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(54) **Title**: METHOD FOR DETECTION OF PYRETHROID RESISTANCE IN CRUSTACEANS AND OLIGONUCLEOTIDE SEQUENCES USEFUL IN DETECTION OF PYRETHROID RESISTANCE.

(57) **Abstract**: The present invention relates to methods for detecting pyrethroid resistance in crustaceans, such as copepods, including copepods belonging to the family Caligidae, and oligonucleotide sequences comprising small nucleotide polymorphism (SNPs) associated therewith.

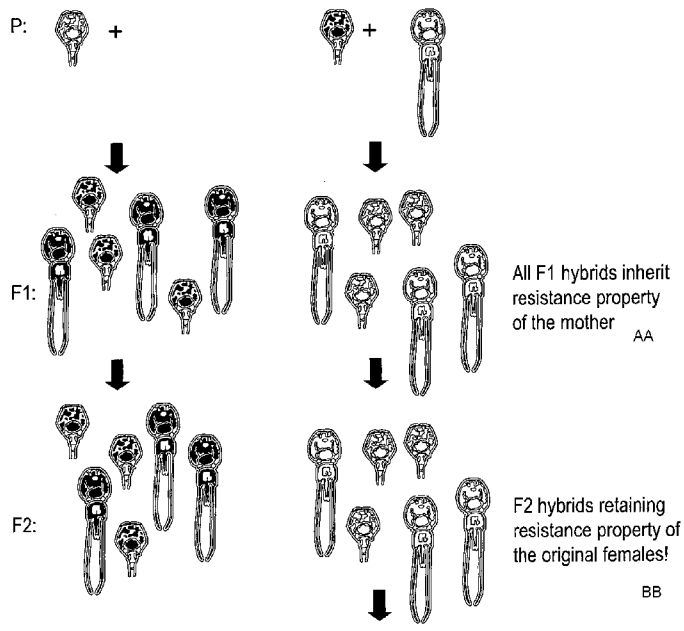

FIG. 1

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Method for detection of pyrethroid resistance in crustaceans and oligonucleotide sequences useful in detection of pyrethroid resistance.

Field of the invention

The present invention relates to a method for detection of single polymorphisms associated with pyrethroid resistant *Lepeophteirus salmonis*, and oligonucleotide sequences and kits useful in the method of the present invention. The present invention furthermore provides kits and reagents useful for the detection of pyrethroid resistance associated SNPs.

Background of the invention

Infestations of the parasitic copepod *Lepeophtheirus salmonis*, commonly referred to as sea lice, represent a major challenge to commercial salmon aquaculture. Control measures have been reliant upon the use of a limited number of chemotherapeutants since the 1970's, and reduced efficacy has now been reported for the majority of these compounds. Reduced sensitivity and potential resistance to currently available medicines are constant threats to maintaining control of sea lice populations in Atlantic salmon farms. Today, resistance against most of the available treatment options is widespread in almost every region where salmonids are cultured in sea water. Double- or triple resistance, in which sea lice is resistant to more than one type of chemotherapeutant, have been verified.

Sea lice (*Lepeophtheirus salmonis* and *Caligus* spp.) are the major pathogens affecting global salmon farming industry and have a significant impact in many areas. The annual loss has recently been estimated to €300 million (**Costello M. J. (2009).** The global economic cost of sea lice to the salmonid farming industry. Journal of Fish Diseases. **32.** 115–118) and the aquaculture industry relays heavily on a few chemotherapeutants for lice control. Emerging resistance development to these drugs increase the necessity to develop new treatment methods (biological, prophylactic and drugs) and tools to avoid increased loss due to sea lice and to ensure a sustainable salmon farming industry in the future. Control measures have relied upon a limited number of chemotherapeutants since the 1970s. Parasite resistance and reduced efficacy have now been reported for the majority of these compounds (Sevatdal S., Copley L., Wallace C., Jackson D., Horsberg T.E. (2005). Monitoring of the sensitivity of sea lice (*Lepeophtheirus salmonis*) to pyrethroids in Norway, Ireland and Scotland using bioassays and probit modeling). Aquaculture 244. 19-27). In many regions, the recent development of resistance to emamectin benzoate has resulted in heavy mortalities, significant economic impact as well as increased prevalence of other infectious diseases (Bravo S., Erranz F., Lagos C. (2009) A comparison of sea lice, *Caligus rogercresseyi*, fecundity in four areas in southern Chile. Journal of Fish Diseases. 32. 107–113).

Although various chemotherapeutants have been in use in the aquaculture industry for more than 30 years, it is only during the last 15 years that such use has been part of some kind of integrated pest management system. Management practices include coordinated salmon production within a defined area, use of single year class of fish, limited production period, fallowing, coordinated restocking, use of wrasse, synchronized treatments during

the winter and targeting female lice to reduce the impact of settlement during the spring (Pike A., Wadsworth S. L., (2000), Sea Lice: A review. Advances in Parasitology. Academic Press. 44. 232-337).

Further controls are required to progress towards a true integrated pest management (IPM) system common to other forms of food production. One key to succeeding with a IPM is to develop tools for the management of resistance to the medicines in use (Brook K. (2009). Considerations in developing an integrated pest management program for control of sea lice on farmed salmon in Pacific Canada. Journal of Fish Diseases. 32. 59–73). So far, drug resistance in sea lice has been detected by various types of bioassays as a significant increase in EC50/LC50. Although the bioassays can detect any type of resistance to a given drug, such methods are not very accurate or sensitive.

The genomes of all organisms undergo spontaneous mutations during their continuing evolution, forming variant forms of progenitor genetic sequences. A mutation may results in an evolutionary advantage or disadvantage relative to a progenitor form or may be neutral. A variant that result in an evolutionary advantage may eventually be incorporated in many members of the species and may thus effectively become the progenitor form. Furthermore, often various variant forms survive and coexist in a species population. The coexistence of multiple forms of a genetic sequence gives rise to genetic polymorphism, including single-nucleotide polymorphisms (SNPs).

A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an organism. For example, two DNA fragments from different individuals, AAGCCTA to AAGCTTA, contain a difference in a single nucleotide, commonly referred to as two alleles. Almost all common SNPs have only two alleles. The genomic distribution of SNPs is not homogenous; SNPs usually occur in non-coding regions more frequently than in coding regions or, in general, where natural selection is acting and fixating the allele of the SNP that constitutes the most favorable genetic adaptation (Adapted from Wikipedia).

Previously, non-mendelian inheritance of resistance by reciprocal crosses between resistant and spider mite susceptible of being resistant towards a hydrazine carbazate derivate have been linked to mutations in mitochondrial DNA of the spider mite (Van Leeuwen, T., Vanholme, B., Van Pottelberge, S., Van Nieuwenhuyse, P., Nauen, R., Tirry, L., & Denholm, I. (2008). Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-Mendelian inheritance in action. Proceedings of the National Academy of Sciences of the United States of America, 105(16), 5980–5). This represents the only other known example of mutations in mitochondrial DNA linked to resistance towards an insecticide apart from the study presented here in sea lice. The spider mite however showed resistance towards a hydrazine carbazate derivate, and not towards pyrethroids as shown for in sea lice in the present application. Spidermite and sea lice are distantly related. Up to date, no SNPs consequently associated with chemotherapeutant resistance in sea lice have been reported, and particularly, no SNPs in the mitochondrial DNA has been linked to

pyrethroid resistance in any species. However, in many arthropods, specific mutations in the gene coding for the voltage gated sodium channel have been reported to result in an altered function of this channel, resulting in a decreased ability for pyrethroids to interfere with its function (Zlotkin E, (1999), "The insect voltage-gated sodium channel as target of insecticides", Annu Rev Entomol. 44:429-55).

Another mechanism which has been suspected to contribute in the resistance towards pyrethroids is enhanced detoxification capability by the parasite. From other arthropods, increased activities of monooxydases and unspecific esterases have been identified as mechanisms in question. However, esterases seem to play an insignificant role in sea lice, as the parasite has a very low background activity of these enzymes. On the other hand, unspecific monooxidases seem to play a significant role, indicated by a correlation between enzyme activity and reduced sensitivity.

The mitochondrial genes COI, A6, Cytb and 16sRNA of *Lepeophtheirus salmonis* has shown to display high levels of polymorphisms (Tjensvoll et al., 2006, Diseases of Aquatic Organisms, vol, 68, pp. 251 – 259), resulting in a significant number of haplotypes. However, the prior art is silent as to whether the large number of polymorphic sites have had any effects on the characteristics of the sea lice.

Pyrethroids are one type of chemotherapeutics used for delousing of commercial salmon aquaculture sites infested with sea lice. In Norway, the synthetic deltamethrin (AlphaMax™) and cis-cypermethrin (BetaMax™) are the most widely used pyrethroids. In Scotland and Ireland, cypermethrin (ExisTM) has been commonly used. The pyrethroids affect the sodium channels thus inhibiting the transmittal of nerve impulses in the synapses. Failure of pyrethroid treatment has been reported in Norway (deltamethrin and cis-cypermethrin) since turn of the century (Sevatdal and Horsberg, 2000, Norsk Fiskeoppdrett, vol. 12, pp. 34-35), and reduced sensitivity towards pyrethroids was documented in 2003 (Sevatdal og Horsberg, 2003, Aquaqulture, vol. 218, pp. 21-31).

Efficient and sensitive methods for diagnosing resistance are crucial in order to manage and control drug resistance. Early detection of reduced sensitivity to a chemical can enable effective countermeasures to be enforced at a time point when these have a greater probability of being effective. Therefore, accurate and speedy identification of pyrethroid resistant *Lepeoptheirus salmonis* (PRLS) is crucial. Detection of PRLS prior to treatment, and the use of such analyses after treatment to evaluate treatment efficacy constitutes an important determinant for the integrated pest management (IPM) in the aquaculture industry.

Summary of invention

The present invention is based on the surprising finding that resistance towards chemotherapeutics commonly used to combat sea lice infestation is linked to mutations in the mitochondrion genome of the sea lice *Lepeophtheirus salmonis*. More particularly, the present invention is based on the identification of novel single-nucleotide polymorphisms (SNPs) in the mitochondrion genome of the sea lice *Lepeophtheirus salmonis* shown to be

involved in the resistance towards pyrethroid-based chemotherapy. Specifically, the present inventors have identified two pyrethroid resistance-associated SNPs located in the mitochondrial CoI gene (T8605C, A9035G) and three pyrethroid resistance-associated SNP in the mitochondrial Cyt b gene (C13957T, A14017G and C14065T).

It is further noted that the identification of the SNPs involved in pyrethroid resistance in the mitochondrial genome proved to be challenging due to unexpected characteristics such as numerous repeats etc. of the non coding parts of the mitochondrial genom (D-loop).

The present invention thus provides an in vitro method for determination of pyrethroid resistance in crustaceans, comprising the steps of detecting single nucleotide polymorphism (SNP) associated with pyrethroid resistance in the mitochondrial genome of the crustaceans to be analyzed. The present invention furthermore provides for novel isolated sequences comprising SNPs involved in the resistance towards pyrethroid-based chemotherapy, and their use in determination of pyrethroid resistance in crustaceans. In particular, said method is useful for detection of pyrethroid resistance in copepods, in particular copepods belonging to the family Caligidae, in particular species selected from the group consisting of *Lepeophteirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*.
According to one embodiment, the present invention provides a method for detection of pyrethroid resistance in sea lice, comprising the steps of detecting single nucleotide polymorphism (SNP) associated with pyrethroid resistance in the mitochondrial genome of a sea lice to be analyzed, wherein said sea lice is resistant to pyrethroids if at least one of the following nucleotides:

i. C in position 8605;
ii. G in position 9035;
iii. T in position 13957;
iv. G in position 14017;
v. T in position 14065;

or the complementary oligonucleotide thereof, is present in the mitochondrial genome sequence of the one or more sea lice to be analyzed, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1. According to one embodiment, the pyrethroid resistance linked SNPs mentioned above is detected in Lepeophteirus salmonis.

According to yet another aspect, a method is provided, comprising the steps of: a) collecting sea lice from infested fish or water samples; b) isolating mitochondrial genomic material from any life stage of collected sea lice; c) determining the nucleotide polymorphic site at the positions 8605, 9035 and 14065 of the isolated mitochondrial DNA compared with of the mitochondrial nucleic acid sequence SEQ ID No. 1, wherein said sea lice is resistant to pyrethroids if at least one of the nucleotide C, G, T, G, and T (U), or a complementary oligonucleotide thereof, is present in position 8605, 9035, 13957, 14017, and 14065, respectively.

According to one embodiment of the above method, said step c) is performed using a primer selected from the group consisting of SEQ ID No. 6-15.

A method according to yet another embodiment, said step c) is performed using at least one probe selected from the group consisting of SEQ ID No. 16-20.

According to yet another embodiment, said step c) comprises nucleic acid amplification, e.g. using polymerase chain reaction.

According to yet another embodiment, said step c) is performed by contacting mitochondrial DNA sequence of the sea lice to be analyzed with a detection reagent, and determining which nucleotide is present in position 8605, 9035, 13957, 14017 and 14065.

According to yet another embodiment, said detection reagent is an oligonucleotide probe.

According to yet another embodiment, said step c) is performed using SNP specific probe hybridization, SNP specific primer extension, SPN specific amplification, sequencing, 5' nuclease digestion, molecular beacon assay, oligonucleotide ligation assay, size analysis, single-stranded conformation polymorphism analysis, denaturing gradient gel electrophoresis.

According to another embodiment of the present method of the invention, the pyrethroid is selected from the group consisting of deltamethrin, and cis-cypermethrin.

According to another embodiment, the present invention provides an isolated oligonucleotide sequence comprising small nucleotide polymorphism (SNP) associated with pyrethroid resistance in crustaceans, such as copepods, in particular copepods belonging to the family Caligidae, in particular species selected from the group consisting of *Lepeophteirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*, , wherein said isolated oligonucleotide sequence comprises nucleotides that distinguishes sea lice which are resistant to pyrethroids from non-resistance to pyrethroids, and wherein the SNP are present in the mitochondrial DNA of the organism.

According to one embodiment, the oligonucleotide of the present invention comprises a single-nucleotide polymorphism (SNP) associated with pyrethroid resistance in sea lice, wherein said isolated oligonucleotide sequence comprises nucleotides that distinguishes sea lice which are resistant to pyrethroids from non-resistant sea lice, and which is identical or has at least 80% sequence identity with a sequence selected from the group consisting of SEQ ID No. 4 and 5 or a fragment thereof, and complementary sequences of SEQ ID No. 4 and 5 and fragments thereof, provided that at least one SNP selected from the group of SNPs consisting of T8605C (U8605C in case of RNA), A9035G, C13957T (U13957T in case of RNA), A14017G, and C14065T (C14065U in case of RNA) is present, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

According to one embodiment, an oligonucleotide probe or oligonucleotide primer is provided comprising a oligonucleotide sequence being homologous to a fragment of an isolated oligonucleotide sequence according to the present invention, said probe or primer being specific for a mitochondrial DNA sequence of sea lice associated with pyrethroid resistance comprising at least one SNPs selected from the group consisting of T8605C and A9035G of SEQ ID No. 4, andC13957T, A14017G, and C14065T of SEQ ID 5, and

wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

According to yet another embodiment, the probe or primer according to the present invention comprising at least 8 contiguous nucleotides of SEQ ID No. 1, including at least one of the nucleotide C, G, T, G, and T (U), or a complementary oligonucleotide thereof in the position corresponding to position 8605, 9035, 13957, 14017 and 14065, respectively, of SEQ ID 1.

According to one embodiment, a probe is provided, wherein the sequence of said probe is selected from the group consisting of SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19 and SEQ ID No. 20, and sequences having at least 80 % sequence identity therewith.

According to one embodiment, a primer is provided, wherein the sequence is selected from the group consisting of SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 and SEQ ID No. 14, and SEQ ID No. 15, and sequences having at least 80 % sequence identity therewith.

Furthermore, the present invention provides a kit for detection of pyrethroid resistance in crustaceans, such as copepods, e.g. in sea lice (Lepeophteirus salmonis), comprising a probe or primers according to the present invention.

Finally, the present invention provides an isolated oligonucleotide sequence comprising at least 8 contiguous nucleotides of the sequence selected from the group consisting of SEQ ID No. 4and SEQ ID No. 5, and wherein said sequence comprises at least one of the nucleotide C, G, C, A, and T (U) or a complementary oligonucleotide thereof in the position corresponding to position 8605, 9035 and 14065, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

Figures

Figure 1 illustrates the hybridization experiment showing that pyrethroid resistance characteristics are transmitted from female sea lice to their progenies.

Figure 2 illustrates the experiment flow of the hybridization experiment. Two field-collected strains of lice were initially used to create two hybrid strains: *Ls*HybridG with *Ls*Gulen as its maternal strain, and *Ls*HybridV with *Ls*Vikna as its maternal strain.

Figure 3 show percent active lice in deltamethrin bioassays 1 and 2. In bioassay 1 the concentrations 0.5 and 20 ppb were not included, and in bioassay 2 *Ls*Gulen F7 was not tested for 20 ppb.

Figure 4 shows the organization of mitochondrial DNA of *L. salmonis.*

Figur 5 shows an alignment of mitochondria sequences from *L. salmonis*. The upper sequence is the reference sequence of the *L. salmonis* mtDNA deposited by Tjensvoll et al (2005) with NCBI accession number NC_007215 (http://www.ncbi.nlm.nih.gov/nuccore/NC_007215.1), corresponding to SEQ ID No. 1. The start point in this sequence is in the D-loop (i.e. the non-coding region of the mtDNA). The sequence named LsGulen2 is isolated from a pyrethroid sensitive strain and is depicted

in SEQ ID No. 2 and 3. The sequence named LsViknal is isolated from a strain resistant to pyrethroids, and is depicted in SEQ ID No. 4 and 5.

The present invention and its various embodiments will be described in more detail in the following.

Detailed description of the invention

The present invention provides an in vitro method for determination of pyrethroid resistance in crustaceans, including copepods, in particular *Lepeophtheirus salmonis*, and novel SNPs, based on the surprising findings that mutations linked with resistance against pyrethroid in *Lepeophtheirus salmonis* were found in mitochondrial DNA.

Although pyrethroid resistance linked SNPs presented in the experimental data was identified in the sea lice species *Lepeophtheirus salmonis,* the skilled person will acknowledge, based on the teaching herein, that the present method and the present oligonucleotides may be used to determine pyrethroid resistance in crustaceans, in particular copepods, in particular copepods belonging to the family Caligidae. In particular, it is to be understood that the present method and the present oligonucleotides may be used to determine pyrethroid resistance in copepods affecting farmed fish, such as fish belonging to the family *Salmonidae*. According to one embodiment, the present method and present oligonucleotides are useful for detection of pyrethroid resistance in copepod selected from the group consisting of *Lepeophteirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*.

Throughout the application, the term "sea lice" is to be understood to mean any copepod belonging to the family Caligidae. However, whenever the term "sea lice" is used in connection with the experimental data in the present application, sea lice refers to the specie *Lepeophtheirus salmonis*.By hybridization of adult male sea lice shown to be sensitive to pyrethroids with preadult female sea lice shown to be resistant towards pyrethroids, the present inventors where able to show that the resistant female sea lice transmitted their pyrethroid resistant characteristics to their progenies (cf. figure 1, example 1). Resistant adult male sea lice did not transfer their pyrethroid resistant characteristics when crossed with sensitive females. The mechanism of action could therefore be linked to the mitochondrion of the sea louse. Based on further extensive sequence analysis of mitochondrial genomes of pyrethroid resistant sea lice, and comparison with the mitochondrial genomic sequence of a known reference strain (GenBank acc. No. NC_007215 (Tjensvoll et al., 2005, Gene, 353 (2), 218-230), SEQ ID No. 1) and mitochondrial sequences of a pyrethroid sensitive sea lice strain (SEQ ID No. 2 and 3), the present inventors have identified five single nucleotide polymorphism sites that are clearly linked to pyrethroid resistance (figure 5) in sea lice.

More particularly, sequences from 180 individual *L. salmonis* obtained from 6 different locations (see Tjensvoll et al 2006, *supra*) from CoI and cyt b were compared to sequences from pyrethroid sensitive and resistant lice and the newly identified SNPs characteristic for resistant strains are only present in the resistant populations. Two of the SNPs uniquely associated with resistance to pyrethroids are found in the CoI gene, more specifically in position 8605 and position 9035 (T8605C, A9035G). Three SNPs found to be uniquely

associated with resistance to pyrethroids is found in the cyt b gene, more specifically in position 13957 (C13957T), 14017 (A14017G), and 14065 (C14065T).

The start point of the mitochondrial DNA sequence of SEQ ID No. 1 is in the D-loop (i.e. the non-coding region of the mtDNA). The numbering of the positions of the identified SNPs are based of the mitochondrial sequence of Tjensvoll et al, 2005, supra, deposited with the GenBank Acc. No. NC_007215 as depicted in SEQ ID No. 1. It is to be understood that whenever referring to the positions of the SNPs identified according to the present invention, the numbering is throughout the present description made according to the numbering of the reference strain sequence (GenBank Acc. No. NC_007215) if not otherwise stated.

Based on the identification of the five SNPs responsible for pyrethroid resistance in sea lice, a method for determination of pyrethroid resistance in crustaceans, such as copepods, in particular in sea lice, e.g. *Lepeophtheirus salmonis* PRLS have been provided. More particular, a method for detection of PRLS is provided comprising the steps of detecting single nucleotide polymorphism (SNP) associated with pyrethroid resistance in the mitochondrial DNA of a sea lice to be analyzed, wherein said sea lice is resistant to pyrethroids if at least one of the following nucleotides:

i. C in position 8605;
ii. G in position 9035;
iii. T in position 13957;
iv. G in position 14017;
v. T in position 14065;

or the complementary oligonucleotide thereof,

is present in the mitochondrial DNA sequence of the sea lice, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

In addition to the qualitative determination of the SNPs involved in pyrethroid resistance according to the present invention, the present invention also provides for a method for gradation of crustaceans population being susceptible of developing resistance, i.e. taking into account the composition of haplotypes that are determined within a crustaceans population (see example 3 for practical details).

According to the present invention, "single-nucleotide polymorphisms (SNP)" is to be understood to refer to a nucleotide sequence variation occurring when a singe nucleotide, A, T (U), C or G in the genome, or other shared sequences (e.g. RNA) or fragments thereof, differs between members of a biological species, such as between variants of *Lepeophtheirus salmonis*. SNPs may fall within coding sequences of genes, or non-coding regions of genes, or in the regions between the genes in a genome. The five SNPs identified according to the present invention fall within the genes encoding the cytochrome oxidase subunit I (CoI) and cytochrome B (Cytb), respectively.

Furthermore, as used herein, an "oligonucleotide sequence" or "nucleic acid sequence" is generally an oligonucleotide sequence or a nucleic acid sequence containing a SNP

described herein, or one that hybridizes to such molecule such as a nucleic acid sequence with a complementary sequence.

The skilled person is well aware of the fact that nucleic acid molecules may be double-stranded or single-stranded, and that reference to a particular site of one strand refers, as well, to the corresponding site on a complementary strand. Thus, when defining a SNP position, reference to an adenine (A), a thymine (T) (uridine (U)), a cytosine (C) or a guanine (G) at a particular site on one strand of a nucleic acid is also to be understood to define a thymine (uridine), adenine, guanine, or cytosine, respectively, at the corresponding site on a complementary strand of the nucleic acid molecule. Thus, reference may be made to either strand in order to refer to a particular SNP position. The oligonucleotide probes and oligonucleotide primers according to the present invention may be designed to hybridize to either strand, and SNP detection methods disclosed herein may thus also in general target either strand.

An "isolated nucleic acid" as used herein is generally one that contains at least one of the SNPs described herein or one that hybridizes to such molecule, e.g. a nucleic acid with a complementary sequence, and is separated from most other nucleic acids present in the natural source of the nucleic acid, and is thus substantially free of other cellular material.

Oligonucleotide probes and oligonucleotide primers

The present invention provides oligonucleotide probes and oligonucleotide primers that may be used for detection of the presence of the SNPs according to the present invention in mitochondrial DNA of a sea lice to be tested, and thus for determination of pyrethroid resistance. The detection of nucleic acids present in a biological sample is widely applied in both human and veterinary diagnosis, wherein nucleic acids from e.g. pathogens present in biological samples are isolated and hybridized to one or more hybridizing probes or primers are used in order to amplify a target sequence.

One or more oligonucleotide probes may be constructed based on the teaching herein and used in hybridization based detection methods where upon the binding of the oligonucleotides to the target sequence enables detection of the presence of at least one of the SNPs described herein if present in the sample to be tested.

The skilled person will acknowledge that an oligonucleotide probe according to the present invention may be a fragment of DNA or RNA of variable length used herein in order to detect an SNP in a target sequence, e.g. single-stranded mitochondrial DNA or RNA, upon hybridization of the oligonucleotide probe to complementary sequence(s) of the said target sequence to be analyzed. The oligonucleotide probe according to the present invention may furthermore be labeled with a molecular marker in order to easily visualize that hybridization, and thus detection of the SNPs disclosed herein, have been achieved. Molecular markers commonly known to the skilled person may be used, e.g. a radiolabel, and more preferably, a luminescent molecule or a fluorescent molecule enabling the visualisation of the binding of the probe(s) to a target sequence.

A oligonucleotide probe according to the present invention is able to hybridize to another nucleic acid molecule, such as the single strand of mitochondrial DNA or RNA originating from a sea lice to be analysed, under appropriate conditions of temperature and solution

ionic strength, cf. e.g. Sambrook et al., Molecular Cloning: A laboratory Manual (third edition), 2001, CSHL Press, (ISBN 978-087969577-4). The condition of temperature and ionic strength determine what the skilled person will recognise as the "stringency" of the hybridization. The suitable stringency for hybridisation of a probe to target nucleic acids depends on inter alia the length of the probe and the degree of complementation, variables well known to the skilled person. A oligonucleotide probe according to the present invention typically comprises a nucleotide sequence which under stringent conditions hybridize to at least 8, 10, 12, 16, 20, 22, 25, 30, 40, 50 (or any other number in-between) or more consecutive nucleotides in a target nucleic acid molecule, e.g. single-stranded mitochondrial DNA or RNA isolated from the sea lice to be analyzed according to the present invention. According to one embodiment, the oligonucleotide probe according to the present invention comprises about 13 to 25 consecutive nucleotides. It is to be understood that the oligonucleotide probe according one embodiment comprise one of the SNPs described herein or the complement thereof. New technology like specific Locked Nucleic Acid (LNA) hybridization probes allows for the use of extremely short oligonucleotide probes (You Y.; Moreira B.G.; Behlke M.A. and Owczarzy R. (2006), "Design of LNA probes that improve mismatch discrimination, *Nucleic Acids Res.* **34** (8): e60) According to one embodiment, probes are provided which are selected from the group consisting of SEQ ID No. 16-20.

The present invention furthermore provides oligonucleotide primers useful for amplification of any given region of a nucleotide sequence, in particular a region containing one of the SNPs described herein. An oligonucleotide primer according to the present invention typically comprises a nucleotide sequence at least 8, 10, 12, 16, 20, 22, 25, 30, 40, 50 (or any other number in-between) or more consecutive nucleotides. According to one embodiment, the oligonucleotide primer according to the present invention comprises about 18 - 25 consecutive nucleotides, more preferably about 20 nucleotides.

As used herein, the term "oligonucleotide primer" is to be understood to refer to a nucleic acid sequence suitable for directing an activity to a region of a nucleic acid, e.g. for amplification of a target nucleic acid sequence by polymerase chain reaction (PCR). According to one embodiment of the present invention, "oligonucleotide primer pairs" is provided suitable for amplification of a region of mitochondrial genome material comprising the SNPs according to the present invention.

The skilled person will acknowledge that an oligonucleotide primer according to the present invention may be a fragment of DNA or RNA of variable length used herein in order to detect an SNP in a target sequence, e.g. single-stranded mitochondrial DNA or RNA, upon alignment of the oligonucleotide probe to complementary sequence(s) of the said target sequence to be analyzed. An oligonucleotide primer according to the present invention may furthermore be labeled with a molecular marker in order to enable visualization of the results obtained. Various molecular markers or labels are available, dependent on the SNP detection method used.

An oligonucleotide primer according to the present invention typically comprises the appropriate number of nucleotides allowing that said primer align with the target sequence

to be analyzed. It is to be understood that the oligonucleotide primer according to the present invention according to one embodiment comprises the SNP described herein or the complement thereof. According to one embodiment, the probes useful in order to determine pyrethroid resistant sea lice is selected from the group consisting of SEQ ID No. 16, SEQ ID No.17, SEQ ID No.18, SEQ ID No.19, and SEQ ID No.20.

According to one embodiment of the present invention, primer pairs are provided selected from the group consisting of SEQ ID No. 6 – SEQ ID No. 15 (see also table 5 below).

Oligonucleotide probes and oligonucleotide primers according to the present invention may be synthesized according to methods well known to the skilled person.

The present invention furthermore relates to isolated nucleic acid sequences and variants or fragments thereof having at least 70% identity with the nucleic acid sequences depicted in SEQ ID NO. 4, or SEQ ID No. 5, or fragments thereof. The term "% identity" is to be understood to refer to the percentage of nucleotides that two or more sequences or fragments thereof contains, that are the same. A specified percentage of nucleotides can be referred to as e.g. 70% identity, 80% identity, 85% identity, 90% identity, 95% identity, 99% identity or more (or any number in between) over a specified region when compared and aligned for maximum correspondence. The skilled person will acknowledge that various means for comparing sequences are available. For example, one non-limiting example of a useful computer homology or identity program useful for determining the percent homology between sequences includes the Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990, J. of Molec. Biol., 215:403-410, "The BLAST Algorithm; Altschul et al., 1997, Nuc. Acids Res. 25:3389-3402, , Karlin and Altschul 1990, Proc. Nat'l Acad. Sci. USA, 87:2264-68; 1993, Proc. Nat'l Acad. Sci. USA 90:5873-77).

SNP identification methods

Upon the identification of the SNPs associated with pyrethroid resistance in sea lice according to the present invention, the skilled person will acknowledge that various methods commonly used in order to detect polymorphisms within a population of sea lice may be used. Both methods based on genome sequencing, hybridization and enzyme based methods are applicable for determining whether a sea louse is pyrethroid resistant in accordance with the present inventions.

Various enzyme based methods are available for the skilled person, of which a number of polymerase chain reaction (PCR) based methods are available.

For example, Perkin Elmer Life Sciences provides SNP detection kit that may be used in order to determine whether a sea louse is pyrethroid resistant (AcycloPrime^{TM}-FP SNP Detection). In said method, a thermostable polymerase is used which extends an oligonucleotide primer according to the present invention by one base, then ending the oligonucleotide primer one nucleotide immediately upstream of the relevant SNP position by the incorporation of fluorescent dye-labeled terminators. The identity of the base added is then determined by the increase fluorescence polarization of its linked dye (http://shop.perkinelmer.com/Content/Manuals/MAN_AcycloPrimeSNPKit.pdf). Oligonucleotide primers according to the present invention useful in such a method would thus be constructed in order to facilitate the extension of the primer by one base in the

position selected from the group 8605, 9035, 13957, 14017, or 14064, relative to SEQ ID No. 1.

Another enzyme based method that may be used is restriction fragment length polymorphism (RLFP), utilizing that various, highly specific endonuclease upon digestion of the target sample, i.e. mitochondrial genome, results in different fragments that may be separated by gel electrophoresis.

Yet another enzyme based method that may be utilized in accordance with the present invention is the flap endocuclease (FEN) method. In said method, a structure-specific endonuclease is used to cleave a three-dimensional complex formed by hybridization with the target DNA, e.g. mitochondrial genomic material from sea lice, and where annealing with a target sequence comprising the SNP of interest triggers cleavage by the endonuclease (Oliver, 2005, Mutat. Res., vol. 573 (1-2), pp. 103-110).

Yet another method applicable in respect of the present invention is based on the use of TaqMan® Assays (Invitrogen). In said assay the oligonucleotide primers used in order to detect an SNP is labeled in both the 5'- and the 3' end, i.e. with a fluorophore at the 5'end of the oligonucleotide primer, and a quencher at the 3'-end of the oligonucleotide primer. Upon annealing of the oligonucleotide primer with a target sequence, the Taq polymerase will extend the oligonucleotide primer and form a nascent strand, followed by degradation of the oligonucleotide primer being annealed to the target, said degradation eventually resulting in the release of the fluorophore and provide a cleavage close to the quencher. The fluorescence signal produced is proportional to the fluorophore released. Various fluorophore labels may be used, such as e.g. 6-carboxyfluorescein, tetrafluorofluorescein. As quenchers, tetramethylrhodamine or dihydrocyclopyrroloindol may be used.

Several hybridization methods for detection of SNPs are available to the skilled person, and which may be utilized in accordance with the method of the present invention. For example, the SNPs according to the present invention may be detected utilizing molecular beacon technology. According to this aspect of the present invention, oligonucleotide primers may be synthesized comprising complementary regions at each end allowing the formation of a hairpin loop, and wherein a fluorophore is attached at one end of the oligonucleotide primer, and a quenching agent is attached to the other end, and wherein fluorescence signal is produced upon binding to a DNA target of interest, i.e. mitochondrial genomic material isolated from the sea louse to be analyzed.

Yet another method applicable in respect of the present invention is based on DNA or RNA sequencing, which is the process of determining the precise order of nucleotides within a molecule. It includes any method or technology that is used to determine the order of the four bases (adenine, guanine, cytosine, and thymine) in a strand of DNA. The skilled person is well known with the various commonly known DNA and RNA sequencing methods that may be used according to the present invention, such as e.g. shotgun sequencing or bridge PCR sequencing.

Isolation of sea lice genomic material

The method according to the present invention may according to one embodiment involve the isolation of a biological sample from a sea lice and testing for the presence of a SNP

associated with PRLS in the mitochondrial genome. The skilled person will acknowledge that the SNPs identified according to the present invention may be detected by analyzing mitochondrial DNA as well as mitochondrial RNA, dependent upon the detection method used.

In order to determine whether a sea louse is pyrethroid resistant in accordance with the present invention, mitochondrial genomic material may be isolated. Various methods for obtaining genomic material well known to the skilled person are available. The skilled person will acknowledge that any tissue (i.e. any part of the sea lice) may be used in order to extract mitochondrial genomic material. Furthermore, the mitochondrial genomic material to be analyzed according to the present invention may be obtained from sea lice of any life stages, e.g. the free swimming stages (nauplius stage I and II), the copepod stage, the pre-adult (chalimus stages 1-4), or the adult stage (adult male or adult female). According to one embodiment, tissue removed from sea lice to be tested is maintained in 70% ethanol or other conservation liquid prior to further isolation of genomic material. DNA may be extracted from the obtained tissue using commonly available DNA extraction/isolation methods, such as e.g. DNeasy DNA Tissue Kit according to the protocol of the manufacturer (http://lycofs01.lycoming.edu/~gcat-seek/protocols/DNeasy_Blood_&_Tissue_Handbook.pdf)

Still another method applicable for detecting SNP is High Resolution Melting Analysis (HRM) enabling rapid detection of SNPs and determination of genetic variation within a population. The first step of a HRM protocol consist often of amplification of the region of interest, using standard nucleotide sequence amplification techniques well known to the skilled person, and wherein the amplification is performed in the presence of a specialized double-stranded DNA binding dye being highly fluorescent when bound to dsDNA and poorly fluorescent in unbound state. This difference provides for the monitoring of the DNA amplification. After amplification, the target is gradually denatured by increasing the temperature in small increments, resulting in a characteristic melting profile. As the amplified DNA is denatured gradually, dye is released, thus resulting in a drop in fluorescence.

SNP detection Kits

Based on the teaching herein, the skilled person will acknowledge that , based on the identified SNPs and associated sequence information disclosed herein, detection reagents can be developed and used to determine any SNP described herein individually or in combination, and that such detection reagents can be readily incorporated into kits used for SNP detection known in the art. The term "kit" as used herein in the context of SNP detection reagents are intended to cover e.g. combinations of multiple SNP detection reagents, or one or more SNP detection reagents, such as oligonucleotide probe(s) and oligonucleotide primer(s) or primer sets, arrays/microarrays of nucleic acid molecules, and beads that contain one ore more oligonucleotide probe(s), oligonucleotide primer(s) or other detection reagents useful in the method of the present invention. It is furthermore to be understood that the SNP detection reagents in a kit according to the present invention may furthermore include other components commonly included in such kits, e.g. such as various types of biochemical reagents (buffers, DNA polymerase, ligase, deoxynucleotide

triphosphates for chain extension/amplification, etc.), containers, packages, substrates to which SNP detection reagents are attached., etc. necessary to carry the method according to the present invention. According to one embodiment of the present invention, a kit is provided which comprises the necessary reagents to carry out one or more assays in order to detect the SNP disclosed herein according to the method of the present invention. A kit according to the present invention may preferably comprise one or more oligonucleotide probes that hybridize to a nucleic acid target molecule (i.e. mitochondrial genetic material) enabling detection of each target SNP position if present in the material analyzed. Multiple pairs of probes may be included in the kit to simultaneously analyze for the presence of the SNP disclosed herein at the same time. The probes contained in the kit according to the present invention may according to one embodiment be immobilized on a carrier, such as e.g. an array or a bead.

According to one embodiment, the oligonucleotide probes are suitable for the detection of the SNP T8605C. According to another embodiment, the oligonucleotide probes are suitable for the detection of the SNP A9035G. According to yet another embodiment, the oligonucleotide probes is suitable for the detection of the SNP C13957T. According to yet another embodiment, the oligonucleotide probes is suitable for the detection of the SNP A14017G. According to yet another embodiment, the oligonucleotide probes is suitable for the detection of the SNP C14065T. According to yet another embodiment, the kit according to the present invention comprises oligonucleotide probes suitable for detection of all the SNPs described herein.

According to one embodiment, a kit according to the present invention comprises oligonucleotide primer(s) and optionally further SNP detection reagents useful in SNP detection methods utilizing oligonucleotide primers or primer pair(s). According to one embodiment, the kit according to the present invention comprises a forward primer and a reverse primer for amplifying a region containing a SNP selected from SNP selected from the group of SNPs consisting of T8605C, (U8605C in case of RNA), A9035G, C13957T (or U13957T in case of RNA), A14017G, or C14065T (C14065U in case of RNA). Said kit may furthermore optionally comprise further SNP detection reagents (enzymes and nucleotide triphosphates) necessary for conducting PCR or real time PCR. According to one embodiment, the primer pairs are suitable for the detection of the SNP T8605C. According to another embodiment, the primer pairs are suitable for the detection of the SNP A9035G. According to yet another embodiment, the primer pairs is suitable for the detection of the SNP C13957T. According to yet another embodiment, the primer pairs is suitable for the detection of the SNP A14017G. According to yet another embodiment, the primer pairs is suitable for the detection of the SNP C14065T. According to yet another embodiment, the kit according to the present invention comprises primer pairs suitable for detection of all the SNPs described herein.

Table 1 Sequences related to the invention

SEQ ID No.	Description of sequence
1	Mitochondrial DNA of Lepeophtheirus salmonis from sea lice (GenBank acc. No. NC_007215 (Tjensvoll et al., 2005, Gene, 353 (2), 218-230)
2	Mitochondrial DNA of Lepeophtheirus salmonis isolated from pyrethroid sensitive strain (Ls_Gulen2 CoI)
3	Mitochondrial DNA of Lepeophtheirus salmonis isolated from pyrethroid sensitive strain (Ls_Gulen2 Cytb)
4	Mitochondrial DNA of Lepeophtheirus salmonis isolated from pyrethroid resistant strain (Ls_Viknal CoI)
5	Mitochondrial DNA of Lepeophtheirus salmonis isolated from pyrethroid resistant strain (Ls_Viknal Cytb)
6	Oligonucleotide (forward) primer useful for detecting SNP T8605C
7	Oligonucleotide (reverse) primer useful for detecting SNP T8605C
8	Oligonucleotide (forward) primer useful for detecting SNP A9035G
9	Oligonucleotide (reverse) primer useful for detecting SNP A9035G
10	Oligonucleotide (forward) primer useful for detecting SNP C13957T
11	Oligonucleotide (reverse) primer useful for detecting SNP C13957T
12	Oligonucleotide (forward) primer useful for detecting SNP A14017G
13	Oligonucleotide (reverse) primer useful for detecting SNP A14017G
14	Oligonucleotide (forward) primer useful for detecting SNPC14065T
15	Oligonucleotide (reverse) primer useful for detecting SNP C14065T
16	Oligonucleotide probe useful for detecting SNP T8605C
17	Oligonucleotide probe useful for detecting SNP A9035G
18	Oligonucleotide probe useful for detecting SNP C13957T
19	Oligonucleotide probe useful for detecting SNP A14017G
20	Oligonucleotide probe useful for detecting SNP T8605C

According to one embodiment of the present invention, a method is provided for detection of the pyrethroids deltamethrin (CAS No. 52918-63-5) and cis-cypermethrin (CAS No. 52315-07-8). It is well known that resistance against one type of pyrethroid in general provides the possibility of resistance also against all type of pyrethroids; see e.g. Sevatdal S, Fallang A, Ingebrigtsen K, Horsberg TE. 2005. Monooxygenase mediated pyrethroid detoxification in sea lice (*Lepeophtheirus salmonis*). Pest Manag Sci. 2005 Aug;61(8):772-8, Du Y, Nomura Y, Satar G, Hu Z, Nauen R, He SY, Zhorov BS, Dong K. 2013. Molecular evidence for dual pyrethroid-receptor sites on a mosquito sodium channel. Proc

Natl Acad Sci U S A. 2013 Jul 2. [Epub ahead of print], and Zhu F, Gujar H, Gordon JR, Haynes KF, Potter MF, Palli SR. 2013. Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. Sci Rep. 2013; 3:1456. doi: 10.1038/srep01456.

The skilled person will thus acknowledge, based on the teaching of the present invention, that the method according to the invention may be used to determine resistance towards various types of pyrethroids in crustaceans, such as e.g. allethrin, bifenthrin, cyfluthrin, cyphenothrin, esfenvalerat, etofenoprox, fenpropathrin, fenvalerate, flumethrin, flucythrinate, imiprothrin, lamda-cyhalothrin, metofluthrin, permethrin, prallethrin, resmethrin, silafluofen, sumithrin, fluvalinate, teflutrhin, and tetramethrin.

Examples

Example 1: Hybridization of sensitive and pyrethroid resistant sea lice

Crossing experiment

In order to investigate the inheritance and mechanism of pyrethroid resistance in *L. salmonis*, two strains (sensitive and resistant) and their reciprocal hybrids were used for a two-generation breeding experiment complimented with bioassays to measure tolerance (Fig. 2). The pyrethroid sensitive strain (hereon also referred to as *Ls*Gulen) was collected in June 2006 from newly slaughtered rainbow trout from Gulen in western Norway. The pyrethroid resistant strain (hereon also referred to as *Ls*Vikna) was collected in February 2009 from a salmon farm in Vikna, North Trøndelag, Norway. The resistant strain was collected on the basis of suspected reduced sensitivity to pyrethroids due to the fact that there had been reported a treatment failure on the farm from which it was collected. This was subsequently confirmed in bioassays with *Ls*Gulen as sensitive control.

Prior to the initiation of the present study, *Ls*Gulen and *Ls*Vikna had been cultivated in laboratory for 13 and 4 generations respectively under standard rearing conditions at the Institute of Marine Research (IMR), Norway (Hamre et al., 2009, Establishment and characterisation of salmon louse (*Lepeophtheirus salmonis* (Krøyer 1837)) laboratory strains. *Parasitology International,* 58, 451-460).

In order to hybridise the lice strains for the inheritance experiment, the parental strains were first synchronised and multiplied up in numbers by simultaneously infecting 20 Atlantic salmon with copepods. In total, 1000 copepods per strain were added. At 32 days post infection (dpi), all salmon were anaesthetised and approximately half of the lice were carefully removed using tweezers. At this stage, lice were predominantly preadult 2 females and adult males, with a few percent adult females and preadult 2 males. The lice removed from the salmon were sexed, and then used to create the reciprocal hybrid strains. This was achieved by combining 4–5 females from the resistant strain and 4-5 males from the sensitive strain (*Ls*HybridV), and 4–5 females from the sensitive strain and 4-5 males from the resistant strain (*Ls*HybridG). Only preadult 2 females were used. These combinations were each placed into 2 replicate tanks in order to make two hybrid strains (4 groups and 8 tanks at this stage). At 67–68 dpi, all lice were removed from the four strains. Egg strings were removed from adult females and incubated in containers containing a

single strain each. The number of adult lice, and harvested egg strings varied, but all were incubated (Table 2).

Table 2. Number of egg strings collected from each strain. Egg strings from replicate tanks were pooled. Most egg strings were part of a pair.

Strain	Generation	Egg strings (n)
*Ls*Gulen	F14	133
*Ls*Vikna	F5	106
*Ls*HybridG	P	96
*Ls*HybridV	P	60

The subsequent generation (F2, Fig. 2) were both produced using the general procedure described above, although the hybrid strains were only crossed back to themselves to create multiple generation-hybrids. Collection of egg strings, which marked the establishment of a new generation, was done at 55 and 56 dpi respectively. Salmon that hosted the previous generation were re-used for reinfection with the next generation, but hosted only the same experimental strain.

Bioassay test

Each strain, including both types of hybrids obtained in example 1 were quantified in their tolerance of deltamethrin at sampling points 1 and 2 representing the F1 and F2 generations for the hybrids (Fig. 1 and 2). Bioassays were performed according to standard guidelines (SEARCH Consortium, 2006, Sea lice resistance to chemotherapeutants: A handbook in resistance management. 2 ed.), with minor modifications. Only adult male lice were used for the bioassay, which after sampling (at 55 or 56 dpi) had been incubated overnight in running seawater (ca. 10 °C). The 30 min exposure to the prepared deltamethrin concentration was also carried out at ca. 10 °C, and higher concentrations than those described in the given protocol were included (Table S3). After exposure to deltamethrin, the lice were kept in running seawater (24 h at ca. 10 °C) until evaluation of the bioassays. At evaluation, lice were characterised as active, or inactive (moribund and dead lice pooled). EC_{50} values were calculated using probit analysis (PoloPlus 2.0; LeOra Software, CA, USA). The results of two deltamethrin bioassay sets are shown in table 3a and 3b below.

Table 3a. Deltamethrin bioassays performed on sampling set 1

Lice: LsHybridG
Generation: F1
Fish tank: 01

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	17	0	0
0.1	16	0	0
0.3	32	9	21
1	0	35	100
3	0	28	100
6	0	18	100
12	0	27	100

Lice: LsHybridV
Generation: F1
Fish tank: 02

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	16	0	0
0.1	17	0	0
0.3	44	0	0
1	56	3	5
3	33	5	13
6	17	1	5
12	15	7	31

Lice: LsGulen
Generation: F15
Fish tank: 04

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	9	0	0
0.1	40	0	0
0.3	29	27	48
1	0	37	100
3	0	20	100
6	0	14	100
12	0	9	100

Lice: LsVikna
Generation: F6
Fish tank: 05

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	12	0	0
0.1	16	0	0
0.3	9	0	0
1	21	3	12
3	29	11	27
6	18	10	35
12	7	5	41

Lice: LsHybridV

Generation: F1
Fish tank: 06

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	26	0	0
0.1	17	0	0
0.3	45	0	0
1	39	3	7
3	36	1	2
6	19	2	9
12	19	9	32

Lice: LsHybridG
Generation: F1
Fish tank: 07

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	14	0	0
0.1	18	4	18
0.3	21	10	32
1	0	38	100
3	0	46	100
6	0	21	100
12	0	20	100

Lice: LsVikna
Generation: F6
Fish tank: 09

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	16	0	0
0.1	21	1	4
0.3	15	0	0
1	26	3	10
3	17	4	19
6	23	7	23
12	5	2	28

Lice: LsGulen
Generation: F15
Fish tank: 10

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	13	0	0
0.1	26	0	0
0.3	10	10	50
1	0	41	100
3	0	9	100
6	0	17	100
12	0	22	100

Table 3b. Deltamethrin bioassays performed on sampling set 2.

Lice: LsGulen
Generation: F16
Fish tank: 03

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	19	0	0
0.1	16	1	5
0.3	28	26	48
0.5	7	43	86
1	0	18	100
3	0	23	100
6	0	7	100

Lice: LsGulen
Generation: F16
Fish tank: 04

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	24	0	0
0.1	23	2	8
0.3	16	20	55
0.5	1	20	95
1	0	16	100
3	0	15	100
6	0	6	100
12	0	3	100

Lice: LsHybridV
Generation: F2
Fish tank: 06

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	19	0	0
0.1	13	0	0
0.3	14	0	0
0.5	15	0	0
1	32	0	0
3	25	5	16
6	22	1	4
12	11	3	21
20	6	4	40

Lice: LsHybridG
Generation: F2
Fish tank: 07

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	22	0	0
0.1	17	0	0
0.3	14	14	50
0.5	1	6	85
1	1	10	90

3	0	18	100
6	0	32	100
12	0	27	100
20	0	32	100

Lice: LsVikna
Generation: F7
Fish tank: 08

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	35	0	0
0.1	9	0	0
0.3	27	0	0
0.5	6	0	0
1	12	0	0
3	14	1	6
6	22	5	18
12	6	0	0
20	17	11	39

Lice: LsHybridV
Generation: F2
Fish tank: 09

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	16	0	0
0.1	12	2	14
0.3	11	0	0
0.5	28	1	3
1	17	1	5
3	17	0	0
6	25	6	19
12	11	1	8
20	14	17	54

Lice: LsHybridG
Generation: F2
Fish tank: 10

Dose (ppb)	Active (n)	Inactive (n)	% Inactive
0	10	0	0
0.1	19	1	5
0.3	25	19	43
0.5	6	16	72
1	0	10	100
3	0	23	100
6	0	19	100
12	0	25	100
20	0	10	100

The four experimental strains were successfully cultured through the two generations and exposed to two separate bioassays. At the control and lowest concentration (0.1ppb), no differences in lice activity was observed between the four strains. This was the case for both sampling 1 and 2. However, at concentrations between 0.3 and 1 ppb, clear

differences were observed between the strains (Fig. 2). Above 1 ppb, only lice from the resistant strain and its maternal hybrid strain survived the bioassay, while the sensitive strain and its associated maternal hybrid strain displayed 100% mortality. No differences between the hybrid strain and its maternal founder strain were observed for either strain, demonstrating that the resistance followed a maternal pattern of inheritance.

Exemple 2: Identification of SNPs associated with pyrethroid resistance

The presented outcome of crossing resistant and sensitive lice (example 1) showed that the mitochondria genome encodes the resistance. The L. salmonis mtDNA was characterized by Tjensvoll et al (2005) and encodes 13 proteins, two rRNAs and 22 tRNA genes.

The first approach to identify genetic differences between the LsSensitive (LsGulen) and Ls Resistent (LsVikna) was by sequencing a 1000 bp fragment from *cytB* from 6 *L. salmonis* specimens (3 sensitive and 3 resistant). The sequencing revealed 3 unique SNPs in the *cytB* from the LsResistent strain. We sequenced the *cytB* from 10 more individual from the LsResistent strain, which revealed that all except 1 individual had the same unique SNP (C14065T) in the *cytB*. This single atypical LsVikna individual was sampled from the control group during the bioassay (i.e. LsVikna incubated without deltamethrin). Tjensvoll et al (2006) sequenced *cytB, A6. coI* and *16S* from 180 *L. salmonis* specimens from six locations in the North Atlantic that were collected in 2000 or 2002. We aligned our cytB (from LsSensitive and LsResistent) sequences to these 180 cytB sequences from Tjensvoll et al (2006). None of the 180 cytB sequences from historic samples had the C14065T SNP. To further validate if the C14065T SNP is unique in resistant lice we obtained samples from 9 different lice strains, including several field strains with reduced sensitivity towards deltamethrin or cis-cypermethrin.

The presented outcome of crossing resistant and sensitive lice showed that the mitochondria genome encodes the resistance. Since *L. salmonis* is ZZ (male)/Z0 (female) the maternally encoded resistance properties must be in the mtDNA. The *L. salmonis* mtDNA was characterized by Tjensvoll et al (2005) and encodes 13 proteins, two rRNAs and 22 tRNA genes.

Genetic variation within the mtDNA

Tjensvoll et al (2006), *supra,* identified 158 haplotypes for cytb and 164 haplotypes for COI. In 19 *L. salmonis* individuals with know deltamethrin resistance only 2 haplotypes for *COI* were identified. The cytB was sequenced from 29 individuals with known deltamethrin resistance and only 1 haplotype was identified. Among 19 sensitive specimens 9 different haplotypes for cytB was present (Table 4).

Table 4. Number of haplotypes identified for each of CytB and CoI in sensitive sea lice and resistant sea lice.

	CytB	CoI
Tjensvoll et al	158 (180)	164 (180)
Sensitive lice	9 (19)	11 (19)
Resistant lice	1 (29)	2 (19)

Example 3: Testing for PRLS

Based on the identification of SNPs responsible for pyrethroid resistance in *L. salmonis*, we developed several sensitive Real-Time PCR (TaqMan) 5′-nuclease assays for single nucleotide polymorphism (SNP) detection (Real-Time PCR SNP-assay) (Table 1), and successfully applied these to differentiate between pyrethroid resistant and sensitive sea lice. Fluorogenic PCR probes, chemically modified with a minor groove binding agent to increase duplex stability, were used in single and multiplex probe closed tube formats. The probes were tested in a commercially available thermocycling fluorimeter (Applied Biosystems 7500 Real-Time PCR System) at PatoGen Analyse AS laboratory in Ålesund. All assays were used qualitatively to determine the relative amount of the SNP- in individual samples, and samples from different life stages of sea lice were use. Comparison of results obtained using sea lice samples with different Real-Time PCR SNP-assays detecting different SNPs specific for pyrethroid resistant sea lice showed no discrepancies from results obtained by genome sequencing. The reported SNPs and the relevant Real-Time PCR SNP-assays are ideal for the differentiating between pyrethroid resistant and sensitive sea lice in the aquaculture industry.

Sampling of sea lice

Sea lice samples were collected from sea lice with known sensitivity status to pyrethroids, cultivated in The Sea Lice Research Centre laboratories at the University of Bergen. The sea lice had been tested with respect to sensitivity to pyrethroids by bioassays as previously described in example 1 above. Also, the genome sequences for the relevant genes were known from previous genomic sequencing performed as described in example 1 above.

In addition, sea lice were sampled from fish farms on the west coast of Norway. Sea lice were collected using forceps, and approximately 10-50 lice per site were conserved in 70% ethanol and kept at 4°C. Samples were sent refrigerated to PatoGen by express mail carrier.

Nucleic acid purification

In PatoGens laboratory, RNA and/or DNA were extracted from samples by methods well known to the skilled person. In short, tissue samples were transferred to Micro Collection Tubes and lysed and homogenized using QIAzol Lysis Reagent, steel beads and vigorous shaking using a TissueLyser system, followed by nucleic acid extraction using an RNAeasy kit (Qiagen) or DNAeasy kit (Qiagen), all according to the manufacturer's instructions, and by methods well known to the skilled person. Chloroform were added to the samples and shaken vigorously. After resting and centrifuging, the relevant liquid phase

were collected for further extraction of either RNA or DNA by vacuum technology using a Qiagen robot system, all according to the manufacturer's instructions, and by methods well known to the skilled person. Finally, nucleic acids were eluted in 25 ml of elution buffer and used for PCR by methods well known to the skilled person.

SNP-detection by Real-Time PCR

The primers and probes listed in table 5 are TaqMan® MGB Probe SNP Genotyping Assays using TaqMan® 5′ nuclease assay chemistry for amplifying and detecting specific SNP alleles in purified genomic DNA or RNA. In this study, primers and probes SNP-14017, SNP-14065, & SNP-9035 listed in table 1 were ordered from Life Technologies Corporation. The primers and probes listed in table 1 for SNP-8605 and SNP-13957 serves as examples of assays for these SNPs, but were not included in this study. One-step amplification (45 cycles) was performed on an Applied Biosystems 7500 Real-Time PCR System performed at PatoGen Analyse AS laboratory in Ålesund, all according to the manufacturer's instructions, and by methods well known to the skilled person. All assays were tested on both RNA and DNA, and used qualitatively to determine the relative amount of the relevant SNPs in individual samples.

Table 5: Primers and probes. Listing of primers and probes for each SNP identified. Probes are both listed with IUPAC nucleotide codes for SNPs, and with the alternative nucleotides for each position as indicated. IUPAC codes used are in accordance with Nomenclature for Incompletely Specified Bases in Nucleic Acid Sequences, of which the following are relevant here: Y = C or T, R = A or G (Nomenclature Committee of the International Union of Biochemistry (NC-IUB) (1984) http://www.chem.qmul.ac.uk/iubmb/misc/naseq.html).

Assay ID	Position AY629857	Forward primer	Revers primer	Probe with IUPAC nucleotide code	Probe	Sensitive	Resistant
SNP-8605	T8605C	TTAAACAATATAAG ATTT (SEQ ID No. 6)	TAGAAAGTGGTGCAG GAACTGGGT (SEQ ID No. 7)	CCTCTTYGAGTTTATT (SEQ ID No. 16)	CCTCTTT/CGAGTT TATT	T	C
SNP-9035	A9035G	GGGCACCCTGAAGT TTATATTCTAAT (SEQ ID No.8)	AACCAAAAGCCTCATC TTTACAAGTT (SEQ ID No. 9)	TTCCAGGGTTTGGRTTA (SEQ ID No. 17)	TTCCAGGGTTTGG A/GTTA	A	G
SNP-13957	C13957T	AGGTTAAAGAAAAA AAATCCC (SEQ ID No. 10)	TAAATGTAGCAGTACG GCGGT (SEQ ID No.11)	CAAAACATAAGYCATTT TC (SEQ ID No.18)	CAAAACATAAGC/ TCATTTTC	C	T
SNP-14017	A14017G	GCAGGTACGGCGGT TCTTT (SEQ ID No. 12)	GCTTTAGCTTTGTCTG TAAGAATTTTGT (SEQ ID No. 13)	CAGCAAAAATGGRAA (SEQ ID No.19)	CAGCAAAAATGGA /GAA	A	G
SNP-14065	C14065T	TTCTTACAGACAAA GCTAAAGCCACTA (SEQ ID No. 14)	AGTAACTCCTGCTCAC ATTCAACCT (SEQ ID No. 15)	CCCCCCYAACTTAT (SEQ ID No 20)	CCCCCCC/TAACTT AT	C	T

Results

The results from Real-Time PCR-assays are interpreted by looking at the deviation in Ct-values between the probes detecting the two variants of the SNP. For the Real-Time PCR-assay towards SNP-14017 performed using RNA or DNA, sensitive lice has a deviation lower than -2, and resistant sea lice has a deviation higher than -2. For the Real-Time PCR-assays towards SNP-14065 & SNP-9035 performed using RNA or DNA, sensitive lice has

a deviation lower than zero, and resistant sea lice has a deviation higher than zero. In addition, there is a quantitative aspect where the highly resistant strains have a higher deviation value than moderately resistant sea lice. Most likely, genetically resistant strains that have not been exposed to pyrethroids for some time has a lower deviation value than genetically resistant strains that been repeatedly and newly exposed to pyrethroids.

The results from the Real-Time PCR SNP-analyses using assays directed towards SNP-14065 & SNP-9035 (according to Table 5) using RNA or DNA from sea lice samples with known sensitivity to pyrethroids showed good correlation with results obtained by genome sequencing, and correlated with the known resistance status in sea lice populations (Table 6 & 7). The Real-Time PCR SNP-assay directed towards SNP-14017 (according to Table 5) gave the same results using DNA or RNA, and correlated with the results from SNP-14065 & SNP-9035 for all but one sea lice sample (Table 6).

Also, analyses performed on a higher number of sampled field strains showed good correlation with the known resistance status and as shown in Table 8. Here, analyses were performed using SNP-14065, and RNA as template.

All tested Real-Time PCR-SNP-assays show very promising results, and we believe that these SNPs can be used separately and/or in combination with other SNPs to serve as a tool in the practical differentiation between pyrethroid- sensitive and resistant sea lice.

Thus the reported SNPs and the relevant Real-Time PCR SNP-assays represent an ideal tool for differentiating between pyrethroid resistant and sensitive sea lice in the aquaculture industry. Also, the prevalence of sensitive versus resistant sea lice in a population, and the deviation value for individual lice or average deviation values for populations, can be used to predict the best possible outcome of a treatment using pyrethroids in the population. Also, the technique can become an important tool for optimizing sea lice treatments using pyrethroids, and to monitor the resistant status of populations of sea lice before treatment.

The Real-Time PCR SNP-assay directed towards SNP-14017 (according to Table 5) gave correlating results using DNA or RNA, and correlated with the results from SNP-14065 & SNP-9035 for all but one sea lice sample (sample 17).

Table 6. Conclusions regarding resistance status showed good correlations between different assays directed towards different SNPs, and regardless of whether RNA or DNA was used as a template. The results from the Real-Time PCR SNP-analyses using assays directed towards SNP-14065 & SNP-9035 (according to Table 5) using RNA or DNA from sea lice samples with known sensitivity to pyrethroids showed full correlation.

Samples from Sea lice lab at the University of Bergen			Concluding results from Real-Time PCR - SNP-analyses using RNA or DNA as template					
Kode	Nr	Lice population label	SNP-14017 - RNA Conclusion	SNP-14017 - DNA Conclusion	SNP-14065 - RNA Conclusion	SNP-14065 - DNA Conclusion	SNP-9035 - RNA Conclusion	SNP-9035 - DNA Conclusion
AB45310871	1	A	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44028166	2	A	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB38571165	3	A	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44589338	4	A	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44028181	5	A	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45310863	6	B	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB38571150	7	B	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44045872	8	B	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44028185	9	B	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45310870	10	B	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45310855	11	B	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45313574	12	C	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB38571164	13	C	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45313582	14	C	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45310865	15	C	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45428417	16	C	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45310852	17	C	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive
AB45310038	18	C	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45666543	19	D	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44045871	20	D	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44589289	21	D	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44506537	22	D	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45108831	23	D	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44028226	24	D	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45312384	25	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44001158	26	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44035432	27	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45312386	28	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44506534	29	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB44589313	30	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45313587	31	E	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive
AB45428414	32	F	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB41018802	33	F	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44589322	34	F	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44035431	35	F	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45310868	36	F	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45309850	37	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB41018811	38	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44594130	39	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45310851	40	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB44506582	41	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB45443862	42	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
AB41018799	43	G	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant

Table 7. SNP-analyses correlate well with known resistance status. SNP-analyses using SNP14065 correlates with known resistance status in sea lice (*L. salmonis*) from the sea lice lab at the University of Bergen (based on bioassays and field observations), and with genotyping performed by the University of Bergen. Bioassays, genotyping and SNP-analyses are all based on different lice from the same populations, and thus the results cannot be correlated on individual sea lice level. Also, since the number of samples is very low, the slight differences with respect to prevalence of resistant genotype observed between sequencing and Real-Time PCR SNP-14065-analysis must be attributed to the low number of samples tested, and this is especially the case for lice population D. Still, the conclusions with respect to population resistance level correlates well. Also, populations A, B and C represent surviving sea lice from the same original population, but A, B and C are surviving lice that has been exposed to different concentrations of pyrethroid in a bioassay. The results show that pyrethroid treatment selects for more resistant sea lice, and this is reflected in both the prevalence of genetically resistant lice, and in the total average deviation in Ct-values between the probes detecting the two variants of the SNP.

Samples from Sea lice lab at the University of Bergen			Results from genotyping CytB - 14065				Results Real-Time PCR - SNP-14065								
Lice population label	Isolate description/ bio-assay conclusions		#tested	#Resistant	#Sensitive	Prevalence of resistant	#tested	#Resistant	#Sensitive	% prevalence sensitive	% prevalence resistant	Average deviation sensitive	Average deviation resistant	Total average deviation	Conclusion resistance status
A	Surviving lice from bioassay	Highest dose	5	5	0	100	5	5	0	0	100	-	12,9	12,9	Resistant
B	Surviving lice from bioassay	Second highest dose	2	1	1	50	6	4	2	33	67	-1,5	13,7	8,63	Resistant
C	Surviving lice from bioassay	Control	6	2	4	33	7	3	4	57	43	-1,6	12,6	4,53	Reduced sensitivity
D	HF	Slightly reduced sensitivity	3	2	1	67	6	1	5	83	17	-1,3	12,9	1,03	Slightly reduced sensitivity
E	LG	Sensitive	3	0	3	0	7	0	7	100	0	-1,4		-1,36	Sensitive
F	LF	Resistant	13	12	1	92	5	5	0	0	100	-	11,8	11,84	Resistant
G	LB	Multi-resistant	10	10	0	100	7	7	0	0	100	-	10,3	10,34	Resistant

Table 8. SNP-analyses correlate well with known resistance status in field strains. Field strains of sea lice (*L. salmonis*) were characterized with respect to sensitivity to pyrethroids at the University of Bergen sea lice lab, and with the exception of strain P, all strains have been cultivated for one or more generations in the lab (up to 34 generations) before sampling. The conclusions from Real-Time PCR analyses towards SNP-14065 correlates very well with the known resistance characteristics from bioassays and field observations.

Samples from Sea lice lab at the University of Bergen		Results Real-Time PCR - SNP-14065								
Lice population label/ bio-assay conclusions		n	# Resistant	# Sensitive	% prevalence sensitive	% prevalence resistant	Average deviation sensitive	Average deviation resistant	Total average deviation	Conclusion resistance status
H	Sensitive	30	0	30	100	0	-1,7	-	-1,7	Sensitive
I	Resistant	30	30	0	0	100	-	5,5	5,5	Reduced sensitivity
J	Resistant	30	30	0	0	100	-	5,1	5,1	Reduced sensitivity
K	Sensitive	30	0	30	100	0	-1,3	-	-1,3	Sensitive
L	Sensitive	30	0	30	100	0	-1,7	-	-1,7	Sensitive
M	Sensitive	30	0	30	100	0	-1,4	-	-1,4	Sensitive
N	Sensitive	7	0	7	100	0	-1,4	-	-1,4	Sensitive
O	Sensitive	28	0	28	100	0	-1,3	-	-1,3	Sensitive
P	Slightly reduced sensitivity	30	1	29	97	3	-0,7	10,7	-0,3	Sensitive (Slightly reduced sensitivity)

SEQUENCE LISTING

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ccccccyaac ttat 14

CLAIMS

1. An in vitro method for detection of pyrethroid resistance in one or more crustaceans, comprising the steps of
    a. analyzing the mitochondrial genomic material of the crustacean,
    b. detecting one or more single nucleotide polymorphisms (SNP) in the mitochondrial DNA of the crustacean,
    c. determining that one or more of the detected SNP's is associated with pyrethroid resistance of the crustacean.

2. A method according to claim 1, wherein the crustaceans is one or more copepods.

3. A method according to claim 2, wherein the copepod belongs to the family Caligidae.

4. A method according to claim 4, wherein the copepod is selected from the group consisting of *Lepeophteirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*.

5. A method according to claim 3, comprising the steps of detecting single nucleotide polymorphism (SNP) associated with pyrethroid resistance in the mitochondrial genome of a sea lice to be analyzed, wherein said sea lice is resistant to pyrethroids if at least one of the following nucleotides:

    i. C in position 8605;
    ii. G in position 9035;
    iii. T in position 13957;
    iv. G in position 14017;
    v. T in position 14065;

or the complementary oligonucleotide thereof,

is present in the mitochondrial genome sequence of the one or more sea lice to be analyzed, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

6. A method according to any of the claims 1-4, wherein the crustacean is a sea lice, and further comprising the steps of:
    a) collecting sea lice from infested fish or water samples;
    b) isolating mitochondrial genomic material from any life stage of collected sea lice
    c) determining the nucleotide polymorphic site at the positions 8605, 9035 and 14065 of the isolated mitochondrial DNA compared with of the mitochondrial nucleic acid sequence SEQ ID No. 1, wherein said sea lice is resistant to pyrethroids if at least one of the nucleotide C, G, T, G and T (U), or a complementary oligonucleotide thereof, is present in position 8605, 9035, 13957, 14017, and 14065, respectively.

7. A method according to claim 6, wherein step c) is performed using a primer selected from the group consisting of SEQ ID No. 6-15.

8. A method according to claim 6, wherein step c) is performed using at least one probe selected from the group consisting of SEQ ID No. 16-20.

9. A method according to claim 6, wherein step c) comprises nucleic acid amplification.

10. A method according to claim 9, wherein the nucleic acid amplification is performed using polymerase chain reaction.

11. A method according to any of claims 6-10, wherein step c) is performed by contacting mitochondrial DNA sequence of the sea lice to be analyzed with a detection reagent, and determining which nucleotide is present in position 8605, 9035, 13957, 14017 and 14065.

12. A method according to claim 11, wherein said detection reagent is an oligonucleotide probe.

13. A method according to claim 6, wherein step c) is performed , wherein step c) is performed using SNP specific probe hybridization, SNP specific primer extension, SNP specific amplification, sequencing, 5' nuclease digestion, molecular beacon assay, oligonucleotide ligation assay, size analysis, single-stranded conformation polymorphism analysis, denaturing gradient gel electrophoresis.

14. A method according to any of the above claims, wherein the pyrethroid is selected from the group consisting of deltamethrin, and cis-cypermethrin.

15. An isolated oligonucleotide sequence comprising small nucleotide polymorphism (SNP) associated with pyrethroid resistance in crustaceans, wherein said isolated oligonucleotide sequence comprises nucleotides that distinguishes crustaceans which are resistant to pyrethroids from non-resistance to pyrethroids, and wherein the SNP are present in the mitochondrial DNA of the crustaceans.

16. An isolated oligonucleotide sequence according to claim 15, wherein the crustacean is a copepod.

17. An isolated oligonucleotide sequence according to claim 16, wherein the copepod belongs to the family Caligidae.

18. An isolated oligonucleotide sequence according to claim 16, wherein the copepod is selected from the group consisting of *Lepeophteirus salmonis*, *Caligus elongatus*, and *Caligus rogercresseyi*.

19. An isolated oligonucleotide sequence according to any of the claim 15-18, comprising a single-nucleotide polymorphism (SNP) associated with pyrethroid resistance in sea lice, wherein said isolated oligonucleotide sequence comprises nucleotides that distinguishes sea lice which are resistant to pyrethroids from non-resistant sea lice, and which is identical or has at least 80% sequence identity with a sequence selected from the group consisting of SEQ ID No. 4 and 5 or a fragment thereof, and complementary sequences of SEQ ID No. 4and 5 and fragments thereof, provided that at least one SNP selected from the group of

SNPs consisting of T8605C (U8605C in case of RNA), A9035G, C13957T (U13957T in case of RNA), A14017G, and C14065T (C14065U in case of RNA) is present, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

20. A oligonucleotide probe or oligonucleotide primer comprising a oligonucleotide sequence being homologous to a fragment of an isolated oligonucleotide sequence according to claim 19, said probe or primer being specific for a mitochondrial DNA sequence of sea lice associated with pyrethroid resistance comprising at least one SNPs selected from the group consisting of T8605C and A9035G of SEQ ID No. 4, and C13957T, A14017G, and C14065T of SEQ ID5, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

21. A probe or primer according to claim 20 comprising at least 8 contiguous nucleotides.

22. A probe according to claim 20, wherein the sequence of said probe is selected from the group consisting of SEQ ID No. 16, SEQ ID No. 17, SEQ ID No. 18, SEQ ID No. 19 and SEQ ID No. 20, and sequences having at least 80 % sequence identity therewith.

23. A oligonucleotide primer, wherein the sequence is selected from the group consisting of SEQ ID No. 6, SEQ ID No. 7, SEQ ID No. 8, SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, SEQ ID No. 12, SEQ ID No. 13 and SEQ ID No. 14, and SEQ ID No. 15, and sequences having at least 80 % sequence identity therewith.

24. A kit for detection of pyrethroid resistance in crustaceans, such as copepods, e.g. in sea lice (Lepeoptheirus salmonis), which comprises at least one oligonucleotide sequence or probe or primer according to any of the claims 15-23.

25. The use of an isolated oligonucleotide sequence comprising at least 8 contiguous nucleotides of the sequence selected from the group consisting of SEQ ID No. 3 or SEQ ID No. 4, and wherein said sequence comprises at least one of the nucleotide C, G, T, G and T (U), or a complementary oligonucleotide thereof, is present in position 8605, 9035, 13957, 14017, and 14065, respectively, and wherein the numbering of said positions is in accordance with the sequence depicted in SEQ ID No. 1.

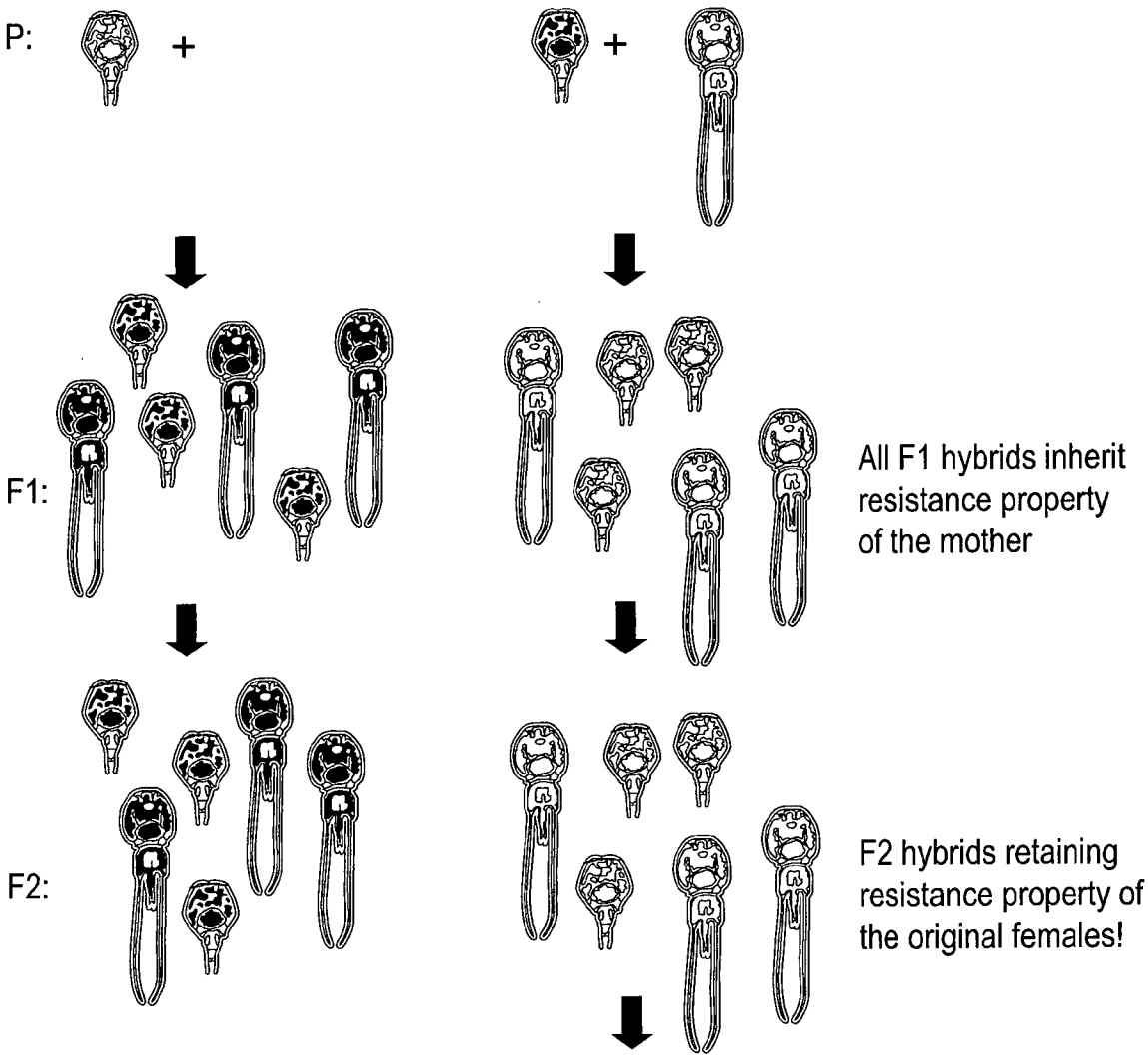
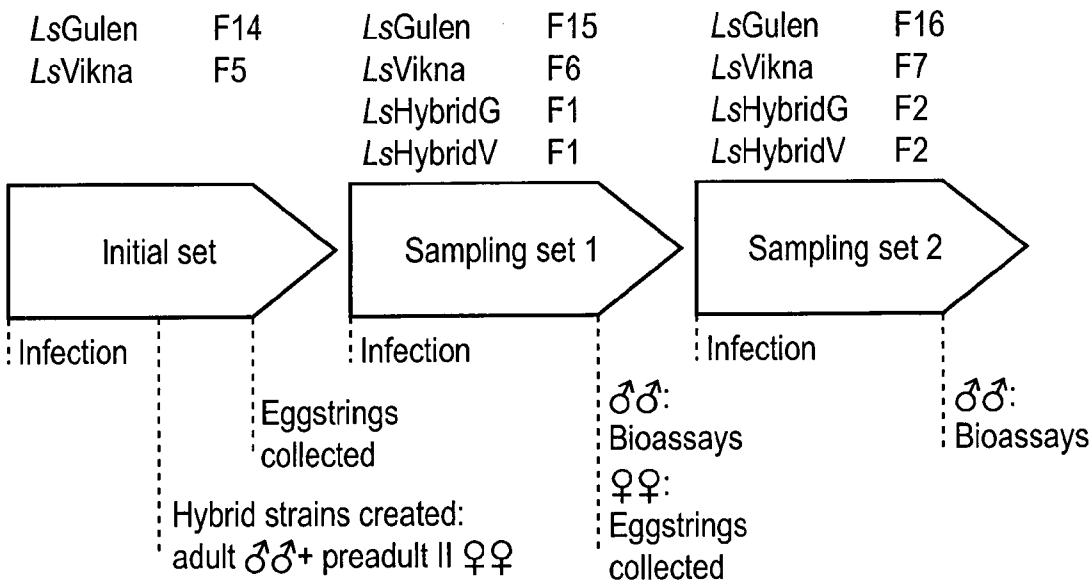

P:
+
+
F1:
All F1 hybrids inherit resistance property of the mother
F2:
F2 hybrids retaining resistance property of the original females!

FIG. 1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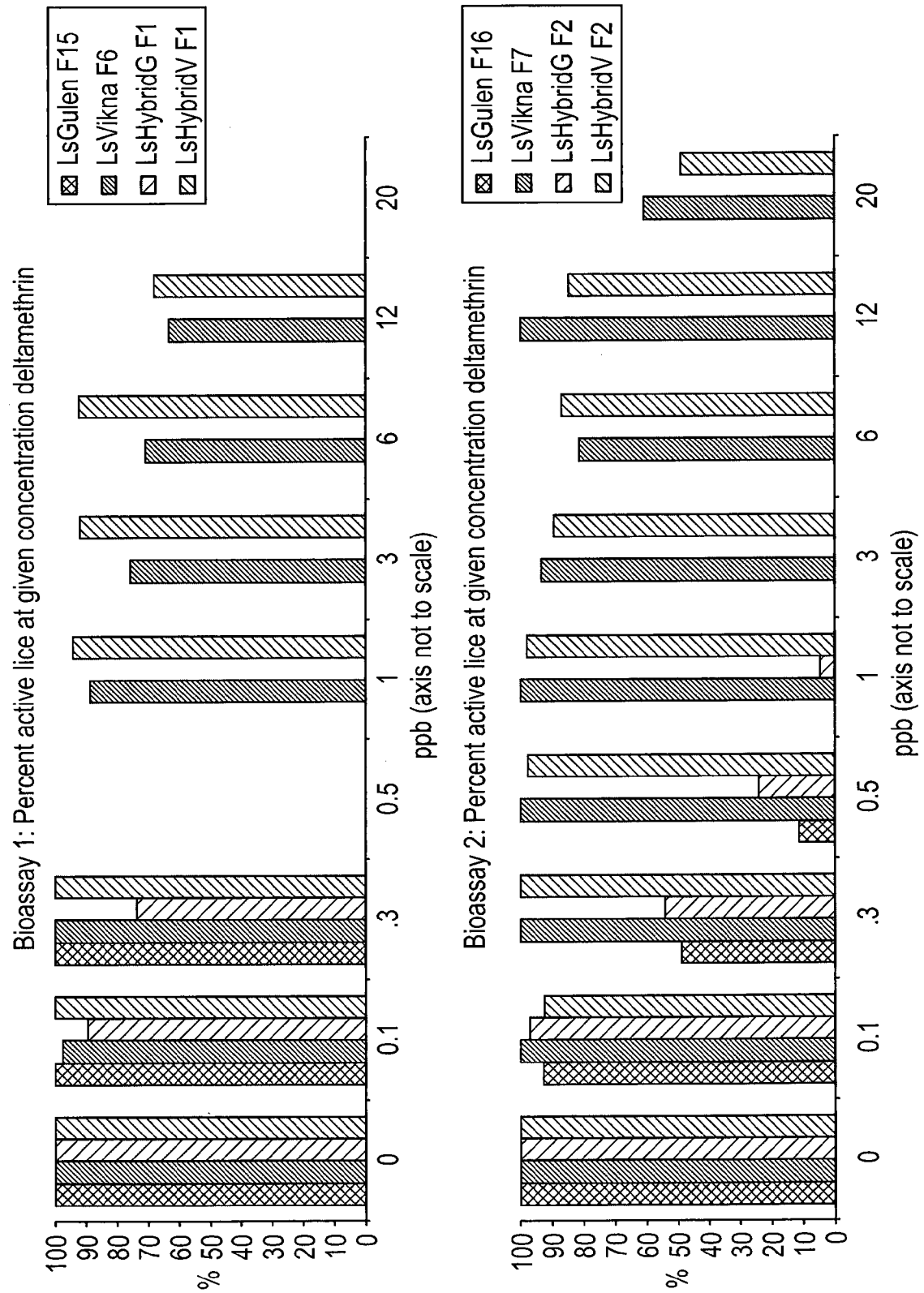
LsVikna F5
LsGulen F15
LsVikna F6
LsHybridG F1
LsHybridV F1
LsGulen F16
LsVikna F7
LsHybridG F2
LsHybridV F2
Initial set
Sampling set 1
Sampling set 2
Infection
Infection
Infection
Eggstrings collected
♂♂: Bioassays
♀♀: Eggstrings collected
♂♂: Bioassays
Hybrid strains created: adult ♂♂+ preadult II ♀♀

FIG. 2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LsVikna F6
LsHybridG F1
LsHybridV F1
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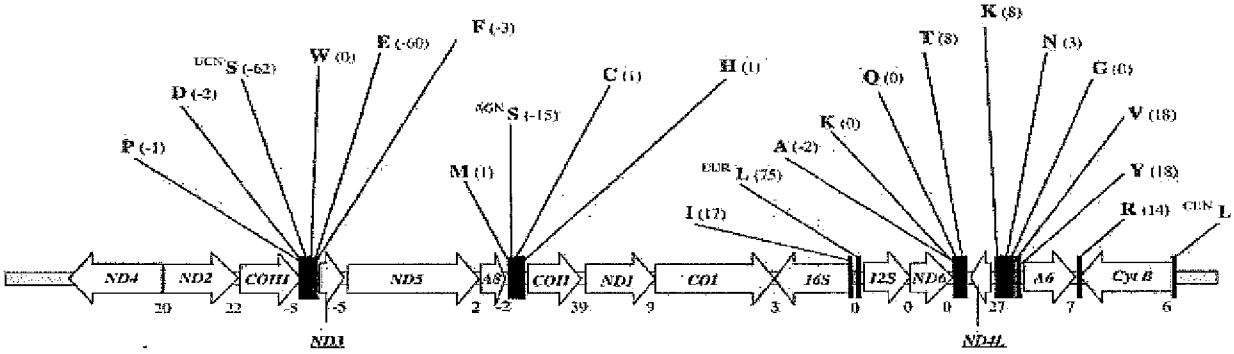
0 0.1 .3 0.5 1 3 6 12 20
ppb (axis not to scale)
Bioassay 2: Percent active lice at given concentration deltamethrin
LsGulen F16
LsVikna F7
LsHybridG F2
LsHybridV F2
% 100 90 80 70 60 50 40 30 20 10 0
0 0.1 .3 0.5 1 3 6 12 20
ppb (axis not to scale)

FIG. 3

Figure 4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AGN S (-15)
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H (1)
I (17)
EUR L (75)
A (-2)
K (0)
Q (0)
T (8)
K (8)
N (3)
G (0)
V (18)
Y (18)
R (14)
CUN L
ND4
ND2
COII
ND5
A8
COII
ND1
CO1
16S
12S
ND6
A6
Cyt B
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22
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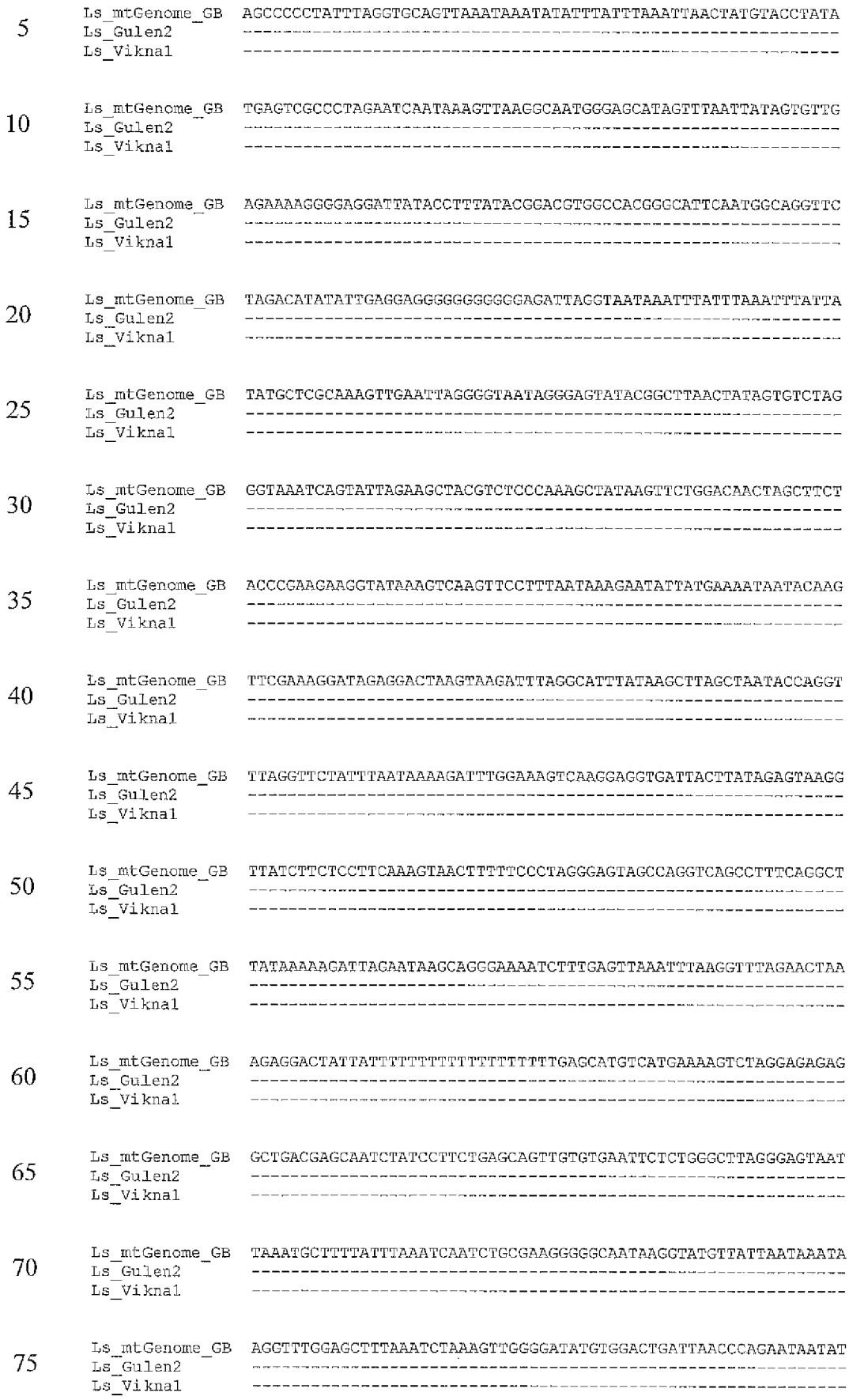
ND4L

Figure 5: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Ls_Viknal ----------
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Ls_Viknal ----------
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Ls_Viknal ----------
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Ls_Viknal ----------
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Ls_Viknal ----------
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Ls_Viknal ----------
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Ls_Viknal ----------

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```

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Ls_Viknal       ------------------------------------------------------------

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Ls_Vikna1       ------------------------------------------------------------

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Ls_Gulen2       ------------------------------------------------------------
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Ls_Gulen2       ------------------------------------------------------------
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Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTTTTAATTTAAATTTCCGGGGGTTATGGCTTGTTTTGAGAAGGTTTTGAGTACTGGGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGGGTTTTAATATTCCTTTCAGCTTTTAGGAGTTTAAAGGAATCCTCAATACTAGTATTG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGAGCAAAACTAAGAATTTCAAATTTGGAACTAGAAGTCAGAGGGTTGGGTGACTTTTAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TCTATATCCTTCAGGGGAGTTGTGATAATAATTAGGGGTTCAGTGTATCTCTACTCCCTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGGTATATAGAAACTGAAAAGTATTTTAATCGATTTATGACTCTCGTTAGGTTGTTTATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTTAGAATGCTAGTGTTAATTTACAGAGGGGATGTGGTACAATTAATTCTAGGGTGGGAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGCTTAGGTCTTAGGTCTTATTTACTAGTTTGTTACTATAATAATAGGAATTCTTCTAAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GCAGGGGCACTAACCCTTATACTAAACCGTTTAGGGGATGTGGGTTTGTTTTTAAGAATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TACGTTATAGTAAATTCCAGGCATTCAATTAACAGGTTCATAAGCATCTCTAGGGTAATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGAAGGCTATTATTATTTGTAGCTTTTACTAAAAGGGCTCAATTGCCCTTTAGGGCTTGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTACCCGCAGCAATAGCTGCTCCTACTCCTGTGTCTTCTCTAGTCCATTCCTCAACTTTG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GTTACTGCAGGAGTCTACGTATTAATTCGATTTTATGACAGGCTAATTTCTGTGTTATGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTAGGTTAATTGTAGGAGTCTCTACTAGAAGATTAGCGACTTTAGTAGCAATAGCTGAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CGCGACATAAAAAAAAATTGTAGCTTTCTCAACTTTAAGACATTTAGGAATTATAATAAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTCTGAGATTAGGTTCAGTCGAACTAGCCTTTAGGCATTTAATTTTCCATGCTTTTTTT
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Ls_Gulen2       ------                                                  ----------
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Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAAGCGTTATTATTTCTAGTAGTGGGGTATTGAATCCACAGCTCCTGCGGTTATCAAGAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CTATTAAAAATTAATTTGTTGAGTAGGCAAGAGCCAGTAATCAGAAGTCTTGGCGGAGTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TCTATAATAAGTCTTTGTGGACTTCCCTATTTAACTGGGTTTTATAGAAAAGACTTATTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATAGAAGCAAGTGTGCAGTTTAGGAGAAGTCTTCTTGGATTAGGTTTTATTTTAAGAAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TGTGTAGGTTCAGTAATGTATAGAATTCGGATGTTTCTAATAGTTAATAAAGTGGGAAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GTCTCAAGGCTTTTAAATTGAGGAGGGTCTTTTAAATTTTACCAGTTAAGTATAAGCTTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTATACTTCTTTTCCATTATAGGAGGTAGATTTTGGAGAAACTTCTGAGTTACTTCCGGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGAGTTTTATATGTCTTTTGAGAAGTAAAAATAGTGATTATTAGGTTAATCACATGCAGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGGTTGCTAGGAGCTTATCTTAGGAATACAGGGGTAAAAAACAGGTTTTTGAGTAAACTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGGTACCTAAGAGACATAAGAATCCGGCTCCCCAACATCCTCAGAAAATCCTTGATAAGC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAAAGAGTAAAATTAGATAATTCTTTTCTTAGCAGCCATTACAATAAATACTACAGAGTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GAAGGAGGAAAATATTTATTAAATAGAGGTAACTTAACGTATTCTGAACTAATCTTAGTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATATTCTTTTTTTATGTGATATTATATGTGCTCAGGGGGTAATTGTTCCTCAAGCAGAAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TAGTATATTGAAACATAATTTTTTGTCTATGTGTCGGTAGTATTGGTGTTAGGGTATTAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AATTATATTTTTTGTAAAGAGGTAAGCTAAAAAAGCTGATAGACTCATAATCTATCTATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------
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Ls_mtGenome_GB  GGATCCCTTCTCTTTAAAGCTTAAAAGAGGAAATTTCTAATTTCTTTATGGCTTAGGTCG
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  AGCTTTTAGCTAATAAAAAGGGAATTGCAAACTCCCTATTCCTCACAGGGTTAGCTTGTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TAGGTAATTTATATAAAATGCCCAATTGTGGAGTGGGTAAAGCATCTTGCCTTAAACATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TGCCTACTTGATCTCAGTTTAGTTTCCAAGATGCTTCCTCCCCAGTAATAGAAGAGTTTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTTTATTCCATGATGTGATTATAGTTTGTTTAGGCCTTATCTTAGTATCCGTAGGGGGTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TATTAGGGGCAGTGTTTGTAGAGGGGCCTTATGTAGGAGGTTTAATCGACGGAGAGTGAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TAGAGTGAATTTGAACAGCTTTACCTGGTTTAGTACTATTTGGGGTAGCTATACCCTCTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TATCTTTATTATATATATACGATGAAATAACAAATTTTGATTTGGGGGTAAAAGTAAATG
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGTATCAGTGATATTGGAGCTACGAGATACCCTTAGCAGAGGGCCTTGTGTTAGATTGTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTATGGTCCCCAGAAGGCATACAAAGTTAGGACAAATGCGCTTACTTGAAAGAGATGTGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  CTTTAACTTTACCTATTAAAAGAATGCTGCAGTTGATAGTGTCTTCAAAGGATGTATTAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  ATGCTTGGGCGGTCCCTAGCTTAGGGGTTAAAATAGACGCTGTGCCAGGACGAATTAATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  CCCTATCAATATATAGATTTCGGCCGGGATTGTATTTTGGGCAATGTTCAGAAATTTGTG
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGGCACATCATAGGTTTATGCCTATTTTACTAGAGATAGTAAGGGTTGACGATTTTGCTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGTGATTAAAGTTAGTGAGAGAAGAATAAGCATAAAGTGCATTGTTATTAAAGTGTTGTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGTTTTTAATCATCAGGATTCCTGTACTTGTAAATGTTGCGTTTATTACCCTACTAGAAC
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Ls_Gulen2       ------                                                  ----------------------
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Ls_Vikna1       ------------------------------------------------------------------------

Ls_mtGenome_GB  GAAAAATTATTGGTTACTCTCAAGCACGTAAAGGACCCAATAAGGTGTCTTTTTTAGGGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTCTTCAGCCTTTCTCCGATGCTATTAAATTATTTGTAAAAGAAGTAGGCAGGTTCAGAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTGTAAATTACAGAGTCTACTGGATTTCTCCCGTAGTGGGTCTAAGGGTGGCACTACTAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TCTGAAATGTCTATCCCTTAGAGATAAGGTTTGCATCTTGAAGAATTTCTTCACTTGGAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TGTTAGCGGGGATAAGAGTAAGAGTTTACCCCTTATTTCTTAGAGGGTGAAGGTCTAATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GTGTGTATTCACATATTGGAGGAGTGCGAGGAGTTGCTCAAGTAATCTCCTACGAAGTAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTTTTACTATCCTAGTATTTTCTTTAATAAGATTAAGAGGAAGCTTAACTTTAGTGGGGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GTATACTATTTAGGGAGTATTTTTATGTTTACATATTAAGTCCTTTATTAGTGTACCTAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GATTCCTAACAGGTCTAGCGGAGACCAACCGAACTCCCTTTGATTTCTCTGAGGGGGAAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GAGAGTTAGTGTCCGGATTCAATACAGAGTATGGGGGCAGGGGGTTTGCTTTAATTTTTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TAGCACAGTATGCTTCGATCTTATTGTTAAGAAGTTTATTTAGATTATTTTTACTTAAAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GAAGAGGACTTTTGTTTTATGTAATTAGAAGGTTAGGAGGGTTCTTGTGAGTGTGAAGGC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGGTTACTTACCCTCGGCACCGGTATGACTGATTAATAAGATTATGTTGAAAATCTCTTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TACCAAGAATTCTACTCGGGGGAGGGGCAATAATTTTAATAATAATAAGAATTTAATTGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  CAACTATAAAAAAGTGATTATGATCTTGCAACCATAAAGATATTGGGACTCTATACCTAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       -------------------------------------------------------------C
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Ls_mtGenome_GB  TAAGTGGATTTTGATCTGGATTAGTGGGTTTAGCTATAAGAGTCATCATCCGTCTTGAGC
Ls_Gulen2       ----------------------------------------------------------C
Ls_Viknal       TAAGTGGATTTTGATCTGGATTAGTGGGTTTAGCTATAAGAGTCATCATCCGTCTTGAGC

Ls_mtGenome_GB  TGTCTCAGCCGGGAGCATATTTAGGGGATTCCCAGGTTTATAATGTTATTGTAACTGCTC
Ls_Gulen2       TGTCTCAGCCGGGGGCATATTTAGGGGATTCCCAGGTTTATAATGTTATTGTAACTGCTC
Ls_Viknal       TGTCTCAGCCGGGAGCATATTTAGGAGATTCCCAGGTTTATAATGTTATTGTAACTGCTC

Ls_mtGenome_GB  ATGCCTTCATTATAATCTTTTTCATAGTAATACCTGTATTAATTGGGGGTTTTGGAAATT
Ls_Gulen2       ATGCCTTCATTATAATCTTTTTCATAGTAATACCTGTATTAATTGGGGGGTTTGGAAATT
Ls_Viknal       ATGCCTTCATTATAATCTTTTTCATAGTAATACCTGTATTAATTGGGGGGTTTGGAAATT

Ls_mtGenome_GB  GATTAGTTCCTTTAATACTGGGGGCACCTGACATAGCTTTCCCCCGCTTAAACAATATAA
Ls_Gulen2       GATTAGTTCCTTTAATACTGGGGGCACCTGACATAGCTTTCCCTCGCTTAAACAATATAA
Ls_Viknal       GATTAGTTCCTTTAATACTGGGGGCACCTGACATAGCTTTCCCCCGCTTAAACAATATAA

                                                T8605C
Ls_mtGenome_GB  GATTTTGGTTTTTAATACCCTCTTTGAGTTTATTGCTTATAAGGGCATTAGTAGAAAGTG
Ls_Gulen2       GATTTTGGTTTTTAATACCCTCTTTGAGTTTATTGGTTATAAGGGCATTAGTAGAAAGTG
Ls_Viknal       GATTTTGGTTTTTAATACCCTCTTCGAGTTTATTACTTATAAGGGCATTAGTAGAAAGTG

Ls_mtGenome_GB  GTGCAGGAACTGGGTGAACAGTATACCCCCCCCTGTCTTCGGGAGTTTTTCACTCCGGGG
Ls_Gulen2       GTGCAGGAACTGGGTGAACAGTATACCCCCCCCTGTCTTCGGGAGTTTTTCACTCCGGGG
Ls_Viknal       GTGCAGGAACTGGGTGAACAGTATATCCCCCCCTGTCTTCGGGAGTTTTTCACTCCGGGG

Ls_mtGenome_GB  CTTCAGTAGATTTTGCGATTTTTTCCCTCCACTTAGCTGGAGTGTCTTCTTTATTAGGGG
Ls_Gulen2       CTTCAGTAGATTTTGCGATTTTTTCCCTCCACTTAGCTGGAGTGTCTTCTTTATTAGGGG
Ls_Viknal       CTTCAGTAGATTTTGCGATTTTTTCCCTCCACTTAGCTGGAGTGTCTTCTTTATTAGGGG

Ls_mtGenome_GB  CTGTAAACTTTATTAGAACTATTACAAATTTACGGTGCTTGGGACTTTTAGTGGGGCAAA
Ls_Gulen2       CTGTAAACTTTATTAGAACTATTACAAATTTACGGTGCTTGGGACTTTTAGTGGGGCAAA
Ls_Viknal       CTGTAAACTTTATTAGAACTATTACAAATTTACGGTGCTTGGGACTTTTAGTGGGGCAAA

Ls_mtGenome_GB  TGCCAATATTCCCCTGATCCGTTTTAATCACTGCTGTGCTTTTACTCTTGTCTTTACCAG
Ls_Gulen2       TGCCAATATTCCCCTGATCCGTTTTAATCACTGCTGTGCTTTTACTCTTGTCTTTACCAG
Ls_Viknal       TGCCAATATTCCCCTGATCCGTTTTAATTACTGCTGTGCTTTTACTCTTGTCTTTACCAG

Ls_mtGenome_GB  TGTTAGCGGGAGCAATTACTATACTTCTTACTGATCGAAATTTAAATACAACTTTTTTTG
Ls_Gulen2       TGTTAGCGGGAGCAATTACTATACTTCTTACTGATCGAAATTTAAATACAACTTTTTTTG
Ls_Viknal       TGTTAGCGGGAGCAATTACTATACTTCTTACTGATCGAAATTTAAATACAACTTTTTTTG

Ls_mtGenome_GB  ACCCTAGAGGTGGAGGGGACCCCATTTTATACCAGCATTTATTTTGATTTTTCGGGCACC
Ls_Gulen2       ACCCTAGAGGTGGAGGGGATCCCATTTTATACCAGCATTTATTTTGATTTTTCGGGCACC
Ls_Viknal       ACCCTAGAGGTGGGGGGGATCCCTATTTTATACCAGCATTTATTTTGATTTTTCGGGCACC

                                                A9035G
Ls_mtGenome_GB  CTGAAGTTTATATTCTAATTCTTTCCAGGGTTTGGATTAATCTCTCAGATTATTACCCAAG
Ls_Gulen2       CTGAAGTTTATATTCTAATTCTTTCCAGGGTTTGGATTAATCTCTCAGATTATTACCCAAG
Ls_Viknal       CTGAAGTTTATATTCTAATTCTTTCCAGGGTTTGGGTTAATCTCTCAGATTATTACCCAAG

Ls_mtGenome_GB  AAACTTGTAAAGATGAGGCTTTTGGTTCGCTTGGTATAATTTATGCAATAGTAGCAATTG
Ls_Gulen2       AAACTTGTAAAGATGAGGCTTTTGGTTCGCTTGGTATAATTTATGCAATAGTAGCAATTG
Ls_Viknal       AAACTTGTAAAGATGAGGCTTTTGGTTCGCTTGGTATAATTTATGCAATAGTAGCAATTG

Ls_mtGenome_GB  GAGTATTGGGATTTATTGTTTGAGCTCATCACATGTTTACAGTTGGGTTAGACTCAGACA
Ls_Gulen2       GAGTATTGGGATTTATTGTTTGAGCTCATCACATGTTTACAGTTGGGTTAGACTCAGACA
Ls_Viknal       GAGTATTGGGGTTTATTGTTTGAGCTCATCACATGTTTACAGTTGGGTTAGACTCAGACA

Ls_mtGenome_GB  CTCGAGCGTATTTTACGGCAGCCACTATAGTAATTGCTATCCCTACAGGGATTAAAGTAT
Ls_Gulen2       CTCGAGCGTATTTTACGGCAGCCACTATAGTAATTGCTATCCCTACAGGGATTAAAGTAT
Ls_Viknal       CTCGAGCGTATTTTACGGCAGCCACTATAGTAATTGCTATCCCTACAGGGATTAAAGTAT

Ls_mtGenome_GB  TTAGTTGATTAGGGACTTTATATGGAAGTAAGATTATTTATACCCCCTCAATTTATTGGG
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Ls_Gulen2
TTAGTTGATTAGGGCCTTTATATGGAAGTAAGATTATTTATACCCCCTCAATTTATTGGG
Ls_Viknal       TTAGTTGATTAGGGACTTTATATGGAAGTAAGATTATTTATACCCCCTCAATTTATTGGG

Ls_mtGenome_GB  TGGTAGGGTTTCTGTTTCTTTTTACAGTGGGAGGATTAACAGGTATTGTATTAGCTAACT
Ls_Gulen2       TGGTAGGGTTTCTGTTTCTTTTTACAGTGGGGGGATTAACAGGTATTGTATTAGCTAACT
Ls_Viknal       TGGTAGGGTTTCTGTTTCTTTTTACAGTGGGAGGATTAACAGGTATTGTATTAGCTAACT

Ls_mtGenome_GB  CTGCCTTGGATATGACTTTGCATGACACTTACTATGTAGTTGCCCACTTTCACTATGTTC
Ls_Gulen2       CTGCCTTGGATATGACTTTGCATGACACTTACTATGTAGTTGCCCACTTTCACTATGTTC
Ls_Viknal       CTGCCTTGGATATGACTTTGCATGACACTTACTACGTAGTTGCCCACTTTCACTATGTTC

Ls_mtGenome_GB  TTAGAATAGGAGCAGTTTTT-GCCCTAATAGCGGGATTCACTCACTGATACCCCCTTTTG
Ls_Gulen2       TTAGAATAGGAGCAGTTTTTCGACCTAGGCTTAGG----------------------
Ls_Viknal       TTAGAATAGGGGCAGTTTTT-GCCCTAATAGCGGG----------------------

Ls_mtGenome_GB  ACAGGTTTAAGAATGAACTTAACTATAAGGAAAGCGCAGTTTATCCTAATATTTATTGGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GTAAATTTAACGTTTTTTCCTATGTACTTCCTAGGGTTAGCAGGTATACCTCGGCGGTAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGGGATTACCCAGACTTCTATTATTTGTGGAATCAGGTAGCTAGTTTCGGTTCTTACCTA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ACTTTATTTTCTGTGGTAGTATTTGTAGGGATAATTTGAGAGTCTATAGTTAGGTTACGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CCAGTATTAGCGGTATTTGATAGGGGAAGATCCATCGAGTTCAAACATACGTGTCCTCCG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TATAACCATTCTTATAACTCCTGCCCCCGACTGACTTTAGCATAAATAAATTAATTTAAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAAATACCTAAAATTAGTTATTTTCCTTTCGTACTAATAAGTAAGTAAAAGCCCTGAGAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGAAACCAATCTGACTTACGTCGATCTTAACTCAAATCATGTAAGTACGAAAAGTCGAAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGACTTAAAATAGGACCTTCTGCAGCACTATATTACTTAATTCAACATCGAGGTCACGAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TATCTTTTAAATAAGAGCTCTAAAAGTATTACGCGCTGTTATCCCTAAGGTACCTATGTG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TAGCAAAATAATTGGTTCAGAATAACTAAGGTTTCTTTATTATATTAAGTGGGCTATCCC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGCCAAACATGGTAAAGGTCTTAGGGTCTTATCGTCTTCGGAGAGTATATCAGCGTTTGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------
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Ls_mtGenome_GB  ACTGATAGCTAAGATTCAAGAGATAAGAATAAACATGATTAACCAATGCTAGCTTCATAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGGTTTACAATTAATAAACTAATTATTGCGCTACTTTAAAGCCATAAAAAATCATTCATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GGGCAGATACATCAGACACAGTCTTACTATGTCTTTGTTAAACAGTTTGGAGAATCTCAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GTTATTTAGTTTATATTTAATTTAAAATTAATAAAAGTTATTTAAGTAAGGAAATAATAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAGTATATTTACTAGTGTAAACAAGTTATAATTCTCAGAATTAACTACCCAAATAATAAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGAAGCTTAATAAATAAATTAGGTCAATAATAATTTATTATAACAGAGGGGACTATTATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CCTGAAATATTTTTAGACTAGTTGCTCACTAATGTATACTCAGCATAAGCTAAATACATT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTTTAAAATTCATTCTAAAATGTTTTATTTCTTTGGGAACCAGATTTTAAAAAGTACCA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TATATCGTGTTCATCCTTGTCTTATAGCTTAGTTTTCTACCTAGGCTTAGAGAGAGGATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GATATTTTTAATCGGGAAAGTTTTTATTGATACTAATTGTTTACCCATGATGCAAAAGGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AACAGTTCTTATACTACTTTGAAGGATTACTAATAGGGGCAGTTCGCATAACTATCCTAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CAAGATGGCCCCTATACTTCGGGCACCCTATATTTTAAAGGGGGTGGCCCATGTCTAGCA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CTTAAAGCTAGTGCACTTTCTCTGCCACTTTAAAAATAATTTATTAAATAAAAGATTAAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GATAATCTACGATAAGCTTCTCCCATAGAGAAGTAAAGCATAAAGCAGATTAAGTTACTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTGAGAATAATAGCCTATATAAAATTCTAATTTACATGCAATGAAATTGGACTCGCAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATATGGATTTACTAAATAAAAAAAGAAGTAGAGATTTTTAAGTTCTGAATAAGAAAGTGC
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Ls_Gulen2       ------                                                  ----------------------
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Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CAGCATTCGCGGTTATACTTTTAGAAAAATAGGCATTTAAATCTCAAAAATTAACATTTC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATGAGGTCAAATTTAAAAAATGTTAGTAAAATTTGATGAAAATAGTCCAGTGAATCGGAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TAGTCCCCGAGTAATAAAGATAAGTTTTTTAGTAGTACTGAATAGAAAACTAAACTCAAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TGGCAGAATTATCTCTCAAATTAGGGAAACCTGTATTAACAGAATTTACTCTTTAGATTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TAATTTTTTTAAAGTATGGCTGTCTTACTTTATTCAGAAATTGTCTTAGTCAAGTAATAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTTATATAATTAATATTTATAGGTTGCATTAAATTATAAAATAGAGTATTTAATAGTAAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CTAAAAAACGCTGAATATTTTAGTAATTAGTTCAAATTGTCCGTCACTCTCTTAGGAGAT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAGTCGTAGCAAAGTAATAGTTATGGAAATAGCTATTAAATTTCTTTTGAAGAAATTTTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTTAAATTAATAGTTATTTCGAGAGTTGCTTGTACTATAATTCTTGGGCTGATTCCTAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  CCTTTCATGTTGTGTTTATTAATTGTAGTATTAACTAGAGTTAGATGTGGGATAGTAAGG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  ATTTTTTGTAGCCAGTGGTTAAGCTACGCTTTAGTAATTGTATTCTTGGGGGGTATAATA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GTATTGTTTACTTACGCTTCAAGAATAAGAGTATCAGATAAATTGACAAAAAGAAGGAGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TGAAAAAGACTATTTGTATTTCTGATTATGGGGTTAACTTCAAGGGCCCCCAGAGGATTT
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AAAGTTTATATAGGAAGACTATATTCAAATAATGGGGGGGTTATACTTTCAATCTTAACC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTTTATTTACTCCTGTGCCTATTTAGAGTGGTAAAAATAGTAGAAGTGAGAAAAGGGGCT
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Ls_Viknal       ------------------------------------------------------------
```

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Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TACTTTTAGAGTGTAATCACGTTATAATTTTATTATAAAAATAGCTTAAAGCTTAAAAGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  ATATTTTAAGGTGTTTAGCACATAGGATTTTGAGTCCTAAGGAGTTAAGAAATTATCTAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  AATAATTATTTGGGCTAAAGCATCTAGTATTTACAAAATACTCATTTTAGTTAAACTACC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  AAATAAAGTTAGCCTAATAAGTAAAAAAAGTGGAGTTGGATAGTTCAGTAGACTTTGAAC
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GGATTGAAGAGATAAGGGCAGCAAGCCCTATACATGCTTCACAGACTATAACTGTCACAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  ATAAAAATCTGATAGTTCAGTAGGTTATACTGAAGACCGAAAAAAAAAATGCAAATAGGAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  TTATGGTAGCTGTAAGAGCTTCTAATAAAAGTAGCCTGATAAGCAAGTGCCCTTTATTAA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  ATAAAAATCACCTAGAGGTGAATAAAGGAGGGAGAGCTAACAAGTATAAACTGCTAAGGT
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  CAGGTAAAAAAAAAAATGAGTACAAGAGTTGTTAAAATAGTTCTCATAGAGTATAAAAACA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  ATAGCTGTTAGCTTAAAAGATAATCTAATAAAGATGTTAAATTTTTACTTTAATTATGGA
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Ls_mtGenome_GB  AGGTCCTCTTTTAATCCCCTAATTATTTAAGGTCTTGCATTAGACGTTTCACTGTTAATG
Ls_Gulen2       ------------------------------------------------------------
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Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  GAGATTTAAAAGTTAAACTGACTATTAATCATAGTAAATTTTATATTTCACTTACAATGA
Ls_Gulen2       ------------------------------------------------------------
Ls_Vikna1       ------------------------------------------------------------

Ls_mtGenome_GB  AAAGGTGCCAATGGCTGATTAATTCTTATCGAGCTCCTATCAATTAAGTAGTTTAATAAA
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Ls_Vikna1       ------------------------------------------------------------

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Ls_Viknal       ------------------------------------------------------------

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Ls_mtGenome_GB  ATGTGAAGAGGGGTTTTTAGGGCTATTCATTCCGATGTAAAAATTATATTTAGTGAAGGG
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Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  GCAGGGTTTATGCTTATGAGATTATTTTTAAGGGTTCTAAGAATGAATTTTCTAGGGCTT
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Ls_mtGenome_GB  TTTCCGTACATTTTTACTAGGAGTAGGCACATCTGTGTGAGATTAGGTATAGGGCTGCCT
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Ls_Viknal       ------------------------------------------------------------

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Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGAAGGTTAATCCGACCTCTAACCCTAAGGGTTCGGCTTATAGCTAATATAATTGCAGGC
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

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Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTAACTATGTTAGGATTATTATTCTTAGTCCTACTAGAGTTAGGGGTGAGTCTAATTCAG
Ls_Gulen2       ------------------------------------------------------------
Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  AGATACGTTTTTATATTATTAAGCTCTTTATATGTTAGGGAGACTCTTCACTAATGAATT
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Ls_Viknal       ------------------------------------------------------------

Ls_mtGenome_GB  TTGTTACTGTCCTAGTTTCGACCTAGGCTTAGGTTTTAACCAGCAAAATAATCTAATTAT
Ls_Gulen2       ---------------------------------TTTTAACCAGCAAAATAATCTAATTAT
Ls_Viknal       -------------------------------------------CAAAATAATCTAATTAT

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Ls_Viknal       TTATGTTTGTGTAATGCGGGCAGTTATTACGCCTGTCAAATGACTTCAAACTAATAAAAG

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Ls_Gulen2       TTTAGCTCACAATCCGTCTGAGTAAGGTAAGGACAGCCCTACTCTGAAGTATAAGACAGT
Ls_Viknal       TTTAGCTCACAATCCGTCTGAGTAAGGTAAGGACAGCCCTACTCTGAAGTATAAGACAGT

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Ls_Viknal       AAAAACTTGGCCCAGGAAAATATAGTTTCCCTCTACAGGTTTTGCTCCAATCCAGGTTAA
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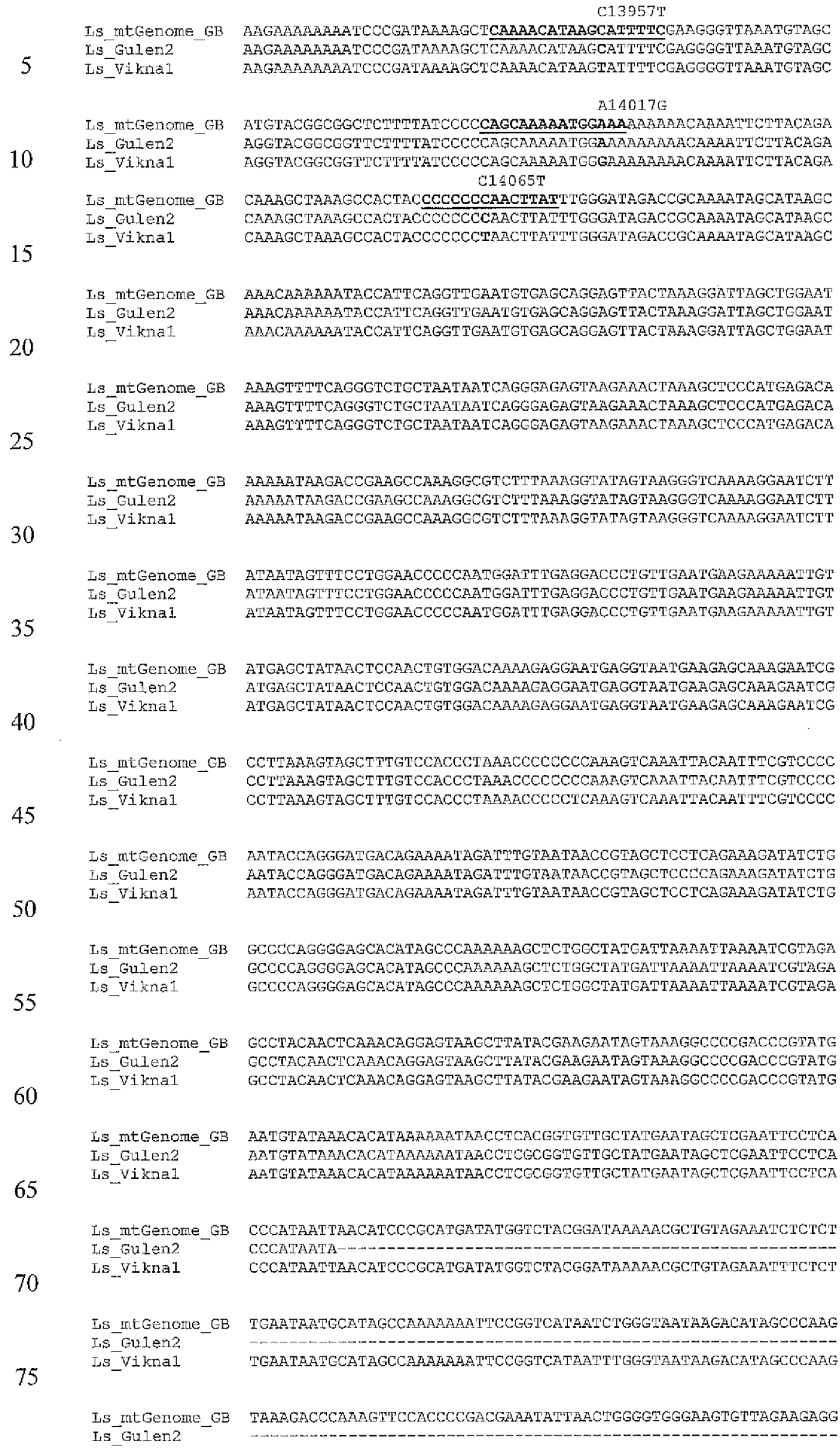
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Ls_Viknal AGGTACGGCGGTTCTTTTATCCCCCAGCAAAAATGGGAAAAAAAACAAAATTCTTACAGA
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Ls_Viknal CAAAGCTAAAGCCACTACCCCCCCCTAACTTATTTGGGATAGACCGCAAAATAGCATAAGC
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Ls_Gulen2 AAACAAAAAATACCATTCAGGTTGAATGTGAGCAGGAGTTACTAAAGGATTAGCTGGAAT
Ls_Viknal AAACAAAAAATACCATTCAGGTTGAATGTGAGCAGGAGTTACTAAAGGATTAGCTGGAAT
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Ls_Viknal AAAGTTTTCAGGGTCTGCTAATAATCAGGGAGAGTAAGAAACTAAAGCTCCCATGAGACA
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Ls_Gulen2 AAAAATAAGACCGAAGCCAAAGGCGTCTTTAAAGGTATAGTAAGGGTCAAAAGGAATCTT
Ls_Viknal AAAAATAAGACCGAAGCCAAAGGCGTCTTTAAAGGTATAGTAAGGGTCAAAAGGAATCTT
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Ls_Viknal AATACCAGGGATGACAGAAAATAGATTTGTAATAACCGTAGCTCCTCAGAAAGATATCTG
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Ls_Viknal GCCCCAGGGGAGCACATAGCCCAAAAAAGCTCTGGCTATGATTAAAATTAAAATCGTAGA
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Ls_mtGenome_GB AATGTATAAACACATAAAAAATAACCTCACGGTGTTGCTATGAATAGCTCGAATTCCTCA
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Ls_Viknal AATGTATAAACACATAAAAAATAACCTCGCGGTGTTGCTATGAATAGCTCGAATTCCTCA
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Ls_Gulen2 CCCATAATA----------
Ls_Viknal CCCATAATTAACATCCCGCATGATATGGTCTACGGATAAAAACGCTGTAGAAATTTCTCT
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Ls_Gulen2 ----------
Ls_Viknal TGAATAATGCATAGCCAAAAAAATTCCGGTCATAATTTGGGTAATAAGACATAGCCCAAG
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Ls_Gulen2 ----------

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Ls_Viknal
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Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       ATTATAGATAGAAAGAGTTTTATAAGAATTCATAAATGGAGCTTTCTGGCAGAAAAAGTG

Ls_mtGenome_GB  CGTTAATTTTAGAAATTAAGTATAAGAGATTCTTGTAAGCTACAACATACTACTTAATAG
Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       CGTTAATTTTAGAAATTAAGTATAAGAGATTCTTGTAA----------------------

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Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  AACGGCTAGATTATTAGTTTAGTATATTAACTAGAACAAAATAGTTAGTTAATATTTATT
Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  AAAAGTTTAACCAAGTTAAGTGGAGGAGGCTTCTGTAGTAACCGCGGAGTTTGGTTTAAT
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Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  GAGAACTCTCTTAAAGCTAACCGAAACTAGGTTGATCTTCAAAATATTTTTTAAGATTGG
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Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  TTTAAGCTATGCACAAGTTGCTAATATTTATTAAAAGATTTAAATAAGTAGAAAGTTTAG
Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  ATTTAATACTAAGAAGAATTACTAGAGAATAAAGCCAGACCAAATATTTATTTAAAGATT
Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  TAGATAAGTAGCTACGGCTAGGTGGTGGGGACAAGGTGCATAATCAATACAAGTGAGTTG
Ls_Gulen2       ----------------------------------------------------------
Ls_Viknal       ----------------------------------------------------------

Ls_mtGenome_GB  GGGGGGGGGGGTATGGGGGGGGATTA
Ls_Gulen2       -------------------------
Ls_Viknal       -------------------------
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2014/066895

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2014/066895

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, EMBL, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 615 976 A1 (AMERICAN CYANAMID CO [US]) 21 September 1994 (1994-09-21) abstract -----	1-25
A	VAN LEEUWEN THOMAS ET AL: "Acaricide resistance mechanisms in the two-spotted spider mite Tetranychus urticae and other important Acari: a review", INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY AUG 2010,, vol. 40, no. 8, 1 August 2010 (2010-08-01) , pages 563-572, XP002731810, ISSN: 1879-0240 abstract paragraphs [03.2], [03.3] ----- -/--	1-25

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2014	18/11/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Celler, Jakub

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VAN LEEUWEN THOMAS ET AL: "Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-Mendelian inheritance in action", NATIONAL ACADEMY OF SCIENCES. PROCEEDINGS, NATIONAL ACADEMY OF SCIENCES, UNITED STATES, vol. 105, no. 16, 22 April 2008 (2008-04-22), pages 5980-5985, XP002731811, ISSN: 1091-6490 abstract	1-25

X	TJENSVOLL KJERSTI ET AL: "Sequence variation in four mitochondrial genes of the salmon louse Lepeophtheirus salmonis", DISEASES OF AQUATIC ORGANISMS, INTER-RESEARCH, DE, vol. 68, no. 3, 2 March 2006 (2006-03-02), pages 251-259, XP002731812, ISSN: 0177-5103	15-22,25
A	abstract page 253, column 2	1-14,23, 24

X	DATABASE EMBL [Online] EBI; 3 February 2006 (2006-02-03), "Lepeophtheirus salmonis isolated cytb gene", XP002731859, Database accession no. AY602377	15-22,25
A	the whole document	1-14,23, 24

X	DATABASE EMBL [Online] EBI; 3 February 2006 (2006-02-03), "Lepeophtheirus salmonis isolated cytb gene", XP002731860, Database accession no. AY602296	15-22,25
A	the whole document	1-14,23, 24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2014/066895

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0615976 A1	21-09-1994	CA 2112445 A1	01-07-1994
		EP 0615976 A1	21-09-1994
		JP H06253849 A	13-09-1994
		US 6027876 A	22-02-2000