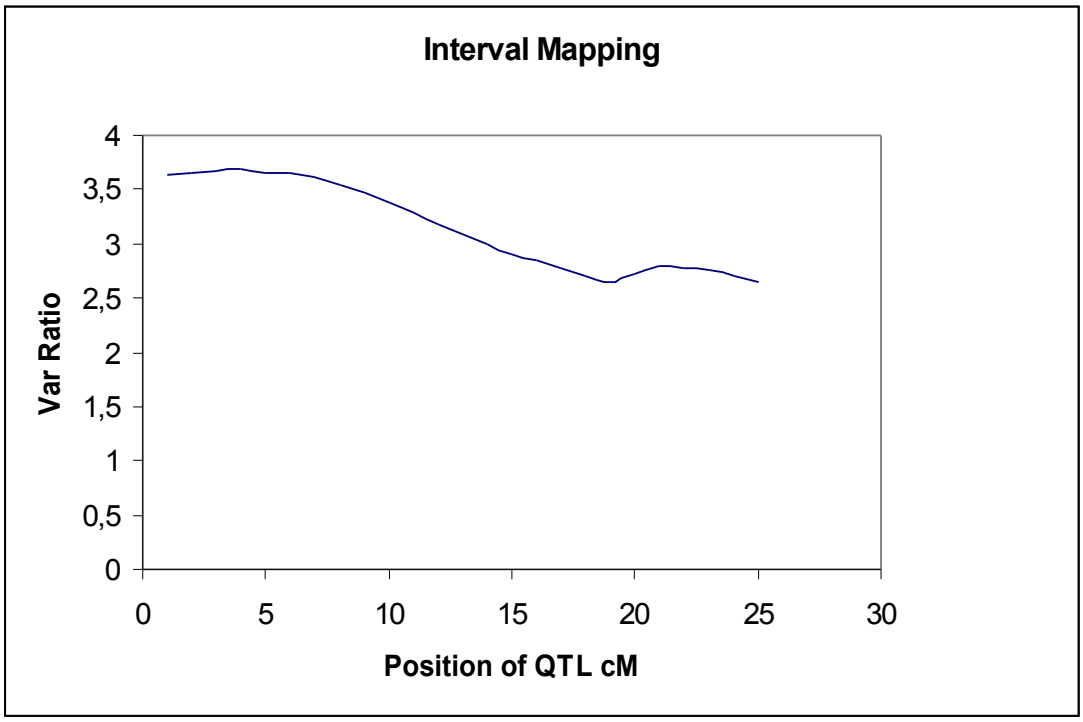


Figure 7: QTL analysis of linkage group LNS16, indication of QTL for fat percentage based on sire analysis.

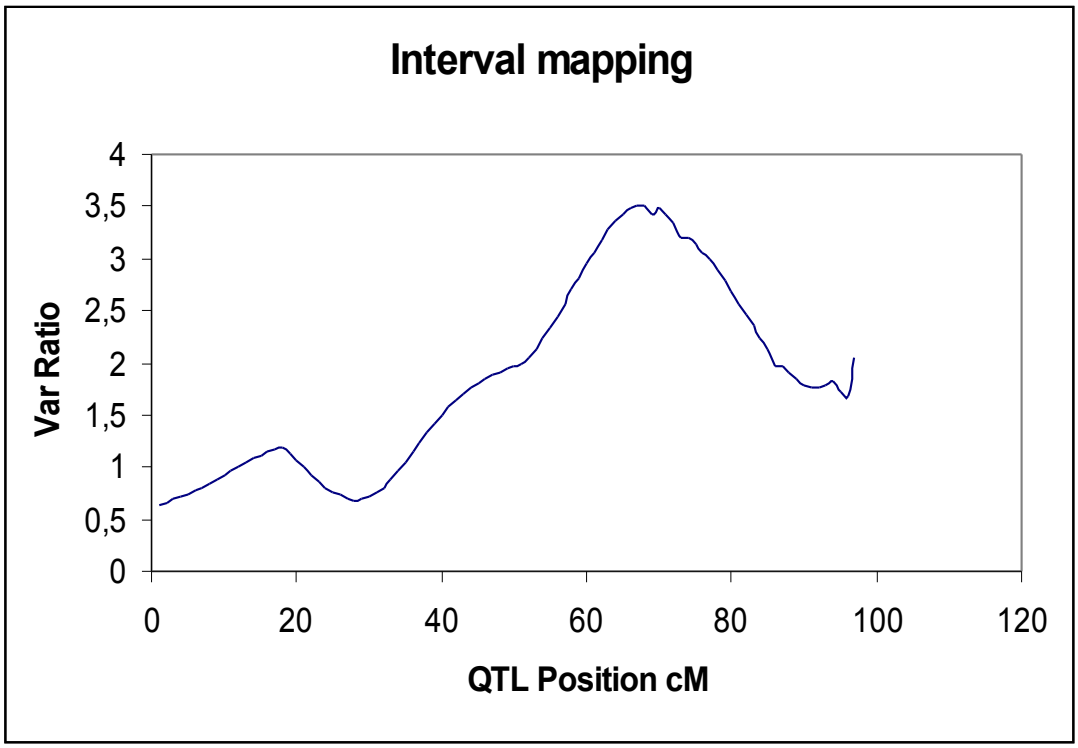


Figure 8: Genetic map of linkage group LNS16 generated based on male analysis.

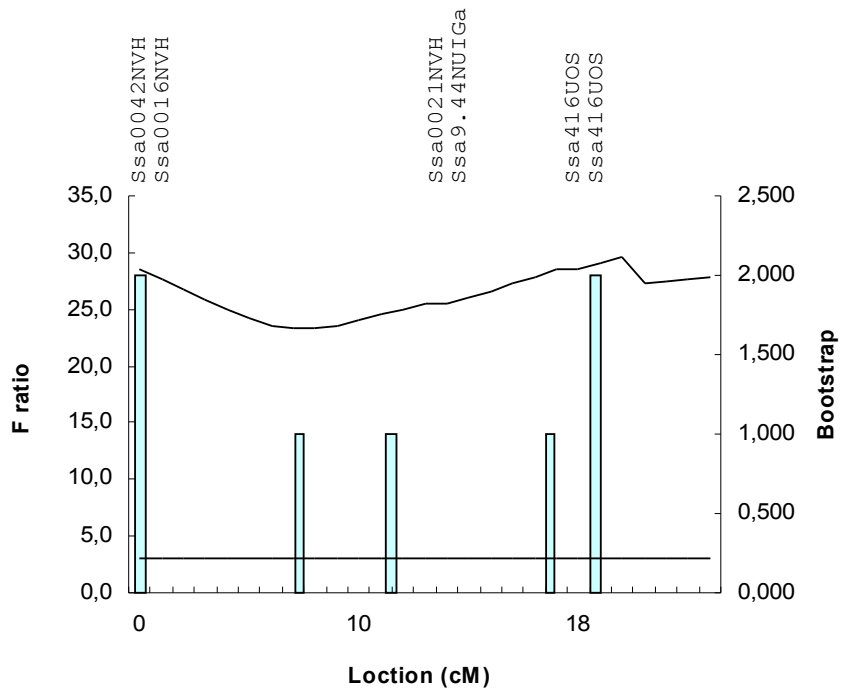
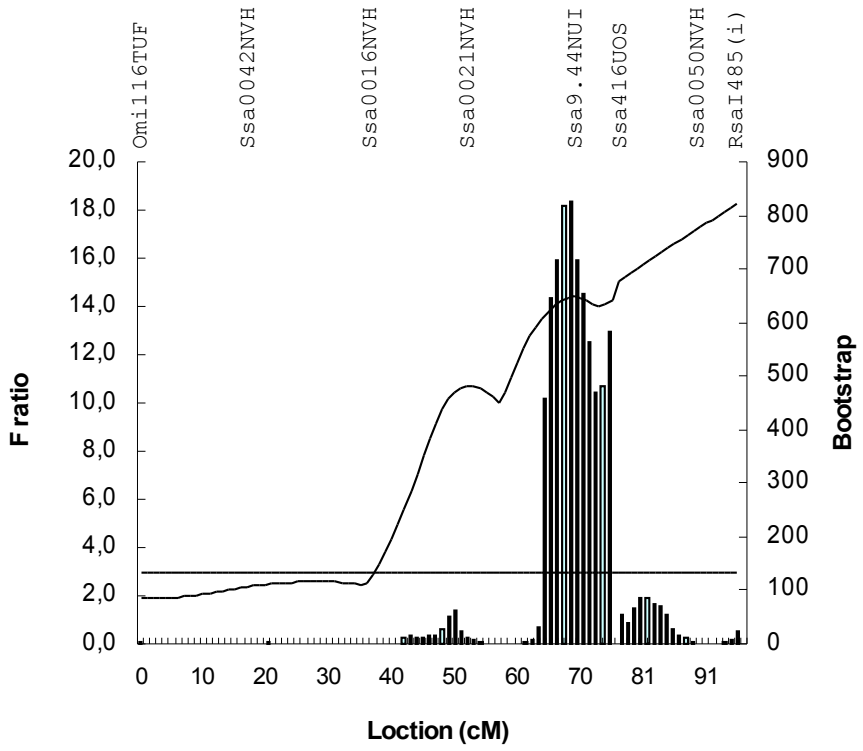


Figure 9: Genetic map of linkage group LNS16 based on female analysis.



3.3.2 Fine Mapping of linkage group LNS1

A suggestive QTL for flesh colour (measured with Chroma) based on dam analysis was found on linkage group LNS1 (Figure 9). This QTL is located at 114 cM on the female map (with F ratio of 2.44). The position of microsatellite markers on the male and female map of linkage group LNS1 is given in Table 17. The closest marker to this putative QTL is microsatellite marker of *Ssa*202 at the location of 139.1 cM. The arbitrary value of the dam based QTL for flesh colour is shown in Table 18. The effect of detected QTL is highest in family five with 9.8% of total variation.

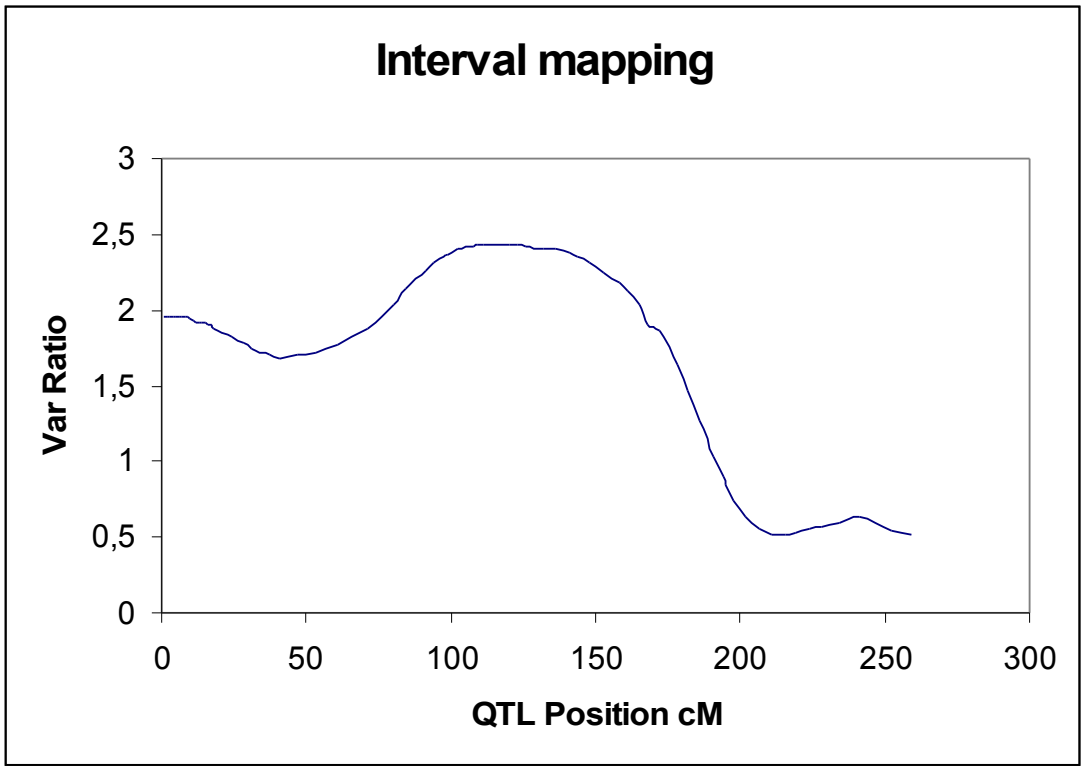
Table 17: Position of microsatellite markers on linkage group of LNS1.

Marker Code	Marker Name	Position on male map cM	Position on female map cM
1	*Oneμ*181	0.0	0.0
2	*Omy*FG8/1TUF	15.5	39.1
3	*Ssa*202DU	26.5	139.1
4	*Ssa*0082NVH	26.5	239.1
5	*Ssa*0244NVH	26.5	245.1
6	*Rsa*I485i	32.9	258.7

Table 18: The arbitrary value of QTL effect on flesh colour in linkage group LNS1 in dam based analysis.

Source	Estimate	S. E.
Dam effect in family 1	4.47	3.38
Dam effect in family 2	5.79	2.34
Dam effect in family 3	0.87	2.23
Dam effect in family 4	3.11	2.32
Dam effect in family 5	9.79	6.32

Figure 10: A suggestive QTL position of flesh colour on linkage group LNS1 based on dam analysis.



3.4 Discussion

This study represents the first quantitative analysis to detect loci linked to flesh quality traits in farmed Atlantic salmon. The result of this study gave evidence for both flesh colour and fat percentage QTL on linkage LNS16. In addition, I found a suggestive QTL linked to linkage group LNS1 affecting flesh colour in Atlantic salmon.

In the current study the microsatellite markers of *One*μ181, *Omy*FG8TUF, *Ssa*202DU, *Ssa*0082, *Ssa*0244, *Rsa*I485i were localised on male and female linkage group one. The linkage map of Atlantic salmon published by Gilbey et al. (2004) consisted of 15 linkage groups, containing 50 microsatellites and 14 unlinked markers. Linkage relationship in the

current study did not show any homologies with the map published by Gilbey et al. (2004). Among the markers that Woram et al. (2003) localised on the linkage group one in Atlantic salmon (One102ADFG, Sal UoG, Omy11/iiNRA, ACC/CAG418, ACT/CAG232, Ssa200DU, Ssa49NVH, Ssa4/iiNVH, Ssa82/iiNVH, Str4/iiNRA, One18/iiASC, OmyFGT8/iiTUF, ACT/CTG71, Ssa-A15/i, Ssa34/iiNVH and Ssa406UoS) none were employed in this study. Gilby et al. (2003) has localized Ssa202DU and Ssleen 17 on the linkage group one in Atlantic salmon and they did not found the linkage between the microsatellites SSOSL34, SSOL85 and SSOSL32 that were already reported by (Slettan et al. 1997) for linkage group one. There are major differences among markers residing on various linkage groups reported for Atlantic salmon. These differences might have arisen from the differences in recombination rate in different strains of fish (Norwegian Scottish or Canadian strains of Atlantic salmon) used in these studies. Likewise, in this study many microsatellites markers failed to show acceptable level of informativeness (markers were homozygous in the both parents), therefore they were discarded. Further studies are needed to accurately determine ordering and localizing the microsatellite markers on the salmon genome.

Among markers residing on linkage group one in Atlantic salmon, microsatellite Ssa202 DU is the most referred one in the mapping studies. The microsatellite Ssa202DU is found to be linked to the sex determining region in male salmon (Reid et al. 2004). The map distance between Ssa202DU and sex determining region is estimated to be 4.2 cM (Woram et al. 2003).

It is expected that a maximum of 29 linkage groups to represent a genetic linkage map of Atlantic salmon (Phillips and Rab 2001) but according to unpublished map at (http://www.asalbase.org/sal-bin/map/index) this species contains 33 linkage groups.

The differences between the actual number of chromosome and the number of linkage groups is not well known but could have arisen because of differences in recombination rate and pseudolinkage phenomenon.

In the recent report published by Moen et al. (2008) a male linkage map of the Atlantic salmon consisted of 29 linkage groups on which the female linkage group one contain the following microsatellite of Ssa202DU, Ssa0166ECIG, Ssa0182ECIG, Ssa406UoS, Ssa0219ECIG and Ssa0114ECIG (Appendix 4). Contrary to the present study, all microsatellite on the male map were very closely linked to each other (due to suboptimal maker coverage in the small region where cross over occurs). In Atlantic salmon, male recombination rate is significantly reduced compared to female recombination rate. The female linkage map reported by Moen et al. (2008) consisted of the microsatellites Ssa202DU, Ssa0166ECIG, Ssa0182ECIG, Ssa406UoS, Ssa0219ECIG and Ssa0114ECIG with the length of 104 cM. Likewise, in this study the female linkage map of LNS1 (with the length of 259 cM) was considerably larger than the male linkage map with the length of 33 cM (Table 17). This can be explained by the fact that due to the lower recombination events microsatellites were localised to a small region. Genotype errors could also led to a significant difference in map distance between male and female fish, particularly for the microsatellite makers that are localized at the end of linkage group.

Linkage relationship in LNS16 did not show a homologies with any linkage group published by Gilbey et al. (2004). None of the microsatellites used in this linkage group were found on the linkage map of Atlantic salmon published by those authors. Differences between microsatellite maps in various studies could also be due to difference in recombination rate between the different populations of fish used in

these studies. On the published linkage map by Moen et al. (2008), the female linkage group of 16 consisted of the microsatellites Ssa0207ECIG, BHMS176, Ssa0230ECIG, Ssa0119ECIG, Ssa0176ECIG, Ssa418UoS/ii, Ssa0229ECIG, CL1721 and Ssa0213ECIG . The male map of the linkage group 16 contained the following microsatellites of Ssa0207ECIG, BHMS176, Ssa0230ECIG, Ssa0119ECIG, Ssa0176ECIG, Ssa418UoS/ii, Ssa0229ECIG, CL17121 and Ssa0213ECIG. In the current study, the male map LNS16 (with the length of 24cM) was considerable smaller than the female map which was spanned 96.4 cM. Similarly, male map of the linkage group 16 was considerably shorter than female map in this linkage group. The microsatellite marker of CL17121 was localised by the end of linkage group 16 in male and female linkage map published by Moen et al. (2008), but in the current study this microsatellite marker was found unlinked on the linkage group LNS16.

In linkage group LNS16, my results of QTL search based on sire analysis showed that the closest marker to QTL for fat percentage is the microsatellite locus *Ssa*0016NVH (1.3 cM), while dam based analysis did not detect any QTL affecting fat percentage on this linkage group. It is not clear why there are such differences in the results between sire and dam based analysis. Differences in recombination rate reported between male and female Atlantic salmon may help to explain the differences in observed marker-trait associations. Recombination rates in males and females are considerably different in all salmonid fishes studied to date (e.g. Sakamoto et al. 2000; Woram et al. 2004; Gharbi et al. 2006). Among salmonids, Atlantic salmon was shown to have the largest sex-specific recombination difference of any vertebrate (Moen et al. 2004a). According to Moen et al. (2004a), recombination differences between female and male has a ratio of 8.26: 1.0 in Atlantic salmon.

Another reason for differences in detected QTL based on male and female analysis could be the effect of pseudolinkage in male salmonids. Pseudolinkage results from pairing and recombination between homeologous chromosomes during meiosis (Allendorf and Danzmann 1997). This phenomenon can explain the discrepancy between linkage maps constructed in male and female salmon, in which the female map represent more accurately the true distance between markers.

In salmonids, the molecular mechanisms responsible for sex-specific differences in recombination rates are still speculative. It has been suggested that in fish species with the XY sex determination system, the female map can usually be longer than the male map because of higher recombination rates in females compared to males (Chistiakov et al. 2006). It is suggested that crossing over is less frequent in the heterogametic sex. In addition to heterogamety, the reduced recombination rate in male salmonids may be the result of a tetraploid event that ancestors of salmonids fishes undergone and have not fully returned to disomy. This phenomenon with formation of multivalents (pairing of both homologous chromosome arm) in male salmonids may explain the greater difference in sex-specific recombination rates seen in the salmonids (Allendorf and Danzmann 1997, Johnson et al. 1987). Greater recombination rate differences between female and male of North American Atlantic salmon compared with female and male of European Atlantic salmon has also been reported by Reid (2003). In addition to that, recombination rates in male salmonids seems to be higher towards the telomeric region than in centromeric regions of chromosome, while in females recombination events appear to be distributed uniformly throughout the chromosome (Sakamoto 2000).

In the current study, microsatellite markers were used to determine the proxy positions of QTL affecting fat percentage and flesh colour traits on linkage groups LNS16 and LNS1. QTL for flesh colour was located on linkage group LNS16, which is flanked by microsatellite *Ssa*0021NVH at the position of 50.6 and *Ssa*9.44NUIG 68.7 at the position of cM.

In the past, quantitative genetic studies have detected a moderate heritability for flesh colour and fat percentage. These studies provided support for potential response to selection for either of these traits; however, they were not useful to examine the number and position of the genes involved. Theoretically, traits such as flesh colour and fat percentage in salmon are controlled by many genes.

New studies are developing towards mapping QTL with large effects, for example, QTL for upper temperature tolerance in rainbow trout accounting for 10% of variation was reported by Perry et al. (2001) and QTL for body weight in rainbow trout accounting for 25 – 30% of total variation of the trait was detected by O'Malley (2001). In this study dam-based detected QTL for flesh colour showed only 5% of total variation of flesh colour in family 3 with no effects on other families. One likely reason for this is that we did not obtain the best families for quality trait (flesh colour and fat percentage) as these families had originally been chosen for an IPN experiment. A selection from all families might have identified a greater range of fillet colour and would have been preferred in order to maximise the chances of identifying a possible QTL but was not possible within this project. This finding also raise important question about the general application of QTL mapping their implications for marker-based breeding program. The cost of genotyping and the

magnitude of genetic improvement are two major issues which must be taken into account before implementation of QTL mapping in salmon industry.

QTL studies in rainbow trout have revealed a great deal about the genetic structure of quantitative traits such as body weight and spawning time (O'Malley 2001), age at maturation (Martyniuk et al. 2003) and resistance to diseases (Ozaki et al. 2001). In Atlantic salmon, Reid (2003) reported the significant and suggestive body weight QTL residing on six linkage groups of one year old fish. Three significant QTL with strong effect on body weight was reported in three different linkage groups; a QTL closely linked to microsatellite *Ssa417UoS* on linkage group AS-11 accounted for (12.7-15.6% of experimental variation) and a significant QTL accounting for 28.6% of the variation in body weight was also reported on linkage group AS-8 closely linked to the microsatellite *Ssa401UoS*. The third significant QTL was found on linkage group 10 closely associated with microsatellite SSOSL85 accounted for 16.6% of the total variation in body weight. Three further suggestive QTL were reported to be on linkage group AS-1 associated to microsatellite Ssa0082NVH with 15.7% of total variation, linkage group AS-5a linked to microsatellite *Str*58CNRS and linkage group AS-17 associated with allele segregation of microsatellite Ssa0104NVH with effect of 12.7% of total variation. However, QTL with the greatest effect in the body weight of Atlantic salmon was reported to be on linkage group AS-8. In addition, this author has located two significant QTL for condition factor on linkage group AS-14 and AS-4. A further two suggestive QTL for condition factor were reported to be on linkage groups AS-1 and AS-11. From these results, Reid (2003) concluded that a large proportion of the quantitative variation for body weight and condition factor in Atlantic salmon is controlled by a few QTL with relatively large effect.

The polygenic basis (single genes are responsible for a small proportion of total phenotypic variance) of resistance to diseases has also been demonstrated by the detection of QTL associated with resistance to parasites in Atlantic salmon. For instance, Gilbey et al. (2006) found 10 QTLs associated with resistance to *Gyrodactylus salaris* in Atlantic salmon. The amount of variance explained by these QTL was from 10.0 to 27.3% of total variation. Microsatellite markers *Ssa*85, *Ssa*77and *SSsp*2216 showed association with infection in the early stages while loci *Ssa*171, *Ssos*1311, *Ssa*42, *Ssa*68 and *Hae*029 were associated with infection in mid and/or later stages.

A suggestive QTL for body weight and condition factor has been identified on linkage group carrying the sex-determining locus in Atlantic salmon (AS-1) (Reid 2003). In previous chapter, I similarly found that suggestive QTLs affecting body weight are residing on linkage LNS1. Sex strongly effects growth and maturation process in salmonids. Furthermore, it has been shown that the loci controlling sex determination in different salmonid fishes are on different linkage group. For example, in Atlantic salmon microsatellite *Ssa 202DU* is tightly linked to sex-determining locus with approximately 4 cM distance in linkage group one (Reid 2003). In rainbow trout, the sex determining locus is on linkage group 18, and is closely associated with microsatellite marker (*Omy*FGT19TUF) which is linked to upper tolerance temperature (Perry et al. 2001). However, I did not use this microsatellite marker because it was not found on linkage group one in the SALMAP (unpublished Atlantic salmon map available at http://www.asalbase.org/sal-bin/map/index).

My results showed evidence for a suggestive QTL affecting the flesh colour, based on dam analysis on linkage group LNS1. Occurrence of a QTL on female based analysis

for flesh colour on sex determining group may suggest that flesh colouration could be sex dependent. Further study is needed to clarify the relationship between this QTL and sex determining region in these families of Atlantic salmon. It is worth bear in mind that males mature one year earlier than females in Atlantic salmon, resulting in migration of carotenoids from muscle to reproductive organ. Therefore, it is suggested that measurements of this trait needs to be done on fish at a similar state of maturity.

The reduced recombination in male salmon results in inheritance of whole chromosome segments. It leads to an increased ability to detect QTL but a decreased ability to localize the QTL to a particular region of the chromosome (Sakamoto et al. 2000). In contrast, detection of QTL is less likely in dam based genome scan approach because of much higher recombination rates. However, once potential linkage groups have been designated, the dam based QTL analysis may be more representative of the actual QTL position because recombination rates are more representative of map distance in female Atlantic salmon compared to males (Somorjai et al. 2003). Thus, detected QTL for flesh colour in the dam on linkage group LNS1 may represent actual location of QTL for this trait in Atlantic salmon. Further study including increasing marker density in the female map and additional QTL analysis should allow for a more detailed investigation to determine the higher number of markers linked to QTL affecting the flesh colour and fat percentage. Although we could have employed more microsatellite markers in the current study (based on SALMAP information of linkage group one and sixteen), because the level of their informativeness in these families was unknown, we decided to apply another type of markers known as Amplified fragment length polymorphism (AFLP).

AFLP markers have previously been used to increase marker density of existing Atlantic salmon linkage map by Moen et al. (2004a). Although, AFLP can provide up to 100 polymorphic bands per primer pair (Liu and Cordes 2004), the major drawbacks for this type of marker are that they are dominant markers (meaning that any single AFLP locus is only informative for one parent) and may exhibit uneven genomic distribution (Ferguson and Danzmann 1998). In next chapter, I intent to employ AFLP markers for localization of the QTLs controlling flesh colour and fat percentage in linkage groups LNS1 and LNS16.

Chapter 4 - Fine mapping of QTL affecting flesh quality traits using AFLP markers

4.1 Introduction

The mapping of QTL with high resolution is often limited by the number of markers available on the linkage map. When a microsatellite genetic map is not dense enough, an alternative strategy for enrichment of the existing genetic map is to use markers that can be developed without prior knowledge of the DNA sequence in the marker region. Among these markers, amplified fragment length polymorphisms (AFLP) markers are the most widely used.

4.1.1 Principle of AFLP generation and detection

AFLP technology has practical applications for DNA fingerprinting, the construction of high density genetic linkage maps and for the positional cloning of genes of interest (Blears et al. 1998). AFLP is selective amplification of restriction fragments from a digest of total genomic DNA. The technique was originally developed by Vos et al. (1995), and ever since it has been applied in various studies in prokaryotes and eukaryotes. The AFLP technology usually comprises of the following steps:

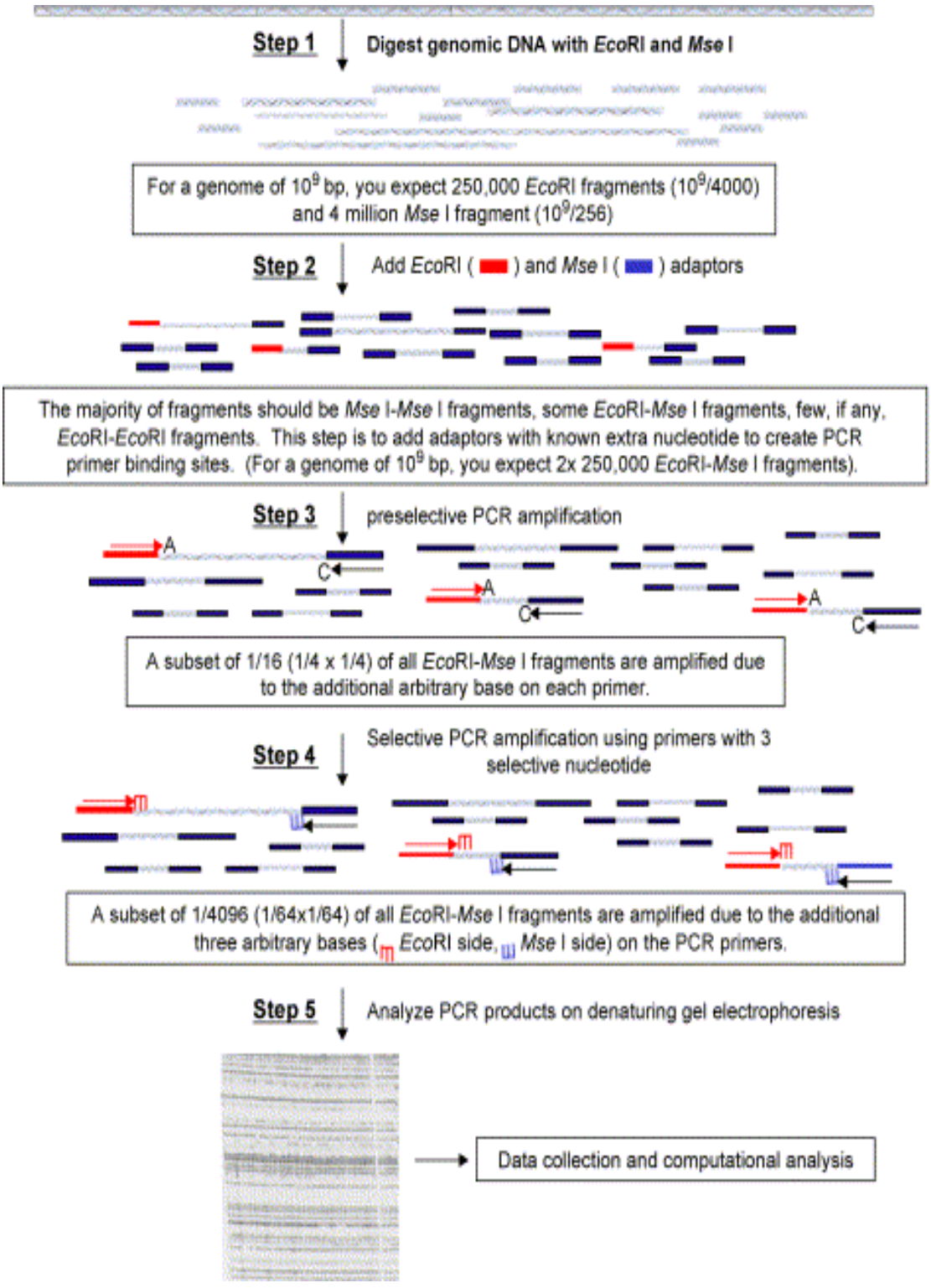
1) The restriction of the DNA with two restriction enzymes as in the case of this study *Eco*RI and *Mse* I.

2) The ligation of double-stranded adapters to the ends of the restriction fragments.

3) The amplification of a subset of the restriction fragments using two primers complementary to the adapter and restriction site sequences, and extended at their 3' ends with additional selective nucleotides.

4) Polymorphisms are revealed by analysis of amplified fragments on a denaturing slab polyacrylamide gel, or more recently, with the capillary electrophoresis technique. Figure 11 shows the schematic representation of AFLP analysis (taken from Liu and Cordes 2004).

Figure 11: Schematic representation of AFLP analysis (taken from Liu and Cordes 2004).



10 to 30 bp long double stranded adapters complementary to the sticky ends of the corresponding restriction site are ligated to restriction fragments using T4 DNA ligase. The adapters and adjacent restriction half-site serve as primer binding sites for the following PCR amplification. AFLP adapters are comprised of a core sequence and enzyme-specific sequences for *Eco*RI and *Mse*I. The structure of the *Eco*RI-adapter is as below:

5-CTCGTAGACTGCGTACC

CATCTGACGCATGGTTAA-5

The structure of the *Mse*I is as below:

5-GACGATGAGTCCTGAG

TACTCAGGACTCAT-5

In selective amplification, two AFLP primers are used; one primer is complementary to the adapter and adjacent rare cutter restriction site sequence with three additional selective nucleotides at the 3'-end, and the second primer is complementary to the adapter and frequent cutter recognition site sequence with three additional selective base extensions (step 2 in figure 1). After the restriction-ligation reaction, a limited number of ligated restriction fragments are amplified by the AFLP pre-selective primers (having a single selective nucleotide). Although frequent-cutter (i.e. *Mse*I-*Mse*I) produces the highest percentage of fragments (90%), fragments cut by both enzymes (i.e. *Eco*RI-*Mse*I fragments) are preferentially amplified. The product from pre-selective amplification is used as templates for a second amplification. The selective amplification takes place with primers having longer selective extension (step 3 and 4 in figure 1). Following amplification reaction, products are visualized by electrophoretic separation of amplified fragments (step 5). The success of the AFLP

procedure can be affected by DNA quality, therefore genomic DNA of high purity is essential for the production of AFLPs to ensure complete digestion by the restriction endonucleases.

4.1.2 Restriction enzymes and their use in AFLP procedure

Restriction enzymes get their names from the fact that they prevent invasion by foreign DNA such as viral DNA, by cutting it up. Bacteria cut foreign DNA at specific sites. Restriction enzymes receive the first three letters of their names from the Latin name of the micro-organism in which they originated. The first letter is the first letter of the genus and the next two letters are the first letter of the species (for example, *Haemophilus influenzae* produces *Hin*). In addition, the strain designation is sometimes included; in this case the d from Rd is used. If the strain of micro-organism produces just one restriction enzyme, the name ends with the Roman numeral I and if more than one enzyme is produced they are numbered II and III and so on (Weaver 2002).

Restriction fragments for AFLP are usually generated using two restriction endonucleases, a rare cutter enzyme (usually with 6 bp recognition such as *Eco*RI, *Hin*dIII or *Pst*I) in combination with a frequent cutter enzyme (4 bp recognition such as *Mse*I and *Taq*I). The frequent cutter produces small fragments within the desired size rage of 100 -1000 bp which is suitable PCR amplification. Restriction enzymes cleave both strands of DNA at highly specific sites resulting in production of a reproducible set of DNA fragments (step 1 Figure 9). Following the enzymatic restriction three type of fragments are generated: a) fragments cut by the rare cutting enzyme on both ends, b) fragments cut with the frequent cutting enzyme on both ends

(more than 90% of fragments are expected to have frequent cutter sites on both ends, for example *Mse*I-*Mse*I fragments), c) AFLP target fragments that have been cut by both the rare-cutter and frequent cutter.

Since a given DNA site can contain one of four bases (A, T, G, or C), adding one known base to one of the primers will, in theory, decrease the number of amplified fragments approximately four fold. Addition of one base to both primers should reduce the PCRed fragment population approximately 16 fold. Adding three bases to each PCR primer should result in a 4096-fold reduction. Digestion with *Eco*RI enzyme should result in approximately 250,000 fragments from a genome of 10^9 bp, or 500,000 *Eco*RI–*Mse*I fragments total since most, if not all, *Eco*RI fragments will be further digested by *Mse*I. Addition of three selective nucleotides to both PCR primers should reduce the *Eco*RI–*Mse*I fragments to about 122 bands on average (500,000/4096 = 120) (Liu and Cordes 2004). Since the genome size of Atlantic salmon is 2.5×10^9 bp, 300 bands are expected to generate from EcoRI / MseI digestion. AFLP technology can be applied using a wide range of restriction enzymes and all feasible combinations of selective nucleotides. Depending on the degree of polymorphism among samples, individual samples can be genotyped using different enzyme and primer combinations.

Table 19 shows the possible primer combination for *Eco*RI primer and *Mse*I digested fragments. The selective amplification bases for *Eco*RI primer are coded by eight letters (a-h) and those of *Mse*I primer are coded with eight numbers (1-8).

Table 19: The possible primer combinations using two restriction enzymes (*Eco*RI primers in the first column and *Mse*I primers are shown in the first row).

***Eco*RI and *Mse*I Combination**	**CAA (1)**	**CAC (2)**	**CAG (3)**	**CAT (4)**	**CTA (5)**	**CTC (6)**	**CTG (7)**	**CTT (8)**
AAC (a)	Primer pair 1	Primer pair 2	Primer pair 3	Primer pair 4	Primer pair 5	Primer pair 6	Primer pair 7	Primer pair 8
AAG (b)	Primer pair 9	Primer pair 10	Primer pair 11	Primer pair 12	Primer pair 13	Primer pair 14	Primer pair 15	Primer pair 16
ACA (c)	Primer pair 17	Primer pair 18	Primer pair 19	Primer pair 20	Primer pair 21	Primer pair 22	Primer pair 23	Primer pair 24
ACC (d)	Primer pair 25	Primer pair 26	Primer pair 27	Primer pair 28	Primer pair 29	Primer pair 30	Primer pair 31	Primer pair 32
ACG (e)	Primer pair 33	Primer pair 34	Primer pair 35	Primer pair 36	Primer pair 37	Primer pair 38	Primer pair 39	Primer pair 40
ACT (f)	Primer pair 41	Primer pair 42	Primer pair 43	Primer pair 44	Primer pair 45	Primer pair 46	Primer pair 47	Primer pair 48
AGC (g)	Primer pair 49	Primer pair 50	Primer pair 51	Primer pair 52	Primer pair 53	Primer pair 54	Primer pair 55	Primer pair 56
AGG (h)	Primer pair 57	Primer pair 58	Primer pair 59	Primer pair 60	Primer pair 61	Primer pair 62	Primer pair 63	Primer pair 64

4.1.3 Advantages and disadvantages of AFLP markers

Advantages and disadvantages of AFLP application as genetic markers have been evaluated in various studies (e.g. Bensch and Akesson (2005); Luccchini (2003). AFLP technique can rapidly generate hundreds of highly replicable markers. AFLP markers are considered useful by many researchers because it is a relatively cheap, easy, fast, and reliable method to generate large number of polymorphic loci. In comparison with other genetic markers such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), the AFLP technique is more easily reproduced across laboratories. More importantly, the large number of markers can be produced by varying the restriction enzymes and number of selective nucleotides.

The time and cost efficiency of AFLPs technology to produce the high marker density is superior in comparison to other markers such RAPD, RFLP and microsatellites. Unlike microsatellite markers, no prior knowledge of the genomic sequence is required for AFLP technology (Blears et al. 1998). The major difference between AFLP and the RFLP screening techniques is PCR amplification of restriction fragments. In addition, RFLP technique only utilize the restriction site for differences in DNA sequence, whereas in AFLP technique the selective nucleotides provide additional possibilities for polymorphisms to be detected beyond the restriction site itself.

Using arbitrary primers, the RAPD technique utilize PCR amplification to randomly amplify segments of the target DNA. During its PCR amplification, fragments of various sizes are produced. Although AFLP and RAPD are both PCR based technique, but AFLP uses primers specific to the adapter and restriction site sequence. RAPD markers are technically simple to work with but can have poor reproducibility.

As a disadvantage, AFLP technique primarily generates dominant rather than co-dominant markers. This means an AA genotype cannot be distinguished from an Aa genotype without further analysis. AFLP is relatively new genetic markers with broad application in systematic, population genetics, DNA fingerprinting and quantitative trait loci mapping.

The frequency with which AFLP markers are detected depends on the level of sequence polymorphism between the tested DNA samples. The molecular basis of AFLP polymorphisms will usually be single nucleotide polymorphisms in the restriction sites, or in the selective nucleotides adjacent to the restriction sites.

Deletions, insertions and rearrangements affecting the presence or size of restriction fragments can result in detectable polymorphisms.

The aim of this study was to apply the AFLP technique to the salmon genome to produce as many markers as possible to saturate linkage groups harbouring QTL for trait quality as was revealed in the previous chapter. Furthermore, I intended to evaluate the associations between the AFLP markers and those microsatellite markers residing on both linkage groups LNS1 and LNS16 used for detection of QTL.

4.2 Material and methods

4.2.1 Family structure

DNA samples from the five full-sib families of Atlantic salmon (as explained in section 2.2.1 of Chapter 2) was used for AFLP genotyping in this study.

4.2.2 Procedure of AFLP genotyping

The chemistry part of the AFLP technique was principally done according to Vos et al., with major changes in gel visualization due to new fluorescent detection hardware. Restriction and ligation took place in single reactions. Genomic DNA was digested with two restriction enzymes, *Eco*RI (a hexanucleotide target sequence 5'-G/AATTC-3') and *Mse*I (a tetranucleotide target sequence 5-'T/TAA-3'). For each enzyme, an adapter pair was ligated to the sticky ends. Single strand adapters were annealed to each other as below:

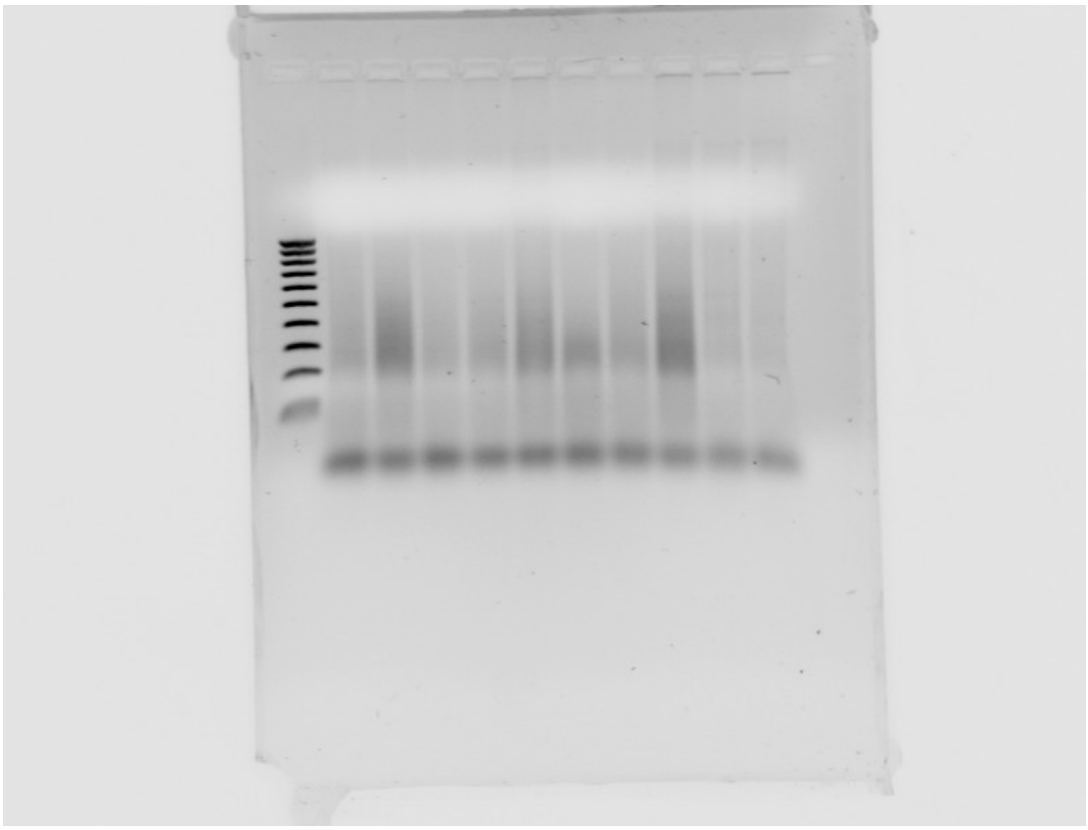
For EcoRI adapter pair with final concentration of 5 µM, 25 µL of EcoRI forward adapter (100µM) was mixed with 25 µL of EcoRI reverse adapter (100 µM), then 450 µL of $TE_{0.1}$ buffer was added for making a total amount of 500 µL.

For *Mse*I adapter pair with final concentration of 50 µM, 250 µL of *Mse*I forward adapter (100µM) was added to 250 µL of *Mse*I reverse adapter (100 µM) to make a total of 500 µL. After vortex and brief centrifuge, adapter were heated at 95 °C for 5 min to denature and then allowed to cool slowly for complete renature. Adapters were stored at -20 °C.

After simultaneous double digestion of ~0.5 µg of genomic DNA by restriction enzymes (*Eco*RI and *Mse*I), adaptors were ligated to the fragments for 2 hours at 37 °C in thermal cycler (TGRADIENT Biometra) with heated lid at 37 °C (in order to avoid evaporation leading to *Eco*RI star activity). Restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (NEB).

Each reaction (11 µl) contained 5.5 µL of diluted DNA in distilled water, 1.0 µl of 10X T4 DNA ligase buffer (50 mM Tris HCl, 10mM $MgCl_2$, 10mM DTT, 1mM ATP, 25 mg/ml BSA at PH 7.5), 1.0 µl of 0.5M NaCl, 0.5 µl of 1.0 mg/ml BSA, 1.0 µL each of *Mse*I (50 µM) and *Eco*RI (5 µM) adaptors, and 1.0 µl enzyme master mix (0.1 µl of 10X T4 DNA ligase buffer 0.1 µl of 0.5 M NaCl, 0.5 µl of 1.0 mg/ml BSA, 1.0 U *Mse*I, 5.0 U *Eco*RI, 1.0 Weiss U T4 DNA ligase (Promega) and appropriate amount of water). After 2 hours incubation at 37 °C in thermal cycler (TGRADIENT Biometra) each reaction was diluted 1:10 with $TE_{0.1}$ buffer (1.5 mM Tris-HCl (pH 8.0), 0.1 mM EDTA). Complete digestions were determined by visualizing the fragments on an agarose electrophoresis gel. DNA digestion of parent fish is shown in Figure 11.

Figure 12: Visualizing the DNA digestion of parent fish using agarose gel. The first lane was loaded with 0.5 µg of 100 bp DNA ladder.



Pre-selective primers complementary to each adaptor sequence were used to amplify the restriction fragments created in the digestion-ligation step. Every 4 µl of diluted digestion-ligation product was amplified in a 20 µl reaction volume containing 1.0 µl of *Eco*RI + A and 1.0 µl of *Mse*I + C pre-selective primers, and 14 µl of PCR Core Mix (200mM of each dNTP, water, 1.5 mM $MgCl_2$, 0.5U Taq DNA polymerase and buffer II (Tris-HCl, $(NH_4)_2SO_4$, Tween 20 at PH 8.8)). Pre-selective PCR was run at a temperature profile of one cycle of 72 °C for 2 minutes, 30 cycles of 94 °C for 25 seconds, 56 °C for 30 seconds, and 72 °C for 2 minutes and a final extension step of 60 °C for 30 minutes. The reactions were checked by visualizing the fragments on an agarose electrophoresis gel. Products from pre-selective PCR were diluted 10 fold with $TE_{0.1}$ buffer (1.5 mM Tris-HCl (pH 8.0), 0.1 mM EDTA) and used as templates

for selective amplification. All samples were stored at 4 °C following dilution with buffer.

Pairs of selective primers, each containing two additional selective nucleotides at their 3' end were used for selective PCR, with the *Eco*RI selective primer being fluorescently labelled. The *Eco*RI selective amplification primer had a sequence of 5′ -GACTGCGTACCAATTCA*NN. For recognition of the *Mse*I adaptor at the other end of the DNA fragment, primers were synthesized with a sequence of 5′ -GATGAGTCCTGAGTAAC*NN. The A* and C* bases represent bases selected for primers in the initial pre-selective amplification and the N's represent user-selected bases amplified in the second selective PCR amplification. Fragments were visualized by attaching a D4, D3 or D2 WELL RED™ dye to the 5′ end of each *Eco*RI selective amplification primer with no modification made to *Mse*I primer.

The *Eco*RI and *Mse*I selective primers were coded with letters and numbers, respectively (Table 20).

Table 20: The list of 24 primer combination of *Mse*I and *Eco*RI which were randomly selected for this study.

*Mse*I and *Eco*RI Combination	CAA (1)	CAC (2)	CAG (3)	CAT (4)	CTA (5)	CTC (6)	CTG (7)	CTT (8)
AGG (a)	Primer pair 1	Primer pair 2	Primer pair 3	Primer pair 4	Primer pair 5	Primer pair 6	Primer pair 7	Primer pair 8
AAG (b)	Primer pair 9	Primer pair 10	Primer pair 11	Primer pair 12	Primer pair 13	Primer pair 14	Primer pair 15	Primer pair 16
ACA (c)	Primer pair 17	Primer pair 18	Primer pair 19	Primer pair 20	Primer pair 21	Primer pair 22	Primer pair 23	Primer pair 24

The second, selective, PCR amplifications were carried out in a 20 µl reaction that was composed of 3.0 µl PCR diluted pre-selective product, 1.0 µl *Mse*I selective primer at 5.0 µM without label, 1.0 µL *Eco*RI selective primer at 2 µM which is dye labelled, and 15 µL PCR Core Mix (as given above). Selective amplifications were

run with a touch-down profile: two minute DNA denaturation at 94 °C followed by 10 cycles of 94 °C for 20 seconds, 66 °C for 30 seconds, and 72 °C for 2 minutes, with a 1 °C decrease in annealing temperature each cycle, followed by 25 cycles of amplification at 94 °C for 20 seconds, 56°C for 30 seconds, and 72 °C for 2 minutes.

Electrophoresis and data collection were carried out using a Beckman Coulter 8800 CEQ genetic analyser. After selective amplification, 0.9 µl of PCR product was added to a cell in a 96-well plate containing 30 µl of deionized formamide and 0.25 µl size standard (600 base pair with WELLRED™ dye D1). The plate was sealed and the solution was thoroughly mixed by vortexing for a minimum of two minutes. Each well was overlaid with a drop of mineral oil before analysis. Prior to running the samples, a sample sheet was created. The gel cartridge and buffer plates were installed in the sequencer according to the instructions in the CEQ 8800 fragment analysis training guide (2004). Raw data were analysed using pre-defined analysis parameters specifying the size standard (600 bp), dye mobility calibration (PA ver.1) and the analysis method (Cubic method).

In order to identify polymorphic AFLP loci, graphs from each pair of parents were overlaid and eye-scanned. The presence or absence of a peak between these parents was scored as possible polymorphism as is shown in Figure 12. After the detection of possible polymorphism loci between the parents, the genotyping graphs of offspring were scanned to detect its segregation as it is shown in Figure 13 (e.g. fragment 89 bp). AFLP markers were scored as dominant markers and recorded as 2-0 for band presence (AA or Aa) and 1-1 for band absence (aa).

Figure 13: Overlaid graphs of both parents shows polymorphism at fragment 139 bp (primer combination AGGCAC).

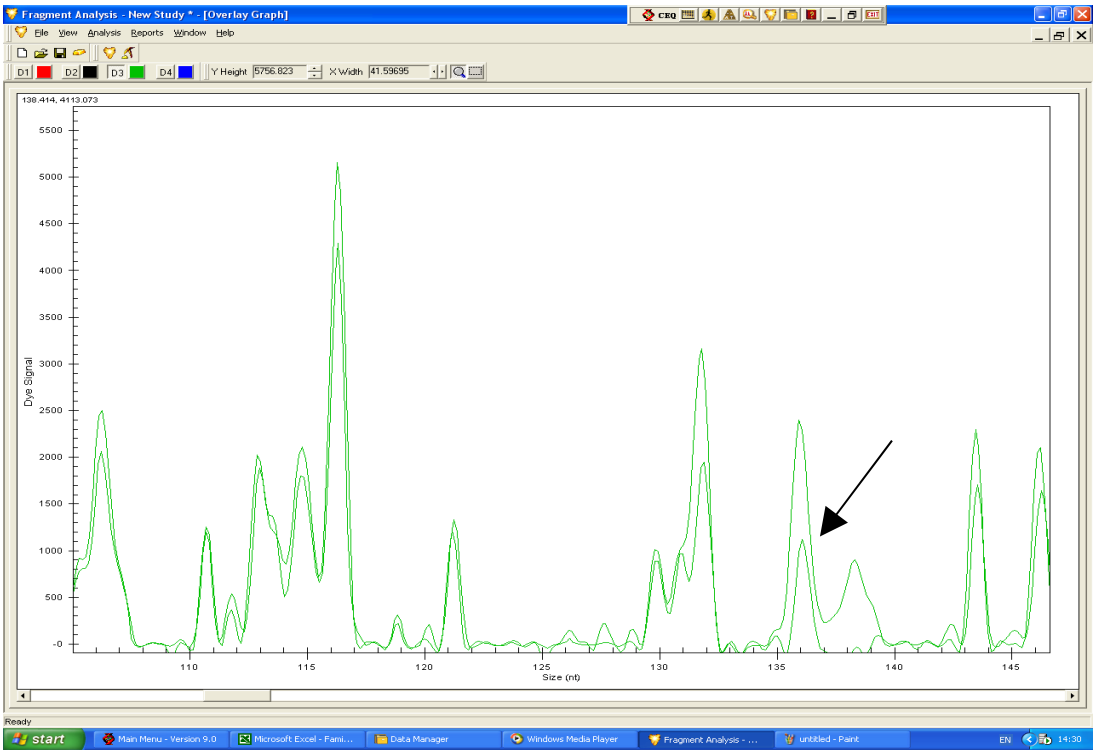


Figure 14: Segregation of polymorphism loci among offspring of known parents.

4.2.3 Genetic nomenclature

Naming of AFLP loci follows the convention used by Young et al. (1998) where the three base selective primer pair extensions used to produce the loci and follows by the base size of the locus. For example, AGGCTT238 displays the three nucleotides (AGG) for the *Eco*RI primer and the three nucleotides (CTT) for the *Mse*I primer that amplified a fragment at 238 bp.

4.2.4 Map construction

AFLP were scored as dominant markers with genotypes indicating the presence or absence of a band. Only clear and unambiguous polymorphisms that were present in one parents (Aa x aa or AA x aa) and segregating in the progeny were scored. The linkage between all AFLP markers with microsatellite markers from linkage group 16 and 1 were examined using software program Crimap 2.4 (Green 1990). Using the 'twopoint' option the linkage analysis for each pair of AFLP and microsatellite loci was performed. This identified which AFLP markers were linked and then mapped to linkage groups of interest. The grouping of markers was carried out with a minimum LOD score of 3.0.

The Crimap 'build' option was then used to determine the most likely order of the AFLP and microsatellites on the linkage groups of interest. The most likely order was checked using the 'flips' option. Once the most likely marker order had been established, the recombination fractions between markers were used to estimate the genetic map distance in centiMorgans (cM), using the Kosambi mapping function (Kosambi 1944). I personally carried out the genotyping of fish samples using 24 AFLP primer combination, then the raw data was represented to Dr. Houston in the

Roslin Institute where he kindly supervised me on the construction of linkage map, using AFLP and microsatellite markers.

4.3 Results

Twenty four AFLP primer combinations were chosen for genotyping the parents and progeny resulted in a total of 392 polymorphic fragments (Appendix 3). Each primer combination resulted in a large number of fragments being produced on each capillary electrophoresis. A total of 59 AFLP markers were detected in family one with the mean of 2.5 markers per primer combination. In family one, primer combination of ACACTC and AAGCTT did not produce any markers, while primer combinations of AGGCTC, AGGCTG and ACACTG generated the highest number of markers (each generated four markers). In family two, 80 AFLP markers were produced with the mean of 3.3 markers per primer combination. In this family, primer combination of AAGCTA did not produce any marker whereas primer combinations of AGGCAT and ACACAG each produced 7 markers. In family three, 115 AFLP markers were found with the mean of 4.8 markers per primer combination. In this family the primer combination of ACACTA had 10 markers, and primer combinations of AGGCAT and ACACAA generated each 8 markers. A total of 119 markers were found in family 4 with the mean of 5.0 markers per primer combination where the primer combination of AAGCAC produced 9 markers. In family five, 115 AFLP markers were produced with the mean of 4.8 markers per primer combination, among them primer combinations of AGGCAG and AGGCAC generating 9 markers.

The high quality chromatograms produced by capillary electrophoresis (CEQ 8800) allowed fragments to be distinguished with high resolution over the entire fragment size range (60 to 600 bp).

The following 9 fragments (AAGCAC181, AAGCAC328, AAGCTA296, AAGCTG67, AGGCAC163, AGGCAC448, AGGCAG447, AGGCTA237, and AGGCTC237) were significantly linked to previously mapped microsatellite markers on linkage group LNS16 (Chapter 3).

Four of these fragments (AAGCAC328, AGGCAG447, AGGCTA237 and AGGCTC237) were significantly linked to microsatellite marker *Ssa*9.44NUIG which in dam based analysis was the closest marker to a QTL for colour trait at position of 68.7 cM (chapter 3). The recombination fraction and LOD between each pair of microsatellites and AFLP markers are shown in Table 21.

Table 21: Recombination fraction and LOD between each pair of microsatellites and AFLP markers.

Marker pairs	Female Recombination Fraction	Male Recombination Fraction	LOD
AAGCAC181 *Omi*116TUF	0.07	0.00	5.27
AAGCAC181 *Rsa*I466	0.07	0.00	4.73
AAGCTA296 OMM1134	0.06	0.00	3.16
AAGCAC328 *Ssa*0050NVH	0.00	0.08	4.20
AAGCAC328 *Ssa*9.44NUIGa	0.00	0.12	5.93
AAGCAC328 *Omi*116TUF	0.00	0.12	5.93
AAGCAC328 *Ssa*416UOS	0.00	0.16	4.35
AAGCTG67 OMM1134	0.00	0.11	3.74
AGGCAC163 *Omi*116TUF	0.14	0.00	5.10
AGGCAC163 *Ssa*416UOS	0.21	0.00	3.06
AGGCAC163 *Rsa*I466	0.14	0.00	4.86
AGGCAC448 *Alu*I387	0.00	0.00	6.62
AGGCAC448 *Omi*116TUF	0.00	0.00	6.62
AGGCAC448 *Rsa*I466	0.00	0.00	6.63
AGGCAG447 *Ssa*9.44NUIGa	0.00	0.00	3.31
AGGCTA237 *Alu*I387	0.00	0.09	5.27
AGGCTA237 *Ssa*9.44NUIGa	0.00	0.07	8.16
AGGCTA237 *Omi*116TUF	0.00	0.15	4.73
AGGCTC237 *Alu*I387	0.00	0.09	5.01
AGGCTC237 *Ssa*9.44NUIGa	0.00	0.10	4.75
AGGCTC237 *Omi*116TUF	0.00	0.09	5.01
AGGCTC237 AGGCTA237	0.00	0.00	9.33

Linkage analysis between microsatellite markers from linkage group LNS1 (*Ssa*202, *Ssa*0244, *Ssa*0082, *Rsa*I485, and *Oneμ*18/1) and AFLP fragments revealed no linkage at all between these two type of markers. However, some of the AFLP fragments (ACACAA125, ACACAA112, ACACAA285, ACACAA125, ACACAA424, ACACTG159, ACACTG155, ACACTT193, and ACACTG273) were significantly linked to each other but lack of linkage to microsatellite markers on linkage group LNS1 prevented further conclusion for these markers being resident in the same linkage group. Table 22 demonstrates the order and the distances between

microsatellite and AFLP markers on linkage group LNS16. The differences between the Table 21 and Table 20 can be explained by differences between a 'twopoint' option and a 'build' analysis in Crimap. Since the 'twopoint' linkage analysis sometimes shows zero recombination but build option puts the markers far apart on the map. In theory, the twopoint option is used to determine which pairs of markers show evidence for linkage but can not be relied upon for the genetic distance between the loci. The zero recombination in Table 20 is probably is due to the fact that there was little opportunity to detect the recombination especially in the case of AFLP markers with their lower level of informativeness. For example, if two markers are both homozygous in one family they will show zero recombination, but this does not mean evidence for linkage and they could be far apart on the chromosome. However, it is worth noting that in the 'build' option, Crimap will calculate the most likely order based on the information provided but a low number of individuals or using AFLP markers (which are quite uninformative) may lead to incorrect order of marker.

Table 22: Position of microsatellite and AFLP markers on male and female based map in linkage group LNS16.

Marker code	Marker name	Male map distance cM	Male map position cM	Female map distance cM	Female map position cM
1	*Ssa*0042NVH	18.6	0.0	7.8	*0.0*
2	*Ssa*0021NVH	21.1	18.6	8.8	*7.8*
3	CL17121	4.2	39.7	92.9	*16.6*
4	*Rsa*I458	50.1	43.9	6.3	*109.5*
5	AGGCTC237	0	94.0	6.3	*115.8*
6	AGGCTA237	6.5	94.0	6.3	*122.1*
7	*Ssa*0042NVH	0.1	100.5	12.3	*128.4*
8	AGGCAG447	0.1	100.6	12.3	*140.7*
9	*Ssa*0021NVH	2.9	100.7	7.2	*153.0*
10	*Ssa*0016NVH	8.6	103.6	13.7	*160.2*
11	*Ssa*0016NVH	3.5	112.2	11.6	*173.9*
12	*Ssa*416UoS	2.6	115.7	11.6	*198.6*
13	*Ssa*9.44NUIGa	2.6	121.1	5.4	*210.2*
14	AGGCAC448	2.6	123.7	0	*215.6*
15	*Omi*116TUF	11.5	126.3	1.4	*215.6*
16	AAGCAC328	15	137.8	1.4	*217.0*
17	CL19368	63	152.8	0	*218.4*
18	*Rsa*I466	0	215.8	7.7	*218.4*
19	AAGCAC181	0	215.8	7.5	*226.1*
20	*AGGCAC163*	*0*	*215.8*	*0*	*233.6*

Dam based QTL analysis on linkage group LNS16, using a constructed map of microsatellites and AFLP markers, revealed a significant QTL for flesh colour at the location of 189 cM with F ratio of 3.73 (Figure 15). Similar to the results from the microsatellite linkage map in the previous study (Chapter 3), no QTL was detected for fat percentage based on dam analysis of the linkage group LNS16. The dam effect of the detected QTL for flesh colour is significant in the family one with 8.8% of the total variation (Table 23).

Figure 15: QTL analysis of linkage group LNS16 (indication of QTL for flesh colour based on female analysis).

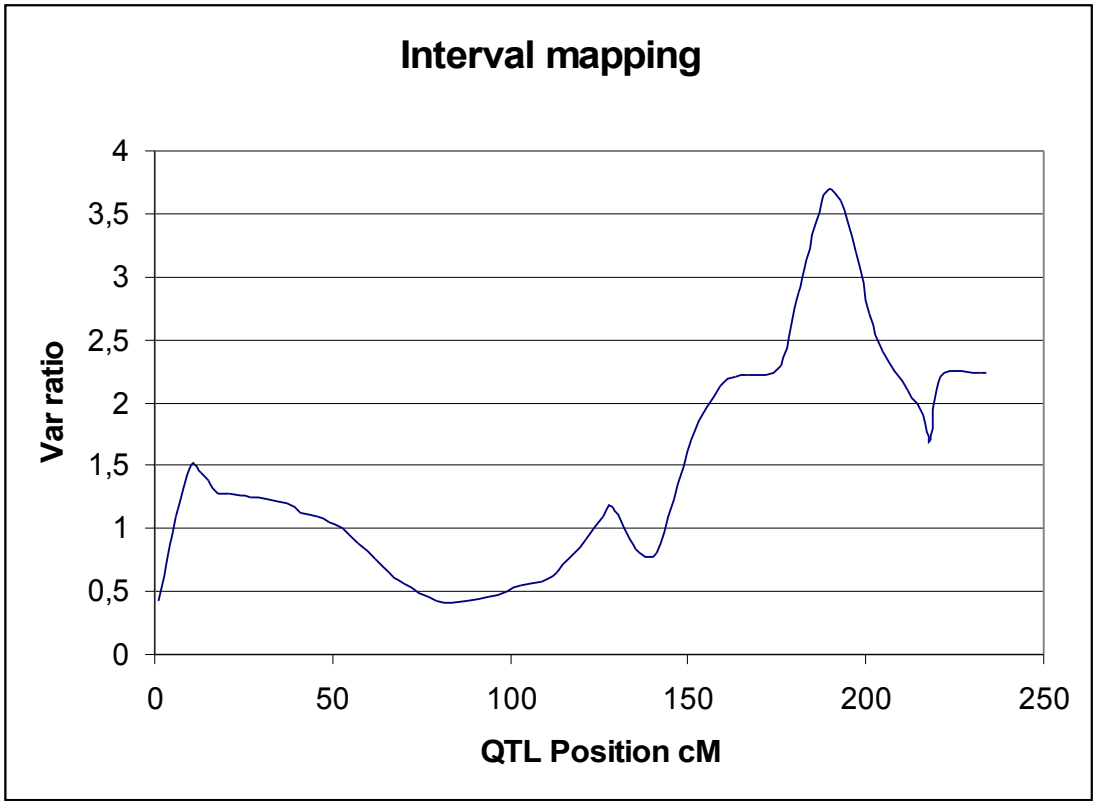


Table 23: The arbitrary value of QTL effects on flesh colour in dam based analysis.

Source	Estimate	S. E.
Dam effect family 1	**8.8**	2.2
Dam effect family 2	0.9	0.5
Dam effect family 3	1.3	0.9
Dam effect family 4	5.9	3.5
Dam effect family 5	0.5	0.2

In sire based analysis on linkage group LNS16, a significant QTL for fat percentage at the location of 80 cM (F ratio of 3.83) was localized (Figure 16). On the male map of linkage group LNS16, the microsatellite *Rsa*I458 at the location of 43.9 is the closest microsatellite marker to this QTL. No QTL for flesh colour was detected on the sire-based analysis of this linkage group. The sire effects of this QTL on total variation of the fat percentage trait are positive on all families except family one (Table 23). A

joint map for the microsatellite and AFLP markers on the linkage group LNS is given in Figure 17.

Figure 16: QTL analysis of linkage group LNS16 (indication of QTL for fat percentage based on male analysis).

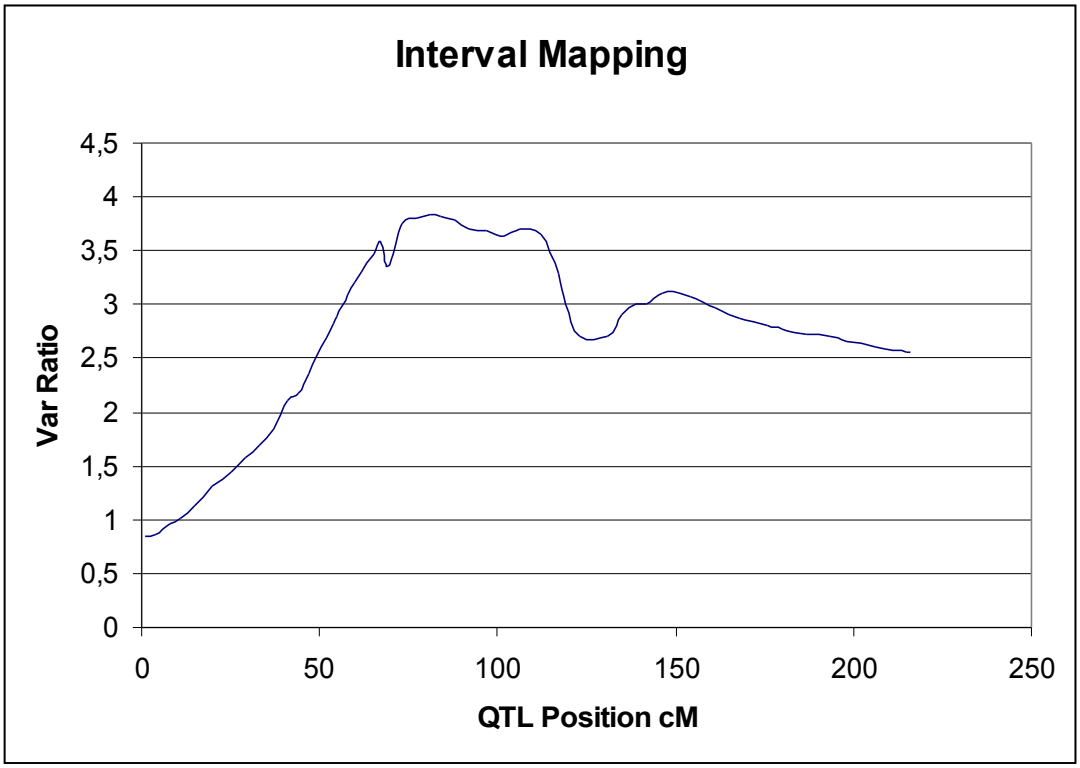
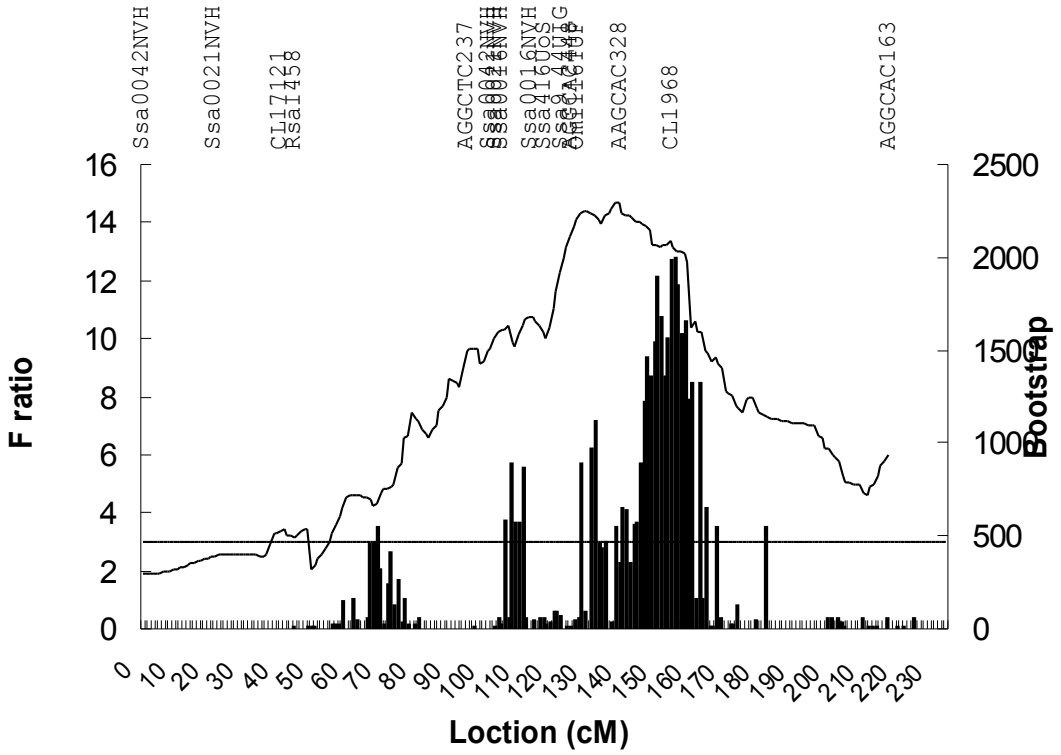


Table 24: The arbitrary value of QTL effects on fat percentage in sire based analysis.

Source	Estimate	S. E.
Sire effect family 1	4.8	4.0
Sire effect family 2	8.4	2.7
Sire effect family 3	5.1	2.4
Sire effect family 4	4.0	2.8
Sire effect family 5	4.4	3.5

Figure 17: A joint map of microsatellite and AFLP markers of male map of linkage group LNS16.



4.4 Discussion

AFLP technique has widely been used for linkage map construction in salmonids for example pink salmon (Linder et al. 2000), Atlantic salmon (Moen et al. 2004a) and rainbow trout (Young et al. 1998). The AFLP technique in combination with a sex-typed pool strategy has revealed that Y-chromosome linked AFLP markers are strain-specific in rainbow trout (Felip et al. 2005).

High density linkage maps facilitate efficient mapping of QTL and complement of marker-assisted selection (Lander and Botstein 1989). Theoretically, a less dense microsatellite linkage map could be saturated by using SNPs and AFLP markers. From previous chapters, I have identified two linkage groups (LNS1 and LNS16) that showed association with flesh colour and fat percentage in this population of Atlantic salmon. In the present chapter, I aimed to map additional markers into the current map and I found 9 AFLP markers which were significantly linked to microsatellite markers on linkage group LNS16.

Unfortunately, I could not find any association between the AFLP markers and microsatellite markers residing on linkage group LNS1. A possible explanation for the lack of linkage between AFLP and microsatellite markers could be the occurrence of an error in AFLP genotyping. The lack of linkage could also be due to abnormal segregation patterns that occur in male salmonids. Moreover, creating linkage between AFLP and microsatellite markers could also be hampered by low information content of dominant markers such as AFLP.

Although AFLP markers have the potential to efficiently construct high resolution linkage maps but it seems that these markers are difficult to transfer among different

populations. For instance, in the current study I could not match any of the detected AFLP fragments on linkage group LNS16 with those of linkage group 16 published by Moen et al. (2004a). And also from a total of 18 AFLP markers (ACGCAA150, AGCCAG49, ACACAC116, AACCTG377, AGCCTA198, AACCTG366, AGGCAT346, ACTCTA476, AGGCAT376, ACTCTA491, CCTCTC112, ACCCTG115, ACACTG299, AACCTG371, AGCCTT413, AACCAT347, ACTCTA485 and ACTCTA479) residing on the sex-specific map of linkage group one published by Moen et al. (2004a), none were found among the entire AFLP markers generated in this study.

From three AFLP markers on the male map (ACCCAG418, ACTCAG232 and ACTCTG71) and nine AFLP fragments on female map of linkage group one (AACCTT97, ACTCTG71, ACTCAC148, AAGCAT218, AGCCTG565 and AACCTA236) published by Woram et al. (2003) only one primer combination (AAGCAT218) was found similar to the primer combination that I employed, but I could not detect a fragment size of 218 among AFLP markers produced in the current study. This experiment showed that such an increase in marker density can be poorly performed with dominant markers like AFLPs.

It should also be mention that neither of the AFLP markers on sex-specific linkage group one published by Moen et al. (2004a) and Woram et al. (2003) corresponded with each other. The difference between the AFLP mapping in this study with those published by Moen et al. (2004a) and Woram et al. (2003) could be due to either a genotyping error or a variable recombination rate between the different strains of fish used in these studies.

From a total of 489 AFLP fragments, only nine fragments (AAGCAC181, AAGCAC328, AAGCTA296, AAGCTG67, AGGCAC163, AGGCAC448, AGGCAG447, AGGCTA237, and AGGCTC237) were found to be linked to microsatellite markers on linkage group LNS16. The strong linkage between the following fragments (AAGCAC328, AGGCAG447, AGGCTA237 and AGGCTC237) with microsatellite marker *Ssa*9.44NUIG suggests that these AFLP markers could possibly be used for improvement of flesh colour in marker assisted selection within the selected families.

From the joint map of microsatellite and AFLP markers, in dam-based QTL analysis on linkage group LNS16 a significant QTL affecting flesh colour was detected. The location of QTL detected for flesh colour in this study was at 189 cM whereas the position of QTL for flesh colour in previous chapter was at 63 cM. As a result, flanking markers for the detected QTLs in these two studies are different as well.

The female based detected QTL on the current study is flanked by microsatellite marker of *Ssa*0016NVH at the location of 173.9 cM and microsatellite marker of *Ssa*416UoS at the location of 198.6 cM, whereas in chapter 3 the detected QTL for flesh colour was flanked by microsatellite markers *Ssa*9.44NUIG at the position of 68.7 cM and *Ssa*0021NVH at the position of 50.6 cM. The lack of linkage between the previous markers with the QTL localized in this study coupled with the position of QTLs on a different location in female maps suggests that a separate QTL for flesh colour might exist on linkage group LNS16. Additional studies are necessary to confirm occurrence of multiple QTLs for flesh colour trait on linkage group LNS16 and clarify their relationships. Another reason for different position of QTLs in these two studies could be the applied method of interval mapping for detection of QTL.

This method can bias identification and estimation of QTLs when multiple QTLs are located in the same linkage group (Lander and Botstein 1989).

The detected QTL for fat percentage on sire based analysis of linkage group LNS16 is positioned at 80 cM of joint map of AFLP and microsatellites in this study. Apart from family one, this QTL explained 4- 8.4 % of phenotypic variances in all other families. This QTL is flanked by the microsatellite marker *Rsa*I458 at the location of 43.9 and two AFLP markers, AGGCTC237 and AGGCTA237, both at the location of 94.0 cM. It is not clear whether these two AFLP markers are the same or an error has led to the identification of either one of them. It should be mentioned that in the sire based analysis of this linkage group in Chapter 3, the detected QTL for fat percentage was found on the location of 3 cM with the closest microsatellite marker of *Ssa*0016NVH at position 1.3 cM.

As it has previously been discussed the recombination rate in males is significantly lower than in females in Atlantic salmon. This phenomenon can perhaps explain the lack of tight linkage between the AFLP and microsatellite markers with QTL for fat percentage in sire based analysis. Ancestors of the current salmonids underwent tetraploidization event (25-100 million years ago) and have not fully returned to diploid state. In particular, the formation of multivalents at meiosis I in the male is thought to be related to the large difference in the recombination rate between the sexes in salmonids (Allendorf and Danzmann 1997; Allendorf and Thorgaard 1984). Therefore, it is more difficult to detect QTL locations based upon male genetic maps than female based genetic maps in salmonids.

In this chapter, the change of QTL profile was observed in linkage group LNS16 with addition of AFLP markers into the existing map of microsatellite markers from

previous chapter. Linkage analyses in several other studies have shown a non-uniform distribution of AFLP based markers, for instance Young et al. (1998), Robison et al. (2001) and Linder et al. (2000). These authors reported a clustering of AFLP markers around centromeric regions both in rainbow trout and pink salmon. These reports were based on the presence of a cluster of tightly linked AFLP markers at the centre of most of linkage groups. They speculated that non-uniform marker distribution of the AFLP markers was the result of the markers being located in regions of reduced recombination (i.e. near centromeres).

In published literatures, AFLP markers are distributed randomly in some species (e. g. Remington et al. 1999) but cluster in others (Sakamoto et al. 2000). In general, AFLP markers tend to cluster around regions where recombination is suppressed (centromere and telomere). The reasons for AFLP clustering are not well known yet. Some potential causes are proposed, which includes the bias in the base composition of certain genomic regions (Linder et al. 2000). The recognition sites *Eco*RI and *Mse*I are highly biased toward A and T and it is known that some centromeric regions in pink salmon are highly saturated (more than 90%) with A/T (Linder et al. 2000). Therefore, it is suggested that the use of *Mse*I (which cuts more frequently in high A/ T regions due to its restriction sequence) may result in an accumulation of AFLPs near the centromere (Linder et al. 2000). In other words, the restriction enzyme used in AFLP analysis can bias marker distribution to different regions. In soybean, AFLP markers generated using *Eco*RI / *Mse*I deviated significantly from a random distribution while markers generated with *Pst*I / *Mse*I did not greatly influence marker placement (Young et al. 1999).

In research carried out by Moen et al. (2004a) it is also shown that a large number of AFLP markers could not be linked to other markers. In addition to 64 *Eco*RI / *Mse*I, they also employed 18 *Pst*I / *Mse*I restriction enzymes leading to higher coverage of the genome. This may also explain the lack of homology between linkage group one in this study and the genetic map of linkage group one reported by Moen et al. (2004a).

Although application of AFLP technology rapidly provides many polymorphic markers and also requires less DNA than other genetic markers, but non-uniform distribution of AFLP markers appears to be a major limiting factor for utility of these markers, in particular for mapping distal regions of chromosomes containing QTLs.

Despite the advantages that AFLP offers in comparison to other DNA marker technologies, it seems that AFLPs are less transferable among labs and populations. Moreover, tendency of AFLP markers to cluster around the centromere is leading to incomplete genome coverage of this type of markers (Young et al. 1998).

Thus, it is concluded that microsatellite markers are better for linkage mapping than AFLP because of their high polymorphism, heterozygosity, co-dominance and wide transportability across different populations.

As a result, addition of microsatellite markers into the linkage groups carrying targeted QTLs may prove more useful than AFLP markers. Effectiveness of marker-assisted selection depends on how closely genetic markers are linked to the genes controlling the phenotype trait. Unlike AFLPs, microsatellite markers are evenly spaced and would enable complete coverage of genome.

The general conclusion of this study is that in this population of Atlantic salmon, AFLP markers generated using *EcoR*I / *Mse*I are probably not very helpful for fine mapping of chromosome regions that carry QTLs. Therefore, it is suggested that different restriction enzymes such as *Pst*I / *Mse*I must also be tested in order to obtain better marker distribution with the AFLP technique. Bottom line, the dominant nature and clustering character of AFLP markers make their use in mapping experiments more limited than microsatellite markers.

Chapter 5 - General Discussion and Conclusion Remarks

5.1 Discussion

In the last two decades, the invention of the polymerase chain reaction (PCR) facilitated the development of new molecular techniques that can be used to identify chromosomal regions carrying quantitative trait loci (QTL). Using a variety of new molecular techniques, many studies have identified several QTLs affecting economic traits in salmonids. Allendorf and Thorgaard (1984) have postulated that salmonid fishes are undergoing a diploidization state after the event of tetraploidization 25-100 million years ago. The re-diploidization process and the phenomenon of pseudolinkage (false linkage that occurs exclusively in males) have generally led to difficulties in interpreting linkage mapping in salmonids.

The prime interest of this study was to search for QTLs controlling quality traits such as flesh colour and fat percentage in Atlantic salmon. A moderate heritability (0.20-0.30) for fat percentage (Rye and Gjerde 1996) and flesh colour (0.12-0.14) (Norris and Cunningham 2004) have been reported in Atlantic salmon. In the population under study, the estimated heritabilities for fat percentage (0.17) and flesh colour (0.15) are well in within the range of those reported by the above mentioned authors. These moderate estimates of heritabilities for quality traits demonstrate that ample genetic variations are available for improvement through traditional breeding programs or in conjunction with the new molecular techniques in the form of marker assisted selection (known as MAS).

In Chapter 2 of this study, a genome-wide scan using microsatellite markers was performed within commercially bred families to search for chromosomal regions

harbouring QTL for a range of commercially important harvest traits. My results in chapter 2 revealed the significant evidence of QTLs for fat percentage and flesh colour on the linkage groups LNS16 and LNS1, respectively. In addition, the results showed that significant QTL for harvest length and suggestive QTL for harvest weight and gutted weight were also residing on the linkage group LNS1. The occurrence of QTLs for harvest weight and harvest length on linkage group LNS1 was suggestive that multiple QTLs might be residing on the linkage group LNS1 in this population of Atlantic salmon. The occurrence of QTL for condition factor on different linkage groups (LNS3, LNS10 and LNS23), suggested that traits of body weight and condition factor are controlled by different set of genes.

In Chapter 3, fine mapping with microsatellite markers located within the region of these linkage groups resulted in identifying the microsatellite markers that were closely linked to the QTL responsible for flesh quality traits. In a sire-based analysis of linkage group LNS16, microsatellite marker *Ssa*0016NVH at position 1.3 cM was found to be closely linked to the QTL affecting fat percentage (the position of the QTL on the linkage group LNS16 was found to be at 3.0 cM).

In dam based analysis, I found evidence for a significant QTL affecting flesh colour at the location of 63.0 cM. On the female map on linkage group LNS16 the closest marker to this QTL are microsatellite markers of *Ssa*0021NVH at position 50.6 cM and *Ssa*9.44NUIG at position 68.7 cM. On sire based analysis of this linkage group, no QTL with effect on flesh colour was detected.

Due to a higher recombination rate in female salmonids, the localized QTL based upon the female map is more representative of a true QTL than the male based QTL (Sakamoto et al. 2000). The very large differences in recombination rate between the

sexes in Atlantic salmon have been reported in different studies. For instance, the female recombination rate vs. male recombination rate reported by Moen et al. (2004a) was 8.26:1.0 whereas the ratio reported by Gilby et al. (2004) was 3.92:1.0.

In dam based analysis of linkage group LNS1, a suggestive QTL affecting flesh colour at the location of 114 cM was found. From the female map of linkage group LNS1 (Table 15 Chapter 3) it is evident that the microsatellite marker *Ssa*202 is the closest marker to this QTL. The detection of a QTL based on dam analysis for flesh colour on a sex determining group may suggest that flesh colouration could be sex dependent. But the long distance between the detected QTL for flesh colour (at 114 cM) and the position of the sex determining locus (at 139.1 cM) suggests that these loci are remotely linked to each other. The map distance between *Ssa*202 and the sex determining region was estimated 4.2 cM by Woram et al. (2003) and 4.81 cM by Gilby et al. (2004). Therefore, it is concluded that the flesh colour QTL found in the current study is not a sex dependent trait. Further studies of the linkage mapping are needed to clarify these relationships.

Sex-linked genetic markers for salmonids have been known for several years. In rainbow trout, the sex determining locus on linkage group 18 is linked to two microsatellite markers, *Omy*FGT19TUF and *Omy*RGT28TUF (Sakamoto et al. 2000).

Recently a dominant SCAR marker *Oki*206 (an RAPD marker that shows a significant association with the trait) linked to QTL associated with flesh colour was reported in Coho salmon (Araneda et al. 2005). Other than this, there seems to be little evidence for sex linked QTL in other fish species.

In Chapter 4 further fine mapping with AFLP markers, with the purpose of increasing marker density in the relevant linkage groups was performed in order to find a closer

marker linked to QTL affecting flesh quality traits. Similar to other fish genome AFLP studies, a combination of *Eco*RI / *Mse*I as restriction enzymes was employed. 24 primer combinations resulted in a total of 489 polymorphic fragments. The microsatellite map constructed in chapter 3 was used as a bridging framework for linkage mapping of AFLP markers to the relevant linkage group. Nine AFLP markers (AAGCAC181, AAGCAC328, AAGCTA296, AAGCTG67, AGGCAC163, AGGCAC448, AGGCAG447, AGGCTA237, and AGGCTC237) were found to be significantly linked to the microsatellite markers that were residing on linkage group LNS16. The dam-based analysis of linkage map LNS16 consisting of microsatellite and AFLP markers revealed a significant QTL affecting flesh colour at different position than the QTL found using only microsatellites markers map (Chapter 3). More research is needed to determine the occurrence of multiple QTLs affecting flesh colour in this linkage group.

Four of the AFLP markers (AAGCAC328, AGGCAG447, AGGCTA237 and AGGCTC237) on linkage group LNS16 were linked to the microsatellite marker *Ssa*9.44NUIG (which in dam based analysis was found to be linked to a QTL for flesh colour trait at position of 68.7 cM). None of these polymorphic fragments were linked to the microsatellite marker *Ssa*0016NVH which in sire-based analysis was linked to a QTL for fat percentage on linkage group LNS16. Since the QTL for fat percentage is localized within the male map, the reduced recombination rate in males can possibly explain the lack of linkage between the microsatellite *Ssa*0016NVH and AFLP markers.

Linkage analysis between microsatellite markers from linkage group LNS1 (*Ssa*202, *Ssa*0244, *Ssa*0082, *Rsa*I485, and *One*μ18/1) and AFLP fragments revealed no linkage

at all between these two type of markers. There are three possible reasons for lack of linkage in between AFLP and microsatellite markers: 1-genotyping error on AFLP data collection, 2- abnormal segregation pattern which is known to occur in male salmonids, 3- due to the mapping families being from a pure strain, whereas most of other mapping experiments are performed on crosses between divergent lines.

Because of very high difference in the recombination rate between the sexes, Moen et al. (2004a) constructed the sex specific maps for male and female with two different methods. Similar to this study, a large proportion of the AFLP markers were unlinked, therefore, they concluded that the low information content of AFLP markers might have been the main reason for lack of linkage in this type of markers.

The comparison between microsatellite marker residing on linkage group LNS1 in this study and those of male map (*Ssa*401UoS, Ssa22, *Omy*301UoG, BHMS313B, *Ssa*87 and Ssa197) and female map (BHMS313B, *Ssa*401UoS, *Ssa*22, *Ssa*87, *Omy*301UoG and *Ssa*197) of linkage group one published by Moen et al. (2004a), did not provide evidence to confirm the homology between these linkage groups in the two separate studies. From 11 AFLP fragments residing on the male map of linkage group one cited by Moen et al. (2004a) only one fragment (AGGCAT376) was found segregating in family three in the current study.

The most noticeable difference between this study and the one carried out by Moen et al. (2004a) is that they employed a total of 82 primer combinations of *EcoR*I / *Mse*I and *Pst*I / *Mse*I whereas in my research I only employed 24 primer combinations of *EcoR*I / *Mse*I. Therefore, further research will be needed to examine a wider range of primer combinations of AFLP markers (for example the use of primer combinations

of *Pst*I / *Mse*I) to draw a firmer conclusion on the true genome coverage of AFLP markers on commercially bred families of Atlantic salmon.

From the male map of linkage group one in Atlantic salmon published by Woram et al. (2003), three AFLP fragments (ACCCAG418, ACTCAG232 and ACTCTG71) residing on this linkage group are of a different primer combination than those I employed. From six AFLP markers residing on female map of linkage group one (AACCTT97, ACTCTG71, ACTCAC148, AAGCAT218, AGCCTG565, AACCTA236) reported by Woram et al. (2003), none was detected among entire AFLP markers in the current study.

Although, in dam based QTL analysis of a joint map of microsatellite and AFLP markers in linkage group LNS16 a significant QTL for flesh colour was detected but the location of this QTL differed significantly based upon the map built from microsatellite markers alone (Chapter 3). The reason for this disparity is not known, but could have risen because of either marker re-arrangement in this linkage group or that two separate QTLs for flesh colour might exist on this linkage group.

From the joint map of AFLP and microsatellite markers, a QTL for fat percentage was detected in sire based analysis of linkage group LNS16. This QTL is flanked by microsatellite *Rsa*I458 at the location of 43.9 cM and two AFLP markers of AGGCTC237 and AGGCTA237 (both at location of 94.0 cM). The only difference between these two AFLP fragments is a single nucleotide of A or C. These two AFLP markers are either located at exactly the same place or an error in data collection may have caused this situation. However, it is more difficult to pinpoint QTL positions based on male linkage map, because of lower recombination rate in the male map.

Despite the various reports implying that AFLP could lead to improved marker densities and can aid in eliminating gaps with no marker converge in the distal parts of chromosomes (for example, Knorr et al. 1999), my results suggest that AFLP markers can be of limited use for fine mapping of chromosomal regions and consequently may have less use for MAS programs in Atlantic salmon. Had I more time and resources I would have applied different combinations of enzymes to draw more comprehensive conclusion about AFLP markers distribution in the genome.

Several other linkage analysis studies have also shown a non-uniform distribution of AFLP based markers (Young et al. 1998; Robison et al. 2001; and Linder et al. 2000). In addition to results form this study, other reports suggest that the clustering character of AFLP markers around centromeric regions in salmonids may limit their utility for identifying QTL. A reduced recombination rate in male salmonids may have further impact on tighter clustering of AFLP markers around the centromeric region in males than in females, especially in Atlantic salmon.

It has been speculated that biases in the base composition of certain genomic regions could be a reason for the clustering character of AFLP markers (Linder et al. 2000). It is therefore suggested that the restriction enzymes used in AFLP analysis may cause uneven distribution of marker to different regions (Young et al. 1999).

In addition to the dominant nature, the non-uniform distribution of AFLP markers appears to be a major limiting factor for the utility of these markers, especially for mapping distal regions of chromosomes and identifying quantitative trait loci residing in these regions.

A primary concern with any genetic marker is reproducibility. My own experience of genotyping with AFLP has been that obtaining complete restriction and ligation is the most important factor to success.

Single nucleotide polymorphisms (SNPs) are the most abundant type of variation in DNA sequences among individuals in agricultural and aquaculture species. As a part of Canadian and Norwegian salmon genome projects, an extensive resource of putative SNPs for Atlantic salmon has been described by Hayes et al. (2007). These putative SNPs are considered as a highly valuable resource for making a dense genetic map and for fine mapping of QTL affecting economically important traits of Atlantic salmon in the near future.

5.2 Prospects for implementation of marker-assisted selection

A marker-assisted selection (MAS) program is the use of genetic markers linked to QTL in selection program. Use of MAS is especially interesting for flesh quality traits because the improvement of flesh quality traits is difficult using conventional selection methods. The relative efficiency of MAS is higher for traits showing both low heritabilities and an inability to measure the trait in the individuals considered for selection (Lande and Thompson 1990). MAS programs have been implemented successfully in a number of plant breeding programs (Kumar 1999).

Microsatellite and AFLP genetic linkage maps available for commercially important salmonids such as Atlantic salmon, rainbow trout and Arctic charr have facilitated a genetic framework for detection of QTL and consequently the development of MAS programs in the near future. Tight linkages between QTL (such as spawning time, upper temperature resistance and resistance to IPN) and several microsatellite markers found in salmonids could be used as candidate traits for marker assisted selection.

Theoretically, genotypic information on spawning time could be used to extend the spawning time in rainbow trout. Disease QTL could also be utilized to produce a disease resistance strain of salmonids. Even more exciting is the possibility of using molecular marker information for delaying or suppressing early sexual maturation which is undesirable because the flesh quality of the individual is reduced, leading to a non-marketable product.

With these new possibilities, many considerations must first be taken before MAS can become a reliable and essential technique used in salmonids breeding programs. The success of MAS program is a function of its predictive reliability. This reliability in turn, depends on many variable factors such as marker efficiency, rates of recombination within each linkage group and interactions between genes and environment.

In the livestock industry, concerns have been expressed that the extensive use of molecular markers has not lived up to initial expectations. For instance, Dekkers (2004) claimed that although opportunities for the use of molecular information exist, their successful implementation requires a comprehensive strategy which must be closely linked to business goals. In salmonids, despite the potential of MAS programs to accelerate the rate of improvement in production traits, so far only little is known about the genetic variability that can be used to improve these traits by selection of favourite alleles.

Today, the general belief is that a combination of traditional selective breeding (for identifying growth traits) and MAS program (designed for improving quality traits and disease resistance) could be more beneficial to the salmon industry (Fjalestad et al. 2003). Growth is relatively easy to measure but meat quality traits are difficult and

usually costly to measure. Therefore, it is hard to improve the quality traits by conventional breeding program.

The cost of implementing a marker based selection must also come into consideration when assessing the program. Walsh and Henderson (2004) suggested that a thorough cost-benefit analysis must be carried out prior to genomics-based approach for any particular selective breeding. However, predictions of the benefits of MAS are that genetic progress may increase by around 11% relative to conventional BULP, under certain circumstances (Gomez-Raya and Klemetsdal 1999).

Locating QTL and markers linked to them is the first step toward the implementation of MAS program (Davis and DeNise 1998). Several factors influence the chance of detecting a QTL including: the size of the effect of the frequency of the alleles, the density of the genetic map, the heritability of the trait, the variation among animals, the number of animals studied and the method of analysis (Beuzen 2000).

Maintenance of linkage between the marker and the QTL across generations is also crucial for an effective implementation of MAS program. The linkage between the marker and the QTL depends on the recombination rate between the QTL and genetic markers. Recombination rates vary considerably between sexes in Atlantic salmon. Recombination rate in female Atlantic salmon is more than eight times greater than in males, suggesting that MAS could be more effective using genomic information from the sire (Moen et al. 2004a).

Moreover, recombination in males is primarily restricted to the telomeric regions (Sakamoto et al. 2000), therefore, the linkage relationship between a telomeric marker and a QTL can be altered more easily across generations than a marker situated closer to the centromere region. As result, using markers closer to the centromere in MAS

programs could be more efficient than telomeric markers. However, QTL are more likely to be detected in centromeric regions in male salmonids compared to females. Such differences in QTL variability between the sexes in salmonids could also act as limiting factor for implementation of MAS programs. As an alternative, if differences in recombination rate among male and female salmonids create difficulties for MAS, sex-specific MAS strategies might be required.

For a successful implementation of MAS, the knowledge of possible epistasis effects (the interaction among QTL and their expression in different genomic backgrounds) that may influence the expression of QTL is also required. For instance, Danzmann et al. (1999) demonstrated that the effects at alleles associated with upper temperature tolerance QTL in rainbow trout varies when they were expressed in a high or low temperature selected background.

Pleiotropy of QLT, or the influence of one gene on multiple traits, must also be investigated. O'Malley (2001) showed that QTL influencing both body weight and spawning time in rainbow trout are located on the same linkage groups suggesting that this may represent a pleiotropic gene or a number of tightly linked genes affecting both traits. Similarly, the hypothesis of a single QTL with pleiotropic effects or gene cluster with individual QTL was suggested when QTL for body weight and condition factor were detected on the same linkage groups in Atlantic salmon (Reid et al. 2004). Increasing marker density (especially in the female parent, since males show greatly reduced recombination rates) or the utilization of larger families for detecting rare recombination events can help to understand the QTL function and interaction.

The use of genetic markers for parental assignment that is currently available in the Atlantic salmon industry could also allow wider application of MAS programs. For

instance, use of genetic markers in parental assignment reduces the pedigree errors and will increase the accuracy of breeding value. In the salmon industry, microsatellite markers are chosen as the marker of the choice for parental assignment, while the most valuable potential contribution of these markers could be in MAS programs in future.

5.3 Conclusion

Although gene technology has opened new opportunities for exploiting genetic variation controlling production traits, selective breeding remains the most successful technique for increasing production in today's salmon industry. I have not come across any report of a selective breeding program that includes molecular information to select fish. Accordingly, all salmon breeding companies are operating their selection program based on phenotypic information. The cost of genotyping and the magnitude of genetic improvement are seemingly the two major factors that could characterize the successful implementation of MAS programs in the salmon industry. Moreover, in the case of Atlantic salmon, a long generation interval of 3-4 years should also be taken into the consideration when planning QTL mapping and their application in MAS program.

Perhaps, the most exciting is the opportunity to utilize MAS programs to maximize genetic improvements in disease and meat quality traits, at the same time using quantitative genetics to address issues surrounding growth.

The genetic improvement of quality traits such as flesh colour could be best served through the careful application of existing approaches modified to incorporate marker information. For instance, co-selection of molecular information associated with flesh colour (such as microsatellite marker *Ssa*9.44NUIG that I detected in the current study

for Atlantic salmon or the marker *Oki*206 reported by Araneda et al. (2005) in Coho salmon) could be experimentally combined with phenotypic data to select superior fish in terms of better flesh colour with higher growth rate. This approach has the advantage of utilizing selection intensity that has not been exploited.

It is also likely that traits such as flesh colour and fillet fat percentage are controlled by a large number of loci, many of them with a small effect on the quantitative trait and relatively few loci with a large effect. Genes with a small effect are difficult to map and it is unlikely that molecular markers linked to those genes could be possibly utilized in MAS programs.

In summary, this study has detected a number of chromosomal regions that influence production traits in hatchery strains of Atlantic salmon. QTL for quality traits (flesh colour and fillet fat) were detected on linkage groups LNS16 (autosomal chromosome) and LNS1 (sex chromosome).

Future studies should continue to investigate the methods for fine mapping of these chromosomal regions. An increase in marker density of the current map and location of functional genes will provide further insight into the genetic structure of quality traits in Atlantic salmon. Identifying major QTL that influence flesh colour and fillet fat percentage in other aquacultureally important fish such as rainbow trout and Arctic charr can also help our understanding of QTL function on closely related members of the Salmonidae family.

Future investigations must also focus on the sex chromosome to examine whether it carries QTL with major affects on fitness or quality related traits in Atlantic salmon. In previous studies, a suggestive QTL for body weight and condition factor has been reported on linkage group carrying the sex-determining locus in Atlantic salmon (Reid

2003). QTL for thermal tolerance in sex linkage groups have also been reported in rainbow trout (Perry 2001) and Arctic charr (Somorjai 2001).

With the regards to the type of molecular markers, the general conclusion of this study is that AFLP markers are not very helpful for fine mapping of QTL especially for those QTL residing on distal regions chromosomes. The dominant nature and clustering character of AFLP markers make their use in mapping experiments more difficult than other markers. Microsatellites genotyping showed satisfactory results for detection of QTL in Atlantic salmon and I suggest that these markers would be the preferable type of markers for detection of QTL, especially if QTL are to be found on distal regions of chromosomes.

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Appendix 1.

Phenotypic data in this study was provided by Landcatch LTD, a commercial breeder of Atlantic salmon in Scotland UK. The raw data utilised in this study was from 5 families of a commercial strain of Atlantic salmon. The broodstock of these families were spawned between October 2001 and December 2001 and the eggs were hatched in the period of two weeks time during March 2002. Each family was reared in the individual tanks for a period of 6 months and then were PIT tagged and moved into the communal rearing unit. Measurements of the body weight and the other characteristics were taken when fish were harvested at approximately 23-24 months of age.

Appendix 2.

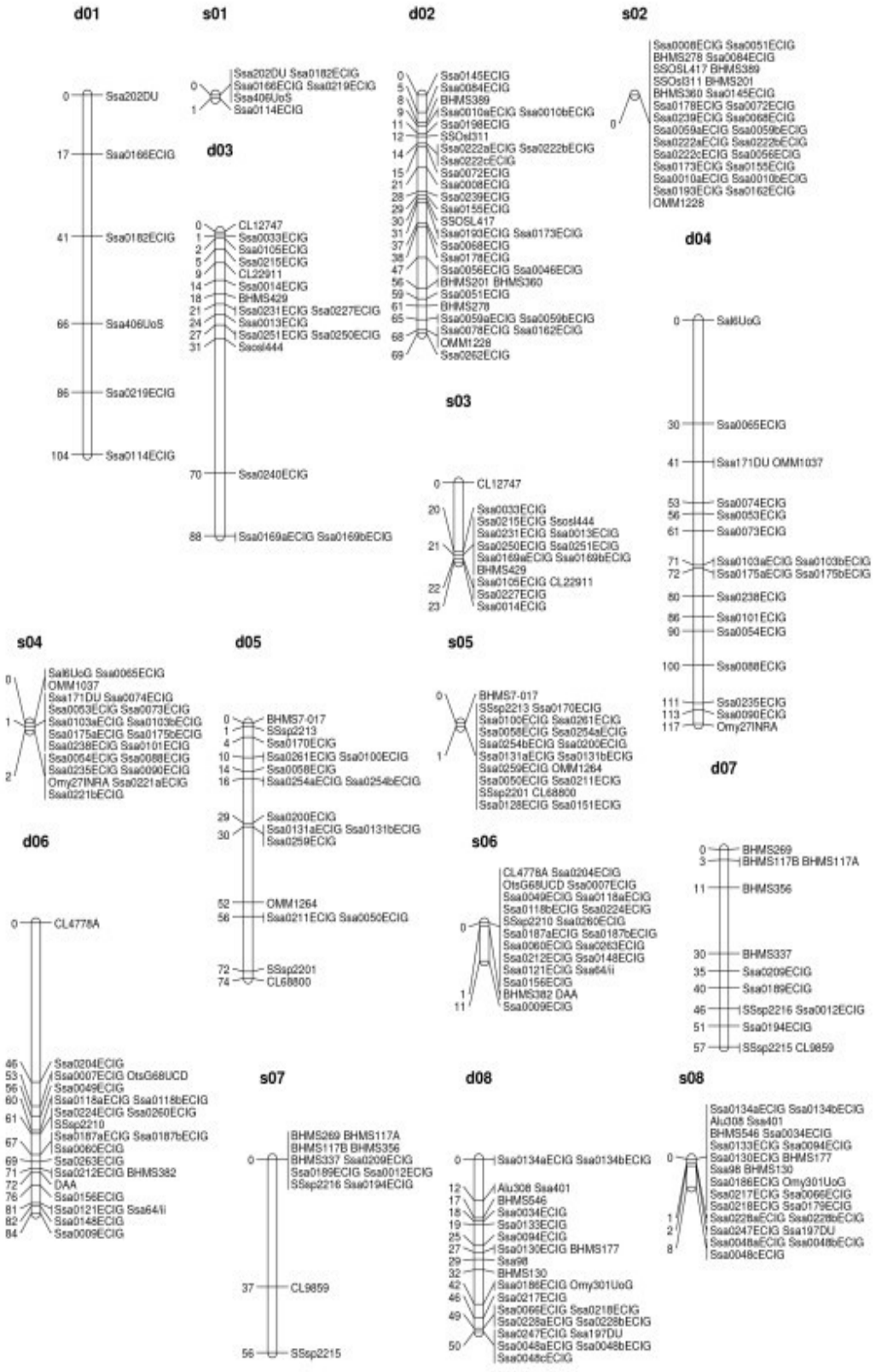
The raw data obtained from microsatellite genotyping of parents and offspring of 5 families. Using various microsatellite loci, parents from each family were first genotyped, then the offspring were genotyped only for those loci that showed acceptable level of informativeness (parents were either heterozygous at the different alleles or homozygous at the same alleles). Non informative microsatellite loci in parents were discarded without further genotyping of the offspring.

Appendix 3.

The raw data obtained from AFLP genotyping of 5 families used in the study. Parents and offspring of these families were genotyped using 24 AFLP primer combinations. The loci were scored as dominant markers. For band presence the code of 2-0 (AA or Aa) and for band absence (aa) the code of 1-1 was given, respectively. Among these families the totals of 392 AFLP markers was detected.

Appendix 4.

Atlantic salmon linkage map. Male and female linkage map of Atlantic salmon published by Moen et al. (2008). Prefix of s and d are given to male and female linkage groups, respectively.


d01
0 Ssa202DU
17 Ssa0166ECIG
41 Ssa0182ECIG
66 Ssa406UoS
86 Ssa0219ECIG
104 Ssa0114ECIG
s01
0 Ssa202DU Ssa0182ECIG
0 Ssa0166ECIG Ssa0219ECIG
Ssa406UoS
1 Ssa0114ECIG
d03
0 CL12747
1 Ssa0033ECIG
2 Ssa0105ECIG
5 Ssa0215ECIG
9 CL22911
14 Ssa0014ECIG
18 BHMS429
21 Ssa0231ECIG Ssa0227ECIG
24 Ssa0013ECIG
27 Ssa0251ECIG Ssa0250ECIG
31 Ssosl444
70 Ssa0240ECIG
88 Ssa0169aECIG Ssa0169bECIG
d02
0 Ssa0145ECIG
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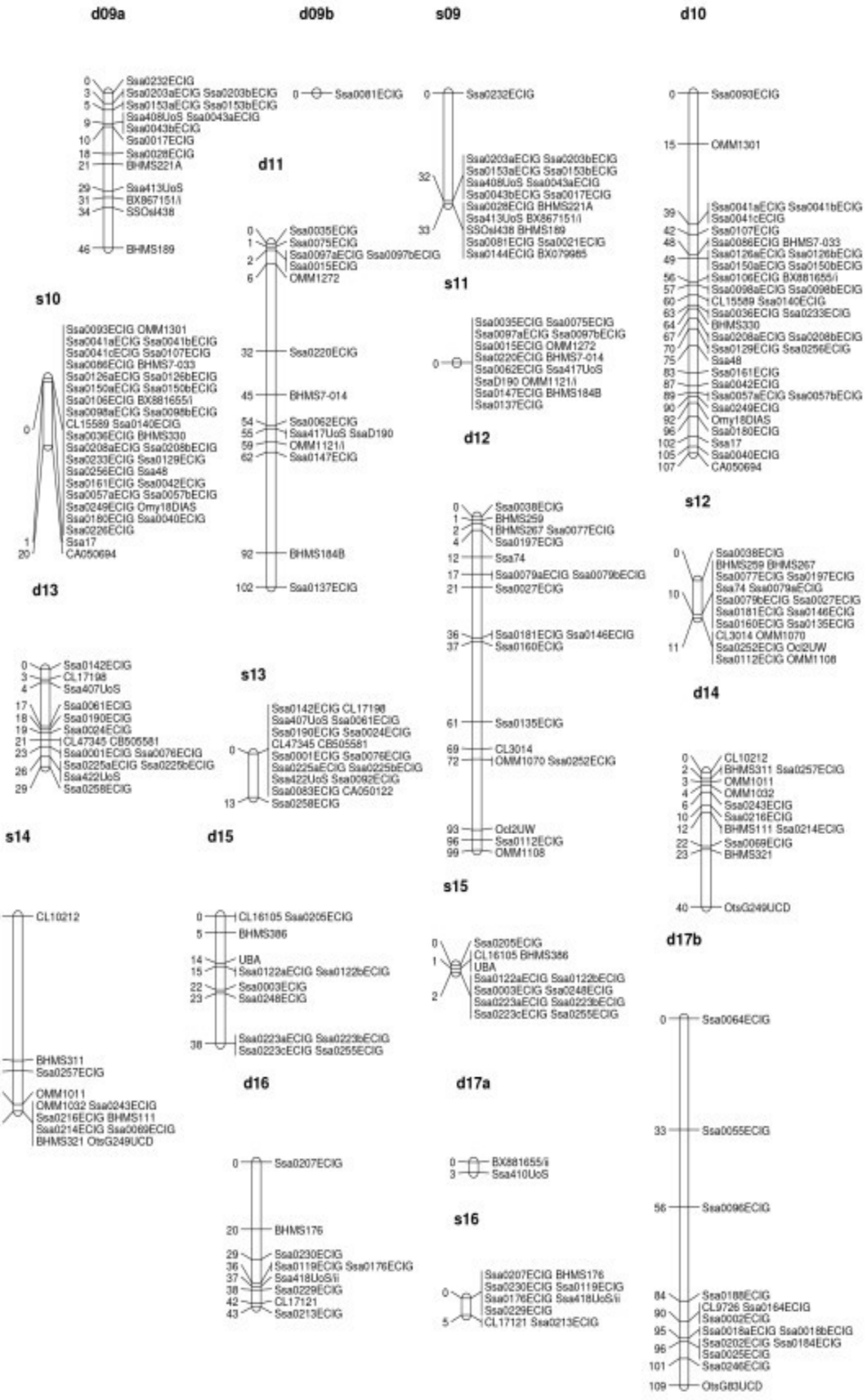
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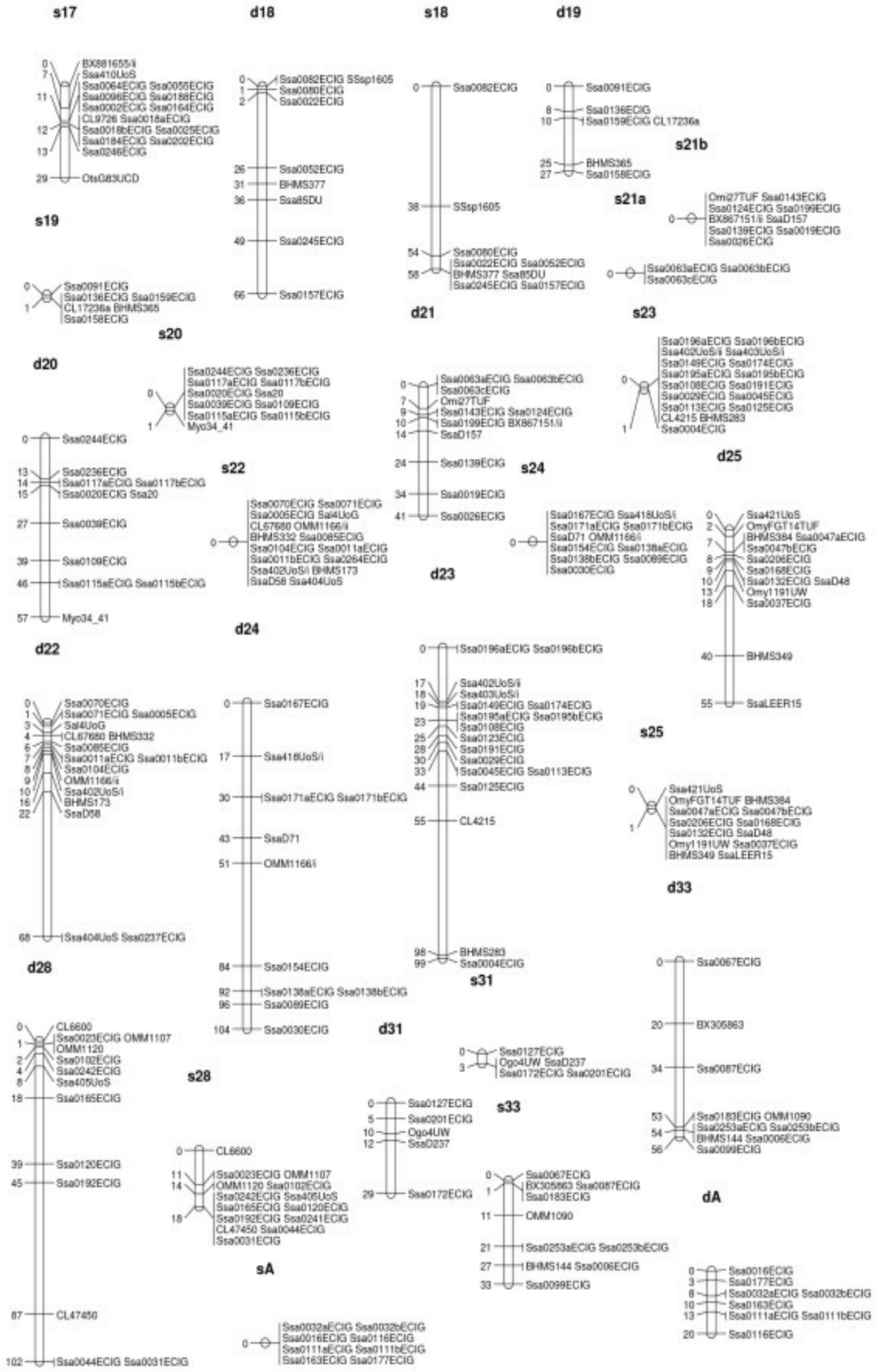
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