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(54) **MODIFIED GENE VACCINES AGAINST
AVIAN CORONAVIRUSES AND METHODS
OF USING THE SAME**

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(57) **ABSTRACT**

The present invention provides both QuilA-loaded chitosan (QAC)-encapsulated NA vaccine compositions and viral vaccine compositions that encode an Infectious Bronchitis Virus (IBV) spike (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Additionally, the present invention provides methods in which the disclosed vaccines are administered to a subject to induce an immune response against IBV or to vaccinate the subject against IBV.

20 Claims, 8 Drawing Sheets

Specification includes a Sequence Listing.

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Fig. 1

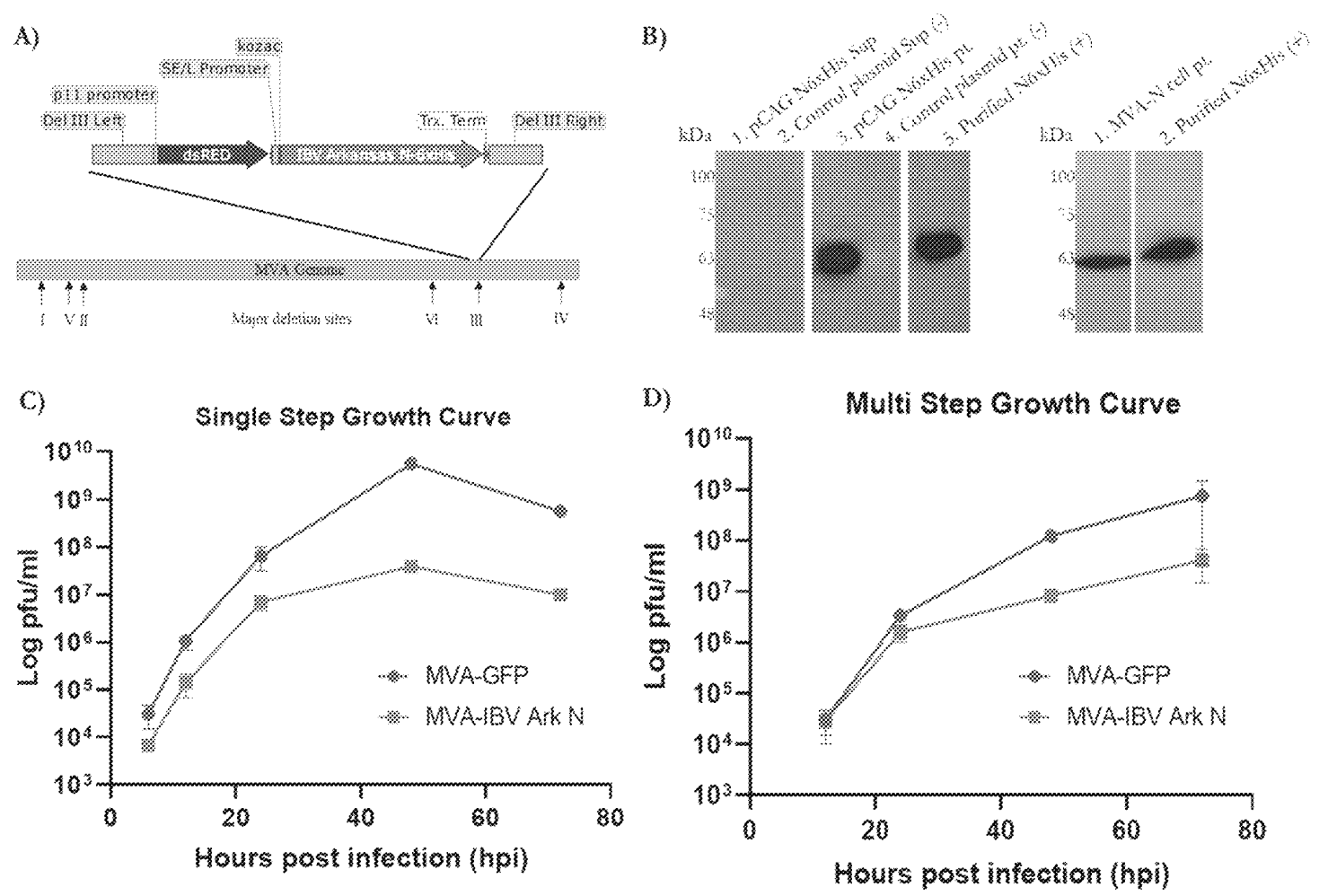


Fig. 2

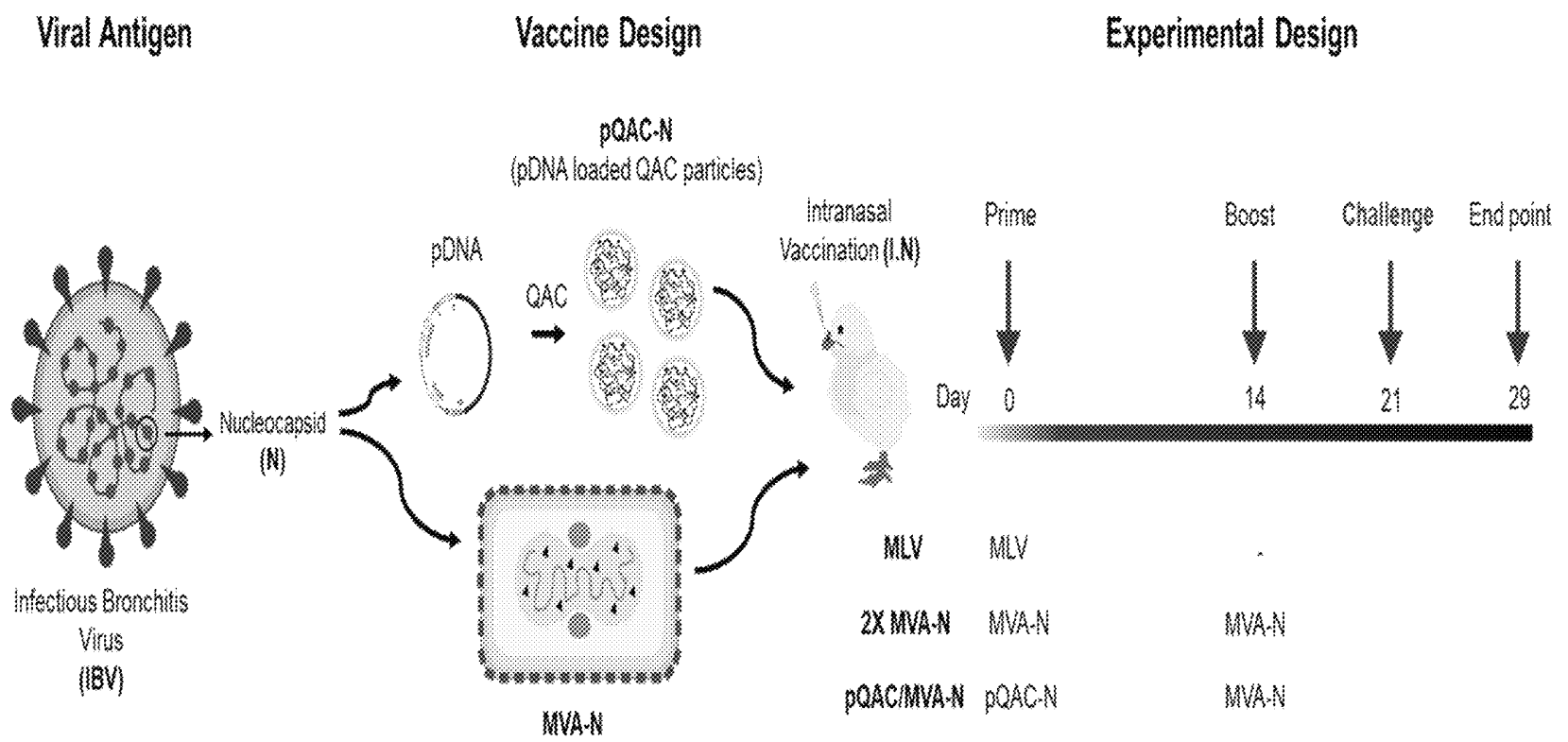
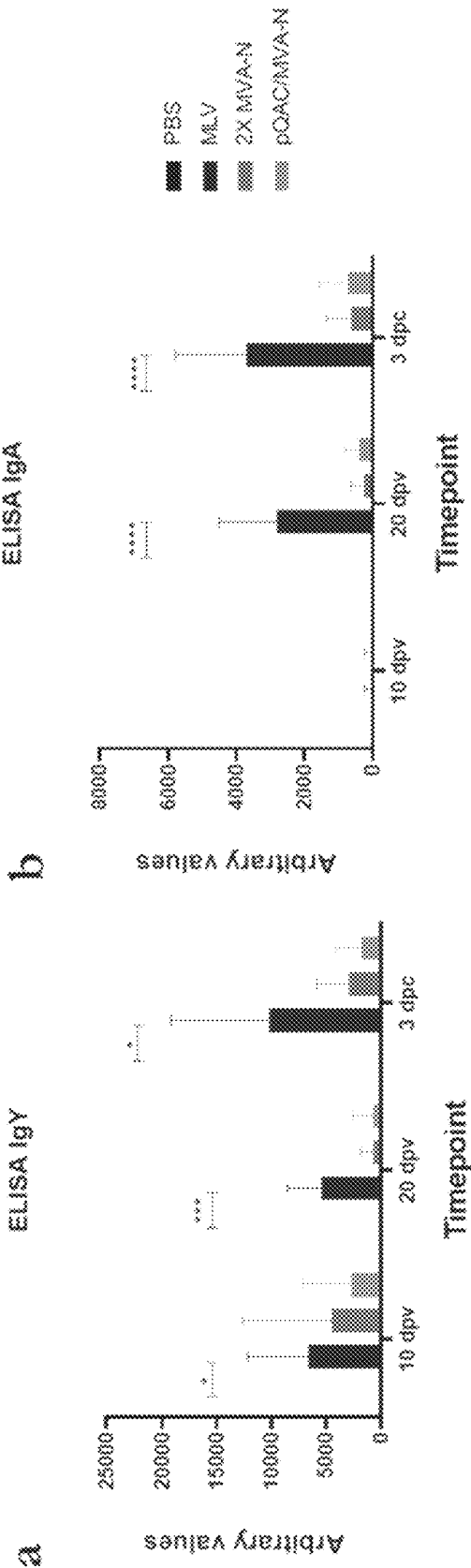


Fig. 3



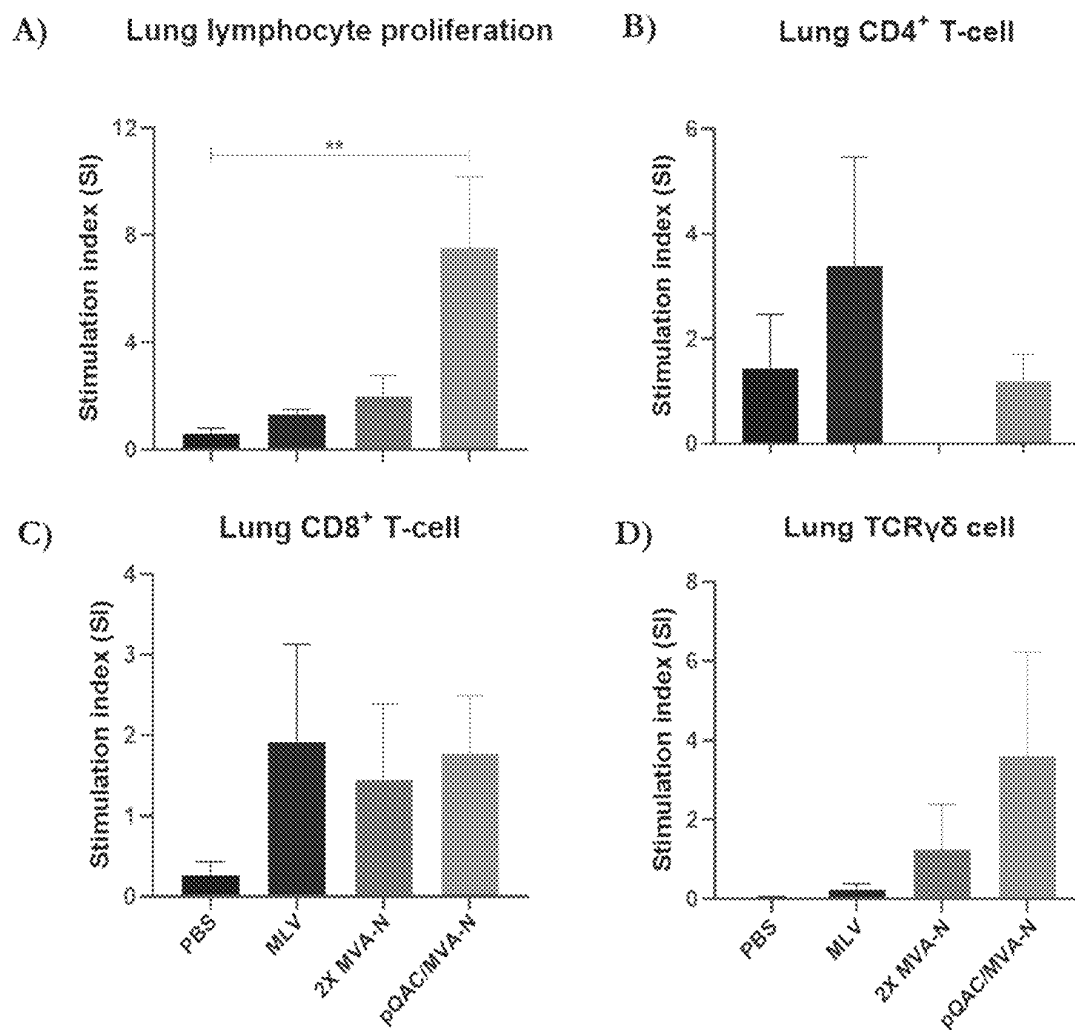


Fig. 4

Fig. 5

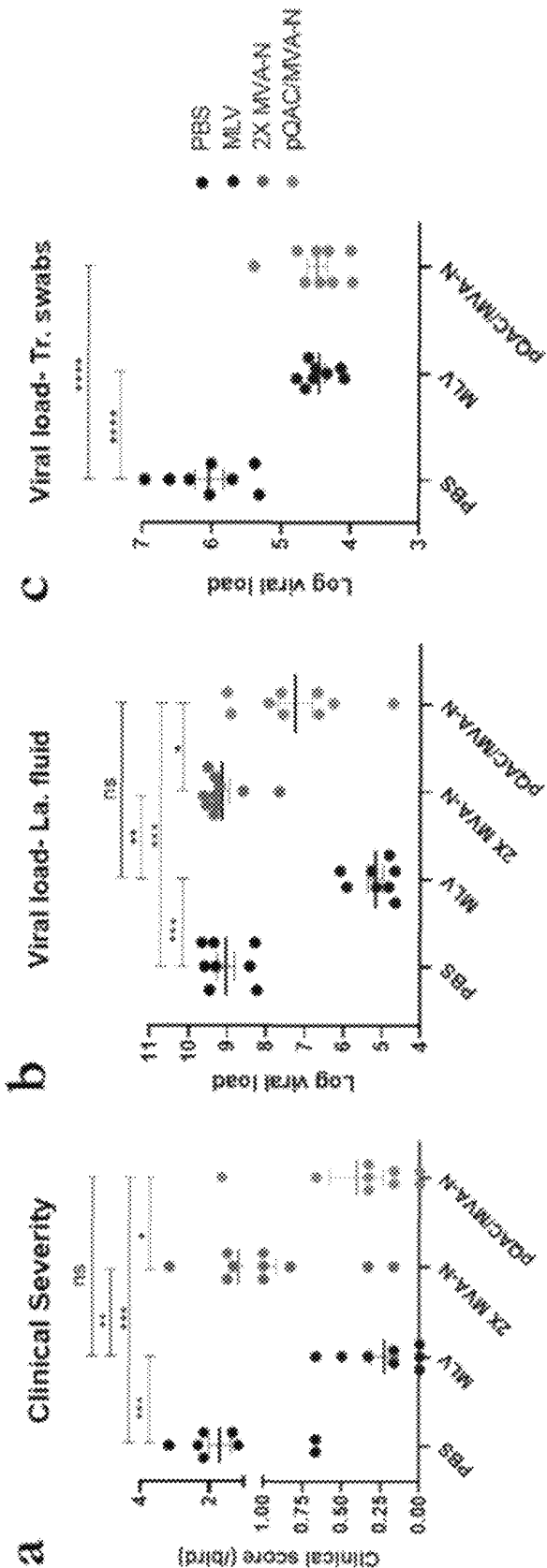


Fig. 6

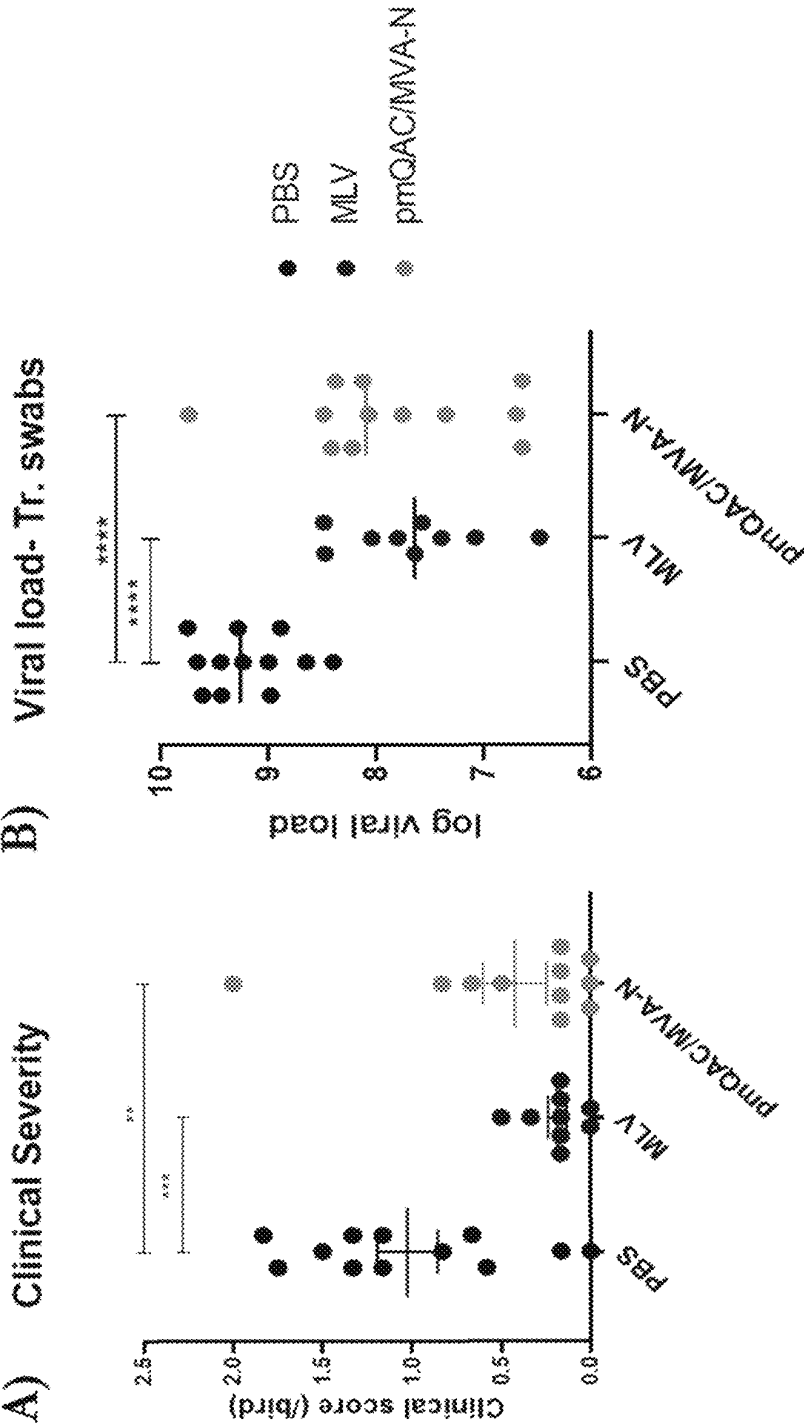
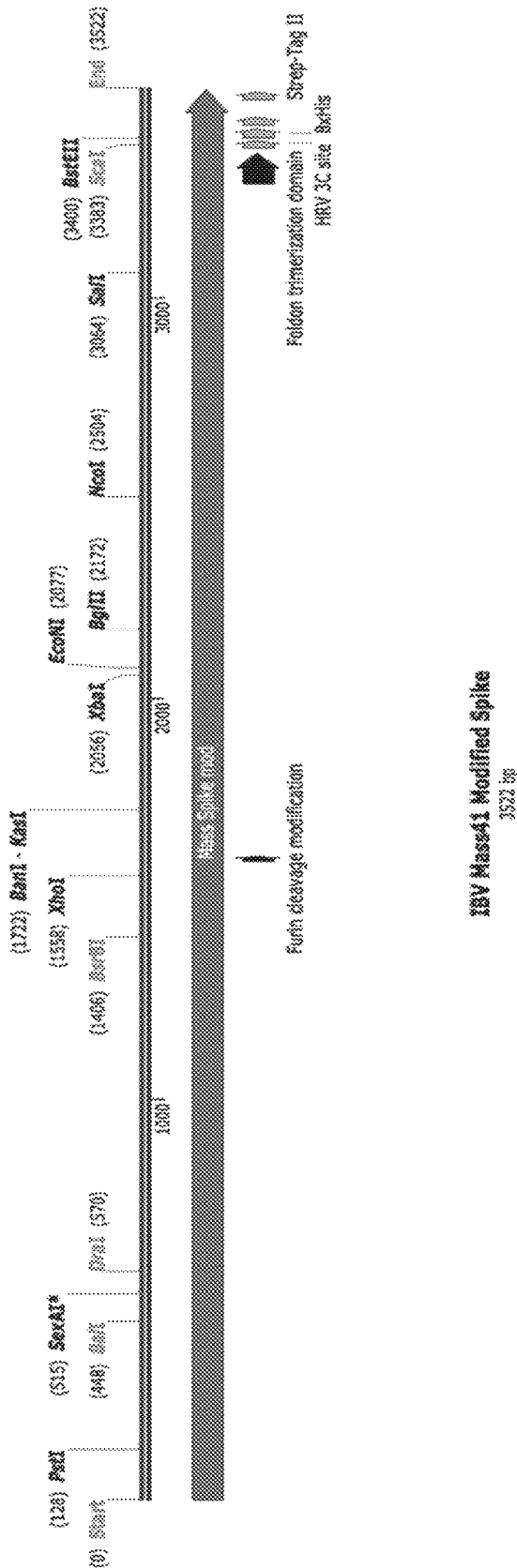


Fig. 7



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MODIFIED GENE VACCINES AGAINST AVIAN CORONAVIRUSES AND METHODS OF USING THE SAME

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under 2016-67021-25042 and 2020-67021-31256 awarded by the USDA/NIFA. The government has certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is being filed electronically via Patent Center and includes an electronically submitted Sequence Listing in .xml format. The .xml file contains a sequence listing entitled "960296_04361" created on Sep. 4, 2024, and is 122,441 bytes in size. The Sequence Listing contained in this .xml file is part of the specification and is hereby incorporated by reference herein in its entirety.

SEQUENCE LISTING

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BACKGROUND

Coronavirus infections, such as infection by infectious bronchitis virus (IBV) in poultry, cause significant health problems for avian subjects as well as economic losses to the poultry industry. A major hurdle to combat these infections is the diversity of viral antigens that can be present in a given outbreak. In addition, a critical failure in preparation for coronavirus infections in avian subjects is the absence of effective vaccines that can be delivered to thousands of animals at the same time. Consequently, there is a dire need for an objective vaccination method that effectively, yet parsimoniously, encompasses existing and emerging isolates of coronavirus, e.g., IBV, to protect against coronavirus infection in avian subjects.

SUMMARY

In a first aspect of the current disclosure, vaccine compositions are provided. In some embodiments, the vaccine compositions comprise a polynucleotide that encodes an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The polynucleotide may be a DNA or RNA and maybe codon optimized for expression in the subject targeted for vaccination. The compositions may further comprise an adjuvant and the adjuvant may include disaggregated spherical nanostructures comprising Quil-A® and chitosan.

In another aspect of the current disclosure, vaccine compositions comprising a viral vector are provided. In some embodiments, the viral vector comprises a polynucleotide

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encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

In still another aspect, a vaccine composition comprising an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The S and N proteins may include one or more of SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, 33, 10, 18, 35, and 37. The vaccine compositions may further comprise an adjuvant such as the Quil-A®-chitosan adjuvant.

In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering the vaccine compositions of current disclosure in an amount effective to induce the immune response against at least one IBV antigen in the subject.

In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering a first vaccine composition comprising a polynucleotide that encodes an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein and a viral vector comprising a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein, wherein administration of the first vaccine composition and the second vaccine composition induces the immune response against at least one IBV antigen in the subject. The first and second vaccine compositions may be administered at separate times with at least two weeks separating the two administrations. In one embodiment the first vaccine composition comprising a polynucleotide is administered prior to the second vaccine composition comprising a viral vector expressing a polypeptide encoded by the polynucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Design and characterization of MVA-IBV vaccine constructs. a) MVA vaccine construct expressing N protein with the addition of C-terminal 6× His tag. Gene map was generated using Snapgene software. b) Western blot analysis with anti 6×His-HRP antibody for pCAG-N plasmid (left) and MVA-N (right) confirming expression of N protein from vaccine constructs. Lanes are as follows: Left, supernatant (lane 2) CEF cells transfected with control plasmid, supernatant (lane 1) CEF cells transfected with pCAG-N plasmid. Cell pellet (lane 4) CEF cells transfected with control plasmid, cell pellet (lane 3) CEF cells transfected with pCAG-N plasmid and control purified N6×His protein (lane 5). Right, cell pellet (lane 1) from CEF cells infected with MVA-TrN and control purified N6×His protein (lane 2). Cell pellet (lane 2) from CEF cells infected with MVA-N. c) Single step and d) Multi step growth curve of parental MVA-GFP and recombinant MVA-N vaccine vectors.

FIG. 2. Vaccine experimental design. Experimental design of IBV immunization and challenge studies. Outline for vaccine construct and immunization protocol using groups of white leghorn SPF birds vaccinated with 2 doses of MVA-N (IN) or pQAC-CoV (I.N) at day -0 followed by boost with MVA-CoV (IN) day -14. Control groups include unvaccinated PBS group and commercial MLV vaccination at day -0 (IN).

FIG. 3. Humoral responses in vaccinated SPF chicks. IBV specific a) IgY in serum and b) IgA in lachrymal fluid,

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significance (*, $P<0.05$; ***, $P<0.001$; ****, $P<0.0001$) was determined by two-way ANOVA. Data show means $\pm$ SEM.

FIG. 4. Localized T-cell immune responses in vaccinated chicks. Lung cell proliferative capacity measured by Cell-Trace Violet dye dilution in unvaccinated, MLV, 2 $\times$ MVA-N and pQAC/MVA-N vaccinated chickens. Proliferation was measured in a) total lung cells, (b) CD4+, (c) CD8+ and (d) TCR $\gamma\delta$ + lung T cells after 4 days in culture post antigen stimulation. Non-significance, ns or significance (*, $P<0.05$; **, $P<0.01$) was determined by one-way ANOVA with multiple comparisons. Data show means $\pm$ SEM.

FIG. 5. Increased protection with heterologous vaccine strategy against IBV. a) Clinical sign severity represented as average score/bird over 8 days post challenge in each group. b) IBV log viral load/10 μ l lachrymal fluid at 6 days post challenge. c) IBV log viral load in tracheal swab at 6 days post challenge. Non-significance, ns or significance (***, $P<0.001$; ****, $P<0.0001$) was determined by one-way ANOVA with multiple comparisons. Data show means $\pm$ SEM.

FIG. 6. Protective efficacy of the MPLA-QAC triple adjuvant system. a) Clinical sign severity represented as average score/bird over 8 days post challenge in each group. b) IBV log viral load in tracheal swab at 6 days post challenge. Significance (***, $P<0.001$; ****, $P<0.0001$) was determined by one-way ANOVA with multiple comparisons. Data show means $\pm$ SEM.

FIG. 7. Shows a map of the Mass41 S antigen with the modified 7 features (codon optimization is not shown with an arrow). All of the other sequences below have the same features.

FIG. 8. Alignment of 7 IBV S protein amino acid sequences. Sequences correspond to, from top to bottom, SEQ ID NOs: 39-45.

DETAILED DESCRIPTION

The present invention provides nucleic acid-based vaccine compositions (DNA vaccines), protein subunit based vaccines and viral vaccine compositions encoding an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Further, the present invention provides methods in which the disclosed vaccines are administered to a subject to induce an immune response directed against IBV.

Compositions:

In a first aspect, the present invention provides vaccine compositions. In some embodiments, the vaccine composition comprises a polynucleotide that encodes an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Alternatively, the compositions may comprise a viral vector encoding an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. As another alternative, protein subunit vaccine compositions comprising an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein are also provided. The nucleic acids encoding the proteins may be RNA or DNA and may be codon optimized for expression in the subject targeted for vaccination. The S and N proteins and nucleic acids encoding the same may be modified to allow for increased inducement of the immune response after administration.

As used herein, the terms “DNA vaccine,” “nucleic acid vaccine,” “NA vaccine” and “plasmid vaccine” are used interchangeably to refer to a polynucleotide encoding at

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least one antigen. Following immunization, a subject's cells take up the polynucleotide and express the encoded antigen from it, inducing an immune response against the antigen. NA vaccines offers several potential advantages over traditional vaccine strategies, including the stimulation of both B- and T-cell responses, improved storage stability, the absence of any infectious agent, and the relative ease of large-scale manufacture. However, NA vaccines also come with several challenges, including in vivo degradation of the construct by DNases or RNases, inefficient uptake by antigen presenting cells, and low immunogenicity. See, for example, P. Cai, X. Zhang, M. Wang, Y. L. Wu, X. Chen, Combinatorial Nano-Bio Interfaces. ACS Nano 12, 5078-5084 (2018); and D. H. a. M. Bros, DNA Vaccines—How Far From Clinical Use? Int J Mol Sci. 19, (2018), both of which are incorporated by reference herein. Nucleic acid-based vaccines generally contain additional elements in addition to the polynucleotide encoding the antigen such as a promoter functional in cells of the subject to be immunized or may be altered to offer increased stability or resistance to degradation in the host cell.

As used herein, “antigen” refers to a substance that induces a targeted immune response in a subject. For example, in some embodiments, the compositions disclosed herein comprise one or more polynucleotides that encode an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. Therefore, in the foregoing example, the antigens are the IBV S and N proteins that are encoded by the one or more polynucleotides. In some embodiments, the S proteins are encoded by one or more of the group consisting of SEQ ID NOs: 1-7, 22, 24, 26, 28, 30, 32, and 34. The S proteins encoded by these polynucleotides are provided as SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33, and any polynucleotide encoding SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31, and 33, is included, as the coding sequence for the proteins may be optimized for expression in particular cell types. In some embodiments, the N proteins are encoded by one or more of SEQ ID NOs: 8, 9, 36 and 38. The N proteins encoded by these polynucleotides are provided as SEQ ID NOs: 10, 18, 35, and 37, respectively, and any polynucleotide encoding SEQ ID NO: 10, 18, 35, or 37 is also encompassed herein. The polynucleotides provided herein may be altered to optimize codon usage for maximal expression in a particular host such as a poultry. Thus, the sequences provided herein also include sequences with 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequences of SEQ ID NO: 1-9, 22, 24, 26, 28, 30, 32, 34, 36, and 38. The proteins encoded by the polynucleotides may also encompass changes especially as these proteins are known to exist in various isoforms and be antigenically diverse in outbreaks of IBV. The sequences provided herein also include sequences with 70%, 75%, 80%, 85%, 90%, 92%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequences of SEQ ID NO: 10-18, 21, 23, 25, 27, 29, 31, 33, 35 or 37. In some embodiments, the polynucleotide encodes both the S and N proteins on a single molecule. As such, in some embodiments, the polynucleotide comprises sequences linking the S and N proteins. The N and S sequences may be linked via a polynucleotide of any length but should be in frame or contain independent regulatory regions such as an internal ribosome entry site to allow for expression of both proteins from the polynucleotide.

As used herein, a “fragment” is a portion of an amino acid sequence which is identical in sequence to, but shorter in length than a reference sequence. A fragment may comprise

up to the entire length of the reference sequence, minus at least one amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous amino acid residues of a reference polypeptide, respectively. In some embodiments, a fragment may comprise at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, or 500 contiguous amino acid residues of a reference polypeptide. Fragments may be preferentially selected from certain regions of a molecule. A fragment may include an N-terminal truncation, a C-terminal truncation, or both N-terminal and C-terminal truncations relative to the full-length reference polypeptide.

The term “recombinant” when used with reference, e.g., to a cell, or nucleic acid, protein, expression cassette, or vector, indicates that the cell, nucleic acid, protein, expression cassette, or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, have higher than normal expression, are under-expressed, or not expressed at all.

The polynucleotide vaccine compositions provided herein may be DNA or RNA and may include regulatory regions to allow for transcription and/or translation of the polynucleotides into polypeptides once in a cell of a vaccinated subject. The polynucleotides may be operably linked to promoters that are capable of recruiting transcriptional machinery in target cells of the vaccinated subject, e.g., cells of the upper respiratory tract, or, in some embodiments, any somatic cell of the subject.

However, as discussed above, NA vaccines can suffer from several drawbacks including in vivo degradation of the construct by DNases or RNases, inefficient uptake by antigen presenting cells, and low immunogenicity. In some embodiments, the vaccine composition further comprises an adjuvant. In some embodiments, the adjuvant comprises disaggregated spherical nanostructures comprising Quil-AR and chitosan, which are present at a ratio between 1:15 and 1:100. As used herein, the term “adjuvant” or “vaccine adjuvant” refers to any substance that enhances the immune response to an antigen. The inventors envision that the use of articulate delivery systems, such as QuilA-loaded Chitosan (QAC) nanoparticles used with the present invention, may overcome these challenges by facilitating a prolonged release of active plasmid. See, for example, S. S. Chandrasekar, B. A. Kingstad-Bakke, C. W. Wu, M. Suresh, A. M. Talaat, A Novel Mucosal Adjuvant System for the Immunization Against Avian Coronavirus Causing Infectious Bronchitis. *J Virol*, (2020), which is incorporated by reference herein. An exemplary adjuvant used with the vaccine compositions disclosed herein is a Quil-AR chitosan (QAC) complex, in which Quil-AR and chitosan are combined to form distinct disaggregated spherical nanostructures. The QAC complexes are loaded with one or more payload molecules (in this case, the antigen-encoding polynucleotide) with which the QAC complex stimulates an immune response. The QAC complex adjuvant was previously described in International Application No. PCT/US2020/037438, which is incorporated by reference, and Chandrasekar et al. 2020, supra. Advantageously, QAC-adjuvanted vaccines appear to target local mucosal immunity, which results in a more effective immune response to IBV given that airway epithelium T cells and IgA humoral responses have been shown to be critical for restricting respiratory viral pathogens. See, for example, N. v. D.

Emmie de Wit, Darryl Falzarano and Vincent J. Munster, SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology* 14, (2016), which is incorporated by reference herein.

“Quil-AR” refers to the powdered saponin fraction isolated from extract of the bark of *Quillaja saponaria* trees. Quil-AR is commercially available, for example from Desert King sold under the product name Vet-Sap™.

“Chitosan” refers to a linear polysaccharide composed of randomly distributed β -linked D-glucosamine and N-acetyl-D-glucosamine. Chitosan can be obtained from the chitin shells of shrimp and other crustaceans by treatment of the shells with an alkaline substance. Chitosan is a non-toxic, naturally occurring cationic polymer that readily complexes with DNA and negatively charged proteins that is biocompatible and biodegradable. Compositions incorporating chitosan have sustained release kinetics and are immunomodulatory, enhancing the T-cell response. In some embodiments, chitosan is deacetylated chitosan, for example deacetylated chitosan (>75%). Deacetylated chitosan is available commercially from Sigma (C3646). Higher deacetylation percentages, for example about 90%, will mediate stronger binding with nucleic acids resulting in slower release kinetics from the nanoparticle structures of the QAC complex. In some embodiments, the chitosan is at least 70%, 75%, 80%, 85%, 90%, or 95% deacetylated. In some embodiments, the chitosan is between about 60% and about 90% deacetylated.

In some embodiments, the chitosan is functionalized. Chitosan may be functionalized with negatively charged sulfonate groups by reaction of the amino group of chitosan with 5-formyl-2-furan sulfonic acid (FFSA) followed by treatment using sodium borohydride to form a negatively charged chitosan surface. Use of the negatively charged chitosan in the formation of the QAC complex will generally be favorable for loading of positively charged payload molecules.

The QAC complex is loaded with the antigen-encoding polynucleotide by mixing a solution of Quil-A® and polynucleotide into a solution of chitosan to form a final mixed solution containing a QAC-polynucleotide complex. In the final mixed solution, the Quil-AR and the chitosan are present at a ratio of between 1:15 to 1:100. In some embodiments, the Quil-A® and the chitosan are present at a ratio of about 1:20 (e.g., 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, or 1:25) in the final mixed solution. In some embodiments, in the final solution Quil-A® is at a concentration of 0.001% and chitosan is at a concentration between about 0.02% and about 0.1%.

In some embodiments, the QAC complex nanostructures are less 100 nm in diameter when measured in the absence of any payload molecules. For example, the nanostructures may be between about 5 nm and about 100 nm, between about 10 nm and about 95 nm, between about 15 nm and about 90 nm, between about 20 nm and about 90 nm, or between about 25 nm and about 85 nm in the absence of a payload molecule. The QAC complex may be loaded with one or more payload molecules such as the polynucleotides described herein encoding an IBV spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The nucleotide-QAC complex may be between about 20 nm and about 1000 nm in diameter. The specific size of the nucleotide-QAC complex will vary depending on the size and amount of payload in the nanostructure. As used herein, “disaggregated,” refers to the formation of discrete observable particles as opposed to aggregated non-discrete assemblies with non-distinct boundaries and “spherical”

means roughly spherical in nature and is not meant to be a precise definition of the structure.

Though the QAC adjuvant strategy significantly improves the immunogenicity and protective immune response generated by the NA vaccine compositions of the current disclosure, the inventors hypothesized that a heterologous vaccine approach may further increase the effectiveness of the compositions. As used herein, “heterologous vaccine approach” refers to practice of inducing a first immune response with a first vaccine composition, then inducing a second immune response with a second different vaccine composition. Accordingly, a “heterologous vaccine” may also refer to the “second different vaccine composition” in the preceding example.

Therefore, in a second aspect, vaccine compositions comprising a viral vector are provided. In some embodiments, the viral vector comprises a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

As used herein, a “viral vector” refers to a virus or viral particle that comprises a polynucleotide encoding at least one antigen. The viral vector delivers the polynucleotide into a subject’s cells. Within the cell, the polynucleotide is transcribed and translated, producing the encoded antigen. Depending on the cell that is expressing the viral antigen, the antigen may be presented on major histocompatibility complex I or II (MHC-I or MHC-II). Thus, the adaptive immune system, e.g., T and B cells, may recognize the antigen and become activated. The viral vectors may be used to induce an immune response to the S or N protein of IBV. The viral vectors of the present invention are “recombinant viruses,” in which foreign genetic material encoding an antigenic protein (i.e., from infectious bronchitis virus) has been inserted into the viral genome.

The viral vectors may be a weakened or killed version of a virus. For example, the viral vector can be based on an attenuated virus, which does not replicate or exhibits very little replication in a host but is able to introduce and express a foreign gene in infected cells. As used herein, an “attenuated virus” is a strain of a virus whose pathogenicity has been reduced compared to its natural counterpart. A virus may be attenuated using serial passaging, plaque purification, or other means. The viruses used herein may be viral like particles (VLP) that are not capable of replication in the subject but do carry the antigenic proteins.

In some embodiments, the viral vector is selected from an adeno-associated virus or a poxvirus. Suitable poxviruses for use with the present invention include, without limitation, canary poxvirus, raccoon poxvirus, vaccinia virus, fowl poxvirus, turkey herpes virus (HVT), and myxoma virus (MYXV). Poxviruses are advantageous for transferring genetic material into new hosts due to their relatively large genome size (approximately 150-200 kb) and because of their ability to replicate in the infected cell’s cytoplasm rather than the nucleus, thereby minimizing the risk of integrating genetic material into the genome of the host cell. Of the poxviruses, the vaccinia and variola species are the two that are most studied. Vaccinia virus is highly immunogenic, provoking strong B-cell (humoral) and T-cell mediated (cellular) immune responses against its encoded gene products. Of these viruses, the modified vaccinia virus Ankara (MVA) is particularly safe, as it has diminished virulence while maintaining good immunogenicity. Thus, in some embodiments, the viral vector is a modified vaccinia Ankara (MVA) virus. Exemplary MVA virus strains include MVA 572, MVA 575, and MVA-BN, which have been deposited at the European Collection of Animal Cell Cul-

tures (ECACC), Salisbury (UK) with the deposition numbers ECACC V94012707, ECACC V00120707 and ECACC V00083008, respectively, and are described in U.S. Pat. Nos. 7,094,412 and 7,189,536, incorporated herein by reference in their entireties.

In yet another embodiment, a vaccine composition including IBV spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein is provided. These proteins may be modified from those found natively in the IBV virus such that the protein subunit vaccine composition comprising these proteins induces an immune response in a subject after administration of the vaccine composition.

Both the NA vaccine compositions and the viral vaccine compositions of the present invention comprise a polynucleotide encoding an IBV spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. The protein subunit vaccine compositions provided herein comprise the IBV S protein, the N protein or a combination thereof. The vaccine compositions may also include more than one of the S proteins or N proteins or nucleic acids encoding more than one S protein or more than one N protein provided herein. Such vaccine compositions would be considered as multivalent vaccine compositions and any combination of S and N protein may be combined and that combination may vary depending on the circulating IBV virus in a particular area or at a particular point in time.

IBV S protein is the major antigen against which neutralizing and protective antibodies are produced. The S protein is partially or completely cleaved into the amino-terminal S1 and into the carboxy-terminal S2 subunits post translationally by a host furin-like protease. Furthermore, the S1 subunit is highly variable among different isolates of IBV and is responsible for viral attachment to host cell and contains major neutralizing epitopes. In some embodiments, the compositions of the current disclosure comprise polynucleotides encoding the S protein selected from the group consisting of SEQ ID NOs: 1-7, 22, 24, 26, 28, 30, 32, and 34 (DNA) or SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33 (amino acid), sequences with 90% or more identity to SEQ ID NO: 1-7, 22, 24, 26, 28, 30, 32, and 34 or SEQ ID NOs: 11-17, 21, 23, 25, 27, 29, 31, and 33 or fragments or portions thereof. The S2 subunit is highly conserved among IBV strains and contributes to viral fusion activity and elicits some minor but broadly reactive neutralizing antibodies. See, for example, Shirvani et al., “A Recombinant Newcastle Disease Virus (NDV) Expressing S Protein of Infectious Bronchitis Virus (IBV) Protects Chickens against IBV and NDV”, Scientific Reports volume 8, Article number: 11951 (2018), incorporated by reference herein in its entirety.

IBV N protein is associated with the RNA genome and forms the ribonucleoprotein. In some embodiments of the disclosed compositions, the N protein is encoded by a sequence selected from the group consisting of SEQ ID NOs: 8, 9, 36, and 38 (DNA) or SEQ ID NOs: 10, 18, 35, and 37 (amino acid), sequences with 90% or more identity to SEQ ID NO: 8-9, 36, and 38, SEQ ID NO: 10, 18, 35, and 37 or fragments or portions thereof.

The compositions of the current disclosure are administered, in some embodiments, intranasally, intramuscularly, or are administered in ovo. In some embodiments, the compositions are administered to greater than one subject at a time through means known in the art, for example, through mass intranasal administration of a group of animals. In some embodiments, the compositions of the current disclosure are administered by aerosol delivery to a flock of birds, for example, chickens.

The vaccine compositions of the present invention may be used as a prophylactic, e.g., to prevent or ameliorate the effects of a future infection by IBV, or may be used as a therapeutic, e.g., to treat IBV. The vaccines provided herein are expected to induce and enhance the immune response of the subject to IBV. The immune response enhanced is suitably a polyfunctional response. As used herein, a “polyfunctional response” refers to an immune response comprising both B and T cells directed to the pathogen.

The vaccine compositions may further comprise other suitable agents or ingredients. Suitable agents may include a suitable carrier or vehicle for delivery. As used herein, the term “carrier” refers to a pharmaceutically acceptable solid or liquid filler, diluent or encapsulating material. A water-containing liquid carrier can contain pharmaceutically acceptable additives such as acidifying agents, alkalizing agents, antimicrobial preservatives, antioxidants, buffering agents, chelating agents, complexing agents, solubilizing agents, humectants, solvents, suspending and/or viscosity-increasing agents, tonicity agents, wetting agents or other biocompatible materials. A tabulation of ingredients listed by the above categories may be found in the U.S. Pharmacopeia National Formulary, 1857-1859, (1990).

The vaccine formulation may be separated into vials or other suitable containers. The vaccine formulation herein described may then be packaged in individual or multi-dose ampoules or be subsequently lyophilized (freeze-dried) before packaging in individual or multi-dose ampoules. The vaccine formulation herein contemplated also includes the lyophilized version. The lyophilized vaccine formulation may be stored for extended periods of time without loss of viability at ambient temperatures. The lyophilized vaccine may be reconstituted by the end user and administered to a patient.

Methods:

In another aspect of the current disclosure, methods of inducing an immune response against infectious bronchitis virus (IBV) in a subject are provided. In some embodiments, the method comprises: administering a first vaccine composition and administering a second vaccine composition wherein administration of the first vaccine composition and the second vaccine composition induces the immune response against at least one IBV antigen in the subject. In some embodiments, a first vaccine compositions comprises a polynucleotide that encodes an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. In some embodiments, the vaccine composition further comprises an adjuvant. In some embodiments, the adjuvant comprises disaggregated spherical nanostructures comprising Quil-AR and chitosan, and wherein the Quil-AR and chitosan are present at a ratio between 1:15 and 1:100. In some embodiments, the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged. In some embodiments, the vaccine composition comprises spherical nanostructures between about 5 nm and about 100 nm in diameter in the absence of a payload molecule.

The vaccine composition may be a polynucleotide. In some embodiments, the S protein is encoded by one or more of the group consisting of SEQ ID NO: 1-7, 22, 24, 26, 28, 30, 32, and 34 or a sequence capable of encoding at least one of SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31, and 33. In some embodiments, the N protein is encoded by SEQ ID NO: 8, 9, 36 or 38, or a sequence capable of encoding at least one of SEQ ID NO: 10, 18, 35, or 37. In some embodiments, the polynucleotide encodes both an S protein and an N protein.

In some embodiments, the vaccine composition comprises a viral vector. In some embodiments, the viral vector comprises a polynucleotide encoding an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein. In some embodiments, the viral vector is selected from an adeno-associated virus or a poxvirus. In some embodiments, the viral vector is a modified vaccinia Ankara (MVA) virus or turkey herpes virus (HVT). In some embodiments, the sequence encoding the S protein comprises one or more of the group consisting of SEQ ID NO: 1-7, 22, 24, 26, 28, 30, 32, and 34 or a sequence encoding SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31. In some embodiments, the sequence encoding the N protein comprises SEQ ID NO: 8, 9, 36 or 38 or a sequence encoding SEQ ID NO: 10, 18, 35, or 37. In some embodiments, the viral vector encodes both the S protein and the N protein.

In other embodiments, the vaccine composition comprises a protein subunit vaccine composition. The protein subunits in the vaccine composition may include one or more of a IBV S protein or an IBV N protein or portion thereof. The vaccine compositions may further comprise an adjuvant and the adjuvant may be a Quil-A® chitosan adjuvant. In one embodiment the the vaccine composition may include both an S protein and an N protein or combinations of more than one S protein and more than on N protein. The S protein may be selected from SEQ ID NO: 11-17, 21, 23, 25, 27, 29, 31 or combinations thereof. The N protein may be selected from SEQ ID NO: 10, 18, 35, or 37 or combinations thereof.

The methods of the current disclosure comprise administration of vaccine composition that elicits an immune response against IBV. The timing of the administration of the vaccine compositions may be varied. Accordingly, in some embodiments, administration of the second vaccine composition occurs at least three weeks after administration of the first vaccine composition. In some embodiments, administration of the second vaccine composition occurs at least six weeks after administration of the first vaccine composition. A hallmark of the QAC adjuvant system is slow release of payload with continual priming of the immune system. Thus, the inventors hypothesize that release of DNA payload can be sustained up to six weeks after which another immunization will further boost immune responses.

The inventors disclose herein that heterologous vaccine strategies for eliciting an immune response against IBV are highly successful. Therefore, in some embodiments, the first vaccine composition comprises a NA vaccine composition, and the second vaccine composition comprises a viral vector or protein subunit vaccine composition.

The methods of the current disclosure comprise administering two vaccine compositions. In some embodiments, both the administration events comprise administering the vaccine compositions via the same route. In other embodiments, the first and second vaccine compositions are administered via different routes. For example, in some embodiments, the vaccine compositions are administered intranasally, intramuscularly, or administered in ovo. Thus, in some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered intranasally. In some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered in ovo. In some embodiments, the first vaccine composition is administered in ovo and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intranasally and the second

vaccine composition is administered intranasally. In some embodiments, the first vaccine composition is administered intranasally and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intramuscularly and the second vaccine composition is administered intramuscularly. In some embodiments, the first vaccine composition is administered intranasally and the second vaccine composition is administered intranasally.

As used herein, "subject" refers to avian and non-avian animals. An "avian subject" may be any member of the class Aves including, but not limited to, chickens, turkeys, ducks, or other fowl. The term "poultry" refers generally to any avian subject that is agriculturally relevant, e.g., chickens, ducks, ostriches, guinea fowl, turkeys, quail, pheasants, Muscovy ducks, and the like. The term "subject" does not denote a particular age or sex. In one embodiment, the subject is a chicken. In a preferred embodiment, the chicken is at risk of being infected IBV.

The phrase "amount effective to induce the immune response," as used herein, refers to an amount of a vaccine composition that would induce a humoral immune response against at least one IBV antigen (e.g., the spike or nucleocapsid protein encoded by the disclosed vaccines) and suitably also induces a polyfunctional T cell response as well. Humoral immunity or cell mediated immunity or both humoral and cell mediated immunity may be induced. The immunogenic response of an animal to a vaccine may be evaluated, e.g., indirectly through measurement of antibody titers, lymphocyte proliferation assays, or directly through monitoring signs and symptoms after challenge with the virus. The protective immunity conferred by a vaccine may also be evaluated by measuring, e.g., clinical signs such as mortality, morbidity, temperature, overall physical condition, overall health, and the performance of the subject. The amount of a vaccine that is therapeutically effective may vary depending on the particular strain of virus used, the antigen used in the vaccine, the species of the subject, the condition of the subject (e.g., age, body weight, gender, health), and should be determined by a veterinarian or physician. The therapeutically effective amount may be administered in one or more doses and is preferably in the range of about 0.01-10 mL, most preferably 0.05-1 mL, containing 1-200 micrograms, most preferably 1-100 micrograms of vaccine formulation/dose.

The present disclosure is not limited to the specific details of construction, arrangement of components, or method steps set forth herein. The compositions and methods disclosed herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating

that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements.

While some claims provided herein are directed to methods of treating a subject, both human and non-human subjects are envisioned. In addition, use of the compositions provided herein as medicaments for uses in therapy or for treating disease are also provided herein. Use of the compositions provided herein in the manufacture of a medicament for the treatment of a disease or condition are also encompassed.

Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about" to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to manufacturing tolerances, instrument and human error in forming measurements, and the like. All percentages referring to amounts are by weight unless indicated otherwise.

No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference, unless explicitly indicated otherwise. The present disclosure shall control in the event there are any disparities between any definitions and/or description found in the cited references.

The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims.

EXAMPLES

Example 1—Heterologous and Homologous DNA Confer Protection Against Avian Coronavirus

Infectious bronchitis (IB) is an acute respiratory disease of chicken caused by the avian coronavirus, Infectious Bronchitis Virus (IBV). Modified Live Virus (MLV) vaccines commercially used can revert to virulence in the field, recombine with circulating serotypes and can cause tissue damage in vaccinated birds. Previously, we showed that a mucosal adjuvant system, QuilA-loaded Chitosan (QAC) nanoparticles encapsulating plasmid vaccine encoding for

IBV Nucleocapsid (N) is protective against IBV. Here, we report a heterologous strategy using QAC encapsulated plasmid vaccine followed by a Modified Vaccinia Ankara (MVA) expressing the same IBV N antigen. Heterologous vaccination led to the development of robust T-cell responses. Heterologous vaccine immunized birds had reduced clinical severity and >2-fold reduction viral burden in lachrymal fluid and tracheal swabs post-challenge in contrast to homologous MVA vaccination where no protection was observed. Outcomes of this study indicate that the heterologous vaccine strategy is more immunogenic and protective than homologous vaccination.

Coronaviruses (CoVs) are enveloped, large viruses with a positive-sense, single-strand, RNA genome ranging from 27-31 Kb in length. They are broadly classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus [1]. CoVs can infect a wide range of hosts, including humans, poultry, mice, pigs, cats, camels, bats, etc. CoVs infections usually cause acute diseases, primarily in the respiratory and gastrointestinal tract [1]. Human CoVs like OC43, 229E, HKU1, and NL63 cause mild respiratory disease. Other CoVs like SARS-COV-2, SARS, MERS in humans, and Avian CoV like Infectious Bronchitis Virus (IBV) in chickens can cause more acute severe respiratory disease [1-3]. IBV is classified within the genus gammacoronavirus encoding for major structural proteins, spike glycoprotein (S), envelope (E), membrane (M), and nucleocapsid (N) [4] and is the etiological agent of infectious bronchitis in chickens. In a typical infectious bronchitis infection, chickens develop respiratory signs, including sneezing, tracheal rales, nasal discharge, and labored breathing[5]. Mortality associated with infectious bronchitis is low; however, concomitant secondary bacterial infections can increase mortality[3]. Infectious bronchitis has a significant economic impact on the commercial US poultry industry, valued at over \$35 billion in the US [6]. Infectious bronchitis infections in broilers can lead to reduced weight gain, and low feed conversion and infections in layers can lead to a drop in egg production and quality[7]. Typically, losses of around \$450,000 per week can be expected due to IB outbreaks in facilities producing about 1 million broilers per week, which is unsustainable in the poultry industry characterized by low-profit margins[8]. IBV control currently revolves around extensive vaccination and acceptable flock management practices like optimal stocking densities, house temperature, water and air quality, etc., to prevent increased mortality due to secondary bacterial infections. Modified live virus (MLV) and inactivated vaccines are the leading vaccine types used against IB. Although effective, MLVs have an inferior safety profile. MLVs have a propensity to persist, revert to virulence in the field, and readily recombine with other circulating serotypes, leading to novel serotypes' emergence due to single mutations as a consequence of lack of polymerase proof-reading activity [9-11]. The emergence of GA98 serotype has been linked to the extensive use of DE072 vaccine [12]. Moreover, current vaccines do not cross-protect against multiple circulating serotypes because of variations in the S protein [13-15]. Unfortunately, safer inactivated vaccines are poorly immunogenic underscoring the need to develop an effective and safe vaccine for IBV control [8].

Experimental plasmid DNA vaccines have been developed against multiple poultry pathogens, and most recently, conditional approval for a DNA vaccine against H5 avian flu was given [16]. Varying protection levels are observed with experimental plasmid DNA vaccines expressing IBV S1, N, and M genes delivered via the intramuscular, intranasal and

in ovo routes[17-25]. DNA vaccines offer several advantages over traditional vaccine approaches; they are safe, thermostable, comparatively inexpensive, and can be rapidly developed in the face of a novel serotype field outbreak [26]. A significant problem with DNA vaccines is their low immunogenicity owing to in vivo degradation leading to reduced cellular uptake and bioavailability. Vaccine hostile surfaces like the nasal mucosa can degrade DNA vaccine before target immune cell uptake[27, 28]. Nanoparticle adjuvant systems like QAC can protect against DNA degradation and boost immune responses observed with DNA vaccines as described by our group previously for the intranasal delivery of DNA immunogens[29, 30].

Similarly, viral vector vaccines against IBV based on Newcastle disease virus, Herpesvirus of turkeys and avian metapneumovirus backbones have been developed with great promise [31, 32]. However, none of them have been licensed for use owing to limited efficacy and regulatory concerns. The heterologous vaccine has been evaluated against viral pathogens like HIV-1, HPV, HCV, and Influenza [33-36]. Although the concept of heterologous vaccine for the poultry industry refers to a broadly cross-protective vaccine, for the purpose of this paper the heterologous vaccination refers to the concept of using a different vaccine platform for boosting from the vaccine that was used for priming. Particularly in this study, we evaluated DNA priming followed by viral vector boosting in comparison to viral vector homologous priming and boosting. The efficacy of heterologous vaccine strategies has been shown with different routes and viral vectors for boosting like vaccinia (e.g. Modified Vaccinia Ankara-MVA), adeno, and VSV (Vesicular Stomatitis Virus) [35]. Heterologous vaccination compared to homologous immunization can lead to a 4 to 10 fold increase in T-cell responses[35]. Previously, we have shown that a heterologous vaccination involving QAC encapsulated plasmid DNA priming followed by MVA boosting was shown to be immunogenic and protective against SARS-COV-2 challenge in transgenic mice[37, 38]. Although the heterologous vaccine approach has been characterized and extensively evaluated for human viral pathogens, not much work has been done in the context of viral poultry pathogens.

We have previously shown that a two-dose QAC encapsulated plasmid DNA (pQAC-N) encoding the N protein was protective against IBV challenge to levels seen with MLV vaccination[30]. We hypothesized that a heterologous vaccine strategy with pQAC-N prime followed by an MVA viral vector boost expressing the N protein (MVA-N) would also protect immunized chicks against IBV challenge similar to our findings with human coronavirus, SARS-CoV-2 [37, 38]. The prime/boost of the experimental vaccines were delivered via the intranasal route and hereafter referred to as either heterologous vaccine or pQAC/MVA-N. Our results indicate that pQAC/MVA-N vaccine elicits a robust IBV specific CD8+ and TCRγδ+ T-cells which protect vaccinated birds against IBV challenge. Levels of protection in vaccinated birds were higher when compared to homologous 2xMVA-N vaccine. Our data demonstrate that intranasal immunization with pQAC/MVA-N protected vaccinated birds with a significant reduction in clinical signs and viral load in trachea and lachrymal fluid to levels on par with commercial MLV vaccinated birds. Also, addition of another adjuvant MPLA (Synthetic Monophosphoryl Lipid A), did not significantly improve protection observed with pQAC/MVA-N.

Materials and Methods

Ethics Statement

All the animals used in this study were cared for in accordance with established guidelines, and the experimen-

tal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Wisconsin at Madison.

Cells and Viruses

Chicken Embryonic Fibroblasts (CEF) were prepared from 9-day-old specific pathogen free (SPF) white leghorn eggs (Charles River Laboratories, Inc., WA, USA) as described previously[39] and used for confirming expression of IBV Ark NoxHis protein from vaccine constructs. The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) at 37° C., 5% CO₂ atmosphere in plastic flasks with ventilated caps. The virulent IBV Arkansas DPI strain (a kind gift from Dr. Ladman and Dr. Gelb) was propagated in 9-day old SPF ECEs and allantoic fluid harvested four days after infection. The stock virus titer was determined using RT-qPCR (see below) and also titrated and expressed as 50% embryo infectious dose (EID₅₀)[40]. IBV S1 gene sequence of Ark DPI challenge isolate is AF006624. Preparation of IBV Vaccine Constructs

pCAG-N encoding IBV Arkansas N protein was constructed and loaded into QAC nanoparticles as described previously[30]. To confirm insertion of genes in the correct orientation, DNA sequencing was performed at the UW-Madison Biotechnology Center with an ABI Prism 3730XL DNA analyzer using BigDye terminators (Applied Biosystems, CA). To confirm expression of N protein, CEF cells seeded in 6-well format was transfected with an optimized ratio of DNA (4 ug): TransIT PRO transfection reagent (2 ul) according to manufacturer's instructions (Mirus Bio, WI, USA). Three days post transfection, cells and supernatant (separately) were harvested for western blot analysis. The MVA expressing N was generated as described before in CEF cells[41]. The cell and supernatant fractions were boiled in Laemmli sample buffer (BioRad, Hercules, CA, USA) and resolved on a 4-20% SDS-PAGE gel by electrophoresis using a Mini-PROTEAN 3 system (BIO-RAD, CA). Polyacrylamide gels were electroblotted onto nitrocellulose membranes using a Turboblot® system. Membranes were blocked in 5% (W/V) skim milk and probed with polyclonal anti-6xHis HRP antibody (ThermoFisher Scientific, MA1-21315-HRP). Membranes were developed using a solid phase 3, 30, 5, 50-tetramethylbenzidine (TMB) substrate system.

Vaccine Efficacy Study.

The protective efficacy of pQAC/MVA-N construct was evaluated in 1-day-old white leghorn SPF chicks (Charles River Laboratories). A total of 40 chicks was divided equally into 4 groups (N=10 each) and used for the efficacy study, first 2 groups were inoculated with PBS (negative control) or commercial Arkansas MLV (Mildvac-Ark®, Merck Animal Health USA, positive control) via direct intranasal instillations (dose according to manufacturer's instructions). The other groups were either vaccinated with MVA-N (10⁸ pfu/bird) at day -1 and followed by a booster dose at day -14 via intranasal (IN) route or pQAC-N (100 ug/bird) at day -1 and followed by a booster MVA-N (10⁸ pfu/bird) dose at day -14 via intranasal (IN) route. Birds were challenged with a dose of 6.5E9 genome copy no or 10^{6.5} EID₅₀/bird of virulent IBV Arkansas DPI strain via direct intranasal instillations at day -21 of age. The challenge dose was determined in an independent infection experiment wherein the challenge dose resulted in discernable clinical signs as early as 3 dpc and peak viral load replication was observed at 6 dpc. At 10, 20 dpv & 3 days post challenge (DPC) serum and lachrymal fluid samples were harvested for ELISA and at 6 DPC for viral load estimation (see below). Lachrymation was induced by placing sodium chlo-

ride (salt) crystals on the eyes and lachrymal fluid were collected using micropipettes [42]. Clinical severity was noted every day post challenge for 8 days, as described before [31]. The severity scores of clinical signs of IBV were as follows; 0=normal, 1=Infrequent sneezing (single event during observation), 2=frequent sneezing (more than one event during observation), 1=mild rales, 2=severe rales, 2=presence of nasal exudate. The severity scores of IBV clinical signs, described in the figure legends were recorded once a day for each chicken for 8 days after challenge. Lachrymal fluid and tracheal swabs harvested at 6 dpc was analyzed for viral RNA using IBV N gene specific qRT-PCR. A similar experimental design was used to test the efficacy of the pmQAC/MVA-N vaccine candidate in a follow-up trial. 10 ug MPLA/bird (PHAD®, Avanti® Polar Lipids) was added to QAC-pCAG-N formulation before IN inoculation and followed by a booster MVA-N (10⁸ pfu/bird) dose at day -14 via intranasal (IN) route. Birds were challenged with a dose of 6.5E9 genome copy no or 10^{6.5} EID₅₀/bird of virulent IBV Arkansas DPI strain via direct intranasal instillations at day -21 of age. Vaccine efficacy read outs including viral shedding and clinical severity scoring as detailed for the previous primary trial were evaluated.

IBV Specific ELISA

Sera and lachrymal fluid from different time-points were screened for humoral response against IBV Arkansas serotype. In order to measure IgY and IgA antibody levels in plasma and lachrymal fluid of chicken respectively, an IBV-specific enzyme-linked immunosorbent assay (ELISA) was developed as described previously with modifications [43]. Briefly, ELISA plates were coated with inactivated IBV Arkansas (100 ng/well, IgY) or IBV Arkansas S1 and N6xHis protein (50 ng total/well, IgA) diluted in carbonate/bicarbonate buffer, pH 9.6 and incubated overnight at 4 C followed by blocking with 5% Skim milk to reduce background. A 50 ul of diluted serum (1/200) or lachrymal fluid (1/50) harvested at different time-points from immunized chickens was added to the wells and incubated at 37 C for 1 hour. Post washing (PBS-TritonX 100, 0.1%), either HRP conjugated anti-chicken IgY (NBP1-74778, NOVUS Bio) or anti-chicken IgA (NB7284, NOVUS Bio) at dilutions of 1/1000 was added to the wells and incubated at 37° C. for 1 hr. Post washing, 50 ul of TMB substrate solution was added and incubated for 20 minutes or until color developed. The reaction was stopped by the addition of IM sulphuric acid and plates are read at 450 nm. To generate standard curves, sera and lachrymal fluid from severely IBV infected chickens from previous experiments was used. Two-fold serial dilutions was assigned and arbitrary value and used for analysis.

Flow Cytometric Assessment of IBV Specific Proliferation

In a separate follow-up study, 16 chicks were divided equally into 4 groups (N=10 each) and used for the flow cytometric assessment, first 2 groups were inoculated with PBS (negative control) or commercial Arkansas MLV (Mildvac-Ark®, Merck Animal Health USA, positive control) via direct intranasal instillations (dose according to manufacturer's instructions). The other groups were either vaccinated with MVA-N (10⁸ pfu/bird) at day -1 and followed by a booster dose at day -14 via intranasal (IN) route or pQAC-N (100 ug/bird) at day -1 and followed by a booster MVA-N (10⁸ pfu/bird) dose at day -14 via intranasal (IN) route. All chicks were euthanized at 20 dpv and single cell suspensions from lungs were prepared using standard techniques and used for T-cell proliferation assay. Briefly, lungs were excised and placed in a gentleMACS dissociator M

tube (Miltenyi 130-093-236) with 5 mL collagenase B (2 mg/mL, Roche). Lung tissue was processed using the gentleMACS dissociator followed by incubation for 30 min at 37° C. with gentle shaking. Single-cell suspensions lung were prepared by gently squeezing through a 70-mm cell strainer (Falcon) after lysing RBCs using 1×BD Biosciences BD Pharm Lyse™. Total of 10⁷ cells/mL were stained with CellTrace™ Violet Cell Proliferation dye (Thermo Scientific C34557) according to manufacturer's instructions and 100 µl of cells plated/well in RPMI 1640 with 10% chicken immune serum. After overnight incubation at 41° C., 5% CO₂, cells were stimulated with 130 ng of IBV Arkansas N6×His protein complexed with chitosan per well in 100 µl of RPMI 1640 with 10% chicken immune serum. Four days post stimulation, cells were stained for surface markers, CD4-AF647 (clone CT-4), CD8α-FITC (clone 3-298) together and TCRγ8-FITC (clone TCR-1) independently for flow cytometry analysis. All antibodies were purchased from SouthernBiotech (Birmingham, AL, USA). All samples were acquired on an BD LSR Fortessa flow cytometer. Data were analyzed with FlowJo software (BD Biosciences). The strategy for gating on proliferating CD4+ and CD8α+ T cells was debris exclusion on the Forward Scatter (FSC)-Side Scatter (SSC) dot plot followed by exclusion of dead cells by fixable viability dye eFluor 780 (Invitrogen™, #65-0865-14) staining. Out of the live cells, total proliferated cells were gated positive using a histogram plot with eF450 on the x-axis (for CellTrace™ Violet). Finally, CD4 cells were gated positive at the AF647 axis and CD8α cells were gated positive at the FITC axis in a FITC-AF647 dot plot. A similar approach was used for identifying proliferating TCRγδ+ T-cells. The output, stimulation index (SI) is the ratio of % proliferating cells post stimulation to the % proliferating cells in unstimulated condition. The chicks from different groups used here were part of another bigger study and the data for only the control groups (PBS and MLV) have already been published[30].

Viral Load Measurement

RNA was extracted from lachrymal fluid (10 µl) or Tracheal swabs (100 µl) collected from chickens using Zymo Direct-Zol™ RNA mini prep kit (Zymo Research, CA, USA) according to manufacturer's instructions. RT-qPCR was conducted in two steps: cDNA synthesis (Invitrogen™ SuperScript™ III First-Strand Synthesis System) and qPCR reactions. cDNA synthesis was performed with 0.5 µl (50 ng/µl) random hexamers, 0.5 µl of 10 mM dNTPs, and 4 µl RNA and heated at 65° C. for 5 min and chilled on ice followed by addition of 1 µl of 10× RT buffer, 1 µl of 0.1 M DTT, 1 µl of 25 mM MgCl₂, 0.5 µl of RNaseOUT and 0.5 µl of SuperScript III enzyme in final volume of 10 µl. The reaction conditions include 25° C. for 5 min, 50° C. for 60 min and 70° C. for 15 min. SYBR green RT-qPCR was performed using an IBV N gene specific primer pair set forward primer: 5' ATGCTCAACCTAGTCCCTAGCA 3' (SEQ ID NO: 46) and reverse primer: 5' TCAAACTGCGGATCATCACGT 3' (SEQ ID NO: 47) amplifying 128 nt of N gene of IBV Arkansas DPI. PCRs were performed using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, U.S.A) under the following conditions: one cycle 95 C for 2 min followed by 40 cycles of 95 C for 3 sec and 60 C for 30 sec. Each 20 µl reaction was carried out using 1 µl of diluted cDNA (1/10), 10 µl of GoTaq® qPCR mastermix (Promega), 2 µl of forward and reverse primers and 7 µl of nuclease free water. A serial 10-fold dilution of pCAG-IBV Ark N6×His plasmid was used to establish the standard curve. Temperature melt curve analysis was used to confirm the specificity of the product.

The challenge dose as estimated with the above-described method was 6.5E9 genome copy no which roughly translated to 10^{6.5} EID₅₀.

Statistical Analysis

Statistical analyses were performed using GraphPad software (La Jolla, CA). Cellular immune assays, clinical severity scoring, viral loads were compared using an ordinary one-way ANOVA test with multiple comparisons where *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 were considered significantly different among groups. Antibody titers were compared using a two-way ANOVA test where *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001 were considered significantly different among groups.

Results:

Design and Construction of MVA-IBV Constructs

The expression of recombinant N from the plasmid DNA vaccine (pCAG-N) was confirmed using western blot analysis on cells and supernatant harvested from transfected chicken embryonic fibroblast (CEF) cells (FIG. 1B). The SE/L promoter controls the expression of the recombinant N-6×His protein in the MVA vaccine candidate (MVA-N, FIG. 1A). As observed with pCAG-N construct, expression of N-6×His antigen was also confirmed using western blot analysis with anti-6×His antibody staining in the cell pellets from MVA-N infected CEF cells (FIG. 1B). To characterize and understand if the expression of IBV N-6×His protein affects MVA replication in cell culture, we evaluated the growth kinetics of MVA-N and parental MVA-GFP in permissive CEF cells. CEF cells were either infected at a MOI of 1 (single step) or 0.1 (multi-step) and viral titers subsequently determined on CEF cells (FIGS. 1C and 1D). MVA-N replicated at rates similar to parental MVA-GFP, although the final titers of the recombinant MVA were about 100-fold lower than that of the parental virus (FIGS. 1C and 1D).

Heterologous Vaccine Strategy Elicits Robust Localized T-Cell Responses

We have previously reported the safety, and protective efficacy of QAC complexed pCAG-N DNA vaccine (pQAC-N) in chickens against IBV Arkansas challenge although no humoral responses were observed [30]. We hypothesized that a heterologous mucosal strategy of priming with pQAC-N followed by boosting with MVA-N would offer a similar or better level of protection than observed with 2-dose intranasal (IN) pQAC-N vaccination with complementing humoral responses. We examined the ability of our experimental vaccines to elicit local and systemic IBV-specific immune responses following IN immunization (FIG. 2). Lachrymal fluid samples and serum harvested at different time points, 10, 20 days post-vaccination (dpv, pre-challenge) and three days post-challenge (dpc) were examined for IBV specific IgA (lachrymal fluid, local) and IgY (serum, systemic) using ELISA. IBV specific IgA and IgY were significantly higher in the MLV groups when compared to the unvaccinated PBS group (FIGS. 3A and 3B). Although detectable at multiple time points, both IgA and IgY levels were not significantly high in birds vaccinated with either the homologous or heterologous vaccine strategy (FIGS. 3A and 3B).

We next evaluated the ability of the experimental vaccines to elicit local (lung) IBV N specific cellular immune responses. Antigen-specific T-cell proliferation assay based on CellTrace™ Violet Cell dye staining of lung cells to trace proliferating T cells was used as described previously[30]. The stimulation index (SI), which is the fold increase in stimulated to unstimulated cells was calculated. Total lung cells from pQAC/MVA-N vaccinated birds had significantly

higher proliferation in response to N antigen stimulation which was higher than the control and 2xMVA-N groups (FIG. 4A). An increase in the stimulation of proliferating TCR $\gamma\delta$ + and CD8+ T-cells was observed in pQAC/MVA-N vaccinated birds in comparison to control birds (FIGS. 4C & 4D) while CD4+ T-cell proliferation was higher in MLV vaccinated birds (FIG. 4B), albeit non-significant. These results highlight the ability of the heterologous pQAC/MVA-N vaccine strategy to elicit robust IBV-specific immune responses.

Heterologous Vaccine is More Effective than the Homologous Vaccine Strategy.

Twenty-one days post initial vaccination (21 dpv) and seven days post final boost, immunized birds were challenged with a virulent strain of IBV Arkansas DPI Serotype via the intranasal route to evaluate vaccine efficacy. Immunization with homologous 2xMVA-N did not confer any protection against the challenge; no reduction in clinical severity was observed (FIG. 5A). In contrast, immunization with heterologous pQAC/MVA-N and MLV resulted in a significant reduction in clinical severity with the birds asymptomatic when compared to unvaccinated PBS group birds (FIG. 5A). Viral RNA in lachrymal fluid and tracheal swabs were evaluated using qRT-PCR. Only the best performing experimental vaccine group as determined by viral shedding in lachrymal fluid along with the control groups was taken for quantifying viral shedding in the tracheal swabs. A significant reduction in viral load was observed both in the lachrymal fluid and swabs of pQAC/MVA-N vaccinated birds in comparison to the unvaccinated and 2xMVA-N vaccinated birds (FIG. 5B). More importantly, reduction in viral load in tracheal swabs was comparable to levels seen in commercial MLV vaccinated birds (FIG. 5C). In contrast, no reduction in viral load was observed in 2xMVA-N vaccinated birds, which correlated well with clinical severity scoring (FIGS. 5A and 5B). Vaccination with the heterologous pQAC/MVA-N confers protection against IBV challenge significantly higher than the homologous 2xMVA-N (FIG. 5B). This protection might be attributed to the induction of CD8+ and TCR $\gamma\delta$ + memory T-cell responses (FIG. 4).

Impact of MPLA Addition on IBV Vaccine Protection.

MPLA is a potent mucosal adjuvant and TLR 4 ligand that stimulates expression of inflammatory-related genes, important of viral control in poultry. We hypothesized that inclusion of MPLA in addition to Quil-A $\text{\textcircled{R}}$ and Chitosan would further improve protection observed with pQAC/MVA-N vaccination. To investigate this, we immunized SPF birds with a triple adjuvant system (MPLA+QAC) loaded with pCAG-N plasmid at day -1 followed by MVA-N immunization (pmQAC/MVA-N) at day -14 similar to the pQAC/MVA-N group in the previous trial. Reduction in clinical severity and viral burden in tracheal swabs was observed comparable to the MLV group (FIGS. 6A-B). Protective efficacy of pmQAC/MVA-N was very similar to and not significantly different from pQAC/MVA-N (FIGS. 6A-B). Our results indicate that addition of MPLA does not improve vaccine performance. Overall, these results highlight the ability of the heterologous vaccine strategy to elicit potent IBV specific T-cell responses and protect vaccinated birds against virulent IBV challenge.

Discussion

Many experimental viral vectored vaccines primarily based on Newcastle Disease Virus (NDV) have been developed against IBV [31, 44, 45]. Recombinant NDV encoding for IBV Spike protects against homologous challenge and resulted in a reduction of clinical severity and viral shedding

[31, 45]. Recombinant MVA based vaccines have been developed for use in chickens against Infectious Bursal Disease Virus (IBDV) and Influenza[46-48]. The heterologous vaccine strategy involving a DNA prime followed by a viral vector booster dose has been evaluated against multiple human and animal viruses with modest success[37, 38, 49-51]. Intranasally administered vaccines are highly favorable for mass vaccinations in the field. Unfortunately, mucosal surfaces are vaccine hostile leading to poor immunogen uptake and bioavailability, rapid degradation and weak immune responses[27]. In a previous study, we demonstrated the ability of a nano adjuvant system, QAC to facilitate the intranasal delivery of DNA immunogens leading to a protection against IBV in poultry and SARS-COV-2 in transgenic mice [30, 37, 38]. In this study we evaluated the efficacy of an intranasally delivered heterologous QAC complexed DNA prime-MVA boost vaccine strategy. To our knowledge, the use of heterologous and MVA based vaccine strategies against IBV infection in chickens have not been extensively studied.

DNA viral vectors like MVA can accommodate and stably express multiple foreign immunogens, making them ideal candidates for vaccine use. In our hands, although the recombinant MVA-N had similar replication rates in cell-culture when compared to the parental MVA-GFP, the titers were 100-fold lower, albeit non-significant. This could mean that constitutive expression of IBV N-6xHis protein potentially weakened the MVA vector replication in permissive CEF cells. The safety and efficacy of MVA-based vaccines in chicken hosts have been well documented[52-54]. Experimental MVA-hemagglutinin based influenza vaccines protect chickens against both lethal high- and low-pathogenicity avian influenza[52, 53]. Furthermore, the safety and replication of MVA in chicken embryos have been extensively characterized with no embryonic death observed even after in ovo inoculation[54]. We have previously shown that QAC based DNA vaccines are well tolerated by chicken hosts when administered via the IN and in ovo routes. Similarly, we observed that chickens intranasally administered MVA-N and pQAC/MVA-N did not show any signs of respiratory distress, in appetite or depression pre-challenge.

Very few studies have investigated the efficacy of MVA based vaccines in poultry. Ocular administration of MVA based flu vaccine protects birds against avian influenza challenge[47]. Mixing and matching viral vector and nucleic acid SARS-COV-2-vaccines also boost the immunogenicity of homologous vaccines[55, 56]. In our hands, the heterologous DNA prime followed by MVA boost was more immunogenic and protective than the homologous MVA vaccination. Reduction in clinical severity and viral burden both in lachrymal fluid and tracheal swabs were observed to levels comparable with MLV vaccination. The protection is most likely due to the induction of local lymphocyte responses by the pQAC-N priming followed by the expansion of T-cells facilitated by the MVA-N boost. We observed a similar phenomenon with our QAC-based COVID-19 vaccines in mice, where the heterologous DNA/MVA vaccine was more immunogenic than the homologous vaccine strategy[37, 38].

In a previous study we showed that 2 doses of pQAC-N vaccine protected vaccinated SPF and commercial birds against IBV challenge comparable to protection observed with MLV[30]. A robust T-cell immune response without a complementing humoral response was induced post vaccination with 2xpQAC-N. We hypothesized that boosting with MVA viral vector instead of DNA vaccine would further expand CD4+ T-cells leading to an induction of comple-

menting humoral responses. We observed that immunization with MVA-N, both in the homologous and heterologous group did not lead to significant induction of both IgY and IgA as assayed using IBV specific binding ELISA. Instead, low level IBV-specific IgA and IgY was observed in the experimental vaccine groups at 3 dpc, indicating presence of an anamnestic response with pQAC—N based vaccines. In contrast, significant induction of humoral responses was observed with commercial MLV vaccine. Irrespective of the vaccine platform used, homologous MVA and heterologous DNA/MVA used in this study and homologous DNA used in the previous study, significant induction of N specific humoral responses are not observed[30]. The absence of humoral responses could be a consequence of using the N immunogen exclusively and not the vaccine platform itself. The N protein here will be intracellularly expressed in cells that take up the vaccine and not secreted. Moreover, it is unlikely that antibodies generated against N will be neutralizing given the intra-virion nature of the protein. With mouse hepatitis virus (MHV), a CoV infecting mice, N specific antibodies fail to neutralize MHV in cell culture[57].

Previously, sequential immunization approach of DNA prime-viral vector boost has led to the initial induction of cell-mediated immune (CMI) responses followed by MVA boost which expands induced CD8+ T-cells and Th1 T-cells [58]. We have previously shown that the potency of unadjuvanted plasmid DNA vaccine was enhanced by QAC nanoparticle formulation leading to induction of robust CD8+ and TCR $\gamma\delta$ + T-cells, potentially a hallmark of the QAC adjuvant system[30]. Similarly, lung cells harvested from pQAC/MVA-N immunized chickens responded well to IBV-N antigen recall stimulation. Furthermore, higher stimulation of TCR $\gamma\delta$ + and CD8+ T-cells was observed in pQAC/MVA-N immunized chickens, albeit non-significant. Although no significance was observed in T-cell specific responses, statistically higher proliferation was observed with total lung cells. This could mean that there are other lymphocytes (non TCR $\gamma\delta$ +, CD8+ or CD4+ T-cells) in the lungs responding to IBV antigen that were not specifically evaluated in this study. We believe that an MVA boost after DNA prime further expanded the lung lymphocytes elicited by the initial DNA vaccination leading to protection. These results are in accordance with our previous data where a similar heterologous DNA/MVA vaccine elicited better local type-1 and type-17 T-cell responses in mice not observed with the homologous vaccine strategy[38]. Further studies are still warranted to evaluate the exact mechanism of action for the pQAC/MVA-N vaccine.

To further improve on the efficacy of the pQAC/MVA-N vaccine we added MPLA to our QAC vaccine formulation. MPLA is a synthetic low toxic form of LPS can engage with TLR4 (toll-like receptor) leading to an enhanced Th1 response[59]. MPLA is the only licensed TLR agonist approved for human use and is currently used as part of AS04 adjuvant in hepatitis B and human papillomavirus vaccines[60, 61]. Engagement of TLRs by agonists like lipopolysaccharides (LPS), Poly I:C and CpG dinucleotides leads to a cascade of intracellular signaling leading to induction of factors and cytokines which enhance immunity [62]. The new tri-adjuvant system based heterologous vaccine dubbed pmQAC/MVA-N with MPLA did not significantly improve protection observed with pQAC/MVA-N when administered intranasally.

Results presented here highlight the utility of a nano-adjuvant complexed DNA prime/viral vector boost vaccine strategy against IBV in chickens which reduces clinical severity and viral load in trachea and lachrymal fluid. The

heterologous vaccine strategy outperformed the homologous MVA/MVA immunization and resulted in the induction of local-IBV specific T-cells in the lungs. Moreover, the protection observed with the heterologous vaccine strategy was very comparable with the commercial MLV vaccine's efficacy.

In general, CD8+ T-cells are important for early protection against IBV infection but CD4+ T-cells and systemic humoral responses are needed for sterilizing long term immunity[63]. We did not observe IBV specific antibody responses with the heterologous vaccine. The use of additional adjuvants and a secreted IBV S protein as an additional immunogen to the pQAC/MVA-N formulation could help in generating a complementing humoral immune response [64]. 2-dose vaccine regimens like the heterologous vaccine strategy described here might also have poor field applicability. Single dose vaccines administered at day -1 are preferred for poultry considering the need for early protection against IBV and the short lifespan of broilers in the poultry industry. Many experimental MVA based vaccines for use in humans are currently undergoing clinical trials. Therefore, use of MVA in poultry might confer people coming in contact with vaccinated birds with pre-existing immunity against the viral vector limiting the efficacy of subsequent human MVA based vaccines. That being said, the utility of this heterologous vaccine platform can be extended for use against other respiratory coronaviruses which necessitate robust local immune responses for protection. As highlighted with the ongoing COVID-19 pandemic, mix and match heterologous vaccines can not only improve immunogenicity, but also help in mitigating global vaccine supply chain shortages.

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SEQUENCE LISTING

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 FEATURE Location/Qualifiers
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 organism = Infectious Bronchitis Virus

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SEQ ID NO: 3      moltype = DNA length = 3552
FEATURE          Location/Qualifiers
source           1..3552
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                 organism = Infectious Bronchitis Virus

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SEQ ID NO: 4 moltype = DNA length = 3534
 FEATURE Location/Qualifiers
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 mol_type = other DNA
 organism = Infectious Bronchitis Virus

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SEQ ID NO: 5 moltype = DNA length = 3543
 FEATURE Location/Qualifiers
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mol_type = other DNA
organism = Infectious Bronchitis Virus

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SEQ ID NO: 6      moltype = DNA length = 3513
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source            1..3513
                  mol_type = other DNA
                  organism = Infectious Bronchitis Virus

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SEQ ID NO: 7      moltype = DNA length = 3513
FEATURE          Location/Qualifiers
source           1..3513
                 mol_type = other DNA
                 organism = Infectious Bronchitis Virus

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FEATURE          Location/Qualifiers
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                 mol_type = other DNA
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SEQ ID NO: 9      moltype = DNA length = 1257
FEATURE          Location/Qualifiers
source           1..1257
                 mol_type = other DNA
                 organism = Infectious Bronchitis Virus

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MASGKATGKT  DAPAPVILKG  GPKPPKVGSS  GNVSWFQAIK  AKKLNPPPK  FEGSGVPDNE  60
NLKPSQQHGY  WRRQARFKPG  KGGKPKVPDA  WYFYTTGTGP  AANLNWGDSC  DGIVWVAGKG  120
ADTKFRSNQG  TRDSKFDQY  PLRFSDDGPD  GNFRWDFIPL  NRGSRGRSTA  ASSAASSRAP  180
SREVSRRGRS  GSEDDLIARA  ARIIQDQKK  GSRITKAKAD  EMAHRRYCKR  TIPPNYKVDQ  240
VFGPRTKGKE  GNFDDKMNNE  EGIKDGRTA  MLNLVPSSHA  CLFGSRVTPR  LQPDGLHLKF  300
EFTTVVPRDD  PQFDNYVKIC  DQCVDPVGT  PTDDEPRPKS  RSSSRPATRG  NSPAPRQQR  360
KKEKPKPKQD  DEVDKALTS  EERNNAQLEF  DDEPKVINWG  DSALGENELG  GG  412

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SEQ ID NO: 11      moltype = AA  length = 1165
FEATURE
source            Location/Qualifiers
                  1..1165
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 11
MLVTPLLLV  LLLCALCSAV  YDSSSYVYYY  QSAFRPPNGW  HLQGGAYAV  NISSEFNAG  60
SSSGCTVGI  HGGRVNVASS  IAMTAPSSGM  AWSSSQFCTA  HCNFSDTTVF  VTHCYKHGGC  120
PITGMLQNL  IRVSAMKNGQ  LFNLTVSVA  KYPTFRSFQC  VMNLTSVYLN  GDLVYTSNET  180
IDVTSAGVY  KAGGPITYKV  MREVKALAY  VNGTAQDVIL  CDGSPRGLLA  CQYNTGNFSD  240
GFYPTNSSL  VQKPIVYRE  NSVNTTCTLH  NFIFHNETGA  NPNPSGVQNI  QTYQTAKTAS  300
GYNPNFSL  SSFVYKESFN  MYGSYHPSCN  FRLETINNG  WFNSLSVISA  YGPLQGGCKQ  360
SVFKGRATC  YAYSYGPSL  CKGVYSGELD  HNFECGLLVY  VTKSGGSRIQ  TATEPPVITQ  420
NNYNNITL  CVDYNIYGR  GQGFITNVT  SAVSYNYLAD  AGLAILDTS  SIDIFVVGQE  480
YGLNYYKVP  CEDVNQFVV  SGGKLVGILT  SRNETGSQLL  ENQFYIKITN  GTGGGVPSIT  540
ENVANCPYV  YGKFCIKPD  SIATIVPKQL  EQFVAPLFNV  TENVLIPNSF  NLTVTDEYIQ  600
TRMDKVQIN  LQYVCGSSL  CRKLFQQYG  VCDNLSVVN  SVGQKEDMEL  LNFYSSTKPA  660
GFNTPVLSN  STGEFNISLL  LTPSSRRKR  SLIEDLLFTS  VESVGLPTND  AYKNCAGPL  720
GFFKDLAC  EYNGLLVLP  IITAEMQALY  TSSLVASMAF  GGITAAGAI  FATQLQARIN  780
HLGITQSL  KQEKIAASF  NKAIGHMQEG  FRSTSLALQ  IQDVVSKQSA  ILTETMASLN  840
KNFGAISSV  QETIQDFDAI  QANAQVDR  TGRLLSSLSV  ASAKQAEYIR  VSQRELATQ  900
KINECVKQS  IRYSPCNGR  HVLTPQNA  NGVFIHFSY  TPDSPVNVTA  IVGFQVCKP  960
ASQYAI  GRGIPQVNG  SYITARDMY  MPRAITAGDV  VTLTSCQANY  VSVNKTIVIT  1020
FVDNDPFD  DELSKWNDT  KHELDPDFK  NYTVPILDID  SEIDRIQVI  QGLNDSLIDL  1080
EKLILKTY  KWPFGSYPE  APRDQYAYR  KDGWVLLST  FLGRSLELV  QQPGSAWSHP  1140
QFEKGGSG  GSGGSAWSH  PQFEK

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SEQ ID NO: 12      moltype = AA  length = 1172
FEATURE
source            Location/Qualifiers
                  1..1172
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 12
MLVKSFLPV  ILFALCSAN  YDNESFVYYY  QSAFRPGHW  HLYGGAYAV  NVSSENNAG  60
TAPSTAGAI  GYSKNLSAAS  VAMTAPLSGM  SWSANSFCTA  HCNFTSYIVF  VTHCYKSGSN  120
SCPLTGLIP  GYIRIAAMKH  GSAMPGLHF  NLTFSVTKYP  KFRSLQCVNN  YTSVYLNGLD  180
VFTSNYTED  VAAGVHFKSG  GPITYKVMRE  VKALAYFVNG  TAHDVILCDD  TPRGLLACQY  240
NTGNFSDGF  PFTNTSIVKD  KFIVYRESS  NTTLTLTNFT  FSNESGAPPN  TGGVDSFILY  300
QTQTAQSGY  NFNFSFLSSF  VYRESYMYG  SYHPRCSFRP  ETLNGLWFN  SLSVSLTYGP  360
IQGGKQSVF  NGKATCCYAY  SYGGPRACKG  VYRGELTQHF  ECGLLVYVK  SDGSRITQAT  420
QPPVLTQNF  NNINLGKVD  YNIYGRIGQ  LITNVTDLAV  SYNLSLSDAG  AILDTSGLD  480
IFVVQGEY  NYYKVNPCED  VNQQFVVS  KLVGILTSRN  ETGSQLENN  FYIKITNGTG  540
GGVPSVTEN  TNCVPVSYG  FCIKPDGSI  VIVPKELDQF  VAPLLNVTEY  VLIPNSFNLT  600
VTDEYIQR  DKIQINCLQY  VCGNSLACRK  LFQYGPVCD  NILSVVNSVG  QKEDMELLNF  660
YSSTKPARF  TPVFSNLSTG  EFNISLLLT  PSSPRRSFI  EDLLFTSVES  VGLPTDDAY  720
MRTAGPLGL  KDLACAREYN  GLLVLPPIIT  AEMQTLTSS  LVASMAFGGI  TAAGAIPFAT  780
QLQARINHL  ITQSLLLKN  EKIAASFNKA  IGHMQEGFRS  TSLALQIQD  VVNKQSAILT  840
ETMLALNKN  GAISSVIQDI  YQQLDSIQAD  AQVDRLITGR  LSSLSVLASA  KQSEYIRVSQ  900
QRELATQIN  ECVKQSIRY  SPCNGRHLV  TIPQNPNGI  VFIHTYTP  SFINVTAVVG  960
FCVSPANAS  YAIVPANGRG  IFIQVNGSVY  ITARDMYMPR  DITAGDIVTL  TSCQANYVSV  1020

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NKTVITTFVD	NDPDFDFDEL	SKWNETKHE	LPDFDKFNYT	VPILDIDSEI	DRIQGVIIQGL	1080
NDSLIDLETL	SILKTYIKWP	GSYIPEAPR	DGQAYVRKD	EWVLLSTFLG	RSLEVLFGQP	1140
GSASHPQFE	KGSGSGSGS	GSASHPQF	EK			1172

SEQ ID NO: 13 moltype = AA length = 1175
 FEATURE Location/Qualifiers
 source 1..1175
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 13

MLVKSPPFIVT	LLCALCSASL	YDNGSYVYYY	QSAFRPSIGW	HLHGGAYAVV	NVTQEYNNAG	60
SASECTAGAI	VSKNFSAA	VAMTAPHSGM	SWSVKQFCTA	HCNFTNFVVF	VTHCFKDG	120
TCPLTGRIDQ	GYIRIAAMKN	TGTGPRDLFY	NFTVSVTKYP	SFKSLQCQVNN	QTSVYVYNGDL	180
VFTSNETVDV	SGAGVHFKAG	GPITYKVMRE	VKALAYFVNG	TAQDVILCDS	SPRGLLACQY	240
NTGNFSDGFY	PFTNSSVVK	KFIVYSENSV	NTTLVLHNFT	FYNESDAPPN	SQQSSAGVGG	300
LTTYQTQTAQ	SGYVNFNFSF	LSSFVYKESN	FMYSYHPQC	NFRPENINNG	LWFNSLSVSI	360
TYGPLQGGCK	QSVFSHRATC	CYAYSNGPH	ICKGVYSGQL	HNNFECGLLV	YITKTDGSRI	420
QTATTPVPRT	QHFYNNITLH	KCVEYNIYGR	VGQGFITNVT	DSVAGVNYLQ	DGGLAILDTS	480
GAIDIFAVQG	GYGLNFYKVN	PCEDVNQQFV	VSGGNLVGIL	TSRNETDSQP	LENQFPVKLI	540
NGTGGGVPSI	SENVTSCSFV	SYGKFCIKPD	GSISTIVPKE	MEQFVAPLLN	VTEHVLIPDS	600
FNLTVTDEYI	QTRMDKQVIN	CLQYVCGNSF	ECRQLFQQYG	PVCDNILSVV	NSVGQKEDME	660
LLSFYSSTKP	AGYNTPVFNI	STGDFNISLL	LPPSSAPSGR	SFIEDLLFTS	VESVGLPTDE	720
AYKKCTAGPL	GFLKDLACAR	EYNGLLVLPP	IITAEMQTLY	TSSLVASMAL	GGITAAGAI	780
FATQLQARIN	HLGITQTULL	KNQEKIAASF	NKAIGHMQEG	FKSTSLALQQ	IQDVVNKQSA	840
ILTETMASLN	KNFGAISSVI	QEIYQQLDAI	QANAQVDRLI	TGRLLSSLVL	ASSKQAEYLR	900
VSQQRELATQ	KINECVKSQS	TRYSFCGNR	HVLTIPQNA	NGIVFIHFTY	TPESFVNVT	960
IVGFCINPAN	ASQYAIIVPAN	GRGIFIQVNG	TYIITARDMF	MPRDIRTAGDV	VTLTSCQAY	1020
VSVNKTIVIT	FVESDDPDFD	DELSKWNNET	KHEFPDFDQF	NYTIPVLNIT	YDIDKIEEVI	1080
KGLNDSLIDL	ETLSILKTYI	KWPGSGYIPE	APRDGQAYVR	KDGEWVLLST	FLGRSLEVL	1140
QPGGSAWSHP	QFEKGGSGSG	GGSGGSAWSH	PQFEK			1175

SEQ ID NO: 14 moltype = AA length = 1169
 FEATURE Location/Qualifiers
 source 1..1169
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 14

MLVKSLLFTVI	PLFALCSATL	YDSGSYVYYY	QSAFRPPNGW	QLHGGAYAVV	NVSTETGSAN	60
RCTAGAISFS	KNFSAASVAM	TAPANGMTWS	DAQFCTAHCN	FTNIVVFVTH	CFKNRPNYCS	120
LTGLLPQVSI	RIAAKMSNGT	GPSDLFYNLT	VPVTYKPKFR	SLQCQVNNQTS	VYVYNGDLVFT	180
SNETVDISGA	GVHFAAGGPI	TYKVMREVKA	LAYFVNGTAQ	DVILCDGTPR	GLLACQYNTG	240
NFSDGFYPPT	NSSLVKERFI	VYRENSVNTT	LVLHNVTFN	ETSAPNGGDL	NANFQIYQTV	300
TAQSGYYNPN	FSFLSGFVYK	ESDFMYGSYH	PNCNFRPENI	NNGLWFNLSL	ISLAYGPLQG	360
GCKQSVFNRR	ATCCYAYSYN	GPHACKGVYR	GQLTQLFECG	LLVYITKSDG	SRIQTATKAL	420
VVTNMFVNNI	TLDRCVEYNI	YGRVGGQFIT	NVTDSTADYN	YLADGGLAIL	DTSGAIDIFV	480
VQGVYGLNFI	KVNPCEDEVQ	QFVVSQGLK	GILTSRNETD	SQFLENQFYI	KLTNETHGGG	540
VPVSENVTS	PVVSYGKFCI	KPDGISTIV	PEELKQFVSP	LLNVTEYVLI	PDSFNLTVTD	600
EYIQTRMDKV	QINCLQYVCG	NSFECRNLFQ	QYGPVCDNIL	SVVNSVGQKE	DMELLTFYSS	660
TKPAGYNTVP	FNNISTGDFN	ISLLLTTPST	PSGRSFIEDL	LFTSVESVGL	PTDEAYKKCT	720
AGPLGFLKDL	ACAREYNGLL	VLPPIITAEM	QTLYTSSLVA	SMALGGITAA	GAIPFATQLQ	780
ARINHLGITQ	TILLKNQEKI	AASFNKAIGH	MQEGFKSTSL	ALQQIQDVVN	KQSAILTETM	840
ASLNKNFGAI	SSVIQEIYQ	LDSIQANAQV	DRIITGRLLS	LSVLASSKQA	EYLRVQQORE	900
LATQKINECV	KSQSTRYSFC	GNGRHVLTIP	QNAPNGIVFI	HFTYTPESFV	NVTAIVGFCV	960
NPANASQYAI	VPANRGRIFI	QVNGSYYITA	RDYMPRDIR	AGDIVTLTSC	QANYVSVNKT	1020
VITTLVDNDD	FDPHDELKSW	WNETKHELDP	FDQFNVTIPV	LNITYDIDKI	EEVIKGLNDS	1080
LIDLETLIL	KTYIKWPGSG	YIPEAPRDGQ	AYVRKDGWV	LLSTPLGRSL	EVLFGQPGSA	1140
WSHPQFEKGG	SGSGGGSGGS	AWSHPQFEK				1169

SEQ ID NO: 15 moltype = AA length = 1172
 FEATURE Location/Qualifiers
 source 1..1172
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 15

MSVLLPLLV	LLCALCSAVL	YDINSYVYYY	QSAFRPSNGW	HLVGGAYAVV	NVSNEMNNAG	60
SASTCTAGAI	GYSKNFSAA	IAMTAPPSGM	AWSTAAPCTA	HCNFTNFVVF	VTHCYKSGSG	120
SCPLTGFIQS	GYIRISAMKK	ECSGPSCLFY	NLTESVSKYP	TFRSLQCQVNN	YTSVYVYNGDL	180
VFTSNYTQDV	VAAGVHFKSG	GPITYKVMRE	VKALAYFVNG	TAQDVILCDD	TPRGLLACQY	240
NTGNFSDGFY	PFTNTSIVKD	KFIVYRESSV	NTTLTLTNFT	FSNESGAPPN	TGGVNSFILI	300
QTQTAQSGYY	NFNFSFLSGF	VYESNMYMG	SYHPLCSFRP	ENINNGLWFN	SLSVSITYGP	360
LQGGCKQSFF	QGRATCCYAY	SYNGPRACKG	VYSGELTQSF	ECGLLVYITK	SDGSRITQAT	420
KAPVVTNFI	NNITLDKCE	YNIYGRVGG	FITNVTDSAF	GYNYLQDGL	AALDTSGLAD	480
IFVVKGVVGL	NYKYKNPCED	VNQQFVVS	TLVGVLTSRN	ETGSQFLENQ	FYIKLTNGTH	540
GGGVVNVN	TSCPVVSYGK	FCIKPDGSTS	VIVPKELEQF	VTPLLNATEY	VPIPDSPNLT	600
VTDEYIQTRM	DKVQINCLQY	VCGNSFECRN	LFQYQGPVCD	NILSIVNSVS	QKEDMELLTF	660
YSSTKPGFEN	TPILSNLSTG	DFNISLLTTP	PSSTTGRSFI	EDLLFTSVES	VGLPTDDAYK	720
KCTAGPLGFL	KDLACAREYN	GLLVLPPIIT	AEMQTMYS	LVASMALGGI	TAGAIPFAT	780
QLQARINHLG	ITQAVLLKNQ	EKIAASFNKA	IGQMGEGRS	TSALALQQIQ	VVNKQSAILT	840
ETMASLNKNF	GAISSVIQDI	YQQLDVIQAD	AQVDRILTGR	LSSLSVLASA	KQSEHIIASQ	900

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SEQ ID NO: 19      multype = DNA  length = 22
FEATURE            Location/Qualifiers
source             1..22
                  mol_type = other DNA
                  organism = synthetic construct
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SEQUENCE: 19
atgctcaacc tagtccctag ca 22

SEQ ID NO: 20 moltype = DNA length = 21
FEATURE Location/Qualifiers
source 1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 20
tcaaaactgcg gatcatcacg t 21

SEQ ID NO: 21 moltype = AA length = 1169
FEATURE Location/Qualifiers
source 1..1169
mol_type = protein
organism = Infectious Bronchitis Virus

SEQUENCE: 21
MLVKSLLPLVT ILFALCSANL YDNESFVYYY QSAFRPGHGW HLYGGAYAVV NVSSENNNAG 60
TAPSTAGAI GYSKNLSAAS VAMTAPLSGM SWSANSFCTA HCNFTSYIVF VTHCYKSGSN 120
SCPLTGLIPS GYIRIAAMKH GSAMPGHLFY NLTVSVTKYP KFRSLQCQVNN YTSVYLLNGDL 180
VFTSNYTEDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAHDVILCDD TPRGLLACQY 240
NTGNFSDGFY PPTNTSIVKD KFIYVRESSV NNTLTLTNFT FSNESGAPPN TGGVDSFILY 300
QTQTAQSGYY NFNFSFLSSF VYRESYMYG SYHPRCSFRP ETLNGLWFN SLSVSLTYGP 360
IQGGCKQSVF NGKATCCYAY SYGGPRACKG VYRGELTQHF ECGLLVYVTK SDGSRITAT 420
QPPVLTONFY NNINLKGKVD YNIYGRIGQG LITNVTDLAV SYNLYSDAGL AILDTSGAID 480
IPVVQGEYGP NYYKVNPCED VNQQFVVSOG KLVGILTSRN ETGSQLENQ FYIKITNGTR 540
RSRRSVTENV TNCPVSVYK FCIKPDGSI VIVPKELDQF VAPLLNVTEY VLIPNSFNLT 600
VTDEYIQTRM DKIQINCLQY VCGNSLACRK LFQQYGPVCD NILSVVNSVG QKEDMELLNF 660
YSSTKPARFN TPVFSNLSTG EFNISLLLT PPSRRRSFI EDLLFTSVES VGLPTDDAYK 720
MRTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTLYTSS LVASMAFGGI TAAGAIPFAT 780
QLQARINHLG ITQSLLLKNQ EKIAASFNKA IGHMQEGFRS TSLALQQIQD VVNKQSAILT 840
ETMLALNKNF GAISSVIQDI YQQLDISIAD AQVDRILITGR LSSLSVLASA KQSEYIRVSQ 900
QRELATQKIN ECVKSQIRY SFCNGRHLV TIPQNA PNGI VFIHFTYTP SFINVTAVVG 960
FCVSPANASQ YAIVPANRGR IFIQVNGSY ITARDMYMPR DITAGDIVTL TSCQANYVSV 1020
NKTVITTFVD NDDPFDFDEL SKWNETKHE LPDFDKFNYT VPILDIDSEI DRIQGVIOQL 1080
NDSLIDLETL SILKYIKWP WYVWLAIAFA TIIFILILGW LFFMTGCCGC CCGCFGIPL 1140
MSKCGKSSY YTFDNDVVT EQYRPKKS 1169

SEQ ID NO: 22 moltype = AA length = 3510
FEATURE Location/Qualifiers
source 1..3510
mol_type = protein
organism = Infectious Bronchitis Virus

SEQUENCE: 22
ATGCTTGTA AATCCCTGTT TTTGGTGACT ATCCTTTTGG CACTGTGCTC CGCTAATCTT 60
TACGACAACG AGTCCTTTGT CTACTACTAC CAGTCCGCCT TCAGGCCAGG CCATGGATGG 120
CACCTCTACG GAGGCGCCTA TGCAGTGGA AATGTGTCTA GTGAAAAACA TAACGCCGGG 180
ACCGCGCCGT CTGCACACG AGGGGCCATT GGCTATTCCA AAAACCTCAG TGCTGCCTCC 240
GTAGCCATGA CAGCTCCCTCT CTCTGGGATG TCCTGGAGTG CCAATTCATT CTGCACCGCT 300
CATTGCAATT TCACATCATA TATTGTGTTT GTTACGCACT GCTACAAGAG CGGCTCTAAC 360
AGTTGCCCCC TCACAGGTTT GATACCTTCT GGATATATCC GAATTGCAGC AATGAAGCAC 420
GGTAGTGCTA TGCTTGGACA TCTTTTGTAC AATCTGACTG TGAGCGTGAC CAAGTATCCA 480
AAATTCCGTT CCCTGCAGTG TGTGAACAAC TACACTTCTG TTTACTTGAA CGGAGACCTA 540
GTCTTCTACT GCAATTATAC CGAAGATGTG GTCGCTGCTG GGGTGCACCT CAAATCAGGG 600
GGCCCAATTA CGTACAAGGT GATGAGAGAA GTGAAGGCAC TAGCCTATT CTGAACCGGT 660
ACTGCACACG ATGTATCCT ATGTGACGAC ACACCTCGCG GATTGTAGC CTGCCAGTAT 720
AACACAGGAA ACTTCTCAGA TGGTTTTTAT CCATTTACGA ACACCTCCAT TGTGAAGGAC 780
AAATTTATTG TTTACCGGGA GAGTTCTGTC AACACAACAC TAACACTCAC AAATTTTACA 840
TTCTCTAATG AATCAGGAGC GCCACCAAT ACGGGTGGAG TGGATTCTTT CATACTCTAT 900
CAGACACAAA CCGCTCAATC AGGGTATTAC AACTTTAATT TCAGCTTTCT CAGTTTCAAT 960
GTTTACAGGG AGTCTTACTA CATGTATGGC TCCTACCACC CACGCTGCAG TTTTACAGCT 1020
GAGACATTGA ACAACGGCCT GTGGTTCAAC TCGTATCGG TATCCCTAAC CTATGGGCGG 1080
ATTCAGGGCG GCTGCAACAC GTCTGTCTTC AACGGAAAAA CTACCTGCTG CTATGCTTAC 1140
TCGTATGGAG GGCACAGAGC TTGCAAGGGA GTGTATCGAG GAGAACTCAC ACAGCACTTT 1200
GAGTGCAGGC TGTTGGTTTA CGTCACTAAA TCCGACGGCA GTAGATTCA GACAGCAACA 1260
CAGCCACCAG GTTTAAACCA GAACCTTTAT AATAATATAA ACCTAGGGAA ATGTGTTGAC 1320
TACAACATTT ATGGAAGAA CCGGCAGGGG CTGATCACC AATGCACAGA TCTTGCTGTT 1380
TCTTATAATT ACCTTTTACA TGCTGGTCTG GCAATCTTGG ACACCAAGTG CGCTATCGAC 1440
ATCTTTGTCT TGCAAGGAGA GTATGGTCCC AATTATTACA AAGTGAACCC CTGTGAGGAG 1500
GTGAACGAGC AGTTTGTGTT TAGCGGGGCG AAGCTTGTG GCATACTCAC CAGCAGAAAT 1560
GAAACTGGAT CCCAGTTACT TGAATAACAG TTCTACATCA AAATCACTAA CGGTACACGA 1620
AGGAGCCGAA GGTCACTCAC GGAGAACGTT ACTAAGTGT CTTATGTGAG TTACGGTAAG 1680
TTCTGCATTA AGCCAGATGG AAGCATTAGC GTGATCGTCC CCAAGGAAGT GGATCAGTTT 1740
GTAGCTCCAT TGCTTAATGT TACAGAATAT GTGCTTATTC CAAACTCCTT CAACCTGACT 1800
GTGACTGATG AATACATTCA GACCAGGATG GACAAGATAC AAATAAACTG CCTGCAATAT 1860
GTTTGTGGGA ATAGTCTAGC ATGCAGGAAA CTGTTTCAGC AGTATGGCCC CGTGTGTGAT 1920
AATATCCTTA GTGTGTTTAA TAGTGTGTTG CAGAAGGAGG ATATGGAGCT GCTCAATTTT 1980
TACAGCAGTA CTAACCCAGC CCGGTTCAAT ACTCCTGTGT TTTCCAACCT CTCACAGGA 2040
GAATTTAATA TCTCTCTGCT GTTAACCCCG CCCTCCAGCC CTCGCGCGGC GTCATTTATC 2100

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GAAGACCTCC	TTTTCACTCC	AGTGGAGTCA	GTAGGTCTGC	CCACGGATGA	TGCATACAAG	2160
ATGAGAACCG	CGGGGCCTCT	GGGCTTCTTG	AAAGATCTCG	CGTGTGCTAG	AGAGTACAAT	2220
GGTCTGCTGG	TGTTGCCTCC	GATTATTACC	GCTGAGATGC	AGACTCTGTA	CACTAGCTCA	2280
CTGGTGGCAT	CAATGGCGTT	CGGAGGAATA	ACCGCCGCCG	GAGCAATTCC	CTTCGCCACA	2340
CAACTGCAGG	CCAGAATCAA	CCATCTGGGG	ATCACTCAGA	GCTTATTGCT	GAAGAACCA	2400
GAGAAGATCG	CTGCATCGTT	CAACAAAGCG	ATAGGCCACA	TGCAGGAAGG	TTTCCGCTCC	2460
ACCTCTTTGG	CTTTGCAACA	GATACAGGAC	GTAGTCAATA	AGCAGTCTGC	AATCTTGACG	2520
GAAACCATGC	TGGCTCTGAA	CAAAAATTTT	GGAGCCATAT	CTAGTGTGAT	TCAAGATATT	2580
TACCAGCAGC	TGGACAGCAT	CCAGGCAGAT	GCACAAGTCG	ATCGTCTGAT	TACCGGTCGG	2640
TTAAGCAGCC	TATCTGTTTT	GGCCAGCGCA	AAACAATCTG	AATATATCCG	GGTCTCCCAA	2700
CAGAGAGAAT	TAGCAACGCA	AAAAATAAAT	GAATGTGTCA	AGAGTCAATC	CATCCGCTAC	2760
TCGTTCTGTG	GGAACGGCAG	GCATGTGCTG	ACTATCCCCC	AGAATGCTCC	TAAACGGTATC	2820
GTATTATATC	ATTTCACATA	TACACCAGAA	TCTTTTATTA	ATGTGACGGC	GGTAGTCGGT	2880
TTTTGTGTGT	CACCTGCCAA	TGCATCTCAG	TACGCTATAG	TGCCCGCAA	TGGGAGAGGG	2940
ATTTTCATTC	AGGTGAACGG	AAGCTATTAC	ATCACGGCCC	GCGACATGTA	CATGCCTCGT	3000
GACATCAGAG	CTGGCGACAT	AGTAACACTT	ACTAGCTGTC	AAGCGAACTA	CGTGAGTGTG	3060
AATAAAACTG	TTATCACGAC	CTTCGTGGAC	AATGATGATT	TTGACTTCGA	CGATGAGCTT	3120
TCTAAATGGT	GGAATGAGAC	TAGCATGAG	CTGCCCGACT	TTGATAAGTT	TAACACACA	3180
GTACCCATTC	TCGATATTGA	TAGTGAGATC	GATCGTATTC	AGGGTGTTAT	CCAAGGACTT	3240
AACGACAGCG	TGATAGACCT	GGAGACGCTG	AGCATTCTGA	AGACATACAT	AAAGTGGCCA	3300
TGGTACGTCT	GGCTGGCCAT	TGCCTTTGCG	ACAATAATTT	TTATTTTGAT	TCTGGGCTGG	3360
CTCTTCTTCA	TGACTGGTTG	CTGTGGCTGT	TGTTGCGGGT	GCTTCGGTAT	CATCCCTCTG	3420
ATGAGCAAGT	GTGGCAAAAA	GTCAAGCTAT	TATACTACCT	TTGATAACGA	TGTGGTAACA	3480
GAGCAGTACC	GCCCGAAAAA	GTCCGTGTGA				3510

SEQ ID NO: 23 moltype = AA length = 1172
 FEATURE Location/Qualifiers
 source 1..1172
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 23

MLVKSPPFIVT	LLCALCSASL	YDNGSYVYYY	QSAFRPSIGW	HLHGGAYAVV	NVTQEYNNAG	60
SASECTAGAI	VWSKNFSAAS	VAMTAPHSGM	SWSVKQFCTA	HCNFTNFVVF	VTHCFKDLGN	120
TCPLTGRIDQ	GYIRIAAMKN	TGTGPRDLFY	NFTVSVTKYP	SPKSLQCVNN	QTSVYLNGLD	180
VFTSNETVDV	SGAGVHFKAG	GPITYKVMRE	VKALAYFVNG	TAQDVILCDS	SPRGLLACQY	240
NTGNFSDGFY	PFTNSSVVEK	KFIVYSENSV	NTTLVLHNF	FYNESDAPPN	SQSSAGVGG	300
LTTYQTQTAQ	SGYVNFNFSF	LSSFVYKESN	FMYGSYHPQC	NFRPENINNG	LWFNSLSVSI	360
TYGPLQGGCK	QSVFSHRATC	CYAYSYNPH	ICKGVYSGQL	HMNFECGLLV	YITKTDGSR	420
QTATTPPVRT	QHFYNNITLH	KCVEYNIYGR	VQGFIITNVT	DSVAGYNYLQ	DGGLAILDTS	480
GAIDIFAVQG	GYGLNFYKVN	PCEDVNQQFV	VSGGNLVGIL	TSRNETDSQP	LENQFFVKLI	540
NGTRRSRRSI	SENVTSCSFV	SYGKFCIKPD	GSISTIVPKE	MEQFVAPLLN	VTEHVLIPDS	600
FNLTVTDEYI	QTRMDKVQIN	CLQYVCGNSF	ECRQLFQQYG	PVCDNILSVV	NSVGQKEDME	660
LLSPYSSTKP	AGYNTPVFNI	STGDFNISLL	LPPSSAPSGR	SFIEDLLFTS	VESVGLPTDE	720
AYKKCTAGPL	GFLKDLACAR	EYNGLLVLPP	IITAEMQTLY	TSSLVASMAL	GGITAAAGAP	780
FATQLQARIN	HLGITQTALL	KNQEKIAASF	NKAIGHMQEG	FKSTSLALQQ	IQDVVNKQSA	840
ILTETMASLN	KNFGAIISSI	QEIYQQQLDAI	QANAQVDRLI	TGRLSSLSVL	ASSKQAEYLR	900
VSQQRELATQ	KINECVKSPQ	TRYSPFCNGR	HVLTIPOQAP	NGIVFIHFTY	TPESFVNVT	960
IVGFCINPAN	ASQYAIVPAN	GRGIFIQVNG	TYIITARDMF	MPRDI TAGDV	VTLTSCQANY	1020
VSVNKTVITT	FVESDDDFDF	DELSKNWNET	KHEFPDPDF	NYTIPVLNIT	YDIDKIEEVI	1080
KGLNDSLIDL	ETLSILKTYI	KWPWYVWLAI	FFAIIIFILV	LGWIFFMTGC	CGCCCGCFGI	1140
IPLMSKCGKK	SSYYTTFDND	VVTEQYRPPK	SV			1172

SEQ ID NO: 24 moltype = DNA length = 3519
 FEATURE Location/Qualifiers
 source 1..3519
 mol_type = other DNA
 organism = Infectious Bronchitis Virus

SEQUENCE: 24

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SEQ ID NO: 25      moltype = AA length = 1166
FEATURE
source            Location/Qualifiers
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                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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LTGLIPQNIY RIAAMKSNGT GPSDLFYNLT VPVTKYPKFR SLQCVNNQTS VYLNGLDVF 180
SNETVDISGA GVHFAAGGPI TYKVMREVKA LAYFVNGTAQ DVILCDGTPR GLLACQYNTG 240
NFSDFGYFFT NSSLVKERFI VYRENSVNTT LVLHNVTFN ETSAPNGDDL NANFQIYQTV 300
TAQSGYVNFN FSFLSGFVYK ESDFMYSYH PNCNFRPENI NNLWFNLSL ISLAYGPLQG 360
GCKQSVFNRR ATCCYAYSYN GPHACKGVYR GQLTQLFECG LLVYITKSDG SRIQTATKAL 420
VVTTFNYNNI TLDRCVEYNI YGRVGQGFIT NVTDSADYN YLADGGLAIL DTSGAIDIFV 480
VQGVYGLNFY KVNPCEDVNO QFVVSQGLV GILTSRNETD SQFLENQFYI KLTNETHRSR 540
RSVSENVTSV PVSYGKFCI KPDGSISTIV PEELKQFVSP LLNVTEYVLI PDSFNLTVTD 600
EYIQTRMDKV QINCLQYVCG NSFECRNLFQ QYGPVCDNIL SVVNSVGQKE DMELLTFYSS 660
TKPAGYNTPV FNNISTGDFN ISLLLTTPST PSGRSFIEDL LFTSVESVGL PTDEAYKKCT 720
AGPLGFLKDL ACAREYNGLL VLPPIITAEM QTLYTSSLVA SMALGGITAA GAIPFATQLQ 780
ARINHLGITQ TILLKNQEKI AASFNKAIGH MQEGFKSTSL ALQQIQDVVN QSAILLTETM 840
ASLNKNFGAI SSVIQEIYQQ LDSIQANAQV DRIITGRLSS LSVLASSKQA EYLRVSQQRE 900
LATQKINECV KSQSTRYSFC GNGRHLVTIP QNAPNGIVFI HFTYTPESFV NVTAVGFCV 960
NPANASQYAI VPANGRGIFI QVNGSYYITA RDMYMPRDI AGDIVTLTSC QANYVSVNKT 1020
VITTLVDNDD FDFHDELSKW WNETKHELDP FDQFNYPITV LNIITYDIDKI EEVIKGLNDS 1080
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SEQ ID NO: 26      moltype = DNA length = 3501
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source            Location/Qualifiers
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                  mol_type = other DNA
                  organism = Infectious Bronchitis Virus

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ttcctaata tgtgtgctct cgtgacacac tgcttcaaga acagacccaa ctactgtagc 360
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aggccaaaga agagtgtgtg a 3501

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SEQ ID NO: 27      moltype = AA      length = 1169
FEATURE
source             Location/Qualifiers
                   1..1169
                   mol_type = protein
                   organism = Infectious Bronchitis Virus

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SCPLTGPIQS GYIRISAMKK ECGSPCLFY NLTESVSKYP TFRSLQCINN YTSVYVYNGDL 180
VFTSNYTDV VAAGVHFKSG GPITYKVMRE VKALAYFVNG TAQDVILCDD TPRGLLACQY 240
NTGNFSDGFY PFTNTSIVKD KFIVYRESSV NTTTLTLNFT FSNESGAPPN TGGVNSFIFY 300
QTQTAQSGYY NFNFSFLSGF VYEESNYMYG SYHPLCSFRP ENININGLWPN SLSVSITYGP 360
LQGGCKQSFY QGRATCCYAY SYNGPRACKG VYSGELTQSF ECGLLVYITK SDGSRITQAT 420
KAPVVTNIFY NITLTDKVE YNIYGRVGGG FITNVTDFAF GYNLYQDGLL AILDTSGLD 480
IFVVKGVYGL NYYKVNPCED VNQQFVVSOG TLVGVLTSRN ETGSQFLENQ FYIKLTNGTH 540
RSRRSVNENV TSCPVSYGK FCIKPDGST VIVPKELEQF VTPLLNATEY VPIPDFFNLT 600
VTDEYIQRN DKVQINCLQY VCGNSFEERN LFQYQGVPCD NILSIVNSVS QKEDMELLTF 660
YSSTKPPGFN TPILSNLSTG DFNISLLLT PSSTTGRSFI EDLLFTSVES VGLPTDDAYK 720
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QRELATQKIN ECVKSQSTRY SPCGNRHLV TIPQNPNGI VFIHFTYTP SFVNVTIAGV 960
FCVKPANASQ YAIVPANRGR IFIQFNSSYY ITARDMYMPR NITAGDIVTL TSCQSNYVS 1020
NKTVITTFVD NDDFDFFDEL SKWWDTKHE LPDFDEFNYT APILDIDSEI DRIQGVIGQL 1080
NDSLIDLETL SILKTYIKWP VYVWLAIFA TIIFILILGW VFFMTGCCG CCGCFGIPL 1140
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SEQ ID NO: 28      moltype = DNA length = 3510
FEATURE           Location/Qualifiers
source            1..3510
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                  organism = Infectious Bronchitis Virus

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SEQ ID NO: 29      moltype = AA length = 1159
FEATURE           Location/Qualifiers
source            1..1159
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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GSIPQHSIRV  SAMKKGRLEF  NLTVSVNKYP  TFKSFQCVNN  FTSVYLNGDL  VYTSNETTDDV  180
TSAGVYFNAG  GPITYKVMRE  VKALAYFVNG  TAQDVILCDG  SPRGLLSQCY  NTGNFSDGFY  240
PFTNSSLVKQ  KFIVYRENSI  NNTLKLHNFT  FHNETGANPN  LSGVQNIQTY  QTQTAQSGYY  300
NPNFNSFLSG  FYKESNFMYG  SYHPSNCFRP  ETINNLWLFN  SLSVSIAYGP  LQGGCKQSVF  360

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TYKVNPCED	VNQQFVSVG	KLVGILTSRN	ETGSQLENQ	FYIKITNGTR	RSRSITANV	540
TNCPYVSYGK	FCIKPDGSVS	AIVPKELEQF	VAPLLNVTEN	VLIIPNSPNT	VTDEYIQRN	600
DKIQINCMQY	VCGNSLDCRK	LFQQYGPVCD	NILSVVNSVG	QKEDMELLNF	YSSTKPSGFN	660
TPVFSNLSGT	DFNISLLTTP	PSSTTGRSFI	EDLLFTSVES	VGLPTDEAYK	KCTAGPLGFL	720
KDLACAREYN	GLLVLPPIIT	AEMQTLTYSS	LVASMAFGGI	TAAGAIPFAT	QLQARINHLG	780
ITQSLLOKNO	EKIAASFNKA	IAVVQEGFRS	TSLALQQVQD	VVNKQSAILT	ETMASLNKNF	840
GAISSVIQDI	YQQLDAIQAN	AQVDRILITGR	LSSLSVLASA	KQAEYIRVSQ	QRELATQKIN	900
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YAIVPANGRG	IFIQVNGSY	ITARDMYMPR	DITAGDIVTL	TSCQANYVSV	NKTVITTFVD	1020
NDDPDDDEL	SKWWDNTHKE	LPDFDEFNYT	VPILDIGSEI	DRIQGVIOGL	NDSLIDLETL	1080
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SEQ ID NO: 30 moltype = AA length = 3480
 FEATURE Location/Qualifiers
 source 1..3480
 mol_type = protein
 organism = Infectious Bronchitis Virus

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CACCTGCATG	CGCGAGCCTA	TGCTGTGGTA	AACACTTCAA	TCGAGTCAAA	CACCTGAGG	180
GAAATGATTG	TAGGGATTAT	AGGTGGTGAC	AGAGTTGTGA	ATGCTAGCAG	CATCGCTATG	240
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ACAAGTGCTG	GTGTTTATT	CAATGCAGGA	GGGCCTATCA	CATATAAAGT	GATGAGGGAG	600
GTGAAAGCGC	TGGCTTACTT	CGTGAACGGG	ACGGCCGAGG	ATGTGATCCT	GTGTGATGGC	660
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SEQ ID NO: 31      moltype = AA  length = 1159
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source            1..1159
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                  organism = Infectious Bronchitis Virus

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NVNINLGLCV DYNVYGITGR GLITNITESV HPGYLDHGGGL VLLDATGSID TFLVHSDKLT 480
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ECVKSQSNRH GFCGEGMHIM SIPQNAPNGI VFLHFTYTPE TYANVTAVVG FCVKPGNGTE 960
YGLVPVVG RG IPIEVNGTYY ITGRDMYSR AITAGDVVKL TPCQANYQSI NRTVITTFVD 1020
EDDFDFDELH SKWNETSRD FPNLDEFNYT IPVLNISNEI DKIQEVIQGL NDSIIDLETL 1080
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YTTFDNDVVT EQYRPKKS 1159

SEQ ID NO: 32      moltype = DNA  length = 3480
FEATURE           Location/Qualifiers
source            1..3480
                  mol_type = other DNA
                  organism = Infectious Bronchitis Virus

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SEQ ID NO: 33 moltype = AA length = 1162
 FEATURE Location/Qualifiers
 source 1..1162
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 33

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SSYYTTFDND	VVTEQNRPKK	SV				1162

SEQ ID NO: 34 moltype = DNA length = 3489
 FEATURE Location/Qualifiers
 source 1..3489
 mol_type = other DNA
 organism = Infectious Bronchitis Virus

SEQUENCE: 34

atgctgtgca	cgccctctgct	gcttgcacaa	ctgttgtgctg	tcttgtgtag	cgcggtctctg	60
tatgattcaa	gtagctatgt	ttactactac	cagtcgcgat	ttaggccacc	taacggctgg	120
cacttacacg	cgaggagctta	tgccgtgggt	aatatttcta	gtgagagcaa	caacgcggcg	180
agctcgcccg	gctgattgt	tggtacgatt	catggagggc	gggtgggttaa	cgctagcagc	240
atagctatga	cagctccatc	cagcgggatg	gcttggctgt	cttcacaatt	ctgcactgca	300
cactgcaatt	tctcagatac	tactgtgttt	gtgacacatt	gttacaagta	cgacggttgt	360
cctatcacgg	gtatgctcca	gaagaacttc	ctgcgcgtca	cgcccatgaa	aaatggccag	420
ttgttctata	acctgacgtg	gtctgtagct	aaatacccta	ccttcaagtc	ttccagtgcc	480
gtcaacaatc	taacaagtgt	gtatttgaac	ggcgatctcg	tatacacttc	caatgaaacc	540
acagatgtaa	catctgcagg	tgtttatttc	aaagccgggtg	gccccattac	atataaagt	600
atgcgcgagg	tgaaagccct	cgcttacttc	gtcaatggca	cgcccaaga	tgtgatactt	660
tgcgacgggt	cacctcgtg	ctgttggtgc	tgccagtaca	atacaggaaa	ttttagcgat	720
ggattttatc	ctttcatcaa	ttcatccctg	gtgaagcaaa	aatattattgt	atatagagaa	780
aacagtgtaa	acaccacctt	tacgtgcgat	aacttcactt	tccacaacga	aactggggca	840
aaccggaatc	caagtgggtg	acagaatata	caaacatacc	agacccaacc	tgcccagctc	900
ggctactaca	actttaattt	ctcttttttg	agcagcttcg	tttataagga	gtccaaactc	960
atgtatggtt	cctatcaccc	cagctgtaat	tttaggctgg	agaccatcaa	taacgggctg	1020
tggtttaact	cccttagtgt	gagcattgca	tatgggcccc	tccaaggggg	ctgtaaaaca	1080
tctgttctca	ggcgagagcg	aacatgttgt	tacgcctatt	cttatggagg	cccgctccct	1140
tgcaaggggag	tatactccgg	tgagcttgat	ctcaattttg	aatgcggctt	attagtatat	1200
gtgacaaagt	caggaggatc	tagaatccag	accgctaccg	aacctcctgt	gatcaccgcg	1260
cataattata	ataattatcac	tttgaatact	tgtgtcgatt	acaacatcta	cggcaggagc	1320
gggcaagggt	ttataaccaa	cgtgacagac	agtgcagttt	cctacaacta	cctggctgac	1380
gcccgcctcg	caatacttga	tacaagtggc	tcgatagata	tcttcgtcgt	gcagggggag	1440
tatgggctga	cttattataa	ggtgaatcct	tgtgaggagc	tgaaccagca	gtttgtggtc	1500
agtggcggca	aactagtcgg	aattctgaca	tctcgtaatg	aaacaggga	ccagcttcta	1560
gaaaatcaat	tctacattaa	aataacaacc	gggaccagac	gcttcgcccg	gagtataacc	1620
gaaaacgtag	ccaactgcgc	ctacgtttcc	tatggaaagt	ctgcataaaa	accgatggga	1680
agtattgcga	ctatcgtccc	aaagcagttg	gagcagttcg	ttgcaccact	gctgaatgtg	1740
actgaaaacg	ttctgatctc	aaactcattt	aatttaacag	tgacagatga	atacatagag	1800
actagaatgg	acaaagtaca	gattaaactgt	ctgcagtacg	tttgggcaaa	ctcactggag	1860
tgcaagggact	tgtttcagca	gtatggggcc	gtgtgtgaca	atattctttc	tgtcgtgaat	1920

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tcgatcggtc agaaggagga catggaactg ctcaacttct actctagcac taaacgggt 1980
ggctttaata cgccattcct gtcaaacgtt tccaccggtg aatttaacat ttcgcttctc 2040
ttgactactc catcttcccc acgtcggagg agctttattg aggatctgct gttcactagt 2100
gtagagtctg tgggtctccc aacggatgat gcatacaaaa attgcaccgc gggggccctt 2160
gggttcctaa aggatcttgc atgcgcgagg gaatacaacg gtctgctcgt actgcccgca 2220
atcattactg cagagatgca aacctctac acgagctcct tgggtggcttc catggccttt 2280
ggagggtatca ctgctgctgg tgctataccg tttgcaacgc agctacaagc ccgaataaac 2340
catctcggca tcacacagtc ttactctta aaaaatcagg agaagatcgc tgcagcttt 2400
aacaaggcca ttggtcgaaat gcaggaaggc tttcgagca caagtctggc cctccagcag 2460
attcaggacg tagtcaacaa acagagcgca atcctgaccg aaaccatggc tagcctcaac 2520
aagaatttcg gagcaatctc ttctgtgatt caggagatct atcagcaatt ggatgccata 2580
caagccaacg cacagggtga caggctaata acgggtagac tgtcatccct gagcgttctt 2640
gcttcagcca agcaggcgga acacattcgg gttagtcaac agagagagct gccactcag 2700
aaaatcaatg agtgcgtgaa aagccagtca atccggtact cctttgtgg aaatggcggt 2760
cacgtgctta ctattctcca aaatgcccc aatggaaattg ttttcttca ctttagttac 2820
acacccgatt cattcgtaa tgctcacagt atagtgggtt tctgtgttaa gccagcgaac 2880
gcctcaaat acgcaattgt gcccgcaaat ggacgtggaa tctttattca agtgaatggg 2940
tcttactata ttaccgcgag agatatgtat atgccaagag caataaccgc aggagacatt 3000
gtcaccattaa ccagctgcca ggctaattac gtaagcgtaa ataagacagt catcacaaac 3060
tttgttgaca acgatgactt cgactttaac gatgagctat caaagtgggtg gaacgatacc 3120
aagcatgagc tgcccgaatt tgacaagttc aactacacag tgccatctct agatatcgat 3180
tcagaaatcg acaggattca aggagtgtac cagggtctta acgactcgt tattgacct 3240
gagaaactga gcataactgaa aacatacatt aaatggccct ggtacgtgtg gctggcgatc 3300
gcgttcgcta ccatcatctt tatactgac ttgggggtggg tgttcttcat gactggctgt 3360
tgggggtgct gctgcggatg ttttgaatc atgccccga tgtccaaatg cggaaagaaag 3420
tcctcatatt atacaacatt tgacaacgat gttgtgacgg aacagaatag acccaagaaa 3480
tctgtttga 3489

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SEQ ID NO: 35      moltype = AA length = 409
FEATURE
source            Location/Qualifiers
                  1..409
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 35
MASGKATGKT DAPAPVIKLG GPKPPKVGSS GNVSWFQAIK AKKLNSPPPK FEGSGVPDNE 60
NLKPSQQHGY WRRQARFKPG KGRKRPVDA WYFYTGTP AANLNWGSQ DGIVWVAGKG 120
ADTKFRNQG TRDSKFDQY PLRFSGDPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP 180
SREVSRRGRS GSEDDLIARA ARIQDQKK GSRITKAKAD EMAHRRYCKR TIPPNYKVDQ 240
VFGPRTKGKE GNFDDKME EGIKDGRTA MLNLVPSHA CLFGSRVTPR LQPDGLHLKF 300
EFTTVVPRDD PQFDNYVKIC DQCVDGVTGR PTDDEPRPKS RSSSRPATRG NSPAPRQQR 360
KKEKKPKKQD DEVDKALTS DERNNAQLEF DDEPKVINWG DSALGENEL 409

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SEQ ID NO: 36      moltype = DNA length = 1230
FEATURE
source            Location/Qualifiers
                  1..1230
                  mol_type = other DNA
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 36
atggcgctcg gtaaagctac aggggaagact gatgctccag ctcccgtaat aaagttagga 60
ggggcaaaag caccaaaagt tggatccagt ggaaatgtta gctggttcca ggcaataaaa 120
gccaaagagc tgaattctcc acccccgaag tttgaagggt ccgggggtccc tgataacgag 180
aaccttaaac ccagccagca gcatggctac tggcgaggc agggccgatt caagcctgga 240
aaagggtgaa ggaagccgct cccagatgcc tgggtactct actacaccgg gactggcccc 300
gcggccaact tgaactgggg agactcccaa gatggcattg tgtgggtggc aggcacaaaga 360
gctgacacca agttcagaag caaccagggg accagggaca gtgacaaatt tgatcaatat 420
cctctgcgct tcagtgtatg gggctctgac ggcaattttc gctgggactt catacctctc 480
aatagaggac gtatgtgtag atctacagca gcattctcag ctgctcttc acgggcgcgc 540
agtagagaag tttcacgggg cagacgtagc ggctctgaag atgacctcat tgcacgggct 600
gcaaggatca tccaggacca acagaagaaa ggcagccgca ttacaaaggc caaagcagat 660
gaaatggctc acagacgcta ctgcaagagg acgatcccc caaattataa agtagaccag 720
gtgtttggac ctagaacaaa agggaaagaa ggaaacttcg gtgatgataa aatgaatgaa 780
gagggcatta aagatggagc tgttactgcc atgctaatac ttgttccttc ctcccatgcc 840
tgctcttttg gtacagagt cacacctcga ctgcagcccg acgggctgca cctgaagttt 900
gagttcacca ctgtgtgccc acgggatgac ccgcagtttg acaactatgt gaagatctgt 960
gatcaatgtg tggatggggg aggcactaga ccaacagacg atgaacctcg acctaaatca 1020
cggtctagtt cccggccagc caccgccgac aactccccag caccgcggca gcaaaaggccc 1080
aaaaaggaga agaaaccaa gaagcaggat gatgaggtgg acaaggcatt gacgtcagat 1140
gaggagagga acaatgtcca gctggagttt gatgacgagc ccaaagtcac caactggggg 1200
gacagcgctt tgggagaaaa tgagctgtga 1230

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SEQ ID NO: 37      moltype = AA length = 409
FEATURE
source            Location/Qualifiers
                  1..409
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 37
MASGKATGKT DAPAPVIKLG GPKPPKVGSS GNASWFQAIK AKKLNSHPPK FEGSGVPDNE 60
NLKTSQQHGY WRRQARFKPV KGRKRPVDA WYFYTGTP AADLNWGSQ DGIVWVAAKG 120
ADVKSRSHQG TRDPDKFDQY PLRFSGDPD GNFRWDFIPL NRGRSGRSTA ASSAASSRAP 180

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SRDGSRRGRS GSEDDLIARA AKIIQDQKK GSRITKVKAD EMAHRRYCKR TIPPYKVDQ 240
VFGPRTKGKE GNFDDDKMNE EGIKDGRTA MLNLVPSHA CLFGSRVTPK LQPDGLHLKF 300
EFTTVVPRDD PQFDNYVKIC DQCVDGVGTR PKDDEPRPKS RSSSRPATRT SSPAPRQQR 360
KKEKPKKKQD DEVDKALTSN EERNNAQLEF DEEPKVINWG DAALGENEL 409

SEQ ID NO: 38 moltype = DNA length = 1230
FEATURE Location/Qualifiers
source 1..1230
 mol_type = other DNA
 organism = Infectious Bronchitis Virus

SEQUENCE: 38

atggccagtg	ggaaggcgac	aggtaaaact	gacgcgccag	ctccagtcac	caaaacttgg	60
ggaccgaagc	ctccaaaggt	gggtccagtc	ggaaatgctt	cttggttcca	ggccattaag	120
gcaaaaaaac	tgaacagtc	tccacctaaa	tttgaggggt	ctggcgctcc	cgacaatgaa	180
aatctcaaaa	cgtctcagca	acatggttac	tggagaagac	aggcacgctt	caagcctgtc	240
aaagggcgta	gaaagccagt	tcttgatgct	tggtacttct	attatactgg	caccggacca	300
gcagctgatt	tgaactgggg	ggatagccag	gatggcattg	tgtgggtggc	agccaaagga	360
gccgacgtaa	aatcccgagg	ccaccagggc	accagagatc	ctgacaagtt	tgaccagtat	420
cctctcggtt	tcagtgacgg	gggtcctgac	ggaaacttcc	gctgggactt	catccccctc	480
aacaggggac	gctcaggacg	ctcaacggct	gccagctctg	cagcgctccag	ccgagcacc	540
agccgcgacg	gctcccgggg	gaggagaagc	gggttcgga	acgatctaat	tgcaagagca	600
gccaaagatc	ttcaggacca	acagaagaaa	ggttctcgga	tcaccaaggt	taaagcagat	660
gagatggcac	acggcggtta	ctgcaagagg	actattcccc	caggctataa	agttgatcag	720
gtcttcgggc	ccagaaccaa	agggaaagaa	ggcaattttg	gagacgacaa	gatgaatgag	780
gaggggaata	aggatgggag	agtaactgcc	atgctgaatc	tggtgccttc	tagccatgct	840
tgctctctcg	gcagtcagat	tacacccaag	cttcagccag	atgggctgca	ectgaagttt	900
gaatttcaaa	cagtggtgcc	gagggatgac	cctcagtttg	ataactacgt	caagatatgt	960
gaccagtggt	tgagtgggcg	gggaacacgg	cctaagatg	acgagcctag	gccccaaatg	1020
cgaagcagtt	cacgtcccg	tactagaaca	tcattccccc	cgccgcgtca	gcaaaggcca	1080
aaaaaggaga	agaagcccaa	gaagcaagat	gatgaagtgg	acaaagctct	tacctcaaat	1140
gaagagcgca	acaaacgctc	attggagttt	gatgaagaac	ccaaagtatt	caattgggga	1200
gatgcccgtt	taggagagaa	cgagctgtga				1230

SEQ ID NO: 39 moltype = AA length = 1173
FEATURE Location/Qualifiers
source 1..1173
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 39

MLVTPLLLV	LLCALCSAVL	YDSSSYVYYY	QSAFRPPNGW	HLQGGAYAVV	NISSEFNAG	60
SSSGCTVGII	HGGRVNVASS	IAMTAPSSGM	AWSSSQFCTA	HCNFSDDTVF	VTHCYKHGGC	120
PITGMLQQL	IRVSAMKNGQ	LFYNLTVSVA	KYPTFRSFQC	VNNLTSVYLN	GDLVYTSNET	180
IDVTSAGVYF	KAGGPITYYK	MREVKALAYF	VNGTAQDVIL	CDGSPRGLLA	CQYNTGNFSD	240
GFYPFTNSSL	VKQKFIIVYR	NSVNTTCTLH	NFIFHNETGA	NPNPSGVQNI	QTYQTKTAQS	300
GYYNFNFSFL	SSFVYKESNF	MYGSYHPSCN	FRLETINNGL	WFNSLSVSIA	YGPLQGGCKQ	360
SVFKGRATCC	YAYSYGGSPL	CKGVYSGELD	HNFECLLVY	VTKSGGSRIQ	TATEPPVITQ	420
NNYNNITLNT	CVDYNIYGR	GQGFITNVD	SAVSNNYLD	AGLAILDTS	SIDIFVVQGE	480
YGLNYYKVP	CEDVNOQFV	SGGKLVGILT	SRNETGSQLL	ENQFYIKITN	GTGGGVPSIT	540
ENVANCPYVS	YKGFCKIPDG	SIATIVPKQL	EQFVAPLFNV	TENVLIPNSF	NLTVTDEYIQ	600
TRMDKQVQIN	LQYVCGSSLD	CRKLQFQYGP	VCDNILSVVN	SVGQKEDMEL	LNFSSTKPA	660
GFNTPVLSNV	STGFNLSLL	LTPSSRRRK	SLIEDLLFTS	VESVGLPTND	AYKNCTAGPL	720
GFFKDLACAR	EYNGLLVLP	IITAEMQALY	TSSLVASMAF	GGITAAGAI	FATQLQARIN	780
HLGITQSLLL	KNQEKIAASF	NKAIGHMQEG	FRSTSLALQQ	IQDVVSKQSA	ILTETMASLN	840
KNFGAIVSSV	QBITYQFDAI	QANAQVDRLI	TGRLLSSLSV	ASAKQAEYIR	VSQRELATQ	900
KINECVKQS	IRYSPCGN	HVLTPQNAP	NGIVFIHFSY	TPDSFVNVT	IVGFQVCKPAN	960
ASQYAIVPAN	GRGIPIQVNG	SYIITARDMY	MPRAITAGDV	VTLTSCQANY	VSVNKTIVIT	1020
FVDNDPDFDN	DELSKWNDT	KHELPDFDKF	NYTVPILDID	SEIDRIQGVI	QGLNDSLIDL	1080
EKLSILKTYI	KWPGSGYIPE	APRDGQAYVR	KDGEVLLST	FLGRSLEVLF	QPGPHHHHHH	1140
HHSASWHPQF	EKGSGSGGGG	SGGSAWSHPQ	FEK			1173

SEQ ID NO: 40 moltype = AA length = 1180
FEATURE Location/Qualifiers
source 1..1180
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 40

MLVKSLLVLT	ILFALCSANL	YDNESFVYYY	QSAFRPGHGW	HLVGGAYAVV	NVSSENNAG	60
TAPSTAGAI	GYSKNLSAAS	VAMTAPLSGM	SWSANSFCTA	HCNFTSYIVF	VTHCYKSGSN	120
SCPLTGLIPS	GYIRIAAMKH	GSAMPGHLPY	NLTVSVTKYP	KFRSLQCQNN	YTSVYLNGLD	180
VFTSNYTEDV	VAAGVHFKSG	GPITYKVMRE	VKALAYFVNG	TAHDVILCDD	TPRGLLACQY	240
NTGNFSDGFY	PFTNTSIVKD	KFIVYRESSV	NTTLTLTNFT	FSNESGAPPN	TGGVDSFILY	300
QTQTAQSGYY	NFNFSFLSSF	VYRESYMYG	SYHPRCSFRP	ETLNNGLWFN	SLSVSLTYGP	360
IQGGCKQSVF	NGKATCCYAY	SYGGPRACKG	VYRGELTQHF	ECGLLVYVTK	SDGSRIQTAT	420
QPPVLTONFY	NNINLGKCDV	YNIYGRIGQG	LITNVTDLAV	SYNYLSDAGL	AILDTSAGID	480
IFVVQGEYGP	NYKYVNPCE	VNQQFVSSG	KLVGILTSRN	ETGSQLLENQ	FYIKITNGTG	540
GGVPSVTENV	TNCPVVSYGK	FCIKPDGSIS	VIVPKELDQF	VAPLLNVTEY	VLIPIVSFNL	600
VTDEYIQR	DKIQINCLQY	VCGNSLACRK	LFQYQGPVCD	NILSVVNSVG	QKEDMELLNF	660
YSSTKPARFN	TPVFNSLSTG	EFNISLLTLP	PSSPRRSFI	EDLLPTSVES	VGLPTDDAYK	720
MRTAGPLGFL	KDLACAREYN	GLLVLPPIIT	AEMQTLTYSS	LVASMAFGGI	TAAGAIPFAT	780

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QLQARINHLG	ITQSLLLKNG	EKIAASFNKA	IGHMQEGFRS	TSLALQQIQD	VVNKQSAILT	840
ETMLALNKNF	GAISSVIODI	YQQLDSTQAD	AQVDRLITGR	LSSLSVLASA	KQSEYIRVSQ	900
QRELATQKIN	ECVKSSQIRY	SFCGNGRHVL	TIPQNA PNGI	VFIHFTYYPE	SFINVTAVVG	960
PCVSPANASQ	YAIVPANGRG	IFIQVNGSY	ITARDMYMPR	DITAGDIVTL	TSCQANYVSV	1020
NKTVITTFVD	NDDPFDDEL	SKWNETKHE	LPDFDKFNYT	VPILDIDSEI	DRIQGVQGL	1080
NDSLIDLETL	SILKTYIKWP	GSGYIPEAPR	DQAYVRKDG	EWVLLSTFLG	RSLEVLFQGP	1140
GHHHHHHHHS	AWSHPQFEKG	GGSGGGSGG	SAWSHPQFEK			1180

SEQ ID NO: 41 moltype = AA length = 1183
 FEATURE Location/Qualifiers
 source 1..1183
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 41

MLVKSPPFIVT	LLCALCSASL	YDNGSYVYYY	QSAFRPSIGW	HLHGGAYAVV	NVTQYNNAG	60
SASCTAGAI	VWSKNFSAAS	VAMTAPHSGM	SWSVKQFCTA	HCNFTNFVVF	VTHCFKDGGLN	120
TCPLTGRIDQ	GYIRIAAMKN	TGTGPRDLFY	NFTVSVTKYP	SFKSLQCVNN	QTSVYLNGLD	180
VFTSNETVDV	SGAGVHFKAG	GPITYKVMRE	VKALAYFVNG	TAQDVILCDS	SPRGLLACQY	240
NTGNFSDGFY	PFTNSSVVEK	KFIVYSENSV	NTTLVLHNFT	FYNESDAPPN	SQQSSAGVGG	300
LTTYQTQTAQ	SGYNNFNFSF	LSSFVYKESN	FMYGSYHPQC	NFRPENINNG	LWFNSLSVSI	360
TYGPLQGGCK	QSVFSHRATC	CYAYSNGPH	ICKGVYSGQL	HNNFECGLLV	YITKTGDSRI	420
QTATTPPVRT	QHFYNNITLH	KCVEYNIYGR	VQGGFITNVT	DSVAGYNYLQ	DGGLAILDTS	480
GAIDIFAVQG	GYGLNFYKVN	PCEDVNQQFV	VSGGNLVGIL	TSRNETDSQP	LENQFFVKLI	540
NGTGGGVPSI	SENVTSCSFV	SYGKFCIKPD	GSISTIVPKE	MEQFVAPLLN	VTEHVLIPDS	600
FNLTVTDEYI	QTRMDKVQIN	CLQYVCGNSF	ECRQLFQQYG	PVCDNILSVV	NSVGQKEDME	660
LLSFYSSTKP	AGYNTVPVNI	STGDFNISLL	LPPSSAPSGR	SFIEDLLFTS	VESVGLPTDE	720
AYKKCTAGPL	GFLKDLACAR	EYNGLLVLP	IITAEMQTLY	TSSLVASMAL	GGITAAGAI	780
FATQLQARIN	HLGITQTVLL	KNQEKIAASF	NKAIGHMQEG	FKSTSLALQQ	IQDVVNKQSA	840
ILTETMASLN	KNFGAISSVI	QEIYQQLDAI	QANAQVDRLI	TGRLLSSVL	ASSKQAEYLR	900
VSQQRELATQ	KINECVKSQS	TRYSFCGNGR	HVLTIPQNA	NGIVFIHFTY	TPESFVNVT	960
IVGFCINPAN	ASQYAIIVAN	GRGIFIQVNG	TYIITARDMF	MPRDIAGDVF	VTLTSCQANY	1020
VSVNKTVITT	FVESDDFDFD	DELSKWNWET	KHEFPDFDQF	NYTIPVLNIT	YDIDKIEEVI	1080
KGLNDSLIDL	ETLSILKTYI	KWPGSGYIPE	APRDGQAYVR	KDGEWVLLST	FLGRSLEVLF	1140
QPGHHHHHHH	HHSAWSHPQF	EKGSGSGGGG	SGGSAWSHPQ	FEK		1183

SEQ ID NO: 42 moltype = AA length = 1177
 FEATURE Location/Qualifiers
 source 1..1177
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 42

MLVKSFLTIV	PLFALCSATL	YDSGSVYVYY	QSAFRPPNGW	QLHGGAYAVV	NVSTETGSAN	60
RCTAGAISFS	KNFSAASVAM	TAPANGMTWS	DAQFCTAHCN	FTNIVVFVTH	CFKNRPNYCS	120
LTGLIPQNYI	RIAAMKSNIGT	GPSDLFYNLT	VPVTKYKPKFR	SLQCVNNQTS	VYLNGLDLVFT	180
SNETVDISGA	GVHFAAGGPI	TYKVMREVKA	LAYFVNGTAQ	DVILCDGTFR	GLLACQYNTG	240
NFSDGFYFPFT	NSSLVKERFI	VYRENSVNTT	LVLHNVTFPN	ETSAPNGGDL	NANFQIYQTV	300
TAQSGYNNFN	FSFLSGFYVK	ESDFMYGSYH	PNCNFRPENI	NNGLWFNLSL	ISLAYGPLQG	360
GCKQSVFNRR	ATCCYAYSYN	GPHACKGVYR	GQLTQLFECG	LLVYITKSDG	SRIQTATKAL	420
VVTNNFYNNI	TLDRCVEYNI	YGRVGGQFIT	NVTDSADYN	YLADGGLAIL	DTSGAIDIVF	480
VQGVYGLNFI	KVNPCEDEVN	QFVVSQGLV	GILTSRNETD	SQFLENQFYI	KLTNETHGGG	540
VPVSENVTS	PVSYGKFCI	KPDGSIISTV	PEELKQFVSP	LLNVTEYVLI	PDSFNLTVTD	600
EYIQTRMDKV	QINCLQYVCG	NSFECRNLFQ	QYGPVCDNIL	SVVNSVGQKE	DMELLTFYSS	660
TKPAGYNTPV	FNNISTGDFN	ISLLLTTPST	PSGRSFIEDL	LFTSVESVGL	PTDEAYKCKT	720
AGPLGFLKDL	ACAREYNGLL	VLPPIITAEM	QTLYTSSLVA	SMALGGITAA	GAIPPATQLQ	780
ARINHLGITQ	TLLLNQEKI	AASFNKAIGH	MQEGFKSTSL	ALQQIQDVVN	KQSAILTETM	840
ASLNKNFGAI	SSVIQEIYQQ	LDSIQANAQV	DRIITGRLSS	LSVLASSKQA	EYLRVSQQRE	900
LATQKINECV	KSQSTRYSFC	GNGRHVLTIP	QNAPNGIVFI	HFTYTPESFV	NVTAIVGFCV	960
NPANASQYAI	VPANGRGIFI	QVNGSYIITA	RDYMPRPDIT	AGDIVTLTSC	QANYVSVNKT	1020
VITTLVDNDD	FDPHDELKSW	WNETKHELDP	FDQFNYTIPV	LNITYDIDKI	EEVIKGLNDS	1080
LIDLETLSIL	KTYIAKPGSG	YIPEAPRDGQ	AYVRKDGWV	LLSTFLGRSL	EVLFGPGGSA	1140
WSHPQFEKGG	GGSGGGSGGH	HHHHHHHSAW	SHPQFEK			1177

SEQ ID NO: 43 moltype = AA length = 1180
 FEATURE Location/Qualifiers
 source 1..1180
 mol_type = protein
 organism = Infectious Bronchitis Virus

SEQUENCE: 43

MSVLLPLLVT	LLCALCSAVL	YDINSYVYYY	QSAFRPSNGW	HLHGGAYAVV	NVSNENNNAG	60
SASTCTAGAI	GYSKNFSAAS	IAMTAPPSGM	AWSTA AFCTA	HCNFTNFVVF	VTHCYKSGSG	120
SCPLTGFQIS	GYIRISAMK	ECSGPSCLPY	NLTESVSKYP	TFRSLQCVNN	YTSVYLNGLD	180
VFTSNYTDV	VAGGVHFKSG	GPITYKVMRE	VKALAYFVNG	TAQDVILCDD	TPRGLLACQY	240
NTGNFSDGFY	PFTNTSIVKD	KFIVYRESSV	NTTLTLTNFT	FSNESGAPPN	TGGVNSFILIY	300
QTQTAQSGYY	NFNFSFLSGF	VYEESENYMY	SYHPLCSFRP	ENINNGLWPN	SLSVSITYGP	360
LQGGCKQSF	QGRATCCYAY	SYNGPRACKG	VYSGELTQSF	ECGLLVYITK	SDGSRIQTAT	420
KAPVVTTNFI	NNITLDKVE	YNIYGRVGG	FITNVTD SAF	GYNYLQDGL	AILDTS GAID	480
IFVVKGVYGL	NYKVNPCED	VNQQFVVS	TLVGLVTSRN	ETGSQFLENQ	FYIKLTNGTH	540
GGGVVNENV	TSCPVSYGK	FCIKPDGSTS	VIVPKELEQF	VTPLLNATEY	VPIPD SFNLT	600
VTDEYIQTMR	DKVQINCLQY	VCGNSFECRN	LFQQYGPVCD	NILSIVNSVS	QKEDMELLTF	660

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YSSTKPPGPN TPILSNLSTG DFNISLLLT PSSTTGRSFI EDLLFTSVES VGLPTDDAYK 720
KCTAGPLGFL KDLACAREYN GLLVLPPIIT AEMQTMYTSS LVASMALGGI TAAGAIPFAT 780
QLQARINHLG ITQAVLLKNQ EKIAASFNKA IGQMQEGFRS TSLALQQIQD VVNKQSAILT 840
ETMASLNKNF GAISSVIQDI YQQLDVIQAD AQVDRILITGR LSSLSVLASA KQSEHIASQ 900
QRELATQKIN ECVKSQSTRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG 960
FCVKPANASQ YAIVPANGRG IFIQFNGSYY ITARDMYMPR NITAGDIVTL TSCQSNYVSV 1020
NKTVITTFVD NDDPFDDEL SKWWDTKHE LPDFDEFNYT APILDIDSEI DRIQGVIGL 1080
NDSLIDLETL SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFGQP 1140
GHHHHHHHHS AWSHPQFEKG GSGGGGSGG SAWSHQFEK 1180

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SEQ ID NO: 44      moltype = AA length = 1170
FEATURE           Location/Qualifiers
source            1..1170
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 44
MLVTPLLLVLT LFLALCSAAL YDNSSVYYYY QSAFRPPNGW HLHGAYAVV NTSIESNNLR 60
ECIVGIIGGD RVVNASSIAM TAPQPGMDWS SRQFCTAHCN FSDITVVFVTH CYKHNGCPIT 120
GSIPQHSIRV SAMKKGRLFY NLTVSVNKYP TFKSFQCVNN FTSVYLNGDL VYTSNETTDV 180
TSAGVYFNAG GPITYKVMRE VKALAYFVNG TAQDVILCDG SPRGLLSCQY NTGNFSDGFY 240
PFTNSSLVKQ KFIVYRENSI NTTLKLHNFT PHNETGANPN LSGVQNIQTY QTQTAQSGYY 300
NFNPSFLSGF VYKSNFMYG SYHPSCNFRP ETINNGLWFN SLSVSIAYGP LQGGCKQSVF 360
SGRATCCYAY SYGSPSLCKG VYLGELKSDF ECGLLVVYTK SDGSRIQTAT EPPVITQHNY 420
NMITLNTCVD YNIYGRGTGG FITNVTDSAV SYNVLADAGM AILDTSGSID IFVVQGEYGL 480
TYYKVNPCED VNQQFVVSOG KLVGILTSRN ETGSQLENQ FYIKITNGTG GGVPSTANV 540
TNCPIVSYGK FCIKPDGVS AIVPKELEQF VAPLLNVTEN VLIPINSFNLT VTDEYIQTRM 600
DKIQINCMQY VCGNSLDCRK LPQQYGPVCD NILSVVNSVG QKEDMELLNF YSSTKPSGFN 660
TPVFSNLSTG DFNISLLLT PSSTTGRSFI EDLLFTSVES VGLPTDEAYK KCTAGPLGFL 720
KDLACAREYN GLLVLPPIIT AEMQTYTSS LVASMALGGI TAAGAIPFAT QLQARINHLG 780
ITQSLQKNQ EKIAASFNKA IAVVQEGFRS TSLALQQVQD VVNKQSAILT ETMASLNKNF 840
GAISSVIQDI YQQLDAIQAN AQVDRILITGR LSSLSVLASA KQAEYIRVSQ QRELATQKIN 900
ECVKQSIRY SFCGNGRHVL TIPQNAPNGI VFIHFTYTPE SFVNVTAIVG FCVKPANASQ 960
YAIVPANGRG IFIQVNGSYY ITARDMYMPR DITAGDIVTL TSCQANYVSV NKTVITTFVD 1020
NDDPFDDEL SKWWDTKHE LPDFDEFNYT VPILDIGSEI DRIQGVIGL NDSLIDLETL 1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFGQP GHHHHHHHHS 1140
AWSHPQFEKG GSGGGGSGG SAWSHQFEK 1180

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SEQ ID NO: 45      moltype = AA length = 1170
FEATURE           Location/Qualifiers
source            1..1170
                  mol_type = protein
                  organism = Infectious Bronchitis Virus

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SEQUENCE: 45
MLGKSLPIVT LLLALCEGGL VGVNYTYYYQ SRYRPPNGWH MQGGAYKVNN KTTISYTSQE 60
CTIGVIRGGV TINQSAIAFT SATGRVGVKK GVCITYCNYT SFYVPVTHCG GTGHNCIVNT 120
KKGVLVFGV KYNNDQFIYN ITLNAAGPYA NFKAWQCLSN YTSVFLNGNL LYTSNYTEDV 180
KAAGVYAKQV NGLERRVMRD TPVMAYFVNG TVQDVILCDD SPKGRALCQY NTGNFSDGLY 240
PVYEEPVASN FTFVPLHTSS TSYGVLHNFT FNNVTGVAPN QEHIAFPNIS TISEGYVNFK 300
FNFLNSTPYV ESDPDRGSYY KPGSRCNFG LESINRGLSF NSLTVSIGYG PISGGCKQSV 360
WKNEATCCFA KYNGGSRNC KGLYTFDRDV NYECVLLVFI SKPDGSRIRT ATSPPVYSNN 420
NVNINLGLCV DYNVYGITGR GLITNITESV HPGYLDHGG LLLDATGSID TFLVHSDKLT 480
SYYKVNPCSD INEQYVVSOG NLVGKLTSSN QTVAQQLGDM FYVKFSTSGG GGVPATSENV 540
TSCPYYTYGK FCIKPDGDIS NIVPEVKDY TSLLLNRTDY VLIPINSFNLT VTDEFIQTF 600
QKIQINCIQY VCGSSIQCK LPQQYGSVCG NILSIVNGIA LQDNAEMLHF YSSTKPRGFD 660
TNSFVNFTAG EFNISLVLPK NGQPTGRCLI EDLLFDKVES LGLPGDSAYQ KCTSGPLGFV 720
KDLVCAQNYN GLLVLPPIIT AEMQTYTSS LVVSMAPGGI TAARAIPFAT QIARINHLG 780
ITQTVLQKNQ EKIAASFNKA MKHMQDGFS TSLALQQVQD VVNEQGAILQ QTMHSLNKNF 840
GAISHVIQDI YQQLDALEAN AQVDRIITGR LSSLSVLASA KQLEYTKVSQ QRELAKEKIN 900
ECVKQSIRY GFCGEGMHM SIPQNAPNGI VFLHFTYTPE TYANVTAVVG FCVKPNGTE 960
YGLVPVVG RG IFIEVNGTY ITGRDMYSPR AITAGDVVKL TPCQANYQSI NRTVITTFVD 1020
EDDPFDDEL SKWWDNSTRD FPNLDEFNYT IPVLNISNEI DKIQEVIQGL NDSIIDLETL 1080
SILKTYIKWP GSGYIPEAPR DGQAYVRKDG EWVLLSTFLG RSLEVLFGQP GHHHHHHHHS 1140
AWSHPQFEKG GSGGGGSGG SAWSHQFEK 1180

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SEQ ID NO: 46      moltype = DNA length = 22
FEATURE           Location/Qualifiers
source            1..22
                  mol_type = other DNA
                  organism = synthetic construct

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SEQUENCE: 46
atgctcaacc tagtccctag ca 22

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-continued

SEQ ID NO: 47 moltype = DNA length = 21
 FEATURE Location/Qualifiers
 source 1..21
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 47
 tcaaaactgcg gatcatcacg t

21

What is claimed:

1. A vaccine composition comprising a polynucleotide that encodes an infectious bronchitis virus (IBV) spike(S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein, wherein the sequence encoding the S protein comprises one of SEQ ID NOs: 22, 24, 26, 28, 30, or 32 or a sequence with at least 90% identity to one of one of SEQ ID NOs: 22, 24, 26, 28, 30, or 32 and, wherein the sequence encoding the N protein comprises one of SEQ ID NOs: 36 or 38 or a sequence with at least 90% identity to one of SEQ ID NOs: 36 or 38.

2. The vaccine composition of claim 1, further comprising an adjuvant.

3. The vaccine composition of claim 2, wherein the adjuvant comprises disaggregated spherical nanostructures comprising a saponin fraction isolated from an extract of the bark of *Quillaja saponaria* (Quil A) and chitosan, and wherein the Quil A and chitosan are present at a ratio between 1:15 and 1:100.

4. The vaccine composition of claim 3, wherein the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged.

5. A method of inducing an immune response against infectious bronchitis virus (IBV) in a subject, the method comprising:

(a) administering a first vaccine composition comprising the vaccine composition of claim 1 in an amount effective to induce the immune response against at least one IBV antigen in the subject; and

(b) administering a second vaccine composition comprising a protein, nucleic acid or viral vectored vaccine composition, the second vaccine composition comprising a polynucleotide encoding an IBV S protein, an IBV N protein, or both or comprising an IBV polypeptide selected from an IBV S protein, an IBV N protein or both, wherein administration of the first vaccine composition and the second vaccine composition induces the immune response against IBV in the subject, wherein administration of the second vaccine composition occurs at least two weeks after administration of the first vaccine composition, and wherein both the first vaccine composition and the second vaccine composition are administered by a route selected from intranasal, intramuscular, oral, or in ovo, and wherein the first vaccine composition and the second vaccine composition are administered via the same route or different routes.

6. The method of claim 5, wherein the first vaccine composition and the second vaccine composition are administered intranasally.

7. The method of claim 5, wherein the subject is an avian subject.

8. The method of claim 7, wherein the avian subject is a chicken.

9. The method of claim 5, wherein the second vaccine composition comprises a protein comprising an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein.

10. A method comprising administering the vaccine composition of claim 1 to a subject.

11. The method of claim 10, wherein the subject is an avian subject.

12. The method of claim 10, wherein the vaccine composition is administered intranasally to the subject.

13. A vaccine composition comprising a viral vector comprising an infectious bronchitis virus (IBV) (S) protein, an IBV nucleocapsid (N) protein, or both the S protein and the N protein, wherein the viral vector further comprises a polynucleotide encoding the S protein, the N protein or both the S protein and the N protein, and wherein the sequence encoding the S protein comprises one of SEQ ID NOs: 22, 24, 26, 28, 30, or 32 or a sequence with at least 90% identity to one of one of SEQ ID NOs: 22, 24, 26, 28, 30, or 32 and, wherein the sequence encoding the N protein comprises one of SEQ ID NOs: 36 or 38 or a sequence with at least 90% identity to one of SEQ ID NOs: 36 or 38.

14. The vaccine composition of claim 13, wherein the viral vector is selected from an adeno-associated virus or a poxvirus.

15. The vaccine composition of claim 13, further comprising an adjuvant.

16. The vaccine composition of claim 15, wherein the adjuvant comprises disaggregated spherical nanostructures comprising a saponin fraction isolated from an extract of the bark of *Quillaja saponaria* (Quil A) and chitosan, and wherein the (Quil A) and chitosan are present at a ratio between 1:15 and 1:100.

17. The vaccine composition of claim 16, wherein the chitosan is functionalized by treatment with 5-formyl-2-furan sulfonic acid and sodium borohydride, such that the chitosan surface is negatively charged.

18. A method comprising administering the vaccine composition of claim 13 to a subject.

19. The method of claim 18, wherein the subject is an avian subject.

20. The method of claim 18, wherein the vaccine composition is administered intranasally to the subject.

* * * * *