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(54) Titre : VACCIN POUR POISSONS CONTRE L'INFECTION PAR PISCIRICKETTSIA SALMONIS
(54) Title: FISH VACCINE AGAINST PISCIRICKETTSIA SALMONIS

(57) **Abrégé/Abstract:**

The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against infection by *Piscirickettsia salmonis*. The invention is based on or derived from the nucleic acid or amino acid sequence of at least a part of the surface antigen present on *Piscirickettsia salmonis*. Nucleic acid and/or amino acid sequences may be used in the preparation of a vaccine to protect against infection by *Piscirickettsia salmonis*.



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(54) Title: FISH VACCINE AGAINST PISCIRICKETTSIA SALMONIS

(57) Abstract: The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against infection by *Piscirickettsia salmonis*. The invention is based on or derived from the nucleic acid or amino acid sequence of at least a part of the surface antigen present on *Piscirickettsia salmonis*. Nucleic acid and/or amino acid sequences may be used in the preparation of a vaccine to protect against infection by *Piscirickettsia salmonis*.



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Fish Vaccine against *Piscirickettsia salmonis*

The present invention relates to a fish vaccine. More specifically the invention relates to a vaccine to protect salmon against piscirickettsiosis also referred to as salmonid rickettsial septicaemia (SRS).

To date no commercially available vaccine is effective against *Piscirickettsia salmonis*, the causative agent of SRS. Accordingly there is a need for an effective vaccine against *Piscirickettsia salmonis*.

It is an object of the present invention to provide a vaccine to protect against SRS.

It is an object of the present invention to provide a vaccine to protect against *Piscirickettsia salmonis*.

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In one aspect, the present invention relates to an isolated nucleic acid molecule consisting of a sequence selected from the group consisting of SEQUENCE ID Numbers: 1, 3, 5, 7, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28, and synthetically
5 prepared analogues thereof.

In another aspect, the present invention relates to an isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQUENCE ID Numbers: 2, 4, 6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 30, and synthetically
10 prepared analogues thereof.

In still another aspect, the present invention relates to an isolated polypeptide consisting of an amino acid sequence selected from the partial amino acid sequence of p45 depicted in SEQ ID No. 9 or the amino acid sequence of HSP70 antigen
15 depicted in SEQ ID No. 30 from *Piscirickettsia salmonis*.

In yet another aspect, the present invention relates to an isolated nucleic acid molecule consisting of a sequence which encodes an amino acid sequence selected from the partial amino acid sequence of p45 depicted in SEQ ID No. 9 or the amino acid
20 sequence of HSP70 antigen depicted in SEQ ID No. 30 from *Piscirickettsia salmonis*.

In another aspect, the present invention relates to a composition comprising at least one nucleic acid molecule as described above and a pharmaceutically acceptable diluent.

25 In another aspect, the present invention relates to a composition comprising at least one polypeptide as described above and a pharmaceutically acceptable diluent.

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- 1b -

In another aspect, the present invention relates to a diagnostic test kit comprising the polypeptide as described above and instructions for use.

5 In another aspect, the present invention relates to the use of the nucleic acid molecule as described above in the preparation of a composition for use as a vaccine to protect against infection by *Piscirickettsia salmonis*.

10 In another aspect, the present invention relates to the use of at least one polypeptide as described above in the preparation of a composition for use as a vaccine to protect fish against *Piscirickettsia salmonis*.

In another aspect, the present invention relates to the use of the polypeptide as described above in the preparation of a diagnostic test for detecting *Piscirickettsia salmonis*.

15 In another aspect, the present invention relates to a nucleic acid PCR primer derived from the open reading frame (ORF) of the nucleic acid molecule as described above.

20 In another aspect, the present invention relates to the use of the nucleic acid PCR primer as described above in the preparation of a diagnostic test for detecting *Piscirickettsia salmonis*.

1 The present invention relates to a fish vaccine.
2 More specifically the invention relates to a nucleic
3 acid vaccine for administration to fish to protect
4 against piscirickettsiosis also referred to as
5 salmonid rickettsial septicaemia (SRS).

6

7 The invention further provides an amino acid
8 sequence for administration to fish to protect
9 against SRS.

10

11 In a further aspect the present invention provides
12 at least one nucleic acid sequence and / or an amino
13 acid sequence which is derived from *Piscirickettsia*
14 *salmonis* or a synthetically prepared analogue
15 thereof or a substantially homologous sequence.

16

17 A substantially homologous nucleic acid sequence is
18 a sequence which can be transcribed and/or
19 translated to provide an amino acid sequence which
20 is substantially homologous to at least a part of a
21 surface antigen present on *Piscirickettsia salmonis*.

22

23 A substantially homologous amino acid sequence
24 encodes at least 70% of a part of a surface antigen
25 and induces an immune response.

26

27 More preferably the homologous amino acid sequence
28 will encode at least 90% of the amino acid sequence.

29

30 A typical nucleic acid sequence is chosen from the
31 sequences of Psclone51A p10.6 (also known as immuno-
32 reactive clone 0110-2-5), IcmE, clone3/original,

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3

clone3/3PST-R, clone3.3APA-F, clone7/original,
clone7/XbaR, clone7/7MunR, clone7/7MunF,
clone20/original, clone20/20VSPF or clone 15.

Typically the amino acid sequence is derived from one of the above-mentioned nucleic acid sequences or is chosen from the sequences of p45 or HSP70 antigen.

Preferably peptide sequences or nucleic acid sequence identified herein as sequence ID numbers 1 to 30 are utilised in a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

The invention provides the use of nucleic acid sequences or peptide sequence as defined herein in the preparation of a vaccine for the protection of fish against *Piscirickettsia salmonis*.

The invention further provides a vaccine to protect fish against *Piscirickettsia salmonis* wherein the vaccine includes nucleic acid or peptide sequences as defined herein.

Figures 1, 2 and 3

A detailed description of the invention, listing the exact nucleotide sequence of Psclone51A and the

1 amino acid sequence deduced for the open reading
2 frame (ORF), is provided in figures 1, 2 and 3.
3 The genetic sequence has been derived from cloned
4 cDNA wherein the cDNA clones were derived from
5 *Piscirickettsia salmonis* type strain (LF-89)
6 messenger RNA (mRNA). The cloned material was
7 sequenced in both directions from the 5' and 3'
8 insertion sites using overlapping amplicons.

9
10 Veracity of the Psclone51A sequence was confirmed by
11 Polymerase Chain Reaction(PCR) and Reverse-
12 Transcriptase PCR (RT-PCR) of appropriate sized
13 amplicons from *Piscirickettsia salmonis* infected
14 Chinook salmon embryonic (CHSE-214) cell line and
15 not from uninfected control material. Expression of
16 the cloned sequence has yielded a protein of
17 approximately 12 kDa.

18
19 The ORF of Psclone51A described in figure 1 does not
20 have any significant homology at the nucleotide
21 level with previous submissions to databases
22 accessible by BLAST. At the protein level, a border
23 line similarity with a hypothetical 21.5 kDa protein
24 of *Escherichia coli* was found.

25
26 The ORF has commercial value for the following
27 reasons:

28
29 There is sufficient reason to believe that the
30 nucleotide corresponding amino acid sequence is of
31 *Piscirickettsia salmonis* origin. As such its
32 incorporation into nucleic acid vaccines may have an

impact on the reduction of mortality of farmed Atlantic salmon caused by *Piscirickettsia salmonis*. Characterisation of the gene product will lead to the identification of key elements in pathogenesis of infection and to the design of more accurate diagnostic tests which will also aid in epidemiological studies documenting the dissemination of different strains of the disease.

The nucleotide sequence Psclone51A and associated derivatives thereof when translated into protein sequence being composed of either identical or equivalent amino acids, should induce a response by the hosts' immune system. This principle can be further expanded to use these proteins in diagnostics tests and vaccination procedures.

Figures 4 and 5

A detailed description of the invention, listing the exact nucleotide sequence of p10.6 and the amino acid sequence deduced for the open reading frame (ORF), is provided in figure 4 and figure 5 respectively.

The genetic sequence of *Piscirickettsia salmonis* (Atcc strain VR-1361), grown in CHSE-214 cells using homologous antisera from *Piscirickettsia salmonis* immunised rabbits, an immuno-reactive clone (0110-2-5) (p10.6) was identified from the expression library. This clone was sequenced and polymerase chain reaction (PCR) primers developed.

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The *Piscirickettsia salmonis* origin of the p10.6 clone was confirmed through PCR amplification of *Piscirickettsia salmonis* and Chinook salmon embryonic cell line (CHSE-214) DNA using clone specific primers. Appropriately sized amplicons were amplified from the *Piscirickettsia salmonis* DNA, but not from the CHSE-214 DNA confirming that the immuno-reactive clone was of *Piscirickettsia salmonis* origin and not from the host cell DNA.

The open reading frame (ORF) for the full gene was completed by inverse PCR from genomic *Piscirickettsia salmonis* DNA.

The ORF of p10.6 described in figure 4 does not have any significant homology at the nucleotide level with previous submissions to databases accessible by BLAST. The derived amino acid sequence, but not the nucleotide sequence, shows significant homology to the 17kDa antigen found in *Rickettsia* of the Spotted Fever Group, where it is considered a group specific, outer membrane protein.

Figure 6

A typical nucleic acid sequence is IcmE (403).

Preferably peptide sequences transcribed or translated from the nucleic acid sequences of IcmE (403) are incorporated into a vaccination strategy

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to induce an immune response to a surface antigen of *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of IcmE (403) and the amino acid sequence deduced for the open reading from (ORF), is provided in figure 6.

The genetic sequence has been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* type strain (LF-89) genomic DNA (gDNA). The IPCR product was sequenced in both direction from the 5' and 3' sides using overlapping amplicons.

The protein encoded by the ORF of IcmE (403) has a 37% significant homology at the protein level to the IcmE protein of *Legionella pneumophila* when compared to previous submissions to databases accessible by BLAST.

Figure 7

A typical amino acid sequence is p45.

Preferably a nucleic acid sequence transcribing the amino acid sequence of p45 are incorporated into a vaccination strategy to induce an immune response to a surface antigen against *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

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A detailed description of the invention, listing the exact amino acid sequence of a portion of the p45 major antigen, is provided in figure 7.

The amino acid sequence has derived from microsequencing of a protein approximately 45 kDa found to be immunoreactive to rabbit anti-P salmonis antibodies. Moreover, p45 was found uniquely in Chinook salmon embryonic (SHSE-214) cells infected with *Piscirickettsia salmonis* and not in infected CHSE-214 cells.

The amino acid sequence of p45 has no significant homology to other bacterial proteins when compared to previous submissions to databases accessible by BLAST.

Figures 8, 9 and 10

A typical nucleic acid sequence is clone3/original, clone3/3PST-R, and clone3.3APA-F.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone3/original, clone3/3PST-R, and clone3.3APA-F are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* genomic DNA (gDNA).

The proteins encoded by the ORF of clone3/original, clone3/3PST-R and clone3.3APA-F have respectively 40%, 38% and 34% significant homology at the protein level to different portion of the transposase

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protein of *Vibrio anguillarum* (gb AAA81776.1) when compared to previous submissions to databases accessible by BLAST.

Figures 11, 12, 13 and 14

A typical nucleic acid sequence is clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the extract nucleotide sequence of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF and the amino acid sequence deduced for their open reading frames (ORF), is provided in figures 11, 12, 13 and 14 respectively.

Some of the genetic sequences have been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* genomic DNA (gDNA).

The peptides encoded by the ORF of clone7/original, clone7/7XbaR, clone7/7MunR, and clone 7/7MunF have a 40% to 44% significant homology at the protein level to different portion of the ABC transporter ATP-

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10

binding protein of the other bacterial species when compared to previous submissions to databases accessible by BLAST.

There is sufficient reason to believe that the nucleotide and corresponding amino acid sequence are of *Piscirickettsia salmonis* origin. Also, part of the ORFs was found in an immuno-reactive clone of an expression library.

Figures 15 and 16

A typical nucleic acid sequence is clone20/original, and clone20/20VSPF.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone20/original, and clone20/20VSPF are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of clone20/original, and clone20/20VSPF and the amino acid sequence deduced for their open reading frames (ORF), is provided in figure 15 and figure 16 respectively.

Some of the genetic sequences have been derived from an inverse polymerase chain reaction (IPCR) product amplified from *Piscirickettsia salmonis* genomic DNA (gDNA).

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The peptides encoded by the ORF of clone20/original, and clone20/20VSPF have a 41% and 51% significant homology at the protein level to an amino acid transporter/permease protein of other organisms when compared to previous submissions to databases accessible by BLAST.

Figure 17

A typical nucleic acid sequence is clone15/original.

Preferably peptide sequences transcribed or translated from the nucleic acid sequence of clone15/original are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact nucleotide sequence of clone15/original and the amino acid sequence deduced for its open reading frames (ORF), is provided in figure 17.

The nucleotide sequence and the peptide encoded by the ORF of clone15/original have no significant homology to proteins of other bacterial species when compared to previous submissions to databases accessible by BLAST.

From the previous information there is sufficient reason to believe that the nucleotide and

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corresponding amino acid sequence are of *Piscirickettsia salmonis* origin. Also, the ORF was found in an immuno-reactive clone of an expression library.

Figure 18

A typical amino acid sequence is HSP70.

Preferably a nucleic acid sequence transcribing the amino acid sequence HSP70 are incorporated into a vaccination strategy to induce an immune response to a surface antigen of *Piscirickettsia salmonis* and thus to *Piscirickettsia salmonis* itself.

A detailed description of the invention, listing the exact amino acid sequence of the major portion of the HSP70 protein is provided in figure 18.

The amino acid sequence of HSP70 has 70% significant homology at the protein level to *Coxiella burnetii* and *Legionella pneumophila* HSP70 proteins when compared to previous submissions to databases accessible by BLAST.

The nucleic acid and amino acid sequences disclosed have commercial value for the following reasons:

From the previous information there is sufficient reason to believe that the nucleotide and corresponding amino-acid sequences are of *Piscirickettsia salmonis* origin. Also, several of the ORF were found in

1 immuno-reactive clones of an expression
2 library. Together, this shows the potential of
3 these ORFs to be incorporated into nucleic acid
4 vaccines that may have an impact on the
5 reduction of mortality of farmed salmonids due
6 to *Rickettsial* Salmonid Septicaemia caused by
7 *Piscirickettsia salmonis*.

8
9 PCR primers derived from the full ORF, used in
10 touchdown PCR are capable of detecting
11 *Piscirickettsia salmonis* in tissues from
12 infected fish. This will lead to more accurate
13 diagnostic tests to be used for clinical as
14 well as epidemiological study purposes.

15
16 The nucleotide sequences associated derivatives
17 thereof when translated into protein sequence being
18 composed of either identical or equivalent amino
19 acids, should induce a response by the hosts' immune
20 system. This principle can be further expanded to
21 use these proteins in diagnostic tests and nucleic
22 acid vaccination procedures.

23

SEQUENCE LISTING

<110> Aqua Health (Europe) Limited
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 Griffiths, Steve
 Brouwers, Huub
 Jones, Simon
 Valenzuela, Pablo
 Burzio, Luis

<120> Fish Vaccine Against *Piscirickettsia salmonis*

<130> P25740A/GST

<140> PCT/GB01/01055

<141> 2001-03-12

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<151> 2000-07-29

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<170> PatentIn version 3.0

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<211> 404

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<220>

<223> Sequence derived from *P. salmonis* type strain (LF-89) mRNA

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attggtatgt tggcgagtca aagtaattta acctgtaaat catggttaga aaaagcgata	180
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tttacttttg ctctgctgtt acctgatggt aatgctgcgt taactgagcg tgcgagttta	360
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<211> 126

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<213> *Piscirickettsia salmonis*

2

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<223> Peptide derived from partial nucleotide sequence 1 (PsClone51A)

<400> 2

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Met Ser Gln Ala Leu Val Glu Phe Lys Thr Val Asp His Val Leu Gln
1           5           10           15
Ala Leu Gly Val Glu Gly Met Ser Ala Ala Asp Val His Gly Leu Leu
           20           25           30
Ile Gly Met Leu Ala Ser Gln Ser Asn Leu Thr Cys Lys Ser Trp Leu
           35           40           45
Glu Lys Ala Ile Phe Met Gly Ala His Leu Asp Ala Glu Ser Asp Leu
           50           55           60
Phe Ser Asn Ile Met Ala Lys Glu Gln Leu Lys Gln Leu Glu Ala Leu
65           70           75           80
Phe Lys Val Ser Trp Glu Gln Leu Ser Ala Gly Asp Phe Thr Phe Ala
           85           90           95
Leu Leu Leu Pro Asp Gly Asn Ala Ala Leu Thr Glu Arg Ala Ser Leu
           100          105          110
Leu Cys Ala Trp Thr Gln Gly Phe Leu Thr Gly Leu His Leu
           115          120          125

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<211> 591

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<213> *Piscirickettsia salmonis*

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<223> Complete coding sequence of PsClone 51A

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aatttaacct gtaaatacatg gttagaaaaa gcgatatatta tgggagctca ccttgatgct      180
gaaagtgatc ttttttagtaa tatcatggcc aaagagcagt taaagcagtt agaggcattg      240
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gatggtaatg ctgcgttaac tgagcgtgcg agtttattat gtgcctggac tcaaggcttt      360
ttaactggct tgcatttata ggggtgtaat attgctaaat ataaagaagg tgaattagcg      420
acgaccttaa aagatttagc tgaagttgcg cagttggatt tagccattga agacagtaat      480
gaaaatgaag cggcatatac tgagattgct gaatatgtac gtatggcggc gctttttgtt      540
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<210> 4

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<212> PRT

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<223> Peptide sequence of PsClone51A complete

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           20           25           30
Ile Gly Met Leu Ala Ser Gln Ser Asn Leu Thr Cys Lys Ser Trp Leu
           35           40           45
Glu Lys Ala Ile Phe Met Gly Ala His Leu Asp Ala Glu Ser Asp Leu
           50           55           60

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Phe Ser Asn Ile Met Ala Lys Glu Gln Leu Lys Gln Leu Glu Ala Leu
65          70          75          80
Phe Lys Val Ser Trp Glu Gln Leu Ser Ala Gly Asp Phe Thr Phe Ala
85          90          95
Leu Leu Leu Pro Asp Gly Asn Ala Ala Leu Thr Glu Arg Ala Ser Leu
100         105         110
Leu Cys Ala Trp Thr Gln Gly Phe Leu Thr Gly Leu His Leu Ser Gly
115         120         125
Val Asn Ile Ala Lys Tyr Lys Glu Gly Glu Leu Ala Thr Thr Leu Lys
130         135         140
Asp Leu Ala Glu Val Ala Gln Leu Asp Leu Ala Ile Glu Asp Ser Asn
145         150         155         160
Glu Asn Glu Ala Ala Tyr Thr Glu Ile Ala Glu Tyr Val Arg Met Ala
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Ala Leu Phe Val His Ser Glu Leu Ala Gly Ser Gly Gln Ala Thr Gln
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Met Thr Val His
195

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<213> Piscirickettsia salmonis

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<223> Nucleotide sequence of P.salmonis (Atcc Strain VR-1361) grown in
C

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gttgctggcc agctgtttgg taaaggtagt ggtcgagttg caatggccat tgggtggtgct 180
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gagcgtcgcc agcaatattg tcgagaattt cagcaaaagg cgatgattgc agggcagaag 420
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<220>
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Met Asn Arg Gly Cys Leu Gln Gly Ser Ser Leu Ile Ile Ile Ser Val
1          5          10          15
Phe Leu Val Gly Cys Ala Gln Asn Phe Ser Arg Gln Glu Val Gly Ala
20         25         30
Ala Thr Gly Ala Val Val Gly Gly Val Ala Gly Gln Leu Phe Gly Lys
35         40         45
Gly Ser Gly Arg Val Ala Met Ala Ile Gly Gly Ala Val Leu Gly Gly
50         55         60
Leu Ile Gly Ser Lys Ile Gly Gln Ser Met Asp Gln Gln Asp Lys Ile
65         70         75         80

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Lys	Leu	Asn	Gln	Ser	Leu	Glu	Lys	Val	Lys	Ala	Gly	Gln	Val	Thr	Arg
				85					90					95	
Trp	Arg	Asn	Pro	Asp	Thr	Gly	Asn	Ser	Tyr	Ser	Val	Glu	Pro	Val	Arg
			100					105					110		
Thr	Tyr	Gln	Arg	Tyr	Asn	Lys	Gln	Glu	Arg	Arg	Gln	Gln	Tyr	Cys	Arg
		115					120					125			
Glu	Phe	Gln	Gln	Lys	Ala	Met	Ile	Ala	Gly	Gln	Lys	Gln	Glu	Ile	Tyr
	130					135					140				
Gly	Thr	Ala	Cys	Arg	Gln	Pro	Asp	Gly	Arg	Trp	Gln	Val	Ile	Ser	Thr
145					150					155					160
Glu	Lys														

<210> 7
 <211> 1098
 <212> DNA
 <213> *Piscirickettsia salmonis*

<220>
 <223> sequence derived from IPCR product obtained from *P.salmonis* strain

<220>
 <221> misc_feature
 <222> (765)..(765)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (1064)..(1064)
 <223> n is any nucleic acid

<400> 7

caaggaatat	tggcaaaatg	ggagaatggt	tctgctcaaa	aagtgatggt	tgccaagggt	60
agtacagtta	ataatgctgg	agataatggt	cagtcaggta	aaaataataa	tgaaaatatt	120
attgaaaaag	caggtgctat	tgtattttgcg	gtactcgata	cacagctaaa	cagtgaccaa	180
cccgggactc	cagtaatggc	aacgattggt	caaggtaa	ttaaaaatgc	caaattgttg	240
ggtagcttta	aaagagagga	tgaaaaacta	gtcattttctt	ttgatcgcat	atctttgcct	300
gaacttgatc	acagtatttc	tattaaggcg	tatgcaatta	atgccacaac	agcacaaaat	360
gcactgtctt	cagatgtaga	taatcattat	ttattacggt	atgggtgggct	ctttgctgct	420
gcgtttttgc	aaggcttttg	cgattatttc	tcccaaaact	catcaagctt	atgtggtggt	480
gcgacaacct	gtattattac	aggcactcaa	tcaactgcag	aacaaaatcg	tacaacgaga	540
aaagccctat	attctgggtt	agggtcaagt	ggaacaactt	tagctggtaa	agcaagcgct	600
gcatttgatc	gccctccaac	ggttacttta	aatcaagggt	ttgggtatggg	gattttattt	660
atgtcggatg	taaagggtga	agttaaaatg	agtaataatc	acacagataa	aaattataat	720
tttgatgatg	atcaagacat	taatgaggat	aaagaagacc	ttgcngctag	ataatgagcc	780
agcagttaaa	agaaataatg	cagatgctgt	ttggcaaggg	acttcactgt	gggataaaat	840
aaggccgatg	ttgcattatt	atatcattgc	tattattgca	ttcgctgtag	caggttatat	900
gatgtataac	gcataccgaa	ctttatatcc	aaagcagtca	gtgcagcagg	ctgaggctaa	960
ccatttaagc	tttagtaatc	agggtgaaac	tggcagtaag	tcagcgaaag	gcttttctcc	1020
cctggctcag	tctcaagaaa	ataagggtcaa	aaataaaagt	gggncttgga	aaaaaagaag	1080
agataaaacc	gaatgcta					1098

<210> 8
 <211> 226
 <212> PRT
 <213> *Piscirickettsia salmonis*

<220>

<223> peptide derived from ORF of IcmE partial sequence

<400> 8

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Gln Gly Ile Leu Ala Lys Trp Glu Asn Val Ser Ala Gln Lys Val Met
1          5          10          15
Val Ala Lys Val Ser Thr Val Asn Asn Ala Gly Asp Asn Gly Gln Ser
20          25          30
Gly Lys Asn Asn Asn Glu Asn Ile Ile Glu Lys Ala Gly Ala Ile Val
35          40          45
Phe Ala Val Leu Asp Thr Gln Leu Asn Ser Asp Gln Pro Gly Thr Pro
50          55          60
Val Met Ala Thr Ile Val Gln Gly Lys Phe Lys Asn Ala Lys Leu Leu
65          70          75          80
Gly Ser Phe Lys Arg Glu Asp Glu Lys Leu Val Ile Ser Phe Asp Arg
85          90          95
Ile Ser Leu Pro Glu Leu Asp His Ser Ile Ser Ile Lys Ala Tyr Ala
100         105         110
Ile Asn Ala Thr Thr Ala Gln Asn Ala Leu Ser Ser Asp Val Asp Asn
115         120         125
His Tyr Leu Leu Arg Tyr Gly Gly Leu Phe Ala Ala Phe Leu Gln
130         135         140
Gly Phe Gly Asp Tyr Phe Ser Gln Asn Ser Ser Ser Leu Cys Gly Gly
145         150         155         160
Ala Thr Thr Cys Ile Ile Thr Gly Thr Gln Ser Thr Ala Glu Gln Asn
165         170         175
Arg Thr Thr Arg Lys Ala Leu Tyr Ser Gly Leu Gly Gln Val Gly Thr
180         185         190
Thr Leu Ala Gly Lys Ala Ser Ala Ala Phe Asp Arg Pro Pro Thr Val
195         200         205
Thr Leu Asn Gln Gly Val Gly Met Gly Ile Leu Phe Met Ser Asp Val
210         215         220
Lys Val
225

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<210> 9

<211> 30

<212> PRT

<213> *Piscirickettsia salmonis*

<220>

<223> Amino acid sequence of *P.salmonis* p45 antigen (N-terminal to C-terminus)

<400> 9

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Ala Asp Asn Gly Lys Leu Gln Leu Gln Ile Ser Gln Leu Lys Ala Gln
1          5          10          15
Gln Thr Gln Leu Gln Gln Gln Val Ala Asn Leu Gln Gly Gln
20          25          30

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<210> 10

<211> 164

<212> DNA

<213> *Piscirickettsia salmonis*

<220>

<223> nucleic acid sequence of *P.salmonis* clone 3/ original

<400> 10
 gatctttaat ctcattaataa tttatgtttc taatcgcggtg ggttattttc atcaaatacg 60
 gcatttttag gaggagggat aacaacatca gcattaggtg agtgggtttaa aacggaatcg 120
 taaacatcat ggctatcgta ggctccgtct gcagtgaagc gatc 164

<210> 11
 <211> 44
 <212> PRT
 <213> Piscirickettsia salmonis

<220>
 <223> 3' to 5' translation of P salmonis clone 3/original nucleic acid
 s

<400> 11
 Asp Arg Phe Thr Ala Asp Gly Ala Tyr Asp Ser His Asp Val Tyr Asp
 1 5 10 15
 Ser Val Leu Asn His Ser Pro Asn Ala Asp Val Val Ile Pro Pro Pro
 20 25 30
 Lys Asn Ala Val Phe Asp Glu Asn Asn Pro Arg Asp
 35 40

<210> 12
 <211> 195
 <212> DNA
 <213> Piscirickettsia salmonis

<220>
 <223> DNA sequence of P salmonis clone 3/ 3 PstR

<220>
 <221> misc_feature
 <222> (31)..(31)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (41)..(41)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (147)..(147)
 <223> n is any nucleic acid

<400> 12
 ataagcctag atgatgatat tgcgggaata nccattgatt nacaggcctt aagcgttttg 60
 gccgtgacga tgtggcacca agaaaaatac aagatatcag caaagcgcag ctggcgtaaa 120
 cttcatgtgg ccgttgatga tgatcantat attcaagccg cactcatcac cgatcgctat 180
 gaagcagatg aggag 195

<210> 13
 <211> 65
 <212> PRT
 <213> Piscirickettsia salmonis

<220>
 <223> 5' to 3' encoded protein of nucleic acid sequence of P salmonis c
 1

<220>
 <221> variant
 <222> (11)..(11)
 <223> Xaa is equivalent any amino acid

<220>
 <221> variant
 <222> (14)..(14)
 <223> Xaa is equivalent any amino acid

<220>
 <221> variant
 <222> (49)..(49)
 <223> Xaa is equivalent any amino acid

<400> 13
 Ile Ser Leu Asp Asp Asp Ile Ala Gly Ile Xaa Ile Asp Xaa Gln Ala
 1 5 10 15
 Leu Ser Val Leu Ala Val Thr Met Trp His Gln Glu Lys Tyr Lys Ile
 20 25 30
 Ser Ala Lys Arg Ser Trp Arg Lys Leu His Val Ala Val Asp Asp Asp
 35 40 45
 Xaa Tyr Ile Gln Ala Ala Leu Ile Thr Asp Arg Tyr Glu Ala Asp Glu
 50 55 60
 Glu
 65

<210> 14
 <211> 213
 <212> DNA
 <213> Piscirickettsia salmonis

<220>
 <223> nucleic acid sequence of P salmonis clone 3/3APA-F

<220>
 <221> misc_feature
 <222> (186)..(186)
 <223> n is any nucleic acid

<400> 14
 tatgagatta aagatcatgg tagaatgcac tggcaaaaga cagacaata cggcaagcgt 60
 aattattctg agttggcgat tcagcggttac aaacgcattt tgggcaacac gatgcagtcc 120
 agagacatat cgcgacagaa aaatgaagga ctaattggcg cgggtatttt aaatagagat 180
 gaccantctc ggcatgccgg tgacaataat gta 213

<210> 15
 <211> 71
 <212> PRT
 <213> Piscirickettsia salmonis

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<400> 17
Ile Xaa Ala Arg Ile Leu Ile Asp Asp His Asp Ile Gln Lys Leu Lys
1          5          10          15
Ile Gln Asn Ile Arg Gln His Ile Ala Tyr Leu Pro Gln His Gly Asp
          20          25          30
Leu Phe Asn Gly Thr Ile
          35
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<210> 18
 <211> 179
 <212> DNA
 <213> *Piscirickettsia salmonis*

<220>
 <223> DNA sequence of P salmonis clone 7/7XbaR

<220>
 <221> misc_feature
 <222> (25)..(25)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (141)..(141)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (164)..(164)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (167)..(167)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (177)..(177)
 <223> n is any nucleic acid

<400> 18
 gcacgcatat cgatacctaa cgtcntttca agaacaaaaa tattataaac aagccataga 60
 agtcagccaa ttacttggcc ttgactcaat tattgagcgc ttgcccaaag gctatcacac 120
 tcctgttgcc aatcatgccg natattaatt gactacgcac gatnttncaa agcttantg 179

<210> 19
 <211> 48
 <212> PRT
 <213> *Piscirickettsia salmonis*

<220>
 <223> 5' to 3' amino acid sequence of Psalmonis clone 7/7XbaR nucleic a
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<220>
 <221> variant
 <222> (47)..(47)
 <223> Xaa is any amino acid

<400> 19
 His Ala Tyr Arg Tyr Leu Thr Ser Phe Gln Glu Gln Lys Tyr Tyr Lys
 1 5 10 15
 Gln Ala Ile Glu Val Ser Gln Leu Leu Gly Leu Asp Ser Ile Ile Glu
 20 25 30
 Arg Leu Pro Lys Gly Tyr His Thr Pro Val Ala Asn His Ala Xaa Tyr
 35 40 45

10

<210> 20
 <211> 390
 <212> DNA
 <213> Piscirickettsia salmonis

<220>
 <223> DNA sequence of Psalmonis clone 7/MunR

<220>
 <221> misc_feature
 <222> (55)..(55)
 <223> n is any nucleic acid

<400> 20
 ttattgagcg cttgccc aaa ggctatcaca ctcctgttgc caatcatgcc atggnagtcg 60
 ctacctcgcg gtatcattca gcgcattgcg attgcccgtg ccctgattca taagccacca 120
 atcgtcctat tcgatgagggc caatacggcc atggacatgc aagggtgatac catcttaatt 180
 aatgtgcttg aacaacttaa aggcacctgc acactcatcc tcgtctctca tcgcccataca 240
 ttgctggcac atgcagataa aatctttatc ctcgagaata aaaatctggg ggagaaagtc 300
 acatgagctc tgcactaacc gcccaggagc ataatatctg cactgcgttt attaacagcc 360
 tcgaaccact gttaactgca ttaggctggc 390

<210> 21
 <211> 101
 <212> PRT
 <213> Piscirickettsia salmonis

<220>
 <223> 5' to 3' encoded protein of Psalmonis clone 7/MunR

<220>
 <221> variant
 <222> (19)..(19)
 <223> Xaa is any amino acid

<400> 21
 Leu Leu Ser Ala Cys Pro Lys Ala Ile Thr Leu Leu Leu Pro Ile Met
 1 5 10 15
 Pro Trp Xaa Ser Leu Pro Arg Gly Ile Ile Gln Arg Ile Ala Ile Ala
 20 25 30
 Arg Ala Leu Ile His Lys Pro Pro Ile Val Leu Phe Asp Glu Ala Asn
 35 40 45
 Thr Ala Met Asp Met Gln Gly Asp Thr Ile Leu Ile Asn Val Leu Glu
 50 55 60
 Gln Leu Lys Gly Thr Cys Thr Leu Ile Leu Val Ser His Arg Pro Ser
 65 70 75 80
 Leu Leu Ala His Ala Asp Lys Ile Phe Ile Leu Glu Asn Lys Asn Leu
 85 90 95
 Val Glu Lys Val Thr
 100

<210> 22
 <211> 280
 <212> DNA
 <213> Piscirickettsia salmonis

<220>
 <223> DNA sequence of Psalmonis clone 7/MunF

<220>
 <221> misc_feature
 <222> (9)..(9)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (38)..(38)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (236)..(236)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (255)..(255)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (256)..(256)
 <223> n is any nucleic acid

<220>
 <221> misc_feature
 <222> (273)..(273)
 <223> n is any nucleic acid

<400> 22
 ggttggtgnt ggaatatcaa ggctaataatt ggtaaatngc caattttcat catcttcatt 60
 tgtgcttgta ttattatatt tatcataacg aaaattgaca tgttcaaagc aaatgtggcc 120
 atcaatatgg gcgagttgct tttgcgtggg aacataactct ggagggagtt taaagacttc 180
 ttgtacattt atcttcagca ctaccaatcg actgtaagcg gttccagacg gaggtngcac 240
 gattaattgg ctgtnnacag cgccctgaca gtnacggtgc 280

<210> 23
 <211> 91
 <212> PRT
 <213> Piscirickettsia salmonis

<220>
 <223> 3' to 5' encoded protein of Psalmonis clone 7/MunF nucleic acid s
 e

<220>
 <221> variant
 <222> (3)..(3)
 <223> Xaa is any amino acid

<220>
 <221> variant
 <222> (9)..(9)
 <223> Xaa is any amino acid

<220>
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 <222> (15)..(15)
 <223> Xaa is any amino acid

<220>
 <221> variant
 <222> (81)..(81)
 <223> Xaa is any amino acid

<220>
 <221> variant
 <222> (91)..(91)
 <223> Xaa is any amino acid

<400> 23
 His Arg Xaa Cys Gln Gly Ala Val Xaa Ser Gln Leu Ile Val Xaa Pro
 1 5 10 15
 Pro Ser Gly Thr Ala Tyr Ser Arg Leu Val Val Leu Lys Ile Asn Val
 20 25 30
 Gln Glu Val Phe Lys Leu Pro Pro Glu Tyr Val Pro Thr Gln Lys Gln
 35 40 45
 Leu Ala His Ile Asp Gly His Ile Cys Phe Glu His Val Asn Phe Arg
 50 55 60
 Tyr Asp Lys Asn Asn Asn Thr Ser Thr Asn Glu Asp Asp Glu Asn Trp
 65 70 75 80
 Xaa Phe Thr Asn Ile Ser Leu Asp Ile Pro Xaa
 85 90

<210> 24
 <211> 170
 <212> DNA
 <213> *Piscirickettsia salmonis*

<220>
 <223> DNA sequence of *Psalmonis* clone 20/original

<400> 24
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 ataccggctc ttctacacgg atgctaacgg ccatgtctcg cgaaaaacag gttccgcggtt 120
 tctttgacca tgtacacccc cactatgggtg tttcccgctcg ttcattgatc 170

<210> 25
 <211> 56
 <212> PRT
 <213> *Piscirickettsia salmonis*

<400> 25
 Ile Met Leu Ile Leu Tyr Val Asp Ala Met Val Ser Pro Leu Gly Thr
 1 5 10 15
 Ala Leu Ala Tyr Thr Gly Ser Ser Thr Arg Met Leu Thr Ala Met Ser
 20 25 30
 Arg Glu Lys Gln Val Pro Arg Phe Phe Asp His Val His Pro His Tyr
 35 40 45
 Gly Val Ser Arg Arg Ser Leu Ile
 50 55

<210> 26
 <211> 199
 <212> DNA
 <213> *Piscirickettsia salmonis*

<220>
 <223> DNA sequence of *Psalmonis* clone 20/20VSPF

<400> 26
 gatgagcatg atcatatgca ggcctaataag accgggctaac tgtaccatgg gtgcttgaaa 60
 gtcaagctgg tgccagccat ttttaagcat acttggcgggt agtgcaccaa taaatgacac 120
 ttgacaagag caagtaaatac actaagcaga ttacaatcga gaggaccaga gagagcggga 180
 tattgcgttt tgggtttga 199

<210> 27
 <211> 38
 <212> PRT
 <213> *Piscirickettsia salmonis*

<220>
 <223> 3' to 5' encoded protein of *Psalmonis* clone20/20VSPF nucleic acid

<400> 27
 Ser Asn Pro Lys Arg Asn Ile Pro Leu Ser Leu Val Leu Ser Ile Val
 1 5 10 15
 Ile Cys Leu Val Ile Tyr Leu Leu Leu Ser Ser Val Ile Tyr Trp Cys
 20 25 30
 Thr Thr Ala Lys Tyr Ala
 35

<210> 28
 <211> 375
 <212> DNA
 <213> *Piscirickettsia salmonis*

<220>
 <223> DNA sequence of *Psalmonis* clone 15/original

<220>
 <221> misc_feature
 <222> (7)..(7)
 <223> n is any nucleic acid

<400> 28
 ggtaaangag tatcgatatt ggcgtttttt ggctgtatatt tatggttcag gttgtgagag 60
 tacggtgccacacagggcgcc ttattctgat taccctcatg tgtatgcatg cccgaataag 120
 ttaagtactt tgtgttatcg tacagcgatt gcaccgggtg gacactgggtc tcagtataat 180
 cagctgagct ttcagttgcc gattgctttg caagtaccat tgcgtcaagg acaattagag 240
 ctacaagagt attatgctaa aaatcccgtg ttgccttcat ctttgccttt atcaggccca 300
 ggcccgttaa cgtcttattt atatccattt ggattgtgtg caacaaaaat aattcgctta 360
 gagagttaa ctgat 375

<210> 29
 <211> 125
 <212> PRT
 <213> *Piscirickettsia salmonis*

<220>

<223> 5' to 3' encoded protein of DNA sequence P.salmonis clone15/origin

<220>

<221> variant

<222> (3)..(3)

<223> Xaa is any amino acid

<400> 29

Gly	Lys	Xaa	Val	Ser	Ile	Leu	Ala	Phe	Phe	Gly	Cys	Ile	Leu	Trp	Phe
1				5				10					15		
Arg	Leu	Cys	Glu	Tyr	Gly	Ala	Asn	Arg	Ala	Pro	Tyr	Ser	Asp	Tyr	Pro
			20					25					30		
His	Val	Tyr	Ala	Cys	Pro	Asn	Lys	Leu	Ser	Thr	Leu	Cys	Tyr	Arg	Thr
		35					40					45			
Ala	Ile	Ala	Pro	Val	Gly	His	Trp	Ser	Gln	Tyr	Asn	Gln	Leu	Ser	Phe
	50					55					60				
Gln	Leu	Pro	Ile	Ala	Leu	Gln	Val	Pro	Leu	Arg	Gln	Gly	Gln	Leu	Glu
65					70				75					80	
Leu	Gln	Glu	Tyr	Tyr	Ala	Lys	Asn	Pro	Val	Leu	Pro	Ser	Ser	Leu	Pro
			85						90					95	
Leu	Ser	Gly	Pro	Gly	Pro	Leu	Thr	Ser	Tyr	Leu	Tyr	Pro	Phe	Gly	Leu
			100					105					110		
Cys	Ala	Thr	Lys	Ile	Ile	Arg	Leu	Glu	Ser	Leu	Thr	Asp			
		115					120					125			

<210> 30

<211> 639

<212> PRT

<213> Piscirickettsia salmonis

<220>

<223> Amino acid sequence of Psalmonis HSP70 protein
(N-terminal to C-terminal)

<220>

<221> Unsure

<222> (251)..(262)

<223> Uncertainties in the sequence

<400> 30

Met	Ala	Glu	Ile	Ile	Gly	Ile	Asp	Leu	Gly	Thr	Thr	Asn	Ser	Cys	Val
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Ala	Val	Leu	Asp	Gly	Asp	Lys	Pro	Arg	Val	Ile	Glu	Ser	Ala	Glu	Gly
			20					25					30		
Asp	Arg	Thr	Thr	Pro	Ser	Ile	Val	Ala	Tyr	Thr	Asn	Asp	Gly	Val	Thr
		35					40					45			
Val	Gly	Gln	Pro	Ala	Lys	Arg	Gln	Ala	Val	Thr	Asn	Pro	Asn	Asn	Thr
	50					55					60				
Leu	Phe	Ala	Val	Lys	Arg	Leu	Ile	Gly	Arg	Lys	Ser	Ser	Asp	Asp	Thr
65					70				75					80	
Val	Gln	Arg	Asp	Ile	Glu	Arg	Leu	Pro	Tyr	Thr	Ile	Ala	Ala	Ala	Asp
			85						90					95	
Asn	Gly	Asp	Ala	Trp	Ile	Asp	Val	Asn	Gly	Glu	Lys	Leu	Ala	Pro	Pro
			100					105					110		
Gln	Ile	Ser	Ala	Gln	Val	Leu	Ala	Lys	Met	Lys	Lys	Thr	Ala	Glu	Asp
		115					120					125			
Tyr	Leu	Gly	Glu	Asp	Val	Lys	Glu	Ala	Val	Ile	Thr	Val	Pro	Ala	Tyr
	130						135					140			

Phe	Asn	Asp	Ala	Gln	Arg	Gln	Ala	Thr	Lys	Asp	Ala	Gly	Arg	Ile	Ala	145	150	155	160
Gly	Leu	Asp	Val	Lys	Arg	Ile	Ile	Asn	Glu	Pro	Thr	Ala	Ala	Ala	Leu	165	170	175	
Ala	Tyr	Gly	Met	Asp	Lys	Lys	Arg	Gly	Asp	Gly	Val	Ile	Ala	Val	Tyr	180	185	190	
Asp	Leu	Gly	Gly	Gly	Thr	Phe	Asp	Ile	Ser	Ile	Ile	Glu	Ile	Ala	Glu	195	200	205	
Val	Asp	Gly	Glu	His	Gln	Phe	Glu	Val	Leu	Ala	Thr	Asn	Gly	Asp	His	210	215	220	
Thr	Leu	Gly	Gly	Glu	Asp	Phe	Asp	Leu	Arg	Leu	Ile	Ser	Tyr	Leu	Val	225	230	235	240
Asp	Glu	Phe	Lys	Lys	Glu	Gln	Gly	Ile	Asp	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	245	250	255	
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Ala	Ser	Glu	Lys	Ala	Lys	Ile	Glu	Leu	260	265	270	
Ser	Ser	Thr	Gln	Gln	Thr	Asp	Val	Asn	Leu	Pro	Tyr	Ile	Thr	Ala	Asp	275	280	285	
Ala	Thr	Gly	Pro	Lys	His	Met	Asn	Ile	Arg	Val	Thr	Arg	Ala	Lys	Phe	290	295	300	
Glu	Ser	Leu	Val	Glu	Asp	Leu	Val	Glu	Gly	Thr	Ile	Glu	Pro	Cys	Arg	305	310	315	320
Val	Ala	Leu	Lys	Asp	Ala	Gly	Leu	Ser	Val	Asn	Asp	Val	Thr	Asp	Val	325	330	335	
Ile	Leu	Val	Gly	Gly	Gln	Thr	Arg	Met	Pro	Lys	Ala	Gln	Ala	Val	Val	340	345	350	
Lys	Asn	Phe	Phe	Gly	Lys	Glu	Pro	Arg	Arg	Asp	Val	Asn	Pro	Asp	Glu	355	360	365	
Ala	Val	Ala	Val	Gly	Ala	Ala	Ile	Gln	Gly	Gly	Val	Leu	Ala	Gly	Asp	370	375	380	
Val	Lys	Asp	Val	Met	Leu	Leu	Asp	Val	Thr	Pro	Leu	Ser	Leu	Gly	Ile	385	390	395	400
Glu	Thr	Met	Gly	Gly	Val	Met	Thr	Lys	Leu	Ile	Glu	Lys	Asn	Thr	Thr	405	410	415	
Ile	Pro	Thr	Lys	Ser	Gln	Thr	Phe	Ser	Thr	Ala	Gln	Asp	Asn	Gln	Asn	420	425	430	
Ala	Val	Thr	Val	His	Val	Leu	Gln	Gly	Glu	Arg	Glu	Val	Ala	Thr	Gly	435	440	445	
Lys	Lys	Leu	Thr	Gly	Arg	Phe	Asp	Leu	Ala	Asp	Ile	Pro	Pro	Ala	Pro	450	455	460	
Arg	Gly	Met	Pro	Leu	Ile	Leu	Arg	Val	His	Phe	Asp	Ile	Asp	Ala	Asn	465	470	475	480
Gly	Thr	Leu	Asn	Val	Ser	Ala	Lys	Asp	Lys	Gly	Thr	Gly	Lys	Glu	Gln	485	490	495	
Ser	Ile	Val	Ile	Arg	Arg	Ser	Ser	Gly	Leu	Ser	Asp	Asp	Glu	Val	Asp	500	505	510	
Ala	Met	Ile	Lys	Asp	Ala	Glu	Asp	His	Ala	Asp	Asp	Asp	Lys	Lys	Phe	515	520	525	
Gln	Glu	Leu	Val	Gly	Ala	Arg	Asn	Asn	Ala	Glu	Ala	Met	Ile	His	Ala	530	535	540	
Thr	Glu	Lys	Gly	Leu	Lys	Glu	Ala	Asp	Gly	Lys	Val	Ala	Ala	Asp	Asp	545	550	555	560
Lys	Thr	Ala	Ile	Glu	Lys	Ala	Ile	Ser	Glu	Leu	Lys	Asp	Val	Val	Ser	565	570	575	
Gly	Leu	Asp	Lys	Ala	Val	Ile	Asp	Glu	Lys	Val	Glu	Ala	Leu	Thr	Gln	580	585	590	
Ala	Ser	Ala	Lys	Met	Ala	Glu	Val	Leu	Tyr	Ala	Asn	Gln	Gly	Ala	Glu	595	600	605	

Ala	Glu	Ala	Ala	Ala	Ala	Gly	Ala	Glu	Gln	Ala	Gln	Ser	Gln	Thr	Asp
610						615					620				
Glu	Lys	Lys	Asp	Asp	Asp	Val	Val	Asp	Ala	Glu	Phe	Glu	Glu	Val	
625						630					635				

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CLAIMS:

1. An isolated nucleic acid molecule consisting of a sequence selected from the group consisting of SEQUENCE ID Numbers: 1, 3, 5, 7, 10, 12, 14, 16, 18, 20, 22, 24, 26 and 28,
5 and synthetically prepared analogues thereof.
2. The nucleic acid molecule according to claim 1, which encodes an amino acid sequence having at least 70% identity to the amino acid sequence of a surface antigen present on *Piscirickettsia salmonis* and which encodes a protein that
10 induces an immune response.
3. The nucleic acid molecule according to claim 1 comprising the IcmE gene of *Piscirickettsia salmonis* type strain LF-89.
4. The nucleic acid molecule according to claim 1
15 wherein the sequence is a cDNA sequence of *Piscirickettsia salmonis* type strain LF-89 which encodes a protein of approximately 12kDa.
5. The nucleic acid molecule according to claim 1 wherein the sequence is a gene sequence of *Piscirickettsia*
20 *salmonis* type strain LF-89, encoding an amino acid sequence showing at least 70% identity to the amino acid sequence of the 17kDa antigen found in *Rickettsia* of the Spotted Fever Group.
6. The nucleic acid molecule according to claim 2, which encodes an amino acid sequence having at least 90% identity to
25 the amino acid sequence of a surface antigen present on

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Piscirickettsia salmonis and which encodes a protein that induces an immune response.

7. Use of the nucleic acid molecule of any one of claims 1 to 6 in the preparation of a composition for use as a vaccine
5 to protect against infection by *Piscirickettsia salmonis*.
8. A composition comprising at least one nucleic acid molecule according to any one of claims 1 to 6 and a pharmaceutically acceptable diluent, for use as a nucleic acid based vaccine against *Piscirickettsia salmonis*.
- 10 9. An isolated polypeptide comprising an amino acid sequence derived from the nucleic acid molecule of any one of claims 1 to 6.
10. The polypeptide according to claim 9 which is translated from the nucleic acid molecule of any one of claims
15 1 to 6.
11. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of SEQUENCE ID Numbers: 2, 4, 6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29 and 30, and synthetically prepared analogues thereof.
- 20 12. The polypeptide according to claim 11, wherein the amino acid sequence has at least 70% identity to the amino acid sequence of a surface antigen of *Piscirickettsia salmonis* and induces an immune response.
13. The polypeptide according to claim 11, wherein the
25 amino acid sequence has at least 90% identity to the amino acid

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sequence of a surface antigen of *Piscirickettsia salmonis* and induces an immune response.

14. An isolated polypeptide consisting of an amino acid sequence selected from the partial amino acid sequence of p45 depicted in SEQ ID No. 9 or the amino acid sequence of HSP70 antigen depicted in SEQ ID No. 30 from *Piscirickettsia salmonis*.

15. An isolated nucleic acid molecule consisting of a sequence which encodes an amino acid sequence selected from the partial amino acid sequence of p45 depicted in SEQ ID No. 9 or the amino acid sequence of HSP70 antigen depicted in SEQ ID No. 30 from *Piscirickettsia salmonis*.

16. Use of at least one polypeptide according to any one of claims 9 to 14 in the preparation of a composition for use as a vaccine to protect fish against *Piscirickettsia salmonis*.

17. A composition comprising at least one polypeptide according to any one of claims 9 to 14 and a pharmaceutically acceptable diluent, for use as an amino acid based vaccine against *Piscirickettsia salmonis*.

18. A diagnostic test kit comprising the polypeptide of any one of claims 9 to 14 and instructions for use.

19. A nucleic acid PCR primer derived from the open reading frame (ORF) of the nucleic acid molecule of any one of claims 1 to 6.

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20. Use of the nucleic acid PCR primer according to claim 19 in the preparation of a diagnostic test for detecting *Piscirickettsia salmonis*.

21. Use of the polypeptide according to any one of claims 5 9 to 14 in the preparation of a diagnostic test for detecting *Piscirickettsia salmonis*.

22. A composition comprising at least one nucleic acid molecule according to any one of claims 1 to 6 and a pharmaceutically acceptable diluent.

10 23. A composition comprising at least one polypeptide according to any one of claims 9 to 14 and a pharmaceutically acceptable diluent.

Figure 1 – SEQ 1 and 2 - Partial nucleotide sequence of Psclone 51 A and the deduced protein

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```

1   atgagtcaag cattagttga atttaaaacg gtagatcatg tgctgcaagc
51  cttagggcgtt gaagggatga gtgcagcgga tgtgcatggc ctgctgattg
101 gtatgttggc gagtcaaagt aatttaacct gtaaatacatg gttagaaaaa
151 gcgatattta tgggagctca ccttgatgct gaaagtgatc tttttagtaa
201 tatcatggcc aaagagcagt taaagcagtt agaggcattg tttaaagtaa
251 gttgggagca gctctctgcg ggtgatttta cttttgctct gctgttacct
301 gatggtaatg ctgcgttaac tgagcgtgcg agtttattat gtgcctggac
351 tcaaggcttt ttaactggct tgcatttatc gggtgttaat attgctaaat
401 ataaagaagg tgaattagcg acgaccttaa aagatttagc tgaagttgcg
451 cagttggatt tagccattga agacagtaat gaaaatgaag cggcatatac
501 tgagattgct gaatatgtac gtatggcggc gctttttggt catagtgaat
551 tagccggttc tgggtcaagcg actcagatga ctgttcatta a

```

Figure 2 – SEQ ID 3 – Complete coding sequence of Psclone51A

```

1   MSQALVEFKT VDHVLQALGV EGMSAADVHG LLIGMLASQS NLTCKSWLEK
51  AIFMGAHLDA ESDLFSNIMA KEQLKQLEAL FKVSWEQLSA GDFTFALLLP
101 DGNAALTERA SLLCAWTQGF LTGLHLSGVN IAKYKEGELA TTLKDLAEVA
151 QLDLAIEDSN ENEAAYTEIA EYVRMAALFV HSELAGSGQA TQMTVH*

```

Figure 3 – SEQ ID 4 - Protein sequence derived from the ORF of the Psclone51A

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atgaacagaggatgtttgcaaggtagtagtctaattattatcagtggtgtt
tttagttggctgtgcccagaacttttagtcgtcaagaagtcggagctgcga
ctgggggctgttggtggcggtgttgctggccagctgtttggtaaaggtagt
ggtcgagttgcaatggccattgggtgggtgctgttttgggtggattaattgg
ttctaaaatcgggtcaatcgatggatcagcaggataaaaataaagctaaacc
agagtttggaaaaggtaaaagcagggcaagtgcacgcttggcgtaatcca
gatacaggcaatagttatagtgttgagccagtgcggtacttaccagcgta
caataagcaagagcgctgccagcaatatgtcgagaatttcagcaaaagg
cgatgattgcagggcagaagcaagagatttacggcactgcatgccggcaa
ccggatggctggttggaagtcatttcaacagaaaaataa

Figure 4 – SEQ ID 5 – Nucleotide sequence of P10.6

MNRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGS
GRVAMAIGGAVLGGLIGSKIGQSMDDQDKIKLNQSLEKVKAGQVTRWRNP
DTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQEIYGTACRQ
PDGRWQVISTEK

Figure 5 – SEQ ID 6 – Amino Acid Sequence of 10.6

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1
 caa gga ata ttg gca aaa tgg gag aat gtt tct gct caa aaa gtg atg gtt
 Q G I L A K W E N V S A Q K V M V
 52
 gcc aag gtt agt aca gtt aat aat gct gga gat aat ggt cag tca ggt aaa
 A K V S T V N N A G D N G Q S G K
 103
 aat aat aat gaa aat att att gaa aaa gca ggt gct att gta ttt gcg gta
 N N N E N I I E K A G A I V F A V

 Xmal
 Smal
 154
 ctc gat aca cag cta aac agt gac caa ccc ggg act cca gta atg gca acg
 L D T Q L N S D Q P G T P V M A T
 205
 att gtt caa ggt aaa ttt aaa aat gcc aaa ttg ttg ggt agc ttt aaa aga
 I V Q G K F K N A K L L G S F K R
 256
 gag gat gaa aaa cta gtc att tct ttt gat cgc ata tct ttg cct gaa ctt
 E D E K L V I S F D R I S L P E L
 307
 gat cac agt att tct att aag gcg tat gca att aat gcc aca aca gca caa
 D H S I S I K A Y A I N A T T A Q
 358
 aat gca ctg tct tca gat gta gat aat cat tat tta tta cgt tat ggt ggg
 N A L S S D V D N H Y L L R Y G G
 409
 ctc ttt gct gct gcg ttt ttg caa ggc ttt ggc gat tat ttc tcc caa aac
 L F A A A F L Q G F G D Y F S Q N

 Hind III
 460
 tca tca agc tta tgt ggt ggt gcg aca acc tgt att att aca ggc act caa
 S S S L C G G A T T C I I T G T Q

 PsII
 511
 tca act gca gaa caa aat cgt aca acg aga aaa gcc cta tat tct ggt tta
 S T A E Q N R T T R K A L Y S G L
 562
 ggt caa gtt gga aca act tta gct ggt aaa gca agc gct gca ttt gat cgc
 G Q V G T T L A G K A S A A F D R

Figure 6 - SEQ ID 7 and 8 - DNA sequence of *P. salmonis* IcmE and encoded protein (5' to 3')

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613

cct cca acg gtt act tta aat caa ggt gtt ggt atg ggg att tta ttt atg
 P P T V T L N Q G V G M G I L F M

664

tcg gat gta aag gtg taa gtt aaa atg agt aat aat cac aca gat aaa aat
 S D V K V

715

tat aat ttt gat gat gat caa gac att aat gag gat aaa gaa gac ctt gcn

766

gct aga taa tga gcc agc agt taa aag aaa taa tgc aga tgc tgt ttg gca

817

agg gac ttc act gtg gga taa aat aag gcc gat gtt gca tta tta tat cat

868

tgc tat tat tgc att cgc tgt agc agg tta tat gat gta taa cgc ata ccg

919

aac ttt ata tcc aaa gca gtc agt gca gca ggc tga ggc taa cca ttt aag

970

ctt tag taa tca ggt tga aac tgg cag taa gtc agc gaa agg ctt ttc tcc

1021

cct ggc tca gtc tca aga aaa taa ggt caa aaa taa aag tgg gnc ttg gaa

1072

aaa aag aag aga taa aac cga atg cta

Figure 6 –(continued) - SEQ ID 7 and 8 DNA sequence of *P. salmonis* IcmE and encoded protein (5' to 3')

ADNGKLQLQI SQLKAQQTQL QQQVANLQGQ

Figure 7 – SEQ ID 9 – Amino acid sequence of *P. salmonis* p45 antigen (N-terminal to C-terminal)

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ga	tct	tta	atc	tca	tta	aaa	ttt	atg	ttt	cta	atc	gcg
											D	R
tgg	ggt	att	ttc	atc	aaa	tac	ggc	att	ttt	agg	agg	agg
P	N	N	E	D	F	V	A	N	K	P	P	P
gat	aac	aac	atc	agc	att	agg	tga	gtg	ggt	taa	aac	gga
I	V	V	D	A	N	P	S	H	N	L	V	S
											PsII	
atc	gta	aac	atc	atg	gct	atc	gta	ggc	tcc	gtc	tgc	agt
D	Y	V	D	H	S	D	Y	A	G	D	A	T
gaa	gcg	atc										
F	R	D										

Figure 8 – SEQ ID 10 and 11 - DNA sequence of P. salmonis clone 3/original and encoded protein (3' to 5')

ata	agc	cta	gat	gat	gat	att	gcg	gga	ata	ncc	att	gat
I	S	L	D	D	D	I	A	G	I	X	I	D
tna	cag	gcc	tta	agc	gtt	ttg	gcc	gtg	acg	atg	tgg	cac
X	Q	A	L	S	V	L	A	V	T	M	W	H
caa	gaa	aaa	tac	aag	ata	tca	gca	aag	cgc	agc	tgg	cgt
Q	E	K	Y	K	I	S	A	K	R	S	W	R
aaa	ctt	cat	gtg	gcc	gtt	gat	gat	gat	can	tat	att	caa
K	L	H	V	A	V	D	D	D	X	Y	I	Q
gcc	gca	ctc	atc	acc	gat	cgc	tat	gaa	gca	gat	gag	gag
A	A	L	I	T	D	R	Y	E	A	D	E	E

Figure 9 – SEQ ID 12 and 13 - DNA sequence of P. salmonis clone 3/3PST-R and encoded protein (5' to 3')

tat	gag	att	aaa	gat	cat	ggt	aga	atg	cac	tgg	caa	aag
Y	E	I	K	D	H	G	R	M	H	W	Q	K
aca	cga	caa	tac	ggc	aag	cgt	aat	tat	tct	gag	tig	gcg
T	R	Q	Y	G	K	R	N	Y	S	E	L	A
att	cag	cgt	tac	aaa	cgc	att	ttg	ggc	aac	acg	atg	cag
I	Q	R	Y	K	R	I	L	G	N	T	M	Q
tcc	aga	gac	ata	tcg	cga	cag	aaa	aat	gaa	gga	cta	att
S	R	D	I	S	R	Q	K	N	E	G	L	I
ggc	gcg	ggt	att	tta	aat	aga	gat	gac	can	tct	cgg	cat
G	A	G	I	L	N	R	D	D	X	S	R	H
gcc	ggt	gac	aat	aat	gta							
A	G	D	N	N	V							

Figure 10 – SEQ ID 14 and 15 - DNA sequence of P. salmonis clone 3/3APA-F and encoded protein (5' to 3')

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g	atc	aan	gcc	cgc	ata	tta	atc	gac	gac	cac	gat	att
	I	X	A	R	I	L	I	D	D	H	D	I
caa	aag	tta	aaa	att	caa	aat	atc	cgc	caa	cat	att	gcc
Q	K	L	K	I	Q	N	I	R	Q	H	I	A
tat	tta	cct	cag	cat	ggc	gac	tta	ttt	aat	ggc	acg	atc
Y	L	P	Q	H	G	D	L	F	N	G	T	I

Figure 11 - SEQ ID 16 and 17 - DNA sequence of *P. salmonis* clone 7/original and encoded protein (5' to 3')

g	cac	gca	tat	cga	tac	cta	acg	tcn	ttt	caa	gaa	caa
	H	A	Y	R	Y	L	T	S	F	Q	E	Q
aaa	tat	tat	aaa	caa	gcc	ata	gaa	gtc	agc	caa	tta	ctt
K	Y	Y	K	Q	A	I	E	V	S	Q	L	L
ggc	ctt	gac	tca	att	att	gag	cgc	ttg	ccc	aaa	ggc	tat
G	L	D	S	I	I	E	R	L	P	K	G	Y
cac	act	cct	ggt	gcc	aat	cat	gcc	gna	tat	taa	ttg	act
H	T	P	V	A	N	H	A	X	Y			
					Hind III							
acg	cac	gat	ntt	nca	aag	ctt	ant	g				

Figure 12 - SEQ ID 18 and 19 - DNA sequence of *P. salmonis* clone 7/Xbar and encoded protein (5' to 3')

tta	ttg	agc	gct	tgc	cca	aag	gct	atc	aca	ctc	ctg	ttg
L	L	S	A	C	P	K	A	I	T	L	L	L
cca	atc	atg	cca	tgg	nag	tcg	cta	cct	cgc	ggc	atc	att
P	I	M	P	W	X	S	L	P	R	G	I	I
cag	cgc	att	gcg	att	gcc	cgt	gcc	ctg	att	cat	aag	cca
Q	R	I	A	I	A	R	A	L	I	H	K	P
cca	atc	gtc	cta	ttc	gat	gag	gcc	aat	acg	gcc	atg	gac
P	I	V	L	F	D	E	A	N	T	A	M	D
atg	caa	ggc	gat	acc	atc	tta	att	aat	gtg	ctt	gaa	caa
M	Q	G	D	T	I	L	I	N	V	L	E	Q
ctt	aaa	ggc	acc	tgc	aca	ctc	atc	ctc	gtc	tct	cat	cgc
L	K	G	T	C	T	L	I	L	V	S	H	R
											XhoI	
											PaeR71	
cca	tca	ttg	ctg	gca	cat	gca	gat	aaa	atc	ttt	atc	ctc
P	S	L	L	A	H	A	D	K	I	F	I	L
gag	aat	aaa	aat	ctg	gtg	gag	aaa	gtc	aca	tga	gct	ctg
E	N	K	N	L	V	E	K	V	T			
cac	taac	cgc	cca	gga	gca	taa	tat	tcg	cac	tgc	gtt	tat
taa	cag	cct	cga	acc	act	gtt	aac	tgc	att	agg	ctg	gc

Figure 13 - SEQ ID 20 and 21 - DNA sequence of *P. salmonis* clone 7/MunR and encoded protein (5' to 3')

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ggt	tgt	tgn	tgg	aat	atc	aag	gct	aat	att	ggt	aaa	tng
		X	P	I	D	L	S	I	N	T	F	X
cca	att	ttc	atc	atc	ttc	att	tgt	gct	tgt	att	att	att
W	N	E	D	D	E	N	T	S	T	N	N	N
ttt	atc	ata	acg	aaa	att	gac	atg	ttc	aaa	gca	aat	gtg
K	D	Y	R	F	N	V	H	E	F	C	I	H
gcc	atc	aat	atg	ggc	gag	ttg	ctt	ttg	cgt	ggg	aac	ata
G	D	I	H	A	L	Q	K	Q	T	P	V	Y
ctc	tgg	agg	gag	ttt	aaa	gac	ttc	ttg	tac	att	tat	ctt
E	P	P	L	K	F	V	E	Q	V	N	I	K
cag	cac	tac	caa	tcg	act	gta	agc	ggc	tcc	aga	cgg	agg
L	V	V	L	R	S	Y	A	T	Q	S	P	P
tng	cac	gat	taa	ttg	gct	gtt	nac	agc	gcc	ctg	aca	gtt
X	V	I	L	Q	S	X	V	A	G	Q	C	X
acg	gtg	c										
R	H											

Figure 14 – SEQ ID 22 and 23 - DNA sequence of P. Salmonis clone 7/MunF and encoded protein (3' to 5')

gg	atc	atg	ctc	atc	ctc	tac	gtc	gat	gcg	atg	gtc	tca
	I	M	L	I	L	Y	V	D	A	M	V	S
cct	tta	gga	acg	gct	tta	gcc	tat	acc	ggc	tct	tct	aca
P	L	G	T	A	L	A	Y	T	G	S	S	T
cgg	atg	cta	acg	gcc	atg	tct	cgc	gaa	aaa	cag	gtt	ccg
R	M	L	T	A	M	S	R	E	K	Q	V	P
cgt	ttc	ttt	gac	cat	gta	cac	ccc	cac	tat	ggc	gtt	tcc
R	F	F	D	H	V	H	P	H	Y	G	V	S
cgt	cgt	tca	ttg	atc								
R	R	S	L	I								

Figure 15 – SEQ ID 24 and 25 - DNA sequence of P. Salmonis clone 20/Original and encoded protein (5' to 3')

g	atg	agc	atg	atc	ata	tgc	agg	cct	aat	aga	ccg	gct
aac	tgt	acc	atg	ggc	gct	tga	aag	tca	agc	tgg	tgc	cag
cca	ttt	tta	agc	ata	ctt	ggc	ggc	agt	gca	cca	ata	aat
			A	Y	K	A	T	T	C	W	Y	I
gac	act	tga	caa	gag	caa	gta	aat	cac	taa	gca	gat	tac
V	S	S	L	L	L	Y	I	V	L	C	I	V
aat	cga	gag	gac	cag	aga	gag	cgg	gat	att	gcg	ttt	tgg
I	S	L	V	L	S	L	P	I	N	R	K	P
gtt	tga											
N	S											

Figure 16 – SEQ ID 26 and 27 - DNA sequence of P. salmonis clone 20/20VSPF and encoded protein (3' to 5')

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Clal												
ggt	aaa	nga	gta	tcg	ata	ttg	gcg	ttt	ttt	ggc	tgt	att
G	K	X	V	S	I	L	A	F	F	G	C	I
tta	tgg	ttc	agg	ttg	tgc	gag	tac	ggt	gcc	aac	agg	gcg
L	W	F	R	L	C	E	Y	G	A	N	R	A
cct	tat	tct	gat	tat	cct	cat	gtg	tat	gca	tgc	ccg	aat
P	Y	S	D	Y	P	H	V	Y	A	C	P	N
aag	tta	agt	act	ttg	tgt	tat	cgt	aca	gcg	att	gca	ccg
K	L	S	T	L	C	Y	R	T	A	I	A	P
gtt	gga	cac	tgg	tct	cag	tat	aat	cag	ctg	agc	ttt	cag
V	G	H	W	S	Q	Y	N	Q	L	S	F	Q
ttg	ccg	att	gct	ttg	caa	gta	cca	ttg	cgt	caa	gga	caa
L	P	I	A	L	Q	V	P	L	R	Q	G	Q
tta	gag	cta	caa	gag	tat	tat	gct	aaa	aat	ccc	gta	ttg
L	E	L	Q	E	Y	Y	A	K	N	P	V	L
cct	tca	tct	ttg	cct	tta	tca	ggc	cca	ggc	ccg	tta	acg
P	S	S	L	P	L	S	G	P	G	P	L	T
tct	tat	tta	tat	cca	ttt	gga	ttg	tgt	gca	aca	aaa	ata
S	Y	L	Y	P	F	G	L	C	A	T	K	I
att	cgc	tta	gag	agt	tta	act	gat					
I	R	L	E	S	L	T	D					

Figure 17 – SEQ ID 28 and 29 - DNA sequence of P. Salmonis clone 15/original and encoded protein (5' to 3')

MAEIIIGIDLG	TTNSCVAVLD	GDKPRVIESA	EGDRTTPSIV
AYTNDGVTVG	QPAKRQAVTN	PNNTLFAVKR	LIGRKSSDDT
VQRDIERLPY	TIAAADNGDA	WIDVNGEKLA	PPQISAQVLA
KMKKTAEDYL	GEDVKEAVIT	VPAYFNDAQR	QATKDAGRIA
GLDVKRIINE	PTAAALAYGM	DKKRGDGVIA	VYDLGGGTFD
ISIIIEIAEVD	GEHQFEVLAT	NGDHTLGGED	FDLRLISYLV
Missing amino acids			
DEFKKEQGID	XXXXXXXXXX	XXEASEKAKI	ELSSTQQTDV
NLPYITADAT	GPKHMNIRVT	RAKFESLVED	LVEGTIEPCR
VALKDAGLSV	NDVTDVILVG	GQTRMPKAQA	VVKNFFGKEP
RRDVNPDEAV	AVGAAIQGGV	LAGDVKDVML	LDVTPLSLGI
ETMGGVMTKL	IEKNTTIPTK	SQTFSTAQDN	QNAVTVHVLQ
GEREVATGKK	LTGRFDLADI	PPAPRGMPLI	LRVHFDIDAN
GTLNVSAKDK	GTGKEQSIVI	RRSSGLSDDE	VDAMIKDAED
HADDDKKFQE	LVGARNNAEA	MIHATEKGLK	EADGKVAADD
KTAIEKAISE	LKDVVSGLDK	AVIDEKVEAL	TQASAKMAEV
LYANQGAEAE	AAAAGAEQAQ	SQTDEKKDDD	VVDAEFEEV

Figure 18 – SEQ ID 30 - Amino acid sequence of P. Salmonis HSP70 protein (N-terminal to C-terminal)