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(54) **Title**: LAWSONIA INTRACELLULARIS VACCINES

(57) **Abstract**: The present invention relates to polynucleotide sequences encoding novel *Lawsonia intracellularis* polypeptides. It also relates to DNA fragments, recombinant plasmid DNA and live recombinant microorganisms comprising these sequences. Moreover, the invention relates to polypeptides encoded by these nucleotide sequences. The invention also relates to immunogenic compositions for prevention of *Lawsonia intracellularis* infections and methods for the preparation of these immunogenic compositions. The invention also relates to diagnostic tests for the detection of *Lawsonia intracellularis* DNA, the detection of *Lawsonia* intracellularis antigens and detection of antibodies against Lawsonia intracellularis.

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LAWSONIA INTRACELLULARIS VACCINES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a non-provisional application that claims priority under 35 U.S.C. § 119(e) of provisional application U.S. Serial No. 61/107,858 filed October 23, 2008, the contents of which are hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0001] This invention relates to immunogenic compositions for immunization against *Lawsonia intracellularis*. Methods of protein expression, purification and use in preparation of immunogenic compositions are provided.

BACKGROUND OF THE INVENTION

[0002] Porcine proliferative enteropathy (PPE or PE), also referred to as Ileitis, is one of the most economically burdensome diseases of the swine industry. The disease has been reported to affect 12% to 50% of pigs on farms world-wide (Diseases of Swine, 7th Edition, 560-569 (1992)). Although the economic impact of PPE is felt largely by the swine industry, the disease has also been shown to affect multiple mammalian species, including monkey, rabbit, hamster, horse, ostrich, fox, ferret, rat and emu (Diseases of Swine, 7th Edition, 560-569 (1992)). In animals exhibiting proliferative hemorrhagic enteropathy (PHE), the acute form of PPE, the death rate is as high as 50 percent, and pregnant animals infected often abort within the first 6 days (Diseases of Swine, 7th Edition, 560-569 (1992)). The causative agent for PPE was first determined and isolated by Lawson et al. (*Journal of Clinical Microbiology* 31(5):1136, (May 1993)). The bacteria isolated by Lawson et al. were obligate, intracellular, gram-negative curved rods which tolerated oxygen only at reduced pressure from atmospheric tension and grew *in vivo* only in enterocytes. Lawson et al. isolated two strains of the bacteria and deposited them as NCTC 12656 and NCTC 12657.

[0003] Further phylogenetic characterization and naming of these bacteria was performed by McOrist et al. (*International Journal of Systematic Bacteriology*, 45(4):820-825, (October 1995)). The causative agent of PPE was determined to be the obligate intracellular bacterium, originally found by Lawson et al., now designated *Lawsonia intracellularis* (LI). This organism is a gram-negative, flagellated bacterium that infects immature epithelial cells of the intestinal crypts, called enterocytes.

[0004] In the early stages of infection, the bacterium associates with the cell membrane and quickly enters the crypt epithelial cells via an entry vacuole (McOrist, S., et al., *The Canadian Journal of Veterinary Research*, 70(2):155-159, (April 2006)). This vacuole rapidly breaks down and the bacteria flourish and multiply freely in the cell cytoplasm. One of the distinguishing features of this disease is the uncontrollable proliferation of crypt epithelial cells which results in thickening of the mucosal lining of the small, and sometimes the large, intestine. In pigs, infection with LI causes diarrhea, stunted growth and in some cases sudden death.

[0005] Proliferative enteropathies are a group of diseases with widely differing appearances and symptoms, one member of which is PPE (Diseases of Swine, 7th Edition, 560-569 (1992)). Several different forms of PPE (acute, chronic, necrotic and subclinical) can also be distinguished; however they all have the common underlying pathogenic cause being *Lawsonia intracellularis*. The disease initially develops as a progressive proliferation of the immature intestinal epithelial cells which are infected by *Lawsonia intracellularis*. This form of the disease is characterized by the presence of bloody or blood-tinged diarrhea, blood clots and sudden death. Porcine intestinal adenomatosis (PIA) is considered the chronic and most common form of the disease, which is seen in growing pigs (nursery to late finishing stage). Proliferative hemorrhagic enteropathy (PHE) is considered the acute form of the diseases, affecting the terminal ileum and colon of adult pigs (4-12 months of age). PHE develops from PIA and is distinguished by severe bleeding into the lumen of the intestine from the pre-existing PIA lesions. This form of the disease is observed in the terminal ileum and upper part of the proximal colon and is characterized by extensive thickening of the mucosal lining of the affected areas with little or no inflammation in the mucosal surface. Necrotic enteritis is another form of the disease, which is characterized by severe thickening of the ileum along with presence of yellowish necrotic lesions in the ileum. Another form of the disease that has been described in pigs with *Lawsonia* infections is a subclinical disease producing carrier animals without overt signs of disease.

[0006] Growth of *Lawsonia intracellularis* in cell culture requires an environment that contains an unusual gas mixture (oxygen, carbon dioxide, nitrogen and hydrogen). In addition, the bacteria do not display observable cytopathic effects in the host cells. These factors underscore the importance of researching unconventional technologies in order to develop efficacious vaccines to protect against the disease caused by *Lawsonia*, but which do not require the cultivation of the bacteria. One such approach involves the application of recombinant DNA technology to develop a subunit vaccine. Previous studies have utilized ARTEMIS and TB-parse to annotate the fully sequenced *Lawsonia* genome (US2006/0024696).

[0007] Despite these advances, development of subunit vaccines has been hampered by the lack of information on the identity of protective antigens that can be used as the basis for a vaccine. The present invention addresses these and other needs.

[0008] The references cited herein are intended to be viewed as references only and are not intended for any other purpose. No inference should be made that the information provided and references cited are prior art merely because they are in this document.

BRIEF SUMMARY OF THE INVENTION

[0009] The present invention is directed toward polypeptides and polynucleotides useful as subunit vaccines for protection against *Lawsonia intracellularis* without the need for *in vitro* culture. The present invention provides for compositions comprising one or more polypeptides of invention (*e.g.*, SEQ ID NOs: 1-13). These compositions can also be immunogenic compositions, which further comprise a pharmaceutically acceptable adjuvant. The present invention also provides the immunogenic compositions that further contain one or more antigenic components of the following microorganisms: *Mycoplasma Hyopneumoniae*, *Erysipelas spp*, *salmonella*, *H. parasuis*, *clostridium spp*, *streptoccous suis*, *brachyspira spp*, *bordetella*, *pasteurella*, *E. coli*, coronavirus, parvovirus, PRRS, Circovirus, and SIV. In other embodiments, the present invention provides for compositions comprising one or more polynucleotides which encode the polypeptides of the invention (*e.g.*, SEQ ID NOs: 27-39). These compositions can also be immunogenic compositions, which further comprise a pharmaceutically acceptable adjuvant. In addition, the present invention provides

immunogenic compositions comprising inactivated *Lawsonia* (bacterin) and an appropriate adjuvant.

[0010] The present invention further provides methods of inducing a protective immune response against *Lawsonia* in an animal, wherein the method comprises administering the composition comprising the bacterin, polypeptides or polynucleotides of the present invention to the animal. In a specific embodiment the animal is a pig. The route of administration is not critical to the invention. Methods of administration useful in the invention include scarification, intramuscular injection, subcutaneous injection, intraperitoneal, intranasal administration or oral administration. The present invention further provides methods for specific detection of antibodies reactive with *Lawsonia* in a biological sample obtained from an animal. The detection method of the present invention comprises the steps of contacting the biological sample with a *Lawsonia* antigen of the present invention and detecting antigen-antibody binding between the *Lawsonia* antigen and an antibody in the biological sample. In a specific embodiment, the biological sample is from blood. The present invention also provides a kit containing one or more *Lawsonia* antigens of the invention, as well as an instruction pamphlet for inducing a protective immune response against *Lawsonia* in pigs.

DETAILED DESCRIPTION

DEFINITIONS OF THE INVENTION

[0011] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry and nucleic acid chemistry and hybridization described below are those well known and commonly employed in the art. Standard techniques are used for nucleic acid and peptide synthesis. Generally, enzymatic reactions and purification steps are performed according to the manufacturer's specifications. The techniques and procedures are generally performed according to conventional methods in the art and various general references (*see generally*, Sambrook *et al.* MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., which is incorporated herein by reference), which are provided throughout this document. The nomenclature used herein and the laboratory procedures in analytical

chemistry, and organic synthetic chemistry described below, are those well known and commonly employed in the art. Standard techniques, or modifications thereof, are used for chemical syntheses and chemical analyses.

[0012] One of skill will recognize that the *Lawsonia* polypeptides of the invention can be varied without loss of immunogenicity. Thus polypeptides of the invention include polymorphic variants, alleles, and mutants that: (1) have an amino acid sequence that has greater than about 80% amino acid sequence identity, 85%, 90%, specifically 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, specifically over a region of at least about 25, 50, 100, 200, 500, 1000, or more amino acids, to the polypeptides exemplified here. Such variants will specifically bind to antibodies, e.g., polyclonal antibodies, raised against an immunogen comprising an amino acid sequence exemplified here. Another means to identify variants of the invention is that the DNA encoding them specifically hybridize under stringent hybridization conditions to the nucleic acid sequences shown here or have a nucleic acid sequence that has greater than about 90%, specifically greater than about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher nucleotide sequence identity to the exemplified sequences.

[0013] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0014] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally, Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0015] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschuel *et al.* (1977) *Nucleic Acids Res.* 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

[0016] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g.*, Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more specifically less than about 0.01, and most specifically less than about 0.001.

[0017] A further indication that two nucleic acid sequences or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with the protein encoded by the second nucleic acid, as described below. Thus, a protein is typically substantially identical to a second protein, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

[0018] The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture of (*e.g.,* total cellular) DNA or RNA.

[0019] The term "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 15° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. (As the target sequences are generally present in excess, at Tm, 50% of the probes are occupied at equilibrium). Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30° C for short probes (*e.g.,* 10 to 50 nucleotides) and at least about 60° C for long probes (*e.g.,* greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is typically at least two times background, more specifically the hybridization is 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5x SSC, and 1% SDS, incubating at 42° C, or, 5x SSC, 1% SDS, incubating at 65° C, with wash in 0.2x SSC, and 0.1% SDS at 65° C. For PCR, a temperature of about 36° C is typical for low stringency amplification, although annealing temperatures may vary between about 32-48° C depending on primer length. For high stringency PCR amplification, a temperature of about 62° C is typical, although high stringency annealing temperatures can range from about 50° C to about

65° C, depending on the primer length and specificity. Typical cycle conditions for both high and low stringency amplifications include a denaturation phase of 90-95° C for 30-120 sec, an annealing phase lasting 30-120 sec, and an extension phase of about 72° C for 1-2 min. Protocols and guidelines for low and high stringency amplification reactions are available, e.g., in Innis, et al. (1990) *PCR Protocols: A Guide to Methods and Applications* Academic Press, N.Y.

[0020] The use of singular terms for convenience is in no way intended to be so limiting. Thus, for example, reference to a composition comprising "a polypeptide" includes reference to one or more of such polypeptides. In addition, reference to an "antibody" includes a reference to a plurality of antibodies. Furthermore, reference to an "organism" includes a reference to a plurality of organisms.

[0021] The use of the term "amplification primer" or "PCR primer" refers to oligonucleotides comprising either natural or synthetic nucleotides that can serve as the basis for amplification of a specific nucleic acid sequence. They include both polymerase chain reaction (PCR) primers and ligase chain reaction primers.

[0022] The term "antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or genes, that is capable of interacting with and binding to a specified protein or antigen contained in a composition comprising, but not limited to, one or more proteins and/or antigens.

[0023] The term "adjuvant" is defined as one or more substances that cause stimulation of the immune system. In the context of the present application, adjuvant is used to enhance or stimulate an immunogenic response to one or more immunogenic composition antigens/isolates.

[0024] The term pig or swine includes all domesticated porcine species, unless otherwise indicated.

METHODS OF GENERATING SYNTHETIC GENES

[0025] The present invention provides novel *Lawsonia* proteins with immunogenic properties. Any of the presently available bioinformatics applications can be employed to determine putative proteins. A further step of a bioinformatics screen using a computer program whose algorithm can be used to predict the cellular location of the putative *Lawsonia* protein sequences was used. While any bioinformatics program that predicts location and/or

function of putative proteins can be employed, PSORT is conveniently used for this purpose. Such programs are useful to obtain information regarding which of the thousands of putative *Lawsonia* proteins are located on the cell surface, and therefore potentially useful in vaccines. The proteins of the present invention are listed as SEQ ID NOs: 1-13. The nucleic acids encoding these proteins are presented in SEQ ID NOs: 14-39.

[0026] The invention further provides the next step of synthesizing the putative genes. Genes can be synthesized via standard methodologies. Once obtained, the sequences are then inserted into expression vectors using standard methods known to those of ordinary skill in the art. It will be appreciated that codon usage can be optimized to obtain optimal protein expression in the desired host cell, *e.g., E. coli.*

METHODS OF AMPLIFICATION

[0027] Once desired sequences are identified, PCR amplification can be used to prepare polynucleotide sequences of interest. PCR amplification can be performed using standard methods by anyone of basic skill in the art. Many methods of PCR amplification can be performed and any of these can be used for the present invention.

METHODS OF CLONING OF *LAWSONIA* GENES

[0028] *Lawsonia* genes can be cloned using standard recombinant DNA technology methods by anyone of basic skill in the art. Many vectors would suffice for the methods of the present invention. The exact vector used is not critical to the invention. Typically, the expression vector is selected and designed to provide efficient protein expression in the desired host cell, *e.g., E. coli.*

EXPRESSION AND PURIFICATION OF PROTEINS

[0029] The present invention provides a method for the expression and purification of novel *Lawsonia* polypeptides. Well known methodologies for protein expression and purification of proteins can be used in the present invention.

[0030] The proteins of the invention can be expressed in a variety of host cells, including *E. coli,* other bacterial hosts, yeast and insect cells. The host cells can be microorganisms, such as, for example, yeast cells, bacterial cells, or filamentous fungal cells. Examples of suitable host cells include, for example, *Pseudomonas sp.*, *Escherichia sp.* (*e.g.*, *E. coli*), *Bacillus,* among many others. Suitable yeast cells can be of any of several genera, including, for

example, *Saccharomyces* (*e.g.*, *S. cerevisiae*), and *Candida.* Suitable insect cells can be of several varieties, including, for example, Sf9 and Hi-5 cells.

[0031] After obtaining the synthetic genes encoding the polypeptides of the present invention, these synthetic genes can be cloned into expression vectors designed for expression in the desired host cell. Cloning of DNA sequences into expression vectors is a common and well know method to those skilled in the art. Any number of specific methods and vectors can be employed. The vector need only contain the polynucleotide sequence encoding the polypeptides of the present invention and the elements necessary for expression in the desired host cell, *e.g., E. coli.* Other specific vectors that can be used for the invention include baculovirus vectors capable of containing the polynucleotide sequence encoding the polypeptides of the present invention and the elements necessary for expression in the desired insect cell type, *e.g.* Sf9 or Hi-5.

[0032] A construct that includes a polynucleotide of interest operably linked to gene expression control signals that, when placed in an appropriate host cell, drive expression of the polynucleotide is termed an "expression cassette." Expression cassettes that encode the proteins of the invention are often placed in expression vectors for introduction into the host cell. The vectors typically include, in addition to an expression cassette, a nucleic acid sequence that enables the vector to replicate independently in one or more selected host cells. Generally, this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria. For instance, the origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria. Alternatively, the vector can replicate by becoming integrated into the host cell genomic complement, being replicated as the cell undergoes DNA replication.

[0033] Selectable markers are often incorporated into the expression vectors used to express the polynucleotides of the invention. These genes can encode a gene product, such as a protein, necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that confer resistance to antibiotics or other toxins, such as ampicillin, neomycin, kanamycin, chloramphenicol, or tetracycline. Alternatively, selectable markers may encode proteins that complement auxotrophic deficiencies or supply critical nutrients not available from complex

media. Often, the vector will have one selectable marker that is functional in, *e.g.*, *E. coli*, or other cells in which the vector is replicated prior to being introduced into the host cell. A number of selectable markers are known to those of skill in the art.

[0034] Expression of the protein can be induced by selecting vectors that comprise inducible promoters.. For example, an IPTG inducible vector (pMAL-c2X and pET-23a(+)) can be employed, transformed into the *E. coli*, and expression induced by IPTG. These methods are well known and have been shown to be highly useful for protein expression.

[0035] In some embodiments, the polynucleotide sequences encoding the polypeptides of the present invention are optimized for codon usage in the desired host in order to optimize protein expression. For example, the original *Lawsonia* genomic polynucleotide sequences are listed as SEQ ID NOs: 27-39; the *E. coli* codon optimized variants of these nucleic acid sequences are listed as SEQ ID NOs: 14-26.

METHODS OF DETECTION AND PURIFICATION OF EXPRESSED PROTEINS

The proteins of the invention can be purified according to standard procedures in the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (*see, generally*, R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982), Deutscher, *Methods in Enzymology Vol. 182: Guide to Protein Purification.*, Academic Press, Inc. N.Y. (1990)). Substantially pure compositions of at least about 70% to 90% homogeneity are envisioned, and more specifically 98% to 99% or are envisioned.

[0036] To facilitate purification, the nucleic acids that encode the proteins can also include a coding sequence for an epitope or "tag" for which an affinity binding reagent is available, *i.e.* a purification tag. Examples of suitable epitopes include the myc and V-5 reporter genes; expression vectors useful for recombinant production of fusion proteins having these epitopes are commercially available (*e.g.*, Invitrogen (Carlsbad CA) vectors pcDNA3.1/Myc-His and pcDNA3.1/V5-His are suitable for expression in mammalian cells). Additional expression vectors suitable for attaching a tag to the proteins of the invention, and corresponding detection systems are known to those of skill in the art, and several are commercially available (e.g., "FLAG" (Kodak, Rochester NY). Another example of a suitable tag is a polyhistidine sequence, which is capable of binding to metal chelate affinity ligands. Typically, six adjacent histidines are used, although one can use more or less than six. Suitable metal chelate affinity ligands that can serve as the binding moiety for a polyhistidine tag include nitrilo-tri-acetic acid (NTA) (Hochuli, E. (1990) "Purification of recombinant proteins with metal chelating

adsorbents" In Genetic Engineering: Principles and Methods, J.K. Setlow, Ed., Plenum Press, NY; commercially available from Qiagen (Santa Clarita, CA)).

[0037] Purification tags also include maltose binding domains and starch binding domains. Purification of maltose binding domain proteins is know to those of skill in the art. Starch binding domains are described in WO 99/15636, herein incorporated by reference. Affinity purification of a fusion protein comprising a starch binding domain using a betacylodextrin (BCD)-derivatized resin is described in USSN 60/468,374, filed May 5, 2003, herein incorporated by reference in its entirety.

[0038] Other haptens that are suitable for use as tags are known to those of skill in the art and are described, for example, in the Handbook of Fluorescent Probes and Research Chemicals (6th Ed., Molecular Probes, Inc., Eugene OR). For example, dinitrophenol (DNP), digoxigenin, barbiturates (see, e.g., US Patent No. 5,414,085), and several types of fluorophores are useful as haptens, as are derivatives of these compounds. Kits are commercially available for linking haptens and other moieties to proteins and other molecules. For example, where the hapten includes a thiol, a heterobifunctional linker such as SMCC can be used to attach the tag to lysine residues present on the capture reagent.

[0039] One of skill would recognize that modifications can be made to the proteins of the invention without diminishing their immunogenic activity. Some modifications may be made to facilitate the cloning or expression. Such modifications are well known to those of skill in the art and include, for example, the addition of codons at either terminus of the polynucleotide to provide, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (*e.g.*, poly His) placed on either terminus to create conveniently located restriction enzyme sites or termination codons or purification sequences.

VACCINE COMPOSITION AND USE:

[0040] Protein immunogens of the present invention may be expressed, concentrated and purified from expression hosts such as *E. coli* using various methods known to one of skill in the art. Purified protein immunogens can be formulated into vaccines containing an immunologically effective amount of one or more of the proteins of the invention and an appropriate pharmaceutical carrier. Thus, proteins of the invention can be administered individually or in combination either in a single composition or multiple compositions. The formulated vaccines may be combined with a pharmaceutically acceptable adjuvant. The formulated vaccines may be an aqueous solution, a suspension or an emulsion. An

immunologically effective amount of each immunogen in the vaccines of the present invention is determinable by methods known in the art without undue experimentation. In a specific embodiment of the invention, the general quantity of each immunogen will be between 5 μg and 500 μg of purified protein. In a specific embodiment of the present invention, the amount will be between 10 μg and 250 μg. In an even more specific embodiment of invention the amount will be between 50 μg and 200 μg of each protein.

[0041] The adjuvant can be any pharmaceutically acceptable adjuvant. In a some embodiments the adjuvant is aluminum hydroxide. In others the adjuvant is aluminum phosphate. In yet another embodiment the adjuvant is Emunade®. Emunade® is an adjuvant consisting of a combination of oil, water and aluminum hydroxide. In yet another embodiment the adjuvant is Quil A. In yet another embodiment the adjuvant is Quil A plus cholesterol.

[0042] In some embodiments, ISCOM is used as an adjuvant. ISCOM is an acronym for Immune Stimulating Complex and the technology was described by Morein *et al.* (Nature 308:457-460 (1984)). ISCOMs are formed as follows. The polypeptides are solubilized using standard methods, such as with a non-ionic detergent (e.g., Mega-9, Triton X-100, Octylglucoside, Digitonin, Nonidet P-40, $C_{12}E_8$, Lubrol, Tween-80). A lipid mixture is added to assist ISCOM formation. The lipid mixture can include a phosphatidyl choline and a synthetic cholesterol. In some embodiments, the mixture is first treated with non-ionic detergent at room temperature with stirring, then the lipid mixture (equal parts phosphatidyl choline and cholesterol, for example) is added and stirring continued. Quil A (a purified glycoside of saponin) is added to polypeptide composition and stirring is continued. Then the non-ionic detergent is removed (for example, by diafiltration with ammonium acetate). The matrix of the ISCOM is formed by Quil A. The morphology of an ISCOM particle, as viewed by electron microscopy, shows a typical cage like structure of approximately 35 nm in size. The ISCOM formation stage can be refined by the use of tangential flow diafiltration. ISCOMs present purified antigens in a multimeric form based on the ability of Quil A to spontaneously form micelles at a critical concentration and by a hydrophobic/hydophilic link that entrap the purified antigens. Formation of ISCOMs can be verified by electron microscopy to verify that the typical cage-like structures have been formed. The Quil A can be added to give a final concentration of about 0.01 to 0.1 %. In some embodiments, the final concentration is about 0.05%.

[0043] The composition containing the proteins of the invention and an adjuvant can be administered through one of the following standard methods: scarification, intramuscular injection, subcutaneous injection, intraperitoneal, intranasal administration or oral administration. In a specified embodiment, the compositions containing one or more of the polypeptides of the present invention is administered by intramuscular injection. In a typical embodiment of the invention, the polypeptides are administered at dosages between 10 μg and 1000 μg. In another embodiment the polypeptides are administered at dosages between 10 μg and 500 μg. In yet another embodiment the polypeptides are administered at dosages between 100 μg and 250 μg.

[0044] After administration of the composition containing one or more of the polypeptides of the present invention, the efficacy of administration of the compositions in eliciting a protective immune response can be examined.

[0045] A further embodiment of the present invention provides for compositions containing the polynucleotides of the present invention. The present invention provides recombinant viral vectors comprising a foreign DNA sequence inserted into the viral vector genome. Viral vectors contemplated by the present invention consist of, but are not limited to, adenoviral vectors, swinepox viruses, PRRS viral vectors and pseudorabis viral vectors. In another embodiment, the present invention provides bacterial vectors comprising a foreign DNA sequence inserted into the bacterial vector genome. Bacterial vectors contemplated by the present invention include, but are not limited to, *Salmonella cholerasuis* vectors, *Salmonella typhimurium* vectors, *E. coli* vectors, and *Lactobacillus* vectors. The polynucleotides of the present invention, listed as SEQ ID NOs:27-39, can be recombinantly inserted into viral and or bacterial vectors through standard procedures well known to one of skill in the art. In another specific embodiment, the recombinant viral vectors are capable of replication in the animal to which the recombinant vector is administered. In a specific embodiment, the vectors are one of those listed above; however, this is not an exhaustive list, and the use of any vector capable of expression in an animal is contemplated by the invention.

[0046] This invention further provides foreign DNA sequences from *Lawsonia*, as listed in SEQ ID NOs:27-39, or foreign RNA derived from these sequences which encodes a polypeptide. In a specific embodiment, the polynucleotides of the invention are recombinantly inserted into an open reading frame (ORF) of the viral or bacterial vector. For purposes of the invention, an ORF is defined as a segment of DNA in the recombinant vector

that contains the codons that can be transcribed into RNA, and that can further be translated into an amino acid sequence or polypeptide; the ORF does not contain a termination codon. The recombinant vectors of the present also contain promoters. For purposes of the invention, a promoter is defined as a specific DNA sequence on the recombinant DNA vector to which the RNA polymerase binds and at which transcription of the foreign and/or recombinantly inserted DNA sequences begins. In a specific embodiment the polynucleotide sequence is under the control of a promoter.

[0047] In one embodiment, the *Lawsonia* polypeptide translated from the RNA transcribed from the recombinant vector is an immunogenically active antigen in the animal in which it is expressed. In a more specific embodiment, this polypeptide is able to induce antibody production in said animal. In a even more specific embodiment of the present invention said animal is a pig.

[0048] The invention further provides for a recombinant adenovirus vector capable of replication and which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant adenovirus vector capable of replication which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0049] The invention further provides for a recombinant swinepox virus vector capable of replication and which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant swinepox virus vector capable of replication which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0050] The invention further provides for a recombinant PRRS virus vector capable of replication and which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant PRRS virus vector capable of replication which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0051] The invention further provides for a recombinant pseudorabies virus vector capable of replication and which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant pseudorabies virus vector capable of replication which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0052] The invention further provides for a recombinant *Salmonella cholerasuis* vector which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant *Salmonella cholerasuis* vector which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0053] The invention further provides for a recombinant *Salmonella typhimurium* vector which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant *Salmonella typhimurium* vector which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0054] The invention further provides for a recombinant *E. coli* vector which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39 that encodes an antigenic polypeptide that is from *Lawsonia.* In some embodiments, SEQ ID NOs:14-26, which are optimized for expression in *E. coli*, are used. The invention further provides for a recombinant *E. coli* vector which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0055] The invention further provides for a recombinant *Lactobacillus* vector which contains a foreign DNA polynucleotide, as listed in SEQ ID NOs:27-39, that encodes an antigenic polypeptide that is from *Lawsonia.* The invention further provides for a recombinant *Lactobacillus* vector which contains a foreign DNA polynucleotide that encodes an antigenic polypeptide, listed as SEQ ID NOs:1-13, that is from *Lawsonia.*

[0056] The polynucleotide containing vector immunogens can be formulated into vaccines containing an immunologically effective amount of one or more of the polynucleotide vectors of the invention and an appropriate pharmaceutical carrier. Thus, polynucleotides of the invention can be administered individually or in combination either in a single composition or

multiple compositions. The formulated vaccines may be combined with a pharmaceutically acceptable adjuvant. The formulated vaccines may be an aqueous solution, a suspension or an emulsion. An immunologically effective amount of each immunogen in the vaccines of the present invention is determinable by methods known in the art without undue experimentation. The polynucleotide immunogens of the present invention can be administered as viral or bacterial particles. In a specific embodiment the viral or bacterial particles containing the polynucleotide sequences of the present invention can be administered at a concentration of 10^4 to 10^9 particles per dose. In a more specific embodiment the viral or bacterial particles containing the polynucleotide sequences of the present invention can be administered at a concentration of 10^5 to 10^8 particles per dose. In an even more specific embodiment, the viral or bacterial particles containing the polynucleotide sequences of the present invention can be administered at a concentration of 10^6 to 10^7 particles per dose.

[0057] The immunogenic compositions of the invention may also comprise nucleic acids encoding the immunogenic polypeptides in the absence of a viral or bacterial vector. See, e.g., Wolff et. al. (1990) Science 247:1465-1468; U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; and WO 98/04720. Examples of DNA-based delivery technologies include "naked DNA", facilitated (bupivicaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated ("gene gun") or pressure-mediated delivery (see, e.g., U.S. Patent No. 5,922,687).

[0058] In another embodiment of the invention, the general quantity of each polynucleotide immunogen will be between 1 μg and 1000 μg of each polynucleotide per dose. In a specific embodiment of the present invention, the amount will be between 10 μg and 500 μg of each polynucleotide per dose. In an even more specific embodiment of invention the amount will be between 25 μg and 250 μg of each polynucleotide per dose.

[0059] One convenient measure of efficacy is based on significant ($p<0.05$) reduction in prevalence and severity of macroscopic and microscopic lesions in the ileum of vaccinated versus control animals. For example, a significant reduction in ileum lesions scores between vaccinated animals and control animals (t-test, $p<0.05$) is one useful measure of efficacy. Furthermore, a significant reduction in the colonization of vaccinated animals versus control as determined by immunohistochemical staining of affected ileal tissues can also be detected. Methdods for carrying out such assays are well known.

[0060] The present invention also encompasses combination vaccines comprising immunogens of the present invention and at least one antigenic component of other pathogens including, but not limited to, *Mycoplasma Hyopneumoniae*, *Erysipelas spp*, *salmonella*, *H. parasuis*, *clostridium spp*, *streptoccous suis*, *brachyspira spp*, *bordetella*, *pasteurella*, *E. coli*, coronavirus, parvovirus, PRRS, Circovirus, and SIV.

[0061] Vaccines of the present invention may be administered to animals using a variety of methods including, but not limited to: scarification, intramuscular, subcutaneous, intranasal, intraperitoneal, or oral methods.

[0062] In another embodiment of the invention, the immunogenic compositions can encompass inactivated bacteria, termed "bacterin." The method of inactivation is not critical to the invention. Inactivation can occur after contaminating or interfering material is removed. Inactivation can include the use of known inactivating agents. Such inactivating agents include, but are not limited to: UV irradiation, formaldehyde, glutaraldehyde, binary ethyleneimine (BEI), and beta-propiolactone. In some embodiments BEI is used because it is known to destroy the viral nucleic acid without damaging the viral proteins. In addition, BEI is not affected by protein content and temperature. Inactivating agents are used at a concentration high enough to inactivate every bacterium in the solution. For example, BEI can be used at a final concentration of between about 0.5 mM and 10 mM. The pH and temperature can be chosen to ensure the resulting inactivated bacteria are still immunogenic. Inactivation can proceed with an appropriate amount of agitation to ensure that the agent contacts all the bacterial particles in the solution.

[0063] After inactivation, the inactivating agents can be removed using methods including, but not limited to, inactivation of the inactivating agent, precipitation of the inactivating agent, filtration of the inactivating agent, and chromatography, or a mixture of these methods. For example, BEI can be inactivated by the addition of sodium thiosulfate. Residual BEI can also be separated from the bacteria using size exclusion methods.

DETECTION OF SEROLOGICAL RESPONSE

[0064] Methods of identifying whether the immunogenic composition induces a serological response are also well known in the art. For example, one can inject a test animal with the immunogenic composition/vaccine and identify antiviral antibodies in the blood serum. Methods of identifying whether the immunogenic composition is protective are well known in the art and include immunization of a test animal with the immunogenic composition,

followed by inoculation with a disease-causing virus and identification of the presence or absence of symptoms of the disease.

[0065] The polypeptides and polynucleotides of the invention can also be used in diagnostic applications for the detection of *Lawsonia* in a biological sample. The presence of parasites can be detected using several well recognized specific binding assays based on immunological results. For instance, labeled monoclonal antibodies to polypeptides of the invention can be used to detect *Lawsonia* in a biological sample. Alternatively, labelled polypeptides of the invention can be used to detect the presence of antibodies to *Lawsonia* antigens in a biological sample. The sample can blood, urine, intestinal mucosa or any other bodily fluid likely to contain antibodies.

[0066] Many assay formats employ labelled assay components. The labelling systems can be in a variety of forms. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels may be used. The component may be labelled by any one of several methods. The most common method of detection is the use of autoradiography with 3H, 125I, 35S, 14C, or 32P labelled compounds or the like. Non radioactive labels include ligands which bind to labelled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labelled ligand. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation.

[0067] The presence of antibodies to *Lawsonia* can be carried out using well known techniques such as Western blots, ELISA, and the like. The polypeptides of the invention can thus also be used to detect *Lawsonia* infection in an animal using these techniques.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention.

EXAMPLE 1: METHODS OF GENERATING SYNTHETIC GENES

[0068] The present invention provides methods for predicting protein sequences based on genomic DNA. The present invention further provides methods for predicting antigenic utility of the aforementioned predicted proteins. Prior to the methods encompassed by the present

invention, the complete DNA sequence of the *Lawsonia intracellularis* chromosome and plasmids has been determined and deposited electronically in Genbank. In order to identify possible protective antigens, bioinformatics methods were applied to the published *Lawsonia* genomic and plasmid DNA sequences.

[0069] Kapur et al. previously reported the use of bioinformatics approaches to obtain putative or hypothetical open reading frames in order to predict *Lawsonia* protein sequences (US2006/0024696). The present invention provides further methods of verification of the hypothetical proteins found. The program PSORT was used to determine the cellular locations of the putative or hypothetical *Lawsonia* proteins. The PSORT program functions by recognizing the localization signal in proteins, and provides information as to whether a given protein may be found intracellularly (i.e. within the cell cytoplasm), in the cytoplasmic membrane, in the outer membrane or in multiple locations. Therefore, the previous methodology was combined with PSORT to obtain sequences that would be potentially immunogenic.

[0070] Based on the putative amino acid sequences obtained from the bioinformatics methodologies, the 13 candidate genes were analyzed using the software, Signal P 3.0, from the Center for Biological Analysis CBS. The software allows identification of the probable location of signal sequence cleavage points. Putative mature *Lawsonia* protein sequences, lacking the proposed putative signal sequence, were provided to DNA 2.0, Inc. (Menlo Park, CA., USA). The DNA encoding each gene was then synthesized, optimizing codon usage for expression in *E. coli*. These synthetic *Lawsonia* genes were then cloned into Invitrogen Gateway Entry Vector, pDONR221. All synthetic gene constructs were sequenced on both strands, to ensure accuracy.

EXAMPLE 2: METHODS OF PCR AMPLIFICATION

[0071] Using the aforementioned methods, 13 putative proteins sequences were selected for further analysis. The DNA encoding the 13 amino acid sequences was isolated and amplified by the polymerase chain reaction methodology. Two PCR reactions were employed for amplification of the DNA encoding the 13 putative protein sequences.

[0072] PCR amplification reactions were performed using KOD Hot Start DNA Polymerase (Novagen), in 50 µL reaction mixtures which contained 100 ng template DNA (synthetic gene sequences listed as SEQ ID NOs:14-26), 15 pM of each primer, 0.2 mM each of dATP, dGTP, dCTP, and dTTP, and 1 mM $MgSO_4$ by 35 cycles of heating and cooling. All primers

used for amplification of the genes are contained in the sequence listing as SEQ ID NOs:40-65.

[0073] PCR amplifications were performed using Accu-Primer GC Rich DNA Polymerase (Invitrogen) in 50 µL reaction mixtures which contained 200-500 ng template DNA (synthetic gene sequences listed as SEQ ID NOs:14-26), 10 pM of each primer, 0.2 mM each of dATP, dGTP, dCTP, and dTTP, and 1.5 mM $MgSO_4$ by 30 cycles of heating and cooling. All primers used for amplification of the genes are contained in the sequence listing as SEQ ID NOs:40-65.

EXAMPLE 3: CLONING OF *LAWSONIA* GENES

[0074] For cloning into pUEX2-M3, an isolated PacI/BamHI fragment from plasmid pDONR-G07501, was digested with EcoRI, and the resulting EcoRI/BamHI fragment purified from a 2.0% agarose gel using QIAquick spin columns (QIAGEN, Valencia, CA). The purified DNA fragment containing the synthetic *Lawsonia* gene Law 0460-PEBP (SEQ ID NO:23), was then inserted into EcoRI/BamHI sites of the pUEX2-M3 plasmid. An N-terminal His-tag, generated by annealing two complimentary synthetic oligomers together, was added to this plasmid at the EcoRI site. The sequence listing contains the sequences of the two DNA oligomers used to create the His-tag adaptor as SEQ ID NOs:66-67.

[0075] For cloning into either pET-23a(+) or pMAL-c2X plasmid vectors, the synthetic *Lawsonia* gene PCR products were first purified from 1.0-2.0 % agarose gels by use of a QIAEX II Gel Extraction Kit or QIAquick spin columns (QIAGEN, Valencia, CA), then digested either with BglII or BamHI and HindIII, and inserted into BamHI/HindIII sites of either plasmid vector (Table 1). The one exception to this was with Law 0050-AsmA (SEQ ID NO:19), which contained an internal HindIII site, so PCR products were digested with BglII and XbaI, and inserted into BamHI/XbaI sites in the plasmid vectors.

[0076] This process produced 13 expression plasmids. These plasmids were confirmed by DNA sequencing. The recombinant proteins expressed by pET-23a(+) and pUEX2-M3/His are His-tag proteins with a six histidine residues at the C-terminus or N-terminus, respectively. The recombinant proteins expressed by pMAL-c2X have a maltose binding protein (MBP) tag at the N terminus.

EXAMPLE 4: EXPRESSION AND PURIFICATION OF PROTEINS

[0077] Competent cells of *E. coli* strain BL21(DE3)/pLysS were purchased from Novagen (San Diego, CA, USA). Competent *E. coli* strains DH5α and TOP 10 were purchased from Invitrogen. Synthetic *Lawsonia* genes were cloned into Invitrogen's GATEWAY entry plasmid, pDONR221, and grown in Invitrogen's *E. coli* strain OmniMAX 2 T1 Phage-Resistant (T1). Expression Plasmids pMAL-c2X and pET-23a(+) were purchased from New England Biolabs and Novagen, respectively. Expression plasmid pUEX2-M3 was obtained from Biostar Inc. (Saskatoon, Saskatchewan, Canada).

[0078] Luria Bertani (LB) broth was prepared according to standard procedures. LB plates were prepared from imMedia Amp (or Kan) Agar (Invitrogen, Carlsbad, CA, USA). Carbenicillin Disodium Salt, Kanamycin Sulfate, and Isopropyl-β-D-thioglactopyranoside (IPTG) were purchased from Thermo Fisher Scientific Inc. (Pittsburgh, PA, USA). All restriction endonucleases, and DNA ligase were purchase from New England Biolabs (Ipswich, MA, USA).

[0079] The expression plasmids derived from pMAL-c2X were transformed into *E. coli* TOP 10 strain. Cells were grown overnight in LB medium at 37 °C. Flasks containing LB medium which contained 100 g/mL carbenicillin were inoculated with a 1/40 volume of the overnight culture and incubated at 37 °C until the absorbance at 600 nm reached 0.6 to 0.8. IPTG was added to a final concentration of 0.25 mM, and incubation was continued for 2-4 hr at 30 C. Two to three liters of cells for each protein were harvested by centrifugation at 7000 **x** g for 10 min and resuspended in 250-300 mL buffered saline solution. The cells were disrupted by APV Homogenizer. The samples were centrifuged at 12,000 **x** g for 10 min to separate soluble and insoluble fractions. Then, Amylose Resin (NEB, Ipswich, MA) equilibrated with a buffered saline solution, were added to the soluble fraction. After the solution were mixed for 1-20 hours at 4 °C, the resin was loaded in a column and washed with 20 column volumes of buffered saline. The recombinant protein was eluted from the column by use of buffered saline with 10 mM maltose.

[0080] The expression plasmids derived from pET-23a(+) were transformed into E.coli BL21(DE3)pLysS strain. Cells were grown overnight in LB medium at 37 °C. Flasks containing LB medium which contained 100 g/mL carbenicillin were inoculated with a 1/40 volume of the overnight culture and incubated at 37 °C until the absorbance at 600 nm reached 0.6 to 0.8. IPTG was added to a final concentration of 0.25 mM, and incubation was continued for 2-4 hr at 30 °C. The pUEX2-M3/His-G07501 plasmid, transformed into DH5α

E. coli strain was grown in a similar manner, and then induced at 42 °C for 2-3 hours. Two to three liters of cells for each protein were harvested by centrifugation at 7000 x g for 10 min and resuspended in 250-300 mL Lysis buffer (50mM phosphate buffer, pH 8.0, 300 mM NaCl, 10 mM imidazole). The cells were disrupted by APV Homogenizer. The samples were centrifuged at 12,000 x g for 10 min to separate soluble and insoluble fractions. Then, Ni-NTA Superflow agarose beads (QIAGEN) equilibrated with Lysis buffer were added to the soluble fraction. After the solution were mixed for 1-20 h at 4 °C, the resin was loaded in a column and washed with 20 column volumes of Wash buffer (50 mM phosphate buffer, pH 8.0, 300 mM NaCl, 20 mM imidazole). The recombinant protein was eluted from the column by use of Elution buffer (50mM phosphate buffer, pH 8.0, 300 mM NaCl, 250 mM imidazole).

[0081] The 13 *Lawsonia* genes of the present invention were successfully expressed in *E. coli* strain TOP10, BL21(DE3)pLysS or DH5α. Nine of the recombinant proteins were expressed as soluble protein (Table 1). Two of the expressed recombinant proteins, Law 0033-OstA (SEQ ID NO:1) and Law C046-SLH (SEQ ID NO:4) are partially soluble, and two, Law 005-AsmA (SEQ ID NO:6) and Law 0043-PtfH (SEQ ID NO:8), are insoluble. The protein size and type of purification tag are listed in Table 1. Western analysis showed that 5 of the 13 proteins reacted with the swine anti-*Lawsonia* sera. Based on the western blot analysis, the 5 proteins that interacted with swine anti-*Lawsonia* sera were Law 0691-PAL (SEQ ID NO:2), Law 0995-OPRM (SEQ ID NO:3), Law 0147-SlyB (SEQ ID NO:5), Law 1082-FeoA (SEQ ID NO:10), and Law 0460-PEBP (SEQ ID NO:11).

Table 1: Recombinant Lawsonia Intracellularis Proteins

Recombinant Protein	Tag	Size (kDa)	Soluble(s) or Insoluble (I)
Law 0033-OstA (SEQ ID NO:1)	MBP	130	partially S
Law 0691-PAL (SEQ ID NO:2)	MBP	57	S
Law0995-OPRM (SEQ ID NO:3)	MBP	80	S
Law C046-SLH (SEQ ID NO:4)	MBP	130	partially S
Law 00147-SlyB (SEQ ID NO:5)	MBP	55	S
Law 0050-AsmA (SEQ ID NO:6)	MBP	160	I
Law 0065-LoIA (SEQ ID NO:7)	MBP	67	S
Law 0043-PtfH (SEQ ID NO:8)	MBP	128	I
Law 0649-OmpB (SEQ ID NO:9)	MBP	131	S
Law 1082-FeoA (SEQ ID NO:10)	MBP	55	S
Law 0460-PEBP (SEQ ID NO:11)	His	20	S
Law B004-Fluf (SEQ ID NO:12)	MBP	100	S
Law 1153-OmpA (SEQ ID NO:13)	His	44	S

EXAMPLE 5: METHODS OF DETECTION OF EXPRESSED PROTEINS

[0082] The recombinant proteins that were boiled for 10 minutes with NuPAGE LDS Sample Buffer (Invitrogen) in the presence of β-mercaptoethanol were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) using 4% to 12% NuPAGE Bis-Tris gel (Invitrogen) at 200 Volts for 35 minutes. The gels were stained with SimplyBlueTM Safe Stain (Invitrogen) or transferred to 0.2 pore size nitrocellulose membranes (Invitrogen) and reacted with swine anti-*Lawsonia* sera, rabbit anti-maltose binding protein sera plus a

phosphatase labeled goat anti- rabbit sera, peroxidase labeled mouse anti-His sera, or phosphatase labeled mouse anti-His(C-term) sera.

[0083] Mouse monoclonal anti-His(C term) antibody was obtained from Invitrogen. Peroxidase labeled mouse monoclonal anti-His antibody was obtained from Roche Applied Science (Indianapolis, IN, USA). Rabbit anti-maltose binding protein antibody was obtained from New England Biolabs. Phosphatase labeled goat anti-rabbit sera was obtained from Kirkegaard & Perry Laboratories, Inc.(Gaithersburg, MD, USA). Swine anti-*Lawsonia* sera was prepared in house by standard methods available to one of skill in the art.

EXAMPLE 6: VACCINE COMPOSITION AND USE

[0084] Protein immunogens of the present invention were expressed, concentrated and purified from expression hosts such as *E. coli* using various methods known in the art. Purified protein immunogens can be formulated into vaccines containing an immunologically effective amount of each immunogen and an appropriate pharmaceutical carrier. The formulated vaccines may be combined with an adjuvant. The formulated vaccines may be an aqueous solution, a suspension or an emulsion. An immunologically effective amount of each immunogen in the vaccines of the present invention is determinable by methods known in the art without undue experimentation. In a specific embodiment of the invention, the general quantity of each immunogen will be between 5 μg and 500 μg of purified protein. In a specified embodiment of the present invention, the amount will be between 10 μg and 250 μg. In an even more specified embodiment of invention the amount will be between 50 μg and 200 μg of each protein.

[0085] The present invention also encompasses combination vaccines comprising immunogens of the present invention and at least one antigenic component of other pathogens including, but not limited to, *Mycoplasma Hyopneumoniae*, *Erysipelas spp*, *salmonella*, *H. parasuis*, *clostridium spp*, *streptoccous suis*, *brachyspira spp*, *bordetella*, *pasteurella*, *E. coli*, coronavirus, parvovirus, PRRS, Circovirus, and SIV.

[0086] Vaccines of the present invention may be administered to animals using a variety of methods including, but not limited to scarification, intramuscular, subcutaneous, intranasal, intraperitoneal, or oral methods.

EXAMPLE 7: RESULTS OF VACCINATION STUDIES

[0087] A study was conducted in pigs to test whether a killed vaccine based on virulent *Lawsonia* micro-organisms could provide protection against the disease caused by *Lawsonia.* In this study, *Lawsonia* organisms used for preparation of the bacteria were grown in a mouse fibroblast (McCoy's) cell line in T-175 cm^2 flasks using the method established in the art (e.g. Lawson et al, Gebhart et al.). Bacteria were harvested form infected McCoy cells as described in the art and live bacteria were inactivated by addition of Binary Ethyleneimine (BEI) and then formulated in the Emunade® adjuvant. The inactivated bacteria was tested at two dose levels; $1x10^8$ and $5x10^8$ bacteria per dose. The primary measure of efficacy was based on significant ($p<0.05$) reduction in prevalence and severity of macroscopic and microscopic lesions in the ileum of vaccinated versus control animals. The study schedule and activities are shown in Table 2. The results of this study are shown in Table 3. The data presented in Table 3 show that there was a significant reduction in ileum lesions scores between vaccinated animals and placebo control (t-test, $p<0.05$). Furthermore, there was a significant reduction in the colonization of vaccinated animals versus placebo control as determined by immunohistochemical staining of affected ileal tissues. These data demonstrate that vaccination of pigs with a bacterin based on killed or inactivated *Lawsonia intracellularis* results in significant protection against the disease caused by this bacteria.

Table 2: Treatment groups and vaccination/challenge activities

Treatment Group	No. of Animals	Vaccine Identification	Dose/Route	Vaccination (Study Day)	Serum Collection Days	Days
A	15	B06-237-01A	2 ml/IM	0, 21	0, 21, 42, 63	63
B	15	B06-237-01B	2 ml/IM	0, 21	0, 21, 42, 63	63
C	15	B06-237-01C	2 ml/IM	0, 21	0, 21, 42, 63	63

TABLE 3: Impact of vaccination with low passage Lawsonia on crypt epithelium colonization and Ileal lesions

Treatment	Ileum lesion score	IHC score	% animals +ve IHC
Vax- $5x10^8$	0.33 ± 0.15	1.1 ± 0.31	54
Vax- $1x10^8$	0.36 ± 0.14	1.56 ± 0.25	91
Placebo	1.0 ± 0.19	2.53 ± 0.18	100

SEQUENCES

SEQ ID NO:1
Name: Law 0033-OstA
Length: 806
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
LYNLFLPFILTNLIYYIIIPSLFIAFFPLQALSIINISEFAQPDINQSQTKWNLEADTLTTLSNNTIIEAKGNII
LTKGQDVFKADFARYYQKTGWLFLKGHVTVKMDENEINADEAEFNLNTKTGWLNNGNIFISSSHVYFSGARITKH
YGDYYTFNNVKVTTCDGPHPAWSISAKEAIVEVDGYAQLYDSTFKIKNIDVMYSPIFTIPAKQTRQSGFLNPNYG
ISQRRGIYYTQPYFLNIDQSSDLTFYAGLMTKIGPLGTVRYRSHKFTNQKTWFAASGIHDKNNIVTPGKDPVYPS
SQLVRNNHQRYWVRGMADGFIGNSTWCYISNLDYVSDQDYLREFDQGITGFSHSRSEMFQMFGRDIQEDDQSRLN
ALLIRKDWQRIGVVGNIRYEQDPTLGHGNHPTSQSELTQRIPQIDMFLYQGKLFQPLSLEGAIHLQSAYMYRAKG
TKGWRTELYPKVTLPIDLKYGSVITTVGLRETYYQTGIKSHTSPVAPHVPDTKTPRQTGQHRSLFNLQLESSTQA
HRIWRLKDKKTINLHSQSIGKTFCTALKHTIQPRICYSFIPREGQEKNPFYTLSDRILPQNDLTYSIVNILTKKN
VTISVDNNNNNNDNSVTPTLITSYYDLLYWNLSTGYDFEEERRKQYVEKYPKRPIKDIYSELELYILSWLTYSGK
TFISPYNGNITRHDHNIIFKSDRFSWKTGLSFRDQYYNYREHLQYRDENNIIMSSRLRLLQNSFSIQLLPNVSVT
LEDFRNLRELGTFGKTNSQLVEVTYLAQCYRIIGRYRYDGYDRSYTVLIEIPGLFE

SEQ ID NO:2
Name: Law 0691-PAL
Length: 162
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MEVFRRYGIVLVLLVVLSAGFGCCKKSVDVEQSLATECIAPAPAINAAAETITDGIIYFDFDKYDIKPEYRDMLQ
KKAELLKEYPCIRVRIEGNCDARGTQEYNLALGERRARAAYEYLVMLGVNPSQLEIISFGKERPAVEGTGPAVWA
KNRRDDFRIIAK

SEQ ID NO:3
Name: Law 0995-OPRM
Length: 459
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MKRLLLCIITCVIVSSCSFAPDYNRPHLELPEVWVSSPETGVPASMQWWKRFNDSTLDILVAEALQHNRDLIAAV
ARVDYAQAQLGVARSDLFPHFSGNAQATPVWVDHKRVTDGQSPYSANFSASWEIDIWGKIRNAKDAAFSQLMATE
AEKEGVFLSIAAQTANAYFLLRSLDLQCSIAERTVKTREDALSIYTAQYQKGFINKLDLTRAKTEVETARTALYQ
KRIAQENAETALSVLLGRSPRLIMDTAIERGVSMKDLSCIPVIPQGIPSELLERRPDIRQAEYTLKATSANIGVA
RAAWLPSISLTGLFGIVSPHLSDLLKNPLKTWSYGETGTVPILDFGQVYYNVEAAQAKEREALANYEKTVQNAFK
DIHDALIRQYESKNIVNSLERMVKELRIAVHLARTLYDNGYTSYLDVLDAERALFQSELDLASAWSDRLSSIVQV
CLALGGSWE

SEQ ID NO:4
Name: Law C046-SLH
Length: 796
Type: Amino Acid Sequence

Organism: *Lawsonia intracellularis*
MYKFIIFYIICIYETIAIYLLLGSFISYFNRVFLKKEVKILDTTKVKKYARIGTFIFLYVIISSQVSLSHNARIG
YEDKNSDRKSKLILHNKLQVNEFDELRSDIVVTMTPLNIYTSTQENKITTIEANLLRLEVIQRTQPVLLVDVESS
LRDIYLLLDEAEGCNREVERCLKIWETASEHTKVLHRQTRERLSNAVIECQPGLPTNNEGKVVSVPEEIVASIEN
KVLHTANQQRTARKMEVAISRHKNKNIFLGNKQKLLRYRIEVLKARVEGNPDPPIPILTPQVLELPLLPDPPPSP
PPLPQQTFQFPDFGDPLPEPLSPLGDDPPQNVLNQEPQPGPSSEIVSTLQPSPSVEDLSSSGVTLECQEELSSSD
EEILDDECLTSGDESSTSDGESQRSSPPTKRRKLTHTPPPSDRGSPPGSSSMLMPYYTYGQVSSLQGLQSTLMSL
EDQLATQLRLSIIRSINVLGVCCKDDNQLQPHTFQSKKQTKIKGGIGRSHSTDNEIRPTSVNNSLFFSQQWHVIA
SMDSRISNLETTISSRQAGVFTTPIDGLCLSLLYSNNKKKTQNFYGVVLDSVDGSAKAQIETDNILATVTWNKEH
QGFSGHLAGCYGWGKITNIRTIHFFDNESVSKGISSIHMSGGFIQLGYNVLLGKNYFLIPYVEYMRLAVAWDPYE
EHTGLIPCKVSGHKVHVCEKSIGLRNQWKITDNSQLQFWGSHIFTNHNTGEIASKPLSLSDYRNKISIPGYKKQY
IHREAGISYESNVMDTLSMELYSKLRVTKSIKDVTSYTSFTIRYVY

SEQ ID NO:5
Name: Law 00147-SlyB
Length: 152
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MQRCGLYIICLVLSIGMISCANFSASSFGGKQIRSAHIVEFGKVVSVKPVELEGNTPILGTITGGAVGGVLGSLI
GGGSGRILSTVVGAGAGAVAGNIAERKITTQQGLEIEVKLDNGQIISIVQGADQSFSPGERVRVLRGSDGSARVS
SI

SEQ ID NO:6
Name: Law 0050-AsmA
Length: 1075
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MRSFLISIVTLFLLGIIVFFGITFYFIKQHPQYITNKILSTISKQLKDTSISANSIGFHIVPFPKLYLTNVILQT
QKGDTIHIKECLITPKITNILSGNISIYSIEVIQPIASIILQNEQKKNSKTTGYAIPKQVSHLLQLITDSKLFIE
NGSITFQNNDYCFKIIGINGKIGVSKTLTSSLKLTADEIIWEIMNTVSNNSSTAQKSIEKVQLHIEDMPYKINTA
LLHDTSPLYDLFTNTKKTTFKVSGAIPTTNTANNITFDFTTKLEKDNSDKLTMHGQLHIEGTLPNGNTSIPILLS
VPFTTTSSEDMTHFPPLLIKNSKLLFDKTHIDLHGTIKNYDTLSNLFFDGTMDVKNFSFPYWFTFARQLPNGIQH
ALNQLSGEIKFTLSPQQVNAQKIIIHSLDTTFQGNGTVNNFLSPTITLSLATKQFNLNTLLPELKGKKSSQLSYP
KETFLTILSNLHNNNNNNNNIKKTINYDITIQADHVTCWKFDGYQFICNIQPKPQGTQIHTNCKNFYDGSLSSSLL
LSNRHTIQLAIENIQLSDITNIITKEYELKGKASGTSRVNGHGDTLASFLSSLKGTIDLYVTDGLVKKTASEAIP
FSMLHLTCDSIGQPSKGNKSSTIPYKGKWSAEISSARWNGSITMDGLIQFSTTDWLSIKAENIPSKVVCSVSGVQ
AVAYGGISFDIDNSFLSFSNFQGEIHPKTALSGTIKTSSSTSNTRQWEGSLTVMTQNLRNLLSKLGYEPKNISPT
MLQYCKLQGDIFISPATIRLTNIQGILDNTLIKFSLNGLQTNPPSWTGDIQLSSLNLDKYLLSINQNKLKKSQEL
WPIELLNKVNIQSTLTLAELIYRKVPYTNVVVPISLNQGTLSITPITASLCDGKTEASFKACPLSTNGNSAQIDF
HYISKGVDMIKLSKKRQQEYLISGLGTFVINIQSIAKSSIDFLKNLQGKWRIFIQNGYFKRNTTTTQQNFSNIGA
TGNIINGIITNNNFAITGPGMVITGSGKIDLPEWNLDYLITIDMEGFPIAIPIKYTGSIDNPKRTINAAKLILST
IGSLGRDTIGLIQDIFSAPLKLLLP

SEQ ID NO:7
Name: Law 0065-LoIA
Length: 249
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
LKNIINTIIFPKQRNKKMSQKFSMLQLILFFLTFIFYSYITDSYAESIPIVKELQQSYQSIKNFSATFTQELTHQ
ESGSKETRIGKLFFKKPLLVRWETNTPHEELLLINTNAVWDYLPDENLVYKYSTDIVKDSTSIIQVITGQVRLDK
NFSIIENNNNNNNELIFLKLYPKEPTTQMVEAFLWINKKSLLIHKVQSIDFYGNTNTITFINITPNTHIDNNIFQ
FTPPKDVTIENLQDSSTPERPLFN

SEQ ID NO:8
Name: Law 0043-PtfH
Length: 900
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MTKVGGSNPFSTLASMVGFSKSSSSSSSSKVAELKSKFEALGTLTKASPRPANGTQDTNRLSDRLFRGHYTVPAN
TASRVGGHLQRVDDNKSSSSAANVAKRAAAHSSFASSNPGLQGASGSSSGGTGGSITDQVKSRGITARNFSDSNN

NSIGSGSSSGSILDQVRSQGITARGSSISSGNASGTSNVNDGTSSRDYGANVRNTRERFEAGPGGAQGESPGVKD
AKEATKGVSVSNLRGIFEGRGGADNNQVGRSSGGVSGASGSGGAQGTGGTSGLGGAQGTLGSGGLDGGGVSGAAG
GTSGASGAGGARGTADGITSPGAREAQETIKNAGVSVRDLAGRFSGAQGTSGASGAAGASGASGAGGAAGAAGGG
GLDRGGVSGSAGGTSGTSGAGGARGTADGITSPGAREAQETIKNAGVSVRDLAGRFSGAQGTSGASGASGAAGVA
GMPAGSGDVVDGLRRGGEDTVDGFGRNQGSGPIPSSADFVDGPIGGIQGAGGASGAAGAAGASGAAGASGAAEPL
PTNGTDQQIAEVVVRNAENGHFDGIDFSTQPGGVESNTGSIPGTDLIVQRDITPGEQGVYNNLSEMGSWMDSPNA
SPTNAPESLTDDHSVLTTLLNNKEGLQDTVPFPLAVGEGTVVTKTLDPDIDTSKIKIPLEIVFGSGSMYGSGIGG
GSSTISSSMSDDGSSSIGSTTRKNRAGEIISRIAMNQSPGAGSGGQTVVGGLGSGSSNINISGGRGGIVYGPMPN
VNVVAGDGLNRLQLGSGINPLALLQERMVNFSAAQLEHLHGQLTGMMAMMEAMPGVAFEGASVQMTLPEPGDTQT
MPSIRLSGFGEPRLRENIGQPGQPPTQEAFDALRNGPLNGVSELMSQVQEMITVRSEGSISLSRSSSLSDLSSEI

SEQ ID NO:9
Name: Law 0649-OmpB
Length: 851
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MAYLSISKNQCKSFLITLVTIFIMTSIPQLAEAVEHFANGVPTVVQDVNVPADSYFGGADSAVGPNPIASTHLTI
STTQGFGQNALEFVVGGSLANGNGNPANINGDIVLIVENTNTQNSIIGGSMANAAPVTIGGSIFMTLRNVTAVDP
IFGGSVDVRFFAQQQPNEDQLVGGDININLENVTTPEFYGLGYANGVIPVNVLNRNFLVAVQGNITTNISNSNIA
TVMLGSHYDTTMAVGGNGTINVDNSTIGYLSASNSSDFVNPDLTNTVTFNIGPNNRIANIFASNNGVIPHFIVNM
DGSGTEIQELTLGNVIRGGLVLTSELNLSQGTINNLITGNEYYDRSGLRTTVNVRGGTIGVLTSGGSDYSELNFI
PGEISTILATNSIGNQDFASLSQVTIHQGAETLWGMRDEVFELQTNNLQLGGELFIPADGTGGVALITNHIIANS
GVITPVNMSPERMTPIIGFLEPTGEVAQLTIYGPLTVNLSHSPEILGKIITQPIPIAVTNSDVFGTSKLFVEHNT
KGLIWSDIIFNPQDKTWYLTNFRGSEDFYGLSAAREASNWLRQQHIWSLQRRSNKLLDHGVDGLWMNVQGGYEKL
DAAIGDAKMPWIMASLGYDFMHKLSDFYNLKALYGFGFGFATGKNKWNTINSTTNDIYMGLVGAYVGLMHEATGL
YGTVSGQFATNRTKTKCTGFDETYNWKENVPTEAIEIGWKWSIDEFKINPRGQVIFEQLSKHHFSLSQEGDTAIL
DKEFLTTTVIGISGEYDLDLRSKIIKLQASVDWIKGISGDFAAKSEVLNMKFKDKNDTSTFRGTLGASAQLLENF
EVHLDIFGDLGNDKGIGGQVGATYRF*

SEQ ID NO:10
Name: Law 1082-FeoA
Length:112
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MSAVVDSMTPFPCSEHDELPVINEATACCVFDKVESYISLRDLKVGQHARVVRVQADGELGRRIRDMGLVPGTEV
TIVGRAPLKDPVALRLLGFTLSLRNSEADYVMVSPIS

SEQ ID NO:11
Name: Law 0460-PEBP
Length: 183
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MKKLILTFALLLVTNITTFASEPFTLSSPQMKNGTIATNQVYNSFGCKGKNISPSLEWKNPPEGTKSFAITMFDI
DAPTGSGWWHWIVYNIPTSTSSLVLGAGNDPKKLPKGAVQSINDFGFIGFGGPCPPVGAKLHHYIFTIYALNVET
MDLPATTMPAAIGFNIHMHMIDKATFTATYSRK

SEQ ID NO:12
Name: Law B004-Fluf
Length: 971
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
MYNIINKHQIIKILLFSLCVFFFTLTEKQKIYAADVFFEGRTETLINVNKPFDSFFGGSDSTIGTLETGPTNLTF
TTVGAFRNSVFRIIGGGRSSFNNPNTVKGNVTLTVYNTDVERIIGAGISNRGLVTVTGSVNMKLENVSVTRGIYG
GVYTQNGHVLGSINMHLKNVQTPLLIGSGVSNGPNRITVNGDINIDVEDSRIQYVNITGEVDAGIKGNATLTVKK
STVELINSGRGNILGNLKISIADSNIRGLSPVDFGSSVYGDTSINVINSQINDITLIPRAGGMLVGPVTLDITSS
TIQNIQCGPVSQNNQLNTLNVTVNTSNITNLNLGSVEGHTISTTATVTDSNITNLNVGTFNGLGVTENASVIINS
GNITNLNVGTNVIAAATTINSSATIHDGLIANLTLGSQGNGRTMIATANVNGGTIGLLTMGSENFIPGTRPITEL
AILNMSGGLIERIIVGNANSSTINFTPGKRSIVKTINGPELPYLVNIQKGAMTQWGTKNMPFLLDTRNLILSGTL
ITSNIQLADLSITNLFVANGGTLVPRKLIPGNQPVIQFLGGPQSLLVIHQPLKVNLSLSPKLIGSSMVPLAFVSQ

SFSSPDLFVKQTRSGLIWSDLEFDPTTSIWYVNNIQASQDFYSFSIARETTNWLRQQHIWTLQNRSSKLLDNEHY
GLWINVQGGHESLDTSIGSKAKMPWIMATAGYDYLQQLPRLDMKALYGLAFGASKGKSKWSSVNSTKNDAELGMV
SGYVGLIHNKTGLYSTLTLQLASSKLHTNSTGFYRNFKWTETTPTEALELGWKYTFNNGIKMNPRGQLIFEQTSK
HHFDLGIQNDKAILDKSQLITSSLGITVEYKLPVTTPINLYAGIERIKGQSGNFAISSQSLQMKFKHDNDTSVVR
ATIGTNILLGEHFNIHCDIFGDKGNDKGIGGQAGFTYKF

SEQ ID NO:13
Name: Law 1153-OmpA
Length: 398
Type: Amino Acid Sequence
Organism: *Lawsonia intracellularis*
EIVMANVSGIPAPRLLSTTNQMTNAAAGNTNRATGSMNGRNLTQIKTPQSMIDNASEELTTSLESKSSDDFAIKD
RKRQGKGSDSLLKMVQEYTELTNDDTRNAKRAMLSQVLRASQSSQDVLEKTLEQFSNKTDAWASLAEIAQEYGAE
SPQPTGLKSVLDAMETLENEFGDEIKAGLKGALNSKEFTDIGSAAQLRDLYTTTVTITAAPDAVLARLLEEYESD
DDLDRAIDFLLSTLGGELESADPSMDKVHLQSVMGDIEKTQQLHSSHKQCTTALSRWKEKHKGGGENSTLTPLEM
MRELIALKNENFISPSSIDKIVDQADPQDIEKEVLFLQEMLAAVRKFPIMVFDNVENRVRVMGAVQDAVDDAVRR
EDEFLFQKEHPDVPLQPDENNIQ

SEQ ID NO:14
Name: Law 0033-OstA
Length: 2322
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
ATTATCAACATCTCTGAATTTGCACAACCGGACATCAACCAGTCTCAGACCAAATGGAACCTGGAAGCCGACACT
CTGACCACTCTGAGCAATAACACTATTATTGAAGCGAAGGGTAACATCATCCTGACCAAAGGCCAGGATGTGTTT
AAAGCTGACTTTGCACGCTACTATCAGAAGACGGGCTGGCTGTTTCTGAAAGGTCACGTTACCGTGAAAATGGAC
GAAAACGAGATCAATGCAGACGAAGCGGAATTCAACCTGAACACCAAAACCGGCTGGCTGAACAACGGCAATATC
TTCATTAGCTCTAGCCATGTGTACTTCAGCGGCGCGCGCATCACTAAACACTACGGCGATTACTACACTTTTAAC
AATGTTAAGGTGACTACCTGCGATGGTCCTCATCCGGCCTGGTCCATCTCTGCGAAAGAAGCTATTGTTGAAGTT
GATGGTTACGCGCAGCTGTATGACAGCACCTTTAAAATCAAGAACATTGATGTTATGTACAGCCCGATCTTCACC
ATCCCGGCGAAACAAACTCGTCAGTCCGGTTTCCTGAATCCGAATTATGGTATCTCTCAACGCCGTGGCATCTAT
TACACGCAGCCGTACTTCCTGAACATCGATCAGTCCTCTGACCTGACTTTCTACGCAGGTCTGATGACCAAAATC
GGTCCTCTGGGCACGGTGCGTTACCGCTCCCACAAATTTACGAACCAGAAGACCTGGTTCGCCGCTTCTGGTATC
CACGACAAAAACAACATCGTTACCCCGGGTAAAGACCCGGTTTATCCGTCTAGCCAACTGGTGCGCAATAATCAC
CAGCGCTACTGGGTTCGTGGTATGGCAGACGGTTTTATCGGTAACTCCACCTGGTGCTATATTTCTAATCTGGAT
TACGTGTCTGATCAGGACTATCTGCGTGAATTCGACCAGGGTATCACCGGTTTCTCTCACAGCCGCTCCGAGATG
TTCCAGATGTTTGGCCGTGACATCCAGGAAGACGATCAGTCCCGCCTGAACGCCCTGCTGATCCGTAAAGACTGG
CAGCGTATCGGCGTAGTGGGTAACATCCGTTACGAACAGGATCCAACTCTGGGTCACGGCAACCACCCGACTTCC
CAAAGCGAGCTGACTCAGCGCATTCCGCAGATTGATATGTTCCTGTACCAGGGTAAGCTGTTTCAGCCGCTGAGC
CTGGAAGGCGCGATCCACCTGCAGAGCGCCTACATGTATCGTGCAAAAGGTACCAAAGGCTGGCGTACCGAACTG
TACCCGAAAGTTACCCTGCCGATCGACCTGAAATACGGCTCTGTAATTACGACGGTTGGTCTGCGCGAAACTTAT
TACCAGACCGGTATCAAATCCCACACCTCTCCGGTAGCTCCGCACGTTCCGGACACCAAGACTCCGCGCCAAACC
GGTCAGCACCGCTCTCTGTTCAACCTGCAGCTGGAATCCAGCACCCAGGCACACCGCATTTGGCGTCTGAAAGAT
AAGAAAACCATCAACCTGCACAGCCAGTCTATTGGCAAAACTTTTTGTACTGCTCTGAAACACACGATCCAGCCA
CGTATCTGCTACTCTTTCATCCCGCGCGAGGGCCAGGAGAAAAACCCGTTCTATACTCTGAGCGATCGCATCCTG
CCGCAGAACGATCTGACCTATTCCATCGTAAACATCCTGACTAAGAAAAATGTCACCATTTCCGTCGACAACAAT
AACAACAATAACGATAATAGCGTTACTCCGACTCTGATTACCTCTTACTACGACCTGCTGTACTGGAACCTGAGC
ACTGGCTATGACTTCGAAGAAGAACGTCGTAAGCAGTACGTAGAAAAATACCCGAAACGCCCTATTAAAGATATC
TACTCCGAACTGGAACTGTACATTCTGTCCTGGCTGACCTACTCTGGCAAAACCTTCATCTCTCCGTACAACGGC
AACATCACCCGTCACGATCACAACATCATCTTCAAGAGCGATCGCTTTTCCTGGAAAACCGGCCTGTCTTTCCGT
GACCAGTATTACAACTACCGTGAACACCTGCAGTACCGTGATGAAAACAATATCATCATGAGCTCCCGTCTGCGT
CTGCTGCAAAACTCTTTCTCTATTCAGCTGCTGCCTAATGTGAGCGTAACCCTGGAGGATTTTCGCAACCTGCGT
GAACTGGGCACCTTCGGTAAGACCAACTCTCAACTGGTGGAAGTTACGTATCTGGCTCAATGCTACCGCATTATT
GGTCGTTATCGCTACGATGGTTACGACCGTAGCTACACTGTCCTGATTGAAATCCCTGGTCTGTTTGAATAA

SEQ ID NO:15
Name: Law 0691-PAL
Length: 414
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
AAAAGCGTAGATGTAGAACAAAGCCTGGCAACCGAATGTATCGCACCGGCGCCGGCAATCAACGCGGCGGCAGAA

ACGATCACGGACGGTATCATTTACTTCGACTTCGATAAATACGACATCAAGCCGGAATACCGTGACATGCTGCAG
AAGAAAGCTGAACTGCTGAAGGAATACCCGTGCATCCGTGTTCGCATCGAAGGCAACTGTGATGCGCGCGGTACT
CAGGAATACAATCTGGCGCTGGGTGAACGTCGTGCGCGTGCGGCCTATGAATACCTGGTTATGCTGGGTGTGAAC
CCGAGCCAGCTGGAAATCATCAGCTTCGGCAAAGAGCGCCCAGCTGTTGAGGGTACCGGTCCGGCTGTGTGGGCG
AAAAACCGTCGTGACGATTTCCGCATCATCGCAAAATAA

SEQ ID NO:16
Name: Law 0995-OPRM
Length: 1326
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTCGCTCCAGATTACAACCGTCCACACCTGGAACTGCCGGAAGTCTGGGTTTCTAGCCCGGAAACTGGCGTGCCG
GCATCCATGCAGTGGTGGAAACGTTTTAATGACTCTACGCTGGACATCCTGGTGGCCGAAGCCCTGCAGCACAAC
CGTGATCTGATCGCGGCGGTTGCGCGTGTAGATTACGCACAGGCTCAGCTGGGTGTTGCGCGCTCTGACCTGTTT
CCGCATTTTAGCGGTAACGCGCAGGCGACTCCTGTTTGGGTTGACCATAAACGTGTAACTGATGGTCAGAGCCCT
TACAGCGCGAACTTCAGCGCTAGCTGGGAGATTGATATCTGGGGTAAAATTCGTAACGCTAAAGATGCTGCGTTC
AGCCAACTGATGGCAACCGAAGCAGAAAAAGAGGGTGTGTTCCTGTCTATCGCAGCACAGACTGCTAACGCATAT
TTCCTGCTGCGCTCTCTGGACCTGCAATGCTCTATCGCTGAACGCACGGTAAAAACTCGTGAAGACGCACTGTCT
ATCTATACCGCGCAGTATCAAAAGGGTTTTATCAACAAACTGGATCTGACGCGCGCGAAAACCGAAGTTGAGACT
GCTCGTACCGCGCTGTACCAGAAACGTATCGCACAGGAGAACGCTGAAACCGCGCTGTCTGTCCTGCTGGGCCGC
AGCCCGCGTCTGATTATGGATACTGCTATTGAACGCGGCGTATCCATGAAAGATCTGAGCTGTATCCCGGTTATC
CCGCAGGGCATTCCGTCCGAACTGCTGGAACGTCGTCCTGATATCCGCCAGGCAGAGTATACCCTGAAAGCAACC
TCCGCGAACATCGGTGTGGCGCGCGGCGTGGCTGCCGTCTATTTCTCTGACCGGCCTGTTTGGTATCGTTTCC
CCGCACCTGTCCGATCTGCTGAAAAATCCTCTGAAAACCTGGAGCTATGGTGAAACCGGCACTGTACCAATCCTG
GACTTCGGTCAGGTTTACTATAACGTTGAAGCGGCCCAGGCGAAGGAACGTGAGGCTCTGGCCAACTACGAGAAA
ACCGTTCAGAACGCCTTCAAAGACATTCACGACGCGCTGATCCGTCAGTATGAATCCAAAAACATCGTCAACAGC
CTGGAACGTATGGTGAAGGAACTGCGTATCGCCGTCCATCTGGCTCGTACTCTGTACGACAACGGCTACACCTCT
TACCTGGACGTTCTGGATGCCGAACGCGCGCTGTTCCAGAGCGAGCTGGATCTGGCGAGCGCGTGGAGCGATCGT
CTGTCTAGCATCGTTCAGGTTTGCCTGGCGCTGGGTGGCTCCTGGGAATAA

SEQ ID NO:17
Name: Law C046-SLH
Length: 2286
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
AAAAAGGAAGTTAAAATCCTGGACACCACTAAAGTAAAAAAGTATGCGCGTATCGGCACGTTTATCTTTCTGTAC
GTAATCATCAGCTCCCAGGTGTCTCTGTCTCACAACGCCCGTATCGGCTATGAAGACAAAAACAGCGACCGCAAG
AGCAAGCTGATCCTGCATAACAAACTGCAGGTCAACGAATTCGACGAACTGCGCAGCGACATCGTTGTGACTATG
ACGCCTCTGAACATCTACACCTCCACCCAGGAAAACAAAATCACCACCATCGAAGCGAATCTGCTGCGTCTGGAA
GTCATTCAACGTACTCAGCCGGTGCTGCTGGTTGATGTTGAATCTTCTCTGCGTGATATCTATCTGCTGCTGGAC
GAAGCGGAAGGTTGCAATCGCGAAGTGGAGCGCTGTCTGAAAATCTGGGAAACCGCGTCCGAACACACTAAGGTA
CTGCACCGTCAGACTCGTGAGCGTCTGAGCAATGCCGTTATCGAATGTCAGCCGGGTCTGCCTACCAACAATGAA
GGTAAAGTAGTCTCTGTTCCGGAGGAAATCGTCGCGTCTATCGAGAACAAAGTTCTGCACACGGCGAACCAGCAG
CGTACGGCACGTAAAATGGAAGTGGCCATTTCCCGTCACAAAAACAAGAATATCTTTCTGGGTAACAAACAAAAG
CTGCTGCGCTATCGCATTGAAGTTCTGAAAGCTCGTGTGGAAGGCAACCCGGATCCGCCGATTCCGATTCTGACC
CCGCAGGTTCTGGAACTGCCGCTGCTGCCGGATCCACCGCCATCTCCGCCGCCTCTGCCGCAGCAAACCTTCCAG
TTCCCGGACTTCGGCGACCCACTGCCGGAACCACTGAGCCCGCTGGGCGATGATCCACCGCAGAACGTACTGAAC
CAGGAACCGCAGCCAGGTCCGTCTTCCGAAATCGTCTCCACTCTGCAGCCATCTCCGTCTGTTGAGGATCTGAGC
TCCTCCGGCGTCACTCTGGAGTGCCAGGAGGAACTGTCTTCCAGCGATGAAGAGATCCTGGATGACGAGTGCCTG
ACCTCCGGCGACGAATCTTCTACTTCCGATGGTGAAAGCCAACGTTCCAGCCCGCCGACCAAACGCCGCAAGCTG
ACCCACACTCCTCCACCATCTGACCGTGGTTCCCCGCCAGGCTCTTCTTCTATGCTGATGCCGTACTATACCTAC
GGTCAGGTGTCTTCTCTGCAGGGTCTGCAGTCTACTCTGATGTCTCTGGAAGACCAGCTGGCTACTCAGCTGCGC
CTGAGCATTATCCGTTCTATTAACGTCCTGGGTGTTTGCTGCAAAGACGACAACCAGCTGCAGCCGCACACCTTC
CAATCCAAGAAACAAACCAAAATCAAGGGTGGTATCGGTCGTTCCCACTCTACCGACAACGAAATTCGTCCGACG
TCCGTTAACAATAGCCTGTTCTTTAGCCAGCAGTGGCATGTGATCGCTTCTATGGACAGCCGTATCTCTAACCTG
GAAACTACTATCTCCTCCCGCCAAGCAGGTGTTTTCACCACTCCGATTGATGGCCTGTGCCTGTCTCTGCTGTAC
TCCAACAACAAGAAAAAAACGCAGAATTTCTACGGCGTTGTTCTGGACTCCGTCGATGGCAGCGCAAAAGCCCAG
ATTGAAACCGACAATATCCTGGCGACGGTAACTTGGAACAAAGAACACCAGGGTTTCAGCGGCCACCTGGCCGGC
TGCTACGGCTGGGGTAAAATCACCAACATCCGCACCATCCACTTCTTTGATAACGAGAGCGTTTCTAAAGGTATC
AGCAGCATCCATATGTCCGGCGGCTTCATCCAGCTGGGTTACAACGTCCTGCTGGGCAAAAACTACTTTCTGATC
CCGTATGTGGAATACATGCGTCTGGCAGTGGCATGGGATCCGTACGAAGAACACACTGGTCTGATTCCGTGTAAA

GTTTCTGGCCACAAAGTTCACGTTTGCGAGAAAAGCATCGGCCTGCGCAACCAATGGAAAATCACTGACAATTCC
CAGCTGCAGTTCTGGGGTAGCCATATTTTCACCAACCATAACACCGGCGAAATCGCCTCCAAACCGCTGTCCCTG
AGCGATTACCGCAACAAAATCTCTATCCCGGGCTACAAAAAGCAGTACATCCACCGCGAGGCGGGCATTTCCTAT
GAGTCTAACGTTATGGATACTCTGTCCATGGAACTGTATTCTAAGCTGCGTGTGACTAAATCCATTAAAGATGTG
ACGAGCTACACCTCTTTTACCATCCGTTATGTGTAC

SEQ ID NO:18
Name: Law 00147-SlyB
Length: 396
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCacGGTAAACAGATCCGTAGCGCACACATCGTTGAATTTGGTAAAGTTGTTTCTGTTAAA
CCGGTTGAACTGGAAGGTAACACCCCGATCCTGGGTACCATCACCGGTGGTGCGGTTGGTGGCGTGCTGGGTTCT
CTGATTGGCGGTGGTTCCGGTCGTATTCTGTCTACTGTTGTTGGTGCAGGTGCTGGCGCCGTCGCGGGCAACATC
GCTGAACGTAAAATCACCACTCAGCAGGGTCTGGAGATCGAAGTTAAACTGGACAATGGCCAGATCATCAGCATC
GTTCAAGGTGCGGATCAGAGCTTCTCCCCGGGTGAACGTGTTCGCGTTCTGCGTGGTTCCGACGGCAGCGCACGC
GTCAGCTCTATCTGAGGATCC

SEQ ID NO:19
Name: Law 0050-AsmA
Length: 3189
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCaCATCACGTTCTACTTCATCAAACAGCACCCGCAGTATATCACCAACAAAATTCTGTCC
ACCATCTCTAAACAGCTGAAGGACACCTCTATCTCTGCGAACAGCATCGGCTTCCACATCGTACCGTTCCCTAAA
CTGTATCTGACCAACGTAATCCTGCAGACCCAGAAAGGCGATACCATCCACATCAAAGAATGCCTGATCACTCCG
AAAATCACGAACATTCTGTCCGGTAACATCAGCATCTACTCCATCGAGGTTATTCAGCCAATTGCATCCATTATC
CTGCAGAACGAGCAGAAAAAGAACTCTAAAACGACTGGTTATGCCATTCCAAAACAGGTGAGCCACCTGCTGCAG
CTGATCACCGATAGCAAACTGTTTATCGAGAACGGCAGCATCACCTTTCAGAACAACGACTACTGCTTTAAAATC
ATTGGTATCAACGGCAAAATTGGTGTTAGCAAAACTCTGACGTCTTCTCTGAAACTGACTGCAGACGAAATCATT
TGGGAGATCATGAACACCGTATCCAACAACTCCAGCACCGCGCAGAAATCTATTGAAAAAGTGCAACTGCACATC
GAAGACATGCCATACAAAATCAACACCGCCCTGCTGCACGACACGAGCCCGCTGTACGATCTGTTTACTAATACC
AAGAAGACCACCTTCAAGGTGTCCGGCGCTATCCCGACCACCAACACCGCAAACAACATTACCTTTGACTTTACC
ACCAAACTGGAAAAAGATAACTCCGACAAGCTGACCATGCACGGTCAGCTGCACATTGAGGGTACTCTGCCGAAC
GGCAACACCTCCATCCCGATCCTGCTGTCCGTTCCGTTTACCACTACTAGCTCCGAAGACATGACGCATTTCCCT
CCGCTGCTGATCAAAAACAGCAAGCTGCTGTTTGATAAAACCCACATCGATCTGCACGGCACCATTAAAAACTAT
GATACCCTGTCCAACCTGTTTTTCGACGGTACCATGGATGTGAAAAACTTCTCTTTTCCGTATTGGTTTACCTTC
GCCCGTCAGCTGCCGAATGGCATTCAGCATGCGCTGAACCAGCTGTCCGGCGAAATTAAATTCACTCTGTCCCCG
CAGCAAGTAAACGCACAGAAAATCATCATTCACAGCCTGGACACTACTTTTCAGGGTAACGGCACCGTTAACAAC
TTCCTGTCCCCTACTATTACTCTGAGCCTGGCTACCAAACAGTTCAACCTGAACACCCTGCTGCCAGAACTGAAA
GGTAAAAAGTCCAGCCAGCTGTCTTACCCTAAGGAAACTTTCCTGACCATCCTGAGCAACCTGCACAACAACAAT
AACAACAACAACATTAAGAAAACGATCAACTACGATATCACCATCCAGGCGGACCATGTGACTTGTTGGAAGTTT
GACGGCTATCAGTTCATTTGTAACATTCAGCCGAAACCGCAGGGCACGCAGATCCATACCAACTGTAAAAACTTC
TACGATGGCTCCCTGTCTTCCTCCCTGCTGCTGAGCAATCGTCACACCATCCAGCTGGCCATTGAGAACATTCAG
CTGTCTGATATCACTAACATTATCACTAAAGAGTACGAGCTGAAAGGCAAAGCCTCCGGCACTTCCCGTGTCAAC
GGTCACGGTGATACTCTGGCTAGCTTTCTGAGCTCTCTGAAAGGCACCATCGACCTGTACGTAACCGACGGCCTG
GTCAAGAAAACCGCGTCTGAAGCCATCCCGTTCTCTATGCTGCACCTGACTTGTGATTCTATCGGCCAGCCGAGC
AAGGGTAACAAGAGCTCCACCATTCCATACAAAGGTAAATGGTCTGCGGAAATTTCTTCTGCTCGCTGGAACGGT
TCCATCACTATGGACGGCCTGATTCAGTTCTCCACCACCGATTGGCTGAGCATCAAAGCAGAAAACATTCCGAGC
AAAGTGGTCTGCAGCGTTAGCGGTGTTCAGGCGGTCGCGTATGGCGGCATCTCTTTTGATATCGATAACAGCTTC
CTGAGCTTCAGCAACTTCCAAGGCGAAATTCACCCGAAAACCGCCCTGAGCGGTACCATCAAGACCAGCTCCTCC
ACCTCTAACACCCGTCAATGGGAAGGTTCCCTGACTGTCATGACCCAGAACCTGCGCAACCTGCTGTCCAAACTG
GGTTATGAACCGAAAAATATCAGCCCAACCATGCTGCAGTATTGCAAGCTGCAAGGTGACATTTTCATTTCCCCT
GCTACTATTCGCCTGACTAACATCCAGGGTATCCTGGACAACACCCTGATTAAGTTTTCCCTGAACGGTCTGCAG
ACTAACCCACCGAGCTGGACCGGCGACATCCAGCTGTCCTCCCTGAACCTGGACAAATATCTGCTGTCTATTAAC
CAAAACAAACTGAAAAAGTCCCAGGAACTGTGGCCTATTGAACTGCTGAACAAAGTTAACATTCAGTCTACTCTG
ACCCTGGCGGAGCTGATCTATCGCAAAGTGCCGTACACCAACGTGGTAGTGCCTATCTCTCTGAATCAGGGCACC
CTGTCTATCACCCCGATCACCGCCAGCCTGTGCGACGGTAAAACCGAAGCGTCCTTCAAAGCTTGCCCACTGTCT
ACTAACGGCAACTCTGCCCAAATCGACTTCCACTATATTTCCAAGGGCGTCGATATGATCAAGCTGTCTAAGAAA
CGTCAGCAGGAGTACCTGATCTCTGGCCTGGGCACCTTCGTAATCAACATCCAGAGCATCGCAAAATCCTCTATT
GACTTCCTGAAAAATCTGCAGGGTAAATGGCGCATTTTTATTCAGAACGGTTACTTTAAACGCAACACTACGACC

ACTCAGCAGAACTTTTCTAACATCGGTGCGACCGGCAATATCATTAACGGCATTATCACCAATAACAACTTCGCG
ATCACTGGTCCGGGTATGGTTATCACCGGTAGCGGCAAGATCGACCTGCCAGAATGGAATCTGGATTACCTGATT
ACTATCGACATGGAAGGTTTTCCAATTGCGATCCCGATCAAATATACTGGTAGCATCGATAATCCAAAACGCACC
ATCAATGCGGCTAAACTGATTCTGTCTACCATCGGTTCTCTGGGTCGCGATACCATTGGTCTGATTCAGGACATT
TTCTCTGCTCCGCTGAAACTGCTGCTGCCGTGAGGATCC

SEQ ID NO:20
Name: Law 0065-LoIA
Length: 639
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCacGAAAGCATCCCGATTGTAAAAGAACTGCAGCAAAGCTACCAGTCTATCAAAAACTTC
AGCGCGACCTTCACCCAAGAGCTGACTCACCAGGAGAGCGGTTCTAAAGAGACTCGTATCGGCAAACTGTTTTTC
AAGAAACCGCTGCTGGTTCGTTGGGAAACCAACACCCCACACGAAGAACTGCTGCTGATCAACACCAACGCCGTG
TGGGACTACCTGCCAGATGAAAACCTGGTCTACAAATACTCTACTGATATCGTTAAAGACAGCACGAGCATTATT
CAAGTGATCACCGGTCAGGTTCGTCTGGACAAAAACTTTAGCATCATTGAAAACAATAACAACAACAATAACGAA
CTGATCTTTCTGAAACTGTACCCGAAAGAACCGACCACCCAGATGGTCGAGGCCTTCCTGTGGATCAACAAAAAG
AGCCTGCTGATCCACAAAGTTCAGAGCATCGATTTCTACGGTAACACTAACACCATCACCTTTATCAACATCACC
CCTAACACCCACATTGATAATAACATCTTCCAGTTTACCCCGCCGAAGGACGTTACTATTGAAAACCTGCAGGAT
AGCTCTACTCCGGAGCGCCCACTGTTCAATTAAGGATCC

SEQ ID NO:21
Name: Law 0649-OmpB
Length: 2472
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCacGTAGAACACTTCGCAAATGGCGTACCGACTGTAGTTCAAGATGTAAACGTGCCGGCC
GATTCTTATTTCGGTGGCGCTGATTCTGCTGTGGGCCCGAACCCGATTGCGTCTACGCACCTGACCATCTCTACT
ACTCAGGGCTTCGGTCAGAACGCCCTGGAGTTCGTAGTAGGTGGTTCTCTGGCAAACGGTAATGGCAACCCAGCA
AACATCAACGGTGATATTGTACTGATCGTTGAAAACACGAACACTCAGAACAGCATCATTGGTGGTTCCATGGCA
AATGCAGCGCCGGTTACCATTGGTGGTAGCATCTTCATGACGCTGCGTAACGTTACCGCTGTTGATCCGATCTTC
GGTGGCTCTGTTGATGTCCGCTTCTTCGCACAGCAGCAGCCGAACGAGGACCAGCTGGTGGGTGGCGATATCAAC
ATTAACCTGGAGAACGTCACTACTCCGGAGTTCTACGGTCTGGGTTATGCGAATGGCGTAATCCCGGTTAATGTT
CTGAATCGTAACTTTCTGGTGGCGGTTCAGGGTAACATCACCACCAATATCTCTAACAGCAACATCGCAACCGTT
ATGCTGGGCTCCCACTACGACACTACCATGGCAGTAGGTGGTAACGGCACTATCAACGTGGACAACAGCACCATT
GGTTACCTGAGCGCGTCTAATTCTTCTGACTTCGTTAATCCGGACCTGACTAACACGGTTACCTTCAACATCGGC
CCGAACAACCGTATTGCAAACATCTTTGCAAGCAACAACGGCGTGATTCCTCATTTCATTGTTAACATGGACGGT
TCCGGCACGGAAATCCAGGAGCTGACCCTGGGTAACGTAATTCGTGGTGGTCTGGTTCTGACGTCCGAACTGAAC
CTGTCCCAGGGTACCATCAACAACCTGATCACCGGCAACGAGTACTATGATCGTTCCGGTCTGCGTACTACCGTA
AACGTGCGTGGTGGTACCATCGGCGTGCTGACGTCCGGCGGTAGCGATTATTCCGAGCTGAACTTCATCCCAGGC
GAAATCAGCACCATTCTGGCTACTAATAGCATTGGTAACCAAGATTTCGCATCCCTGAGCCAGGTAACTATCCAC
CAAGGCGCCGAAACCCTGTGGGGTATGCGTGATGAAGTGTTCGAGCTGCAGACTAACAACCTGCAGCTGGGTGGC
GAACTGTTCATTCCGGCAGATGGTACCGGTGGTGTAGCCCTGATCACCAACCATATCATTGCAAACAGCGGTGTA
ATCACGCCGGTGAACATGTCCCCGGAGCGCATGACGCCGATCATCGGTTTCCTGGAGCCTACCGGCGAAGTTGCT
CAGCTGACCATCTACGGTCCTCTGACCGTAAACCTGTCCCACTCTCCTGAGATCCTGGGCAAGATCATCACGCAG
CCGATCCCGATCGCTGTGACTAATTCTGATGTGTTCGGCACTTCCAAACTGTTTGTGGAGCACAATACCAAGGGT
CTGATTTGGTCTGATATCATCTTTAATCCGCAGGACAAGACCTGGTACCTGACCAACTTCCGTGGCTCTGAAGAT
TTCTACGGCCTGTCTGCCGCTCGTGAAGCCTCTAACTGGCTGCGTCAACAACACATCTGGTCCCTGCAACGTCGT
TCTAACAAACTGCTGGATCACGGTGTGGACGGTCTGTGGATGAACGTGCAAGGTGGTTATGAAAAGCTGGATGCT
GCTATTGGTGATGCCAAGATGCCGTGGATTATGGCCTCCCTGGGCTATGACTTCATGCACAAACTGAGCGACTTC
TATAATCTGAAGGCGCTGTACGGTTTCGGCTTCGGTTTCGCTACCGGCAAGAACAAATGGAACACTATCAACTCT
ACCACTAATGACATTTACATGGGTCTGGTCGGTGCGTACGTTGGTCTGATGCATGAAGCCACGGGCCTGTATGGT
ACCGTGAGCGGTCAGTTCGCGACGAACCGCACCAAAACCAAATGCACTGGCTTCGACGAAACCTACAATTGGAAA
GAAAACGTTCCGACTGAAGCCATTGAAATCGGTTGGAAATGGTCCATTGATGAGTTTAAAATTAACCCTCGCGGT
CAGGTAATCTTTGAACAGCTGAGCAAACATCATTTTTCCCTGTCTCAGGAAGGTGATACTGCGATCCTGGATAAG
GAGTTCCTGACGACCACTGTTATCGGTATTTCCGGCGAATATGACCTGGACCTGCGCTCCAAAATCATTAAACTG
CAGGCCAGCGTTGACTGGATTAAAGGCATCTCTGGTGATTTCGCAGCTAAAAGCGAAGTTCTGAACATGAAATTC
AAAGACAAAAACGATACTTCTACCTTCCGTGGCACTCTGGGTGCTAGCGCCCAGCTGCTGGAGAACTTTGAAGTT
CACCTGGATATCTTCGGTGATCTGGGTAACGACAAAGGTATTGGCGGCCAGGTGGGTGCTACCTACCGCTTC

SEQ ID NO:22
Name: Law 0043-PtfH
Length: 2628
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TCTAGCAGCTCTAAGGTTGCAGAACTGAAATCTAAATTCGAAGCGCTGGGTACGCTGACCAAGGCTTCCCCACGT
CCGGCGAACGGTACCCAGGACACCAATCGCCTGTCCGACCGCCTGTTCCGTGGCCATTATACTGTCCCTGCAAAC
ACCGCAAGCCGTGTTGGCGGTCATCTGCAGCGCGTCGATGATAACAAATCCTCTTCTAGCGCTGCGAACGTAGCA
AAACGTGCTGCTGCTCACTCCTCTTTTGCGTCTAGCAACCCGGGTCTGCAAGGCGCGTCTGGCTCTTCTAGCGGT
GGTACCGGCGGTAGCATTACCGACCAGGTGAAAAGCCGTGGCATCACTGCTCGTAATTTCTCTGACTCCAACAAC
AACTCCATCGGCAGCGGCTCTAGCTCCGGCTCTATCCTGGACCAGGTCCGTAGCCAGGGTATTACCGCACGTGGC
TCCAGCATCTCTTCTGGCAACGCTTCCGGCACCTCCAACGTTAACGATGGCACCAGCTCCCGTGATTACGGTGCG
AACGTACGTAACACCCGTGAACGTTTTGAAGCAGGTCCGGGCGGTGCTCAGGGTGAAAGCCCGGGTGTTAAAGAC
GCTAAAGAAGCCACCAAAGGTGTAAGCGTCTCCAACCTGCGTGGTATCTTTGAAGGTCGTGGTGGCGCGGATAAT
AACCAAGTTGGTCGTTCTTCTGGCGGCGTCTCCGGCGCATCCGGCTCTGGCGGCGCCCAGGGCACCGGTGGTACC
TCCGGTCTGGGCGGTGCTCAGGGCACCCTGGGTTCTGGTGGCCTGGACGGTGGCGGTGTAAGCGGTGCCGCGGGC
GGTACTTCCGGCGCCAGCGGCGCTGGCGGTGCGCGTGGCACCGCTGATGGCATCACTTCTCCGGGTGCACGTGAA
GCGCAGGAAACCATCAAAAACGCCGGTGTATCCGTTCGTGATCTGGCAGGCCGTTTTTCTGGTGCGCAGGGTACT
AGCGGTGCTTCTGGTGCTGCCGGCGCCTCCGGTGCATCTGGTGCGGGTGGTGCGGCAGGTGCAGCTGGCGGTGGT
GGTCTGGACCGCGGTGGCGTTTCTGGCAGCGCTGGCGGTACCAGCGGCACCAGCGGTGCCGGTGGCGCGCGTGGT
ACCGCCGACGGTATCACTTCCCCGGGTGCTCGTGAAGCACAGGAAACCATTAAAAACGCTGGTGTTTCCGTTCGT
GACCTGGCCGGTCGTTTCAGCGGCGCTCAAGGTACTTCTGGTGCATCTGGCGCGTCCGGTGCGGCAGGCGTGGCA
GGCATGCCGGCGGGCTCCGGCGACGTGGTAGACGGTCTGCGTCGCGGTGGCGAAGACACCGTTGACGGTTTCGGT
CGTAACCAAGGTTCCGGCCCTATCCCATCTTCCGCCGACTTTGTTGACGGCCCGATCGGTGGTATTCAGGGCGCG
GGTGGCGCTTCTGGTGCAGCGGGTGCTGCGGGTGCTTCCGGTGCTGCTGGCGCAAGCGGTGCGGCGGAACCGCTG
CCGACGAACGGTACCGACCAGCAAATCGCCGAGGTGGTTGTTCGTAACGCGGAGAACGGCCACTTCGATGGCATC
GACTTCTCCACGCAGCCGGGTGGCGTTGAATCTAACACTGGTTCTATCCCAGGCACCGATCTGATTGTACAGCGT
GACATCACCCCGGGTGAACAGGGTGTATATAACAACCTGTCTGAAATGGGTTCCTGGATGGACAGCCCAAACGCT
TCCCCGACCAACGCACCAGAATCTCTGACCGATGACCACTCTGTTCTGACCACCCTGCTGAACAACAAGGAAGGT
CTGCAGGATACGGTTCCGTTCCCGCTGGCAGTAGGTGAAGGCACGGTCGTGACTAAAACCCTGGATCCGGATATT
GATACCTCCAAAATCAAAATTCCACTGGAGATTGTATTCGGTTCTGGCTCTATGTACGGTTCCGGTATCGGTGGT
GGCTCTAGCACCATCTCCTCTTCTATGTCTGATGACGGTTCCTCTTCTATTGGTTCTACCACCCGTAAAAACCGC
GCTGGCGAAATTATCAGCCGCATCGCCATGAACCAGAGCCCAGGCGCGGGTTCTGGCGGTCAAACTGTGGTGGGC
GGCCTGGGTAGCGGCTCTTCTAACATTAATATCTCTGGCGGTCGTGGTGGTATTGTTTACGGTCCAATGCCTAAC
GTTAACGTGGTGGCAGGTGACGGCCTGAACCGCCTGCAACTGGGCAGCGGCATCAACCCGCTGGCGCTGCTGCAG
GAACGTATGGTGAACTTCAGCGCGGCACAGCTGGAACATCTGCATGGCCAGCTGACTGGTATGATGGCAATGATG
GAGGCAATGCCGGGCGTGGCTTTCGAGGGTGCTTCCGTACAGATGACCCTGCCTGAACCTGGTGATACCCAAACC
ATGCCGTCCATTCGTCTGTCCGGTTTCGGTGAACCGCGCCTGCGTGAAAACATCGGCCAGCCGGGCCAACCGCCT
ACTCAGGAAGCGTTCGACGCACTGCGTAACGGCCCGCTGAACGGTGTGTCTGAACTGATGAGCCAAGTTCAGGAA
ATGATCACGGTACGCTCTGAAGGCTCTATCTCCCTGTCTCGCTCCAGCAGCCTGTCTGACCTGTCCTCTGAAATC
TAA

SEQ ID NO:23
Name: Law 0460-PEBP
Length: 516
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCacTCTGAACCGTTTACTCTGTCTAGCCCTCAAATGAAAAATGGCACTATCGCAACCAAC
CAGGTTTACAACAGCTTTGGCTGTAAGGGCAAGAACATCTCTCCGTCTCTGGAATGGAAAAACCCACCGGAAGGC
ACGAAAAGCTTCGCCATCACCATGTTCGATATTGATGCCCCGACGGGCAGCGGCTGGTGGCATTGGATCGTTTAT
AACATTCCGACTTCTACCAGCTCTCTGGTCCTGGGCGCAGGCAACGACCCGAAAAAGCTGCCGAAAGGCGCTGTT
CAATCTATTAACGATTTCGGCTTCATCGGCTTCGGTGGCCCGTGTCCTCCGGTCGGCGCGAAACTGCACCACTAT
ATCTTCACTATCTACGCTCTGAACGTGGAAACTATGGATCTGCCGGCAACGACTATGCCAGCTGCTATTGGTTTT
AACATCCACATGCATATGATTGATAAAGCTACTTTCACCGCGACCTATAGCCGCAAGTAAGGATCC

SEQ ID NO:24
Name: Law 1082-FeoA
Length: 363
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic

TTAATTAAtaGAATTCacATGTCTGCCGTTGTTGATTCTATGACTCCTTTTCCGTGCTCTGAACACGATGAGCTG
CCTGTTATTAACGAAGCCACCGCATGCTGTGTTTTTGACAAGGTTGAGAGCTACATTTCCCTGCGCGACCTGAAG
GTGGGCCAGCATGCCCGTGTGGTTCGTGTGCAAGCGGACGGCGAACTGGGTCGCCGTATTCGTGACATGGGTCTG
GTCCCGGGCACCGAAGTAACTATCGTAGGCCGCGCGCCACTGAAAGATCCGGTTGCCCTGCGTCTGCTGGGTTTT
ACTCTGAGCCTGCGTAACTCTGAAGCTGATTACGTTATGGTGTCTCCGATTTCTTAAGGATCC

SEQ ID NO:25
Name: Law B004-Fluf
Length: 2739
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
AAACAAAAGATCTACGCGGCGGATGTATTCTTTGAAGGTCGTACCGAAACCCTGATCAACGTGAACAAGCCGTTC
GACTCTTTCTTTGGCGGTAGCGATTCTACCATTGGCACCCTGGAAACCGGCCCGACTAACCTGACTTTCACCACC
GTTGGCGCCTTTCGTAACTCCGTATTCCGTATCATCGGCGGTGGTCGTTCCAGCTTCAACAACCCGAACACTGTG
AAAGGTAACGTGACCCTGACTGTTTACAACACCGACGTTGAACGTATCATCGGTGCTGGCATCTCCAACCGTGGT
CTGGTGACCGTGACCGGTTCCGTTAACATGAAACTGGAGAACGTGTCCGTCACGCGTGGTATCTACGGTGGCGTT
TACACCCAGAACGGTCACGTGCTGGGTTCTATCAACATGCACCTGAAAAACGTGCAGACCCCGCTGCTGATCGGT
TCTGGCGTATCTAACGGCCCGAACCGTATTACCGTAAACGGCGACATTAACATTGACGTCGAGGATAGCCGTATC
CAGTACGTGAACATCACCGGCGAGGTAGATGCAGGCATTAAAGGTAACGCAACCCTGACCGTTAAAAAGAGCACC
GTGGAACTGATCAACAGCGGTCGCGGCAACATCCTGGGTAATCTGAAAATCTCTATCGCAGACAGCAACATTCGT
GGCCTGTCTCCGGTCGACTTCGGCAGCTCCGTATACGGTGACACGAGCATCAACGTTATTAACTCCCAGATCAAC
GACATCACCCTGATTCCGCGCGCAGGCGGCATGCTGGTTGGCCCGGTCACTCTGGACATTACCTCTTCCACGATT
CAGAACATTCAGTGTGGCCCGGTGTCCCAGAACAACCAGCTGAATACTCTGAATGTGACCGTAAACACGTCCAAT
ATCACCAACCTGAACCTGGGCTCTGTAGAAGGCCATACCATCAGCACCACCGCGACCGTAACGGATTCCAACATC
ACCAATCTGAACGTCGGCACCTTCAACGGCCTGGGTGTTACCGAGAATGCATCTGTCATTATTAACAGCGGTAAC
ATTACTAATCTGAACGTAGGCACTAACGTTATTGCGGCTGCGACTACGATCAATTCTTCCGCTACCATCCACGAC
GGCCTGATCGCAAACCTGACTCTGGGCAGCCAGGGTAACGGTCGTACTATGATCGCTACCGCTAACGTCAACGGT
GGCACCATTGGTCTGCTGACCATGGGTTCTGAAAACTTCATTCCGGGTACCCGTCCGATCACCGAACTGGCGATC
CTGAACATGAGCGGTGGTCTGATCGAACGTATCATTGTGGGTAACGCTAATTCTTCTACTATTAACTTCACGCCA
GGTAAACGTTCCATCGTAAAAACCATCAATGGCCCGGAACTGCCGTACCTGGTTAATATCCAGAAAGGCGCGATG
ACTCAATGGGGCACCAAAAACATGCCGTTCCTGCTGGACACCCGTAACCTGATTCTGTCCGGTACTCTGATCACT
AGCAACATCCAGCTGGCAGACCTGAGCATTACCAATCTGTTTGTGGCTAATGGTGGCACCCTGGTTCCTCGCAAA
CTGATTCCGGGTAACCAGCCGGTTATTCAGTTCCTGGGTGGCCCGCAGTCTCTGCTGGTGATCCATCAGCCGCTG
AAAGTAAACCTGAGCCTGTCTCCAAAGCTGATCGGCTCCAGCATGGTCCCTCTGGCGTTCGTATCCCAGTCTTTC
AGCTCTCCAGATCTGTTTGTGAAACAGACCCGCTCCGGCCTGATTTGGTCCGATCTGGAATTTGACCCAACGACT
AGCATCTGGTACGTTAACAACATTCAGGCGTCCCAGGATTTCTATAGCTTCAGCATTGCGCGTGAAACCACCAAC
TGGCTGCGTCAACAGCATATTTGGACCCTGCAGAACCGTTCCTCCAAACTGCTGGACAACGAACACTACGGCCTG
TGGATTAACGTACAAGGCGGCCACGAGTCTCTGGACACTTCCATCGGTAGCAAGGCAAAAATGCCATGGATCATG
GCTACCGCGGGTTATGATTATCTGCAGCAACTGCCGCGTCTGGACATGAAAGCACTGTACGGCCTGGCATTTGGT
GCCTCTAAAGGTAAAAGCAAGTGGTCTTCCGTAAATAGCACTAAGAACGATGCGGAACTGGGCATGGTTTCTGGT
TACGTCGGTCTGATCCACAACAAAACTGGCCTGTATAGCACTCTGACTCTGCAGCTGGCTTCTTCTAAACTGCAT
ACCAATAGCACGGGTTTTTATCGTAACTTTAAATGGACGGAAACCACTCCAACTGAGGCGCTGGAGCTGGGTTGG
AAATACACCTTTAACAATGGCATCAAAATGAACCCTCGTGGTCAGCTGATTTTCGAACAGACTAGCAAACACCAC
TTCGACCTGGGTATCCAGAACGACAAAGCGATCCTGGACAAAAGCCAGCTGATCACCAGCTCCCTGGGTATTACG
GTTGAGTACAAACTGCCGGTCACCACGCCTATCAACCTGTACGCAGGTATCGAGCGTATTAAAGGCCAATCCGGC
AACTTTGCGATTTCCAGCCAGTCCCTGCAGATGAAGTTCAAGCATGATAACGACACCAGCGTGGTCCGTGCTACC
ATCGGTACGAACATTCTGCTGGGCGAGCACTTCAACATCCACTGCGATATCTTCGGTGACAAAGGCAATGACAAG
GGTATCGGCGGTCAGGCTGGTTTCACCTACAAATTCTAA

SEQ ID NO:26
Name: Law 1153-OmpA
Length: 1140
Type: Synthetic Nucleic Acid Sequence
Organism: Synthetic
TTAATTAAtaGAATTCacGGTAACACTAATCGTGCTACTGGCTCTATGAATGGTCGTAATCTGACCCAGATCAAA
ACGCCGCAGAGCATGATCGACAACGCGTCTGAGGAACTGACCACCTCTCTGGAAAGCAAATCTTCCGACGACTTC
GCGATTAAGGATCGTAAACGTCAGGGCAAGGGCTCCGACAGCCTGCTGAAAATGGTTCAGGAATATACTGAGCTG
ACGAATGATGACACCCGTAACGCGAAACGTGCGATGCTGTCTCAAGTGCTGCGTGCTAGCCAGTCTAGCCAGGAC
GTGCTGGAGAAAACCCTGGAGCAGTTCTCTAACAAAACTGACGCCTGGGCGTCTCTGGCCGAAATCGCACAAGAA
TACGGCGCGGAATCTCCGCAGCCGACTGGTCTGAAAAGCGTACTGGACGCCATGGAAACGCTGGAAAACGAGTTC
GGCGACGAGATCAAAGCAGGTCTGAAGGGCGCCCTGAACTCCAAAGAGTTCACTGATATCGGTAGCGCTGCTCAG

CTGCGTGACCTGTACACTACTACCGTCACCATTACCGCTGCACCTGATGCAGTACTGGCCCGCCTGCTGGAAGAA
TATGAATCCGATGACGACCTGGACCGCGCGATCGATTTCCTGCTGTCTACTCTGGGTGGCGAACTGGAAAGCGCA
GATCCGAGCATGGATAAAGTCCACCTGCAATCTGTGATGGGTGATATCGAGAAAACTCAACAGCTGCACAGCTCC
CACAAGCAGTGCACTACTGCTCTGAGCCGTTGGAAGGAAAAACACAAGGGCGGTGGTGAGAACTCTACTCTGACC
CCGCTGGAAATGATGCGCGAGCTGATCGCACTGAAAAACGAAAACTTCATTTCTCCTTCCTCCATCGACAAAATT
GTGGACCAGGCAGACCCGCAGGACATCGAGAAAGAAGTTCTGTTCCTGCAAGAAATGCTGGCTGCCGTGCGTAAA
TTTCCGATTATGGTATTCGATAACGTTGAAAACCGCGTTCGTGTAATGGGTGCCGTTCAGGATGCGGTGGACGAT
GCCGTACGTCGTGAAGATGAGTTCCTGTTCCAGAAAGAGCATCCAGATGTGCCGCTGCAGCCGGATGAAAACAAC
ATCCAGTAAGGATCC

SEQ ID NO:27
Name: Law 0033-OstA
Length: 2421
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
ttgtataatctttttttaccatttattctaactaaccttatatattatataattattccatccttatttatagca
ttttttcctctacaagctttatctattataaacatatccgaatttgcacaaccagatattaaccaatctcaaact
aaatggaaccttgaagctgatacgttaactacactatcaaataatactattattgaagctaaaggaaacattatc
cttacaaaaggacaggatgtatttaaagctgactttgcaaggtattatcaaaaaacaggttggcttttccttaaa
ggtcatgtcactgtaaagatggatgaaaatgaaatcaatgctgacgaagcagaatttaatttgaatactaaaaca
ggctggcttaataatgggaacatatttatctcttcatcacatgtttacttttctggtgcacgtattaccaaacat
tacggagattattatacttttaataatgtcaaagttactacatgtgacggacctcatccagcatggtcaatatca
gctaaagaggcaatagtagaagttgatggatatgcacaattatatgattctacctttaaaattaaaaacatagat
gtaatgtatagccctatttttacaatacctgcaaaacaaacaagacaatcaggtttttaaatcctaattatgga
atcagtcagcgacgtggaatttattatactcaaccatacttttaaatattgatcaaagcagcgatctaacattt
tatgctggactgatgacaaaaataggtccattaggaactgtaagatatcgttcacacaaatttacaaatcaaaaa
acatggtttgctgctagtggcattcatgataaaaataatattgtcacaccagggaaagatcctgtctatccatca
agccaacttgtacgtaataaccatcaacgttattgggttcgtggaatggctgatggttttattggaaactcaact
tggtgctatatatctaatttagactatgtatcagaccaagattatcttagagaatttgatcaaggtataacaggc
ttttcacactcccgtagtgaaatgtttcaaatgtttggcagagatatccaagaagatgaccaatctcgattaaat
gctttacttattagaaaagattggcagcgtataggggtagtaggaaatattagatatgaacaagatccaacatta
ggacatgggaatcatcctactagtcaaagtgagttaacgcaacgaattccacaaattgacatgtttctttaccaa
ggaaaactatttcaacctctttcattagagggtgccattcatttacagtctgcttatatgtatcgtgctaaaggc
actaaaggttggagaacagaactttatccaaaagttacattaccaatcgatctcaaatatggatctgttataaca
actgttgggctacgtgaaacttactatcaaacaggtataaaatcacacacaagtcctgtagcaccacatgtccct
gatactaaaacaccacgtcaaacaggtcagcatcgttcacttttaacttacaactagaaagtagtacacaagct
caccgaatatggcgactaaaggataaaaaaactattaatcttcattctcaaagtataggaaaaacctttgtaca
gcactcaagcatacaatccaaccacgtatatgctatagctttatacctagagaaggccaagaaaaaaatccattt
tatacactatcagataggatccttccccaaaatgaccttacttattcaatagtaaatattctcacaaaaaagaat
gttactattagtgtagataataataataataataatgataatagtgttacaccaacacttattacttcctactat
gatcttctctactggaacttatcaacaggatatgattttgaagaagaacgccgaaaacaatatgtagaaaaatat
ccaaagcgccctataaaagacatctattctgaactagaactttatatactatcttggttaacttattcaggtaag
acatttatttctccatataacggtaatattactagacatgatcataatatcatattcaaatcagacagattttct
tggaaaacaggccttagctttcgtgatcaatactataattaccgtgagcatcttcaatatcgagatgaaaataat
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ttagaagatttccgaaacctacgagaacttggaacttttggtaaaacaaactctcaactagttgaagttacatat
ttagcacaatgttatcgtattattggtagataccgatatgacggctatgatcgtagctatacagtattaatagaa
atacctggattatttgaataa

SEQ ID NO:28
Name: Law 0691-PAL
Length: 489
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
atggaagtatttagacgttatggcatagttctggttctgttagtagtattaagtgcaggttttggttgttgtaaa
aagagtgttgatgtagaacaatctttagcaacggagtgtattgctccagcaccagcaattaatgcagctgcagaa
actataactgatgggattatttatttgattttgataaatatgatattaaacctgaatatcgtgatatgttgcag
aagaaagctgaacttttaaaagaatatccttgtattcgtgtccgtatagaaggtaattgtgacgctcgtggtact
caagagtataatttagcacttggagagcgtcgtgcacgtgcagcatatgaatatttagtcatgcttggagtaaat
ccatctcagcttgagataataagttttgggaaagagcgtccagctgttgaaggaacagggccagctgtatgggca
aaaaatcgtcgtgatgattttcgtattattgccaagtaa

SEQ ID NO:29
Name: Law 0995-OPRM
Length: 1380
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*

atgaaaagattgttactctgtattataacatgtgttattgtatcaagttgctcttttgctccagattacaatcga
ccacatttagagttacctgaggtatgggttagttcaccagagacaggagttcctgcttctatgcagtggtggaaa
cgctttaatgattctacacttgatattttagtagcagaagctttgcagcataatagagacttaattgcagctgtg
gccagagtagattatgcacaagctcagttaggagttgctcgatcagatttgtttccacatttttcaggaaatgct
caagcaacacctgtatgggtagatcataagagggttacagatggacaatctccatatagcgcaaacttttcagca
agttgggaaattgatatttggggaaagatacggaatgccaaagatgctgcattttctcaattaatggctacagaa
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ttacaatgttctattgcagagaggacagtaaaaacacgtgaagatgcattaagtatctatactgcacaatatcaa
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aaacgtattgcacaagagaatgctgaaacagctttatctgttttgttaggccgttctccacggttaattatggac
acagcaattgagcgtggggtatctatgaaagatttatcttgtattcctgttattcctcaaggtattccttcagag
cttttagaaagacgtcctgatatacgtcaagctgaatatacgttaaaagctactagtgcaaatattggtgtggca
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aatgtggaagcagcccaggcaaaagaacgtgaagcattagctaactatgaaaaaacagtacaaaatgcttttaaa
gatattcatgatgctcttatacggcaatatgaatcaaagaatatagttaattcacttgaacgaatggtgaaagag
ttacgtatagctgtacatcttgcacgtactctttacgataatggttatacatcttatcttgatgtgttagatgca
gaacgtgctctttttcaaagtgaactagatcttgctagtgcttggagtgatcgcctatcttctattgtacaagtt
tgtttagcattaggaggcagttgggaataa

SEQ ID NO:30
Name: Law C046-SLH
Length: 2391
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*

atgtataaatttattatatttatattatatgtatatatgaaacaatagccatatatttactcttaggtagtttt
atatcctattttaatagagtctttcttaaaaaagaagtaaaaatattagatactactaaagtaaaaaagtatgct
agaataggaacatttatttttttgtatgtaataataagttctcaagtatcgttatctcataatgctcgtataggc
tatgaagataaaaatagtgataggaagagtaaactgattttacataacaaattacaagtaaatgagtttgatgag
cttcgttcagatattgtagttacaatgacaccattaaatatatatacttctactcaggaaaataaaattacaact
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aatattttcttggggaataaacaaaaactacttaggtatcgaattgaagtactgaaagcgcgtgtagaaggtaat
cctgatcctcctatacctatacttacaccacaagtgcttgaattaccacttttacctgatccaccaccatcacca
ccaccattgccacagcagacttttcaattcccagattttggagatcctcttccggaaccattgtctcctttagga
gatgatcctccccaaaatgtactaaatcaagagcctcagccaggaccatcttctgagattgtctcaactttacag
ccttcaccttcagtagaggatctatctagttcaggagtgactttagaatgtcaagaagagctttctagcagtgat
gaggagatattagatgatgagtgtttaacctctggcgatgaatcttcaacatcagatggtgagtctcaaaggtca
tcaccgccaacaaaacgtaggaagctaactcatactccaccaccttctgatagaggctctcctccagggagttct
tctatgcttatgccatattacacatatggacaagtcagttctcttcaaggattacaaagtacgttaatgagttta
gaggatcagttagcaacacaattacgactatctattattagatccattaatgttttaggtgtttgttgtaaggac
gacaatcagttacaacctcatacttttcaaagtaaaaaacagactaaaattaaaggaggaataggaagaagtcac
tcaacagataatgaaatacgtccaacttctgtgaataattcattatttttttcgcagcaatggcatgttattgca
tctatggatagtcgtatatctaatttagagactactatttcttcaagacaagcaggggtttttacaacacctatc
gatggactctgtttatctttattgtatagtaataacaaaaagaaaactcaaaatttctatggagttgtactagac
tcagtagatggttctgcaaaagctcagatagaaacagataacattttagcaacagtgacatggaataaagaacat
caaggtttttcaggtcatttagcaggttgttatgggtgggggaaaataacaaatattcgtacaatacatttttt
gataacgaaagtgtttctaaaggtatttcaagtatacatatgagtggtggatttattcagctaggatataacgtt
ttactaggaaaaaattattttcttattccatatgttgaatatatgagattagcagtagcatgggatccatatgaa
gaacatacaggtcttattccttgtaaagtgagtggacataaagttcatgtttgtgagaaaagtataggtttgcgt

aatcaatggaaaattacagataattcccagctacagttttggggttctcatatttttacaaatcataatacaggt
gaaatagcttctaaaccacttagtttatctgactataggaataaaatatctattcctggttataagaagcaatat
atccatagggaggcagggatttcttatgagtcaaatgtaatggatacattatctatggagctttatagtaagtta
agggtaactaaaagcataaaagatgttactagttatacaagttttacaataagatacgtttattaa

SEQ ID NO:31
Name: Law 00147-SlyB
Length: 459
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
atgcagcgttgtggtctttatatcatttgtttagtgttatctataggtatgataagttgtgcaaattttagtgct
tcttcttttggagggaagcaaattcgtagtgcacatattgttgaatttggaaaagtcgtttctgtaaaacctgtt
gaactagaaggaaatacaccaattctaggtactattactggtggagctgttggtggtgttcttggtagtttaatc
ggtggaggatcaggaagaatattatcaactgttgttggtgctggagcaggggctgttgctggaaacattgctgaa
agaaaaattacaacgcaacaaggtctcgaaatagaagtgaaacttgataatggtcaaattatttctatagttcaa
ggtgctgatcaatcttttagtcctggcgaacgtgttcgagtactacgtggaagtgatggttcggctcgagtatct
tcaatatag

SEQ ID NO:32
Name: Law 0050-AsmA
Length: 3228
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
atgagatcttttctcattagtatagttactctctttctattaggaattatagtatttttggtattacattctac
tttatcaaacaacatccacaatatattacaaataaaatactttctactatttcaaaacaattaaaggatacttct
atttctgctaattctattgggtttcatattgtacctttcctaagctttacttaacaaatgttatacttcaaaca
caaaaaggtgatacaattcatattaaagaatgcttgattactccaaaaatcaccaacatattatcaggaaacatt
tctatatattctattgaggtaatccaaccaatagcctccattattcttcaaaatgaacaaaaaaaaaatagtaaa
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aatggtagtattacctttcaaaataatgactattgttttaagataataggtattaatggtaaaattggtgtttct
aagacacttacaagctcattaaagctcactgcagatgaaataatctgggaaataatgaatactgtaagtaataat
tctagcactgcacaaaaaagtattgaaaaagtacagctccacatagaggatatgccgtataaaataaatacagca
ttacttcatgatacttctcctttatacgatttatttacaaatactaaaaaaactacatttaaggtttcaggggct
atacctacaaccaatacagctaataacattacattcgacttcacgacaaaactagaaaaagataactctgataaa
cttacaatgcatgggcagttacatattgaaggaacactccctaatggtaatacatccattcctatactattatct
gttccgtttacaacaacatcttctgaggacatgacacactttcctccactgctgataaaaaactcaaaactactt
tttgataaaactcatatagatcttcatggaacaataaaaaattatgatacactatcaaatctctttttgatgga
acaatggatgtaaaaaattttagttttccctattggtttacttttgcaaggcaactaccaaatggtattcaacat
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cattctttagatactacatttcaagggaatggaactgtcaataatttttatccccaacgattacattatccctt
gccacaaaacagttcaaccttaatacacttcttccagaactaaagggtaaaaaatcttcacaactatcctatcct
aaagaaacgtttttaactatcttaagtaatctccataataataataataataatatataaaaaaaacaatcaac
tatgacattactattcaggctgatcatgtaacatgttggaaatttgatggatatcagtttatatgtaacatacaa
cctaaacctcagggtactcagattcatactaactgtaaaaatttttatgatggcagtttatcttcctcactttta
ctatctaataggcacacaatacaattagctatagaaaatattcaactgtctgatataacaaatattattacaaaa
gaatatgaactaaaaggtaaagcttctggcacaagtcgtgttaatggacatggtgatactttagcatcatttctt
tcaagtctaaaaggtacaattgatttatatgtaactgatggattagtaaaaaaaacagcgtctgaagctattcct
ttttctatgcttcatctaacatgtgacagtattggtcaaccatcaaagggaaataaatcctctacaattccatat
aaagggaagtggagtgcagagatatcatcagcaagatggaatggctcaattacaatggatgggttaatacaattt
tcaaccactgactggctttctattaaagcagaaaacataccatcaaaagttgtatgttctgtatctggcgtacaa
gctgtggcttatggaggaatatcttttgacatagataatagttttcttagcttctctaattttcaaggtgaaata
catcctaaaacagccctatctggaacaatcaaaactagttctagtacaagtaacacaagacaatgggagggttct
ctcactgtcatgacacaaaatcttagaaatttattaagtaaacttggttatgaaccaaaaaatatttctccaact
atgttgcaatattgtaaattgcaaggagatatctttatttctcctgcaacaattcgccttacaaatatacaggga
atattagataatacactgattaaatttttctctcaatggattacagacaaaccctccaagttggacaggagatatc
caactaagttcattaaacttagataaatatttactatcaattaaccaaaataaactgaaaaaatcacaggaactc
tggccgatagaattgctgaataaagtaaatattcaatctacactgactctagctgagcttatctatagaaaagtt
ccatatactaatgtagtagtacccattagtcttaatcaaggaacattatctatcactccaattacagcatcacta
tgtgatggaaaaacagaagcaagctttaaggcttgccctctttcaactaatggcaatagtgcacagatagatttt
cattatatttcaaaaggtgtggatatgattaaactaagtaaaaaacgtcaacaagaatatcttatttcaggcctt
ggtacatttgttattaatatacagtcaatagcaaaatcatctatagatttccttaaaaatcttcaagggaaatgg

cgtatttttatccagaatggctatttcaaaagaaacaccactactactcaacaaaattttagtaacattggtgca
acaggaaatattataaatggtattattacaaataataattttgcaataacagggcctggaatggtaataacaggg
agtggaaaaattgatcttcctgaatggaatcttgactacctcatcactatagatatggaaggatttcctatagct
atccccataaaatatacaggaagtatagataacccaaaaagaacaattaatgcggccaagttaattcttagtact
ataggttctcttgggagagatactataggattaatacaagatatttttcagctccactgaagttacttcttccc
taa

SEQ ID NO:33
Name: Law 0065-LoIA
Length: 750
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
ttgaagaatattataaatactataatttttcctaagcaaaggaacaaaaaaatgtcacaaaaattttctatgcta
caactaatattatttttttttaacttttatatttttattcatatataacagatagttatgcagagtctataccaata
gtaaaagaattacagcaatcatatcaatccattaaaaacttttctgcaacatttactcaagaacttactcatcaa
gaaagtgggtccaaagaaacacgtattgggaaactctttttttaaaaaaccacttcttgtcagatgggaaacaaac
acacctcatgaagaacttcttcttattaataccaatgctgtatgggattatcttcctgatgaaaaccttgtttat
aagtactctacagatattgtaaaagattccacatctattattcaggttattactggacaagtacgacttgataaa
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ttttatggaaatacaaatactattacttttattaatattacacccaatactcatattgataataacatatttcaa
tttactccacctaaagatgttactatagaaaatttacaagattcatctacaccagaacgaccactatttaattag

SEQ ID NO:34
Name: Law 0649-OmpB
Length: 2466
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
atggcatacctatctatttcaaaaaatcaatgtaagtctttttttaataactctagtaactatatttataatgaca
tcaataccacaactagctgaggctgttgaacactttgctaatggtgtccccacagtagtacaagatgttaatgtc
ccagctgactcatactttggtggtgctgactcagctgtaggtccaaacccaatagcttctactcaccttacaatt
tctacaactcaagggtttggacaaaatgcactagaatttgttgtgggtggtagccttgcaaatggtaatggaaat
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atggcaaatgctgcccctgtaactattggtggctctatttttatgactcttagaaatgttactgcagtagatcca
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attaatataaatctagagaatgtaacaactccagagttttatggtctaggctacgcaaatggtgtaatacctgtt
aacgtcctcaatagaaatttttttggtagctgttcagggaaatataactacaaatatttctaattctaacattgca
accgttatgttaggttcacactatgatacaactatggctgtaggaggtaatggaacaataaatgttgataattca
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gatggatctggaacagaaatacaagagttaacacttggtaatgtaattcgaggtggccttgtacttacttcagag
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actgtaaatgttagaggaggtacaattggcgtattgacttcaggaggttctgactattcagaattaaacttcatt
cctggagaaatatccactatattagcaacaaattcaataggtaaccaagattttgcatctctttctcaagttaca
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ggtggtgaactatttattcctgctgatggaactggaggagtagccctaatcacaaatcatattattgcaaattca
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gtagcacagttaacaatatatggaccacttacagttaaccttagccattctccagaaattcttgggaaaattatt
acacaacctatccctattgcagttactaatagtgatgtttttggtacttctaaactatttgtggaacataacaca
aaaggactaatttggagtgatatcatttttaatcctcaagataaaacatggtatctaactaactttagaggttct
gaagacttctacggactttcagcagcacgggaagcatctaattggttaagacaacaacatatctggagcctacaa
cgtcgctctaataaattattagatcatggtgtagatgggttatggatgaatgttcaaggtggttatgaaaagctt
gatgcagcaattggtgatgctaagatgccttggattatggcaagcttaggatatgattttatgcataagttaagt
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aactcaactactaatgatatctacatggggctggttggtgcctatgttggccttatgcatgaagccacaggcctt
tatggtacagtatccggacagtttgcaactaaccgtacaaaaacaaaatgtacaggctttgatgaaacctataac
gaaatggagcattgatgagtttaaaataaacccacgtggacaagttatttttgaacaattatctaaacatcactt
tagtcaaccgttataggtatctctggagaatatgatttagatttaagaagtaaaataataaagcttcaagctagt
gttgactggattaaaggcatctctggtgactttgcagctaaatccgaagttcttaatatgaagtttaaagataaa
aatgatactagtacatttagaggaacactgggtgctagtgcacaacttctagaaaactttgaagttcaccttgat
atttttggtgatcttggcaatgataaaggtattggtggacaggtaggagctacttatagattctaa

SEQ ID NO:35
Name: Law 0043-PtfH
Length: 2703
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*

atgactaaagttggtggtagtaatccttttcaacacttgcatcaatggttggctttagcaaatcttcttccagc
tcatcttcaagtaaggtagctgaattaaaaagtaaatttgaagcacttgggacactaacaaaagcaagtcctcgt
cctgcaaatggcactcaggatactaatcgattaagtgatagactatttagaggacactatacagttcctgctaat
acagcttcaagagtaggtggtcatcttcagcgggttgatgacaataaaagtagcagttcagcagctaatgttgca
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ggaacaggcggaagtataacagatcaagttaaaagtaggggaattactgcaaggaatttttcggattctaataat
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tcatctatttcttctggtaatgcatcaggtactagcaatgttaatgatggaacaagttcaagagattatggagct
aatgtccgtaatactcgtgaaagatttgaagctggtcctggaggtgctcaaggagagtctcctggagtaaaagat
gctaaagaagctactaaaggagttagtgtttctaatttaagaggaatatttgaaggaagaggtggtgcagataat
aatcaagtaggtagaagctctggtggagtttcaggagcatcaggatcaggaggagctcaagggacaggaggaaca
tcgggattaggtggagcacaaggtacattaggaagtggaggtcttgatggaggaggagtttcaggtgctgctggt
ggaacttcaggcgcctcaggagccggaggagcaagaggtactgctgatggaataacgtctcctggagcaagagaa
gcacaagaaactattaaaaatgctggtgttagtgtaagagatttagctggaagatttagtggagctcaaggtaca
agtggagcttcaggagcagcgggagcttcaggagcctcaggagccggaggagcagctggtgcggcaggaggtgga
ggtcttgatagaggaggagtttcaggttctgctggtggaacttcaggcacctcaggagccggaggagcaagaggt
actgctgatggaataacatctcctggagcaagagaagcacaagaaactattaaaaatgctggtgttagtgtaaga
gatttagctggaagatttagtggagctcaaggtacaagtggagcttcaggagcttcaggagcagctggtgtggca
ggaatgccagcaggtagtggagatgttgttgatggactaagaagaggaggagaagatactgtcgatggatttgga
agaaatcagggtagtggtcctatccctagttctgcagattttgtagatggtcctataggcggtatacaaggagct
ggaggagcttcaggagcagcgggagcagctggtgcttcaggagcagcgggagcttcaggagcagcagagccactt
cctactaatggtacagatcagcagatagctgaagtcgttgtaagaaatgcagaaaatggtcattttgatggtatt
gatttctcgacacagcctggtggtgtagaatcaaatacaggtagtatccctggaacagatcttattgtccaacga
gacatcactcctggagaacaaggagtgtataataatctcagtgagatgggatcatggatggatagccctaatgct
agtcctacaaatgcaccagagtctcttacagatgatcatagtgttttgactacactacttaataataaagaaggc
ctacaagatactgttccttttcctcttgctgtgggtgaaggcactgttgttactaaaactcttgatccagacatt
gatacaagtaagattaaaatcccacttgaaattgtttttggttctggaagtatgtatggttctggtattggagga
ggtagtagtacaattagctcctcaatgtctgatgatggaagttctagcataggatcaactacacgtaaaaatagа
gcaggtgaaataataagtagaattgcaatgaatcaatcacctggcgctggtagcgggggtcaaactgtagttggt
ggtcttggttctggtagtagtaatatcaatataagtggtggacgtggtggtattgtttatggaccaatgcctaat
gtgaatgtagtagctggagatggccttaatagactacagcttggtagtggaataaatccattagcacttcttcag
gaaagaatggttaatttttctgctgctcaactagagcatttacatggccaacttacaggtatgatggctatgatg
gaagctatgcctggcgtagcatttgaaggtgcaagtgtccaaatgacattacctgaaccaggtgatacacagact
atgcctagtataaggctatcaggttttggagagccaaggttgcgggaaaatataggacagccagggcaacctcca
acacaagaagcatttgatgcattaaggaatggtccactaaatggagtaagcgagttaatgagccaagttcaagaa
atgataactgtaaggagtgaaggaagtatttcactaagcagaagtagtagtttatcagatttaagttcagaaata
taa

SEQ ID NO:36
Name: Law 0460-PEBP
Length: 552
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*

ATGAAAAAACTGATTCTAACTTTTGCTTTATTATTAGTAACAAATATAACTACTTTTGCTTCTGAGCCTTTTACT
CTATCAAGCCCACAAATGAAAAATGGAACTATTGCAACTAATCAAGTTTATAATAGTTTTGGATGCAAAGGAAAA
AATATTTCTCCAAGTCTAGAGTGGAAAAATCCTCCTGAAGGAACTAAAAGCTTTGCTATAACTATGTTTGATATA
GATGCACCAACAGGTAGTGGATGGTGGCATTGGATAGTTTATAACATTCCTACATCCACTTCCTCATTAGTACTA
GGAGCAGGAAATGATCCTAAAAAACTTCCCAAAGGTGCAGTTCAATCAATAAATGACTTTGGTTTTATTGGATTT
GGAGGACCTTGTCCTCCAGTTGGTGCAAAACTTCATCACTATATTTTTACTATCTATGCTCTCAATGTAGAGACA
ATGGATTTACCAGCAACAACTATGCCTGCAGCTATTGGATTCAATATTCATATGCATATGATTGACAAAGCTACT
TTTACTGCTACTTATTCTCGTAAGTAA

SEQ ID NO:37

Name: Law 1082-FeoA
Length: 339
Type: *Lawsonia* Nucleic Acid Sequence
Organism: *Lawsonia intracellularis*
ATGTCTGCAGTTGTTGATAGTATGACACCATTTCCTTGTTCAGAACATGATGAGCTTCCAGTTATAAATGAAGCA
ACAGCATGCTGTGTCTTTGATAAGGTAGAGAGTTATATTTCATTGCGTGATTTAAAAGTAGGACAACATGCTCGT
GTTGTACGTGTGCAAGCAGATGGAGAGCTAGGAAGACGTATCCGTGATATGGGTCTTGTCCCAGGGACAGAGGTA
ACTATTGTTGGGAGGGCACCTCTTAAAGATCCAGTTGCATTAAGGTTACTAGGTTTTACTCTTAGTCTTAGAAAT
AGTGAAGCAGACTATGTGATGGTATCACCTATTTCATAA

SEQ ID NO:38
Name: Law 1153-OmpA
Length: 1197
Type: *Lawsonia* Nucleic Acid Sequence
GAGATAGTTATGGCTAATGTTAGTGGAATCCCTGCACCACGATTACTTTCCACAACAAATCAAATGACCAATGCA
GCTGCTGGTAATACTAATAGAGCTACCGGTAGTATGAACGGTCGTAATCTCACACAAATAAAAACACCTCAGTCC
ATGATTGATAATGCTTCAGAAGAATTAACAACTTCTCTTGAATCTAAAAGCAGTGACGACTTTGCAATTAAAGAT
CGTAAAAGACAAGGGAAAGGATCTGATTCTCTATTAAAAATGGTTCAAGAATATACAGAGCTGACGAATGATGAT
ACCCGTAATGCTAAAAGAGCTATGTTATCCCAGGTATTACGTGCAAGTCAAAGTTCACAAGATGTACTCGAAAAA
ACATTAGAACAATTTTCTAATAAAACAGATGCTTGGGCTTCTCTTGCAGAAATTGCACAAGAATATGGTGCAGAA
TCTCCACAGCCAACAGGATTAAAATCTGTATTAGATGCTATGGAGACATTAGAAAATGAGTTTGGTGATGAAATT
AAAGCAGGACTAAAAGGAGCTCTAAATTCAAAAGAATTTACTGATATAGGCAGTGCAGCACAGTTAAGAGATCTT
TATACAACAACAGTAACTATAACAGCTGCACCTGATGCAGTGTTAGCAAGACTTCTTGAAGAATATGAGAGTGAT
GATGATCTGGATAGAGCCATTGATTTCCTTCTATCTACACTTGGTGGAGAGCTTGAATCAGCTGATCCAAGTATG
GATAAAGTACATCTTCAAAGTGTAATGGGTGATATTGAAAAAACACAACAACTTCATAGCTCTCATAAACAATGT
ACTACAGCCCTTAGCAGGTGGAAAGAGAAACATAAAGGTGGGGGGGAAAATAGTACACTAACTCCTTTAGAAATG
ATGCGTGAACTAATTGCACTAAAAAATGAAAATTTTATTTCTCCTTCCTCTATAGATAAAATTGTTGATCAAGCT
GATCCCCAAGATATTGAAAAAGAAGTCCTTTTTTTACAAGAGATGTTAGCTGCTGTAAGAAAATTTCCCATTATG
GTATTTGATAATGTCGAAAATCGTGTAAGAGTTATGGGTGCTGTACAAGATGCTGTTGACGATGCTGTAAGAAGA
GAAGATGAATTCCTCTTTCAAAAAGAACATCCTGATGTACCACTACAACCAGATGAAAATAATATACAATAA

SEQ ID NO:39
Name: Law B004-Fluf
Length: 2820
Type: *Lawsonia* Nucleic Acid Sequence
atgtataatataattaataagcatcaaatcataaaaatttattatttccttatgtgttttctttttacactt
acagaaaaacaaaaaatttatgctgcagacgtctttttgagggcagaaccgaaaccttaatcaatgtaaacaaa
ccatttgattctttttttggaggttctgactctacaataggaacccttgaaacaggacctactaatcttaccttc
acaacagtaggagccttccgcaattctgttttcagaattattggtggtggtaggtctagttttaacaacccaaat
acagttaaaggcaatgttactctaactgtttataatactgatgtagaaagaataattggtgcaggtatcagcaat
agaggacttgtaaccgttactggctcagtaaatatgaagctagaaaatgtttctgttactagaggaatttatggt
ggtgtctatactcaaaatggacatgtactaggctctatcaacatgcatttgaaaaacgtccaaactccactatta
ataggttctggagtaagcaatggacctaatcgtattactgtaaatggagacataaacattgatgttgaagactct
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cacgacggacttattgcaaaccttaccttaggctcacaaggtaatggtcgtactatgatagctacagcaaatgtt
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gctatgacacaatggggcactaaaaatatgccctttttattggatacaagaaatttaatcttgtccggaactctg
attacctcaaatattcaactagctgatttatctataaccaatctatttgttgctaatggcggtacactagtacct
agaaaattaatacctgggaaccaacctgttatacagtttcttggaggtcctcaatcactcttagttatccatcaa
ccattaaaagtaaatttaagcttatcaccaaaacttattggaagtagcatggtgccacttgcttttgtctctcaa
tctttttcatcaccagatctttttgttaaacaaactagaagtggtctcatttggagtgatcttgagtttgatcca
acaacatctatttggtatgttaataatatccaagcatctcaagattttactctttctctattgctcgtgagact

actaactggctaagacaacaacatatatggactctacaaaaccgttcaagtaaacttttagacaacgaacattat
ggactatggataaatgttcaaggtggacatgaaagtcttgatacttctattggtagcaaagcaaaaatgccatgg
ataatggcaacagcaggatatgactatcttcaacaactaccaaggttagatatgaaagccctttatggtcttgct
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agtggttatgtaggtcttatccataacaaaactgggctctatagtacattgaccttacaacttgcgtctagtaaa
ttacatactaattctacagggttctatagaaattttaaatggacagaaacaactccaacagaagcacttgaactt
ggatggaaatacactttcaacaacggtattaaaatgaatcctcgtggacaacttatttttgaacaaacatctaaa
caccattttgatttaggaattcaaaatgataaggctatattagataaaagccagttaataacaagttctcttggt
attaccgttgaatataagctaccagttaccacacctattaatctttatgctggtattgaaaggataaaaggtcag
tctggaaactttgcaattagttcccagagccttcaaatgaagttcaagcatgacaatgatacaagtgtagttaga
gcaacaataggtacaaatatattattgggagaacattttaatattcactgtgatatatttggagataaaggaaat
gataaaggcattggtgggcaagcaggatttacatacaaattttaa

SEQ ID NO:40
Name: Law 0033-OstA
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTATTATCAACATCTCTGAA-3'

SEQ ID NO:41
Name: Law 0033-OstA
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTATTCAAACAGACCAGGGAT-3'

SEQ ID NO:42
Name: Law 0691-PAL
Vector: pET23a+
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTGGATCCAAAAGCGTAGATGTAGAA-3'

SEQ ID NO:43
Name: Law 0691-PAL
Vector: pET23a+
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTTTGCGATGATGCGGAA-3'

SEQ ID NO:44
Name: Law 0691-PAL
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTGGATCCAAAAGCGTAGATGTAGAA-3'

SEQ ID NO:45
Name: Law 0691-PAL
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTATTTTGCGATGATGCGGAA-3'

SEQ ID NO:46
Name: Law0995-OPRM
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AAACTAGGATCCTTCGCTCCAGATTACAAC-3'

SEQ ID NO:47
Name: Law0995-OPRM
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCAAAAGCTTTTATTCCCAGGAGCCACCCAG-3'

SEQ ID NO:48
Name: Law C046-SLH
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTAAAAAGGAAGTTAAAATC-3'

SEQ ID NO:49
Name: Law C046-SLH
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTAGTACACATAACGGATGGT-3'

SEQ ID NO:50
Name: Law00147-SlyB
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTGGTAAACAGATCCGTAGC-3'

SEQ ID NO:51
Name: Law00147-SlyB
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTCAGATAGAGCTGACGCGTGC-3'

SEQ ID NO:52
Name: Law 0050-AsmA
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTATCACGTTCTACTTCATC-3'

SEQ ID NO:53
Name: Law 0050-AsmA
Vector: pMAL-c2X

Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTTCTAGACGGCAGCAGCAGTTTCAGCGG-3'

SEQ ID NO:54
Name: Law 0065-LoIA
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTGAAAGCATCCCGATTGTA-3'

SEQ ID NO:55
Name: Law 0065-LoIA
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTAATTGAACAGTGGGCGCTC-3'

SEQ ID NO:56
Name: Law 0043-PtfH
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTTCTAGCAGCTCTAAGGTT-3'

SEQ ID NO:57
Name: Law 0043-PtfH
Vector: pMAL-c2X
Length: 34
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTAGATTTCAGAGGACAGGTCAGA-3'

SEQ ID NO:58
Name: Law 0649-OmpB
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTGTAGAACACTTCGCAAAT-3'

SEQ ID NO:59
Name: Law 0649-OmpB
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTCAGAAGCGGTAGGTAGCACC-3'

SEQ ID NO:60
Name: Law 1082-FeoA
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTATGTCTGCCGTTGTTGAT-3'

SEQ ID NO:61
Name: Law 1082-FeoA
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTAAGAAATCGGAGACACCAT-3'

SEQ ID NO:62
Name: LawB004-Fluf
Vector: pMAL-c2X
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTGGATCCAAACAAAAGATCTACGCG-3'

SEQ ID NO:63
Name: LawB004-Fluf
Vector: pMAL-c2X
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTTTAGAATTTGTAGGTGAAACC-3'

SEQ ID NO:64
Name: Law 1153-OmpA
Vector: pET23a+
Length: 33
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAGATCTGGTAACACTAATCGTGCTACT-3'

SEQ ID NO:65
Name: Law 1153-OmpA
Vector: pET23a+
Length: 30
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AACCTTAAGCTTCTGGATGTTGTTTTCATC-3'

SEQ ID NO:66
Name: N-terminal HIS tag adaptor
Vector: pUEX2-M3
Length: 60
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AATTCATCATCATCATCATAGCAGCGGCATCGAAGGCCGCGGCCGCTTAATTAATAG-3'

SEQ ID NO:67
Name: N-terminal HIS tag adaptor
Vector: pUEX2-M3
Length: 60
Type: Synthetic Nucleic Acid Sequence
Organism: Primer
5'-AATTCTATTAATTAAGCGGCCGCGGCCTTCGATGCCGCTGCTATGATGATGATGATGATG-3'

[0088] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be

suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising one or more polypeptides, each polypeptide having an amino acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs:1-11 and an adjuvant.

2. An immunogenic composition of claim 1 further comprising a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs:12 and 13.

3. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:1.

4. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:2.

5. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:3.

6. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:4.

7. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:5.

8. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:6.

9. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:7.

10. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:8.

11. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:9.

12. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:10.

13. An immunogenic composition according to claim 1 wherein the polypeptide has an amino acid sequence at least 90% identical to SEQ ID NO:11.

14. The immunogenic composition of claim 1 wherein the adjuvant is an adjuvant suitable for pharmaceutical use in an animal.

15. The immunogenic composition of claim 14 wherein the adjuvant is aluminum hydroxide.

16. The immunogenic composition of claim 14 wherein the adjuvant is aluminum phosphate.

17. The immunogenic composition of claim 14 wherein the adjuvant comprises oil, water and aluminum hydroxide.

18 The immunogenic composition of claim 14 wherein the adjuvant is ISCOM.

19. An immunogenic composition of claim 1 further comprising one or more antigenic components of *Mycoplasma Hyopneumoniae*, *Erysipelas spp*, *salmonella*, *H. parasuis*, *clostridium spp*, *stretoccous suis*, *brachyspira spp*, *bordetella*, *pasteurella*, *E. coli*, coronavirus, parvovirus, PRRS, Circovirus, or SIV.

20. An immunogenic composition comprising one or more polynucleotides, each polynucleotide having a nucleotide sequence encoding a polypeptide having an amino acid sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs:1-11 and an adjuvant.

21. An immunogenic composition of claim 20 further comprising a polynucleotide having a nucleotide sequence at least 90% identical to a sequence selected from the group consisting of SEQ ID NOs:38 and 39.

22. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:27.

23. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:28.

24. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:29.

25. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:30.

26. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:31.

27. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:32.

28. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:33.

29. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:34.

30. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:35.

31. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:36.

32. An immunogenic composition according to claim 20 wherein the polynucleotide has a nucleotide sequence at least 90% identical to SEQ ID NO:37.

33. An immunogenic composition of claim 20 further comprising a polynucleotide encoding one or more antigenic components of *Mycoplasma Hyopneumoniae*, *Erysipelas spp*, *salmonella*, *H. parasuis*, *clostridium spp*, *stretoccous suis*, *brachyspira spp*, *bordetella*, *pasteurella*, *E. coli*, coronavirus, parvovirus, PRRS, Circovirus, or SIV.

34. A method of inducing a protective immune response against *Lawsonia intracellularis* in an animal, wherein the method comprises administering the composition of claim 1 to said animal.

35. The method of claim 34 wherein the animal is a pig.

36. The method of claim 34, wherein the immunogenic composition is administered by a route selected from the group consisting of scarification, intramuscular injection, subcutaneous injection, intraperitoneal, intranasal administration and oral administration.

37. A method of inducing a protective immune response against *Lawsonia intracellularis* in an animal, wherein the method comprises administering the composition of claim 20 to said animal.

38. The method of claim 37 wherein the animal is a pig.

39. The method of claim 37, wherein the immunogenic composition is administered by a route selected from the group consisting of scarification, intramuscular injection, subcutaneous injection, intraperitoneal, intranasal administration and oral administration.

40. A method for specific detection of antibodies reactive with *Lawsonia* in a biological sample from an animal, the method comprising the steps of:

a) contacting the biological sample with a *Lawsonia* antigen having a sequence as shown in SEQ ID NOs:1-11, or an immunogenic fragment thereof;

b) detecting antigen-antibody binding between the *Lawsonia* antigen and an antibody in the biological sample, in which the detection of antigen-antibody binding indicates the presence of antibodies reactive with *Lawsonia* in the biological sample.

41. The method of claim 40 wherein the biological sample comprises blood.

42. A kit containing one or more *Lawsonia* antigens having a sequence as shown in SEQ ID NOs:1-11 or 27-37.

43. The kit according to claim 42, wherein the kit further comprises an instruction pamphlet for inducing a protective immune response against *Lawsonia* in pigs.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/061429

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/195 C12Q1/68 G01N33/68

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)
C07K

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 practical, search terms used)
EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/033631 A (UNIV MINNESOTA [US]; KAPUR VIVEK [US]; GEBHART CONNIE J [US]) 22 April 2004 (2004-04-22) page 29, line 14 - page 30, line 29 -& DATABASE JPO Proteins [Online] 16 August 2006 (2006-08-16), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002560456 retrieved from EBI accession no. JPOP:BD894600 Database accession no. BD894600 the whole document -/--	1-3, 14-22, 33-43

[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 document 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
26 February 2010	16/03/2010

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	Turri, Matteo

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE EMBL [Online] 17 August 2006 (2006-08-17), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002560660 retrieved from EBI accession no. EMBL:DD307274 Database accession no. DD307274 -& DATABASE JPO Proteins [Online] 16 August 2006 (2006-08-16), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING. 720" XP002570348 retrieved from EBI accession no. JPOP:BD895216 Database accession no. BD895216 -& DATABASE EMBL [Online] 17 August 2006 (2006-08-17), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002570349 retrieved from EBI accession no. EMBL:DD307890 Database accession no. DD307890 -& DATABASE JPO Proteins [Online] 16 August 2006 (2006-08-16), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002570350 retrieved from EBI accession no. JPOP:BD894443 Database accession no. BD894443 -& DATABASE EMBL [Online] 17 August 2006 (2006-08-17), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002570351 retrieved from EBI accession no. EMBL:DD307117 Database accession no. DD307117 -& DATABASE JPO Proteins [Online] 16 August 2006 (2006-08-16), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002570352 retrieved from EBI accession no. JPOP:BD895720 Database accession no. BD895720 -& DATABASE EMBL [Online] 17 August 2006 (2006-08-17), "NUCLEIC ACID AND POLYPEPTIDE SEQUENCES FROM LAWSONIA INTRACELLULARIS AND METHODS OF USING." XP002570353 retrieved from EBI accession no. EMBL:DD308693 Database accession no. DD308693 -/--	

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/061429

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& JP 2006 501846 T 19 January 2006 (2006-01-19)	
X	WO 2006/116763 A (BOEHRINGER INGELHEIM VETMED [US]; VAUGHN ERIC [US]; SCHAEFFER MERRILL) 2 November 2006 (2006-11-02) sequence 208	1,3, 14-18, 22,34-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/061429

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 34-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

1-3,14-22,33-43 (partially)

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:1 and 27

Invention 2: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:2 and 28

Invention 3: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:3 and 29

Invention 4: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:4 and 30

Invention 5: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:5 and 31

Invention 6: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:6 and 32

Invention 7: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:7 and 33

Invention 8: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:8 and 35

Invention 9: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:9 and 34

Invention 10: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

All subject-matter relating to SEQ ID NO:10 and 37

Invention 11: claims 1, 3, 14-18, 20, 22, 34-43 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

All subject-matter relating to SEQ ID NO:11 and 36

Inventions 12-22: claims 1-43 (partially)

As inventions 1-11 further comprising the subject-matter of claims 2, 19, 21 and 33

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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