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(54) Title: CHLAMYDIA ANTIGENS AND CORRESPONDING DNA FRAGMENTS AND USES THEREOF

(57) Abstract
The present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by a strain of Chlamydia, specifically C. pneumoniae, employing a vector containing a nucleotide sequence encoding full-length, 5'-truncated or 3'-truncated 76kDa protein of a strain of Chlamydia pneumoniae and a promoter to effect expression of the 76kDa protein gene in the host. Modifications are possible within the scope of this invention.
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TITLE OF INVENTION

CHLAMYDIA ANTIGENS AND CORRESPONDING DNA FRAGMENTS
AND USES THEREOF

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S.
Provisional Application No. 60/132,270, filed May 3, 1999, and
U.S. Provisional Application No. 60/141,276 filed June 30,
1999.

FIELD OF INVENTION

The present invention relates to the Chlamydia 76kDa
protein and corresponding DNA molecules, which can be used to
prevent and treat Chlamydia infection in mammals, such as
humans.

BACKGROUND OF THE INVENTION

Chlamydiae are prokaryotes. They exhibit morphologic
and structural similarities to gram-negative bacteria including
a trilaminar outer membrane, which contains lipopolysaccharide
and several membrane proteins that are structurally and
functionally analogous to proteins found in E coli. They are
obligate intra-cellular parasites with a unique biphasic life
cycle consisting of a metabolically inactive but infectious
extracellular stage and a replicating but non-infectious
intracellular stage. The replicative stage of the life-cycle
takes place within a membrane-bound inclusion which sequesters
the bacteria away from the cytoplasm of the infected host cell.

C. pneumoniae is a common human pathogen, originally
described as the TWAR strain of Chlamydia psittaci but
subsequently recognised to be a new species. C. pneumoniae is
antigenically, genetically and morphologically distinct from other Chlamydia species (C. trachomatis, C. pecorum and C. psittaci). It shows 10% or less DNA sequence homology with either of C. trachomatis or C. psittaci.


C. pneumoniae infection usually presents as an acute respiratory disease (i.e., cough, sore throat, hoarseness, and fever; abnormal chest sounds on auscultation). For most patients, the cough persists for 2 to 6 weeks, and recovery is slow. In approximately 10% of these cases, upper respiratory tract infection is followed by bronchitis or pneumonia. Furthermore, during a C. pneumoniae epidemic, subsequent co-infection with pneumococcus has been noted in about half of these pneumonia patients, particularly in the infirm and the elderly. As noted above, there is more and more evidence that C. pneumoniae infection is also linked to diseases other than respiratory infections.

The reservoir for the organism is presumably people. In contrast to C. psittaci infections, there is no known bird
or animal reservoir. Transmission has not been clearly defined. It may result from direct contact with secretions, from fomites, or from airborne spread. There is a long incubation period, which may last for many months. Based on analysis of epidemics, *C. pneumoniae* appears to spread slowly through a population (case-to-case interval averaging 30 days) because infected persons are inefficient transmitters of the organism. Susceptibility to *C. pneumoniae* is universal. Reinfections occur during adulthood, following the primary infection as a child. *C. pneumoniae* appears to be an endemic disease throughout the world, noteworthy for superimposed intervals of increased incidence (epidemics) that persist for 2 to 3 years. *C. trachomatis* infection does not confer cross-immunity to *C. pneumoniae*. Infections are easily treated with oral antibiotics, tetracycline or erythromycin (2 g/d, for at least 10 to 14 d). A recently developed drug, azithromycin, is highly effective as a single-dose therapy against Chlamydial infections.

In most instances, *C. pneumoniae* infection is often mild and without complications, and up to 90% of infections are subacute or unrecognized. Among children in industrialized countries, infections have been thought to be rare up to the age of 5 y, although a recent study (E Normann et al, Chlamydia pneumoniae in children with acute respiratory tract infections, Acta Paediatrica, 1998, Vol 87, Iss 1, pp 23-27) has reported that many children in this age group show PCR evidence of infection despite being seronegative, and estimates a prevalence of 17-19% in 2-4 y olds. In developing countries, the seroprevalence of *C. pneumoniae* antibodies among young children is elevated, and there are suspicions that *C. pneumoniae* may be an important cause of acute lower respiratory tract disease and mortality for infants and children in tropical regions of the world.
From seroprevalence studies and studies of local epidemics, the initial \textit{C. pneumoniae} infection usually happens between the ages of 5 and 20 y. In the USA, for example, there are estimated to be 30,000 cases of childhood pneumonia each year caused by \textit{C. pneumoniae}. Infections may cluster among groups of children or young adults (e.g., school pupils or military conscripts).

\textit{C. pneumoniae} causes 10 to 25\% of community-acquired lower respiratory tract infections (as reported from Sweden, Italy, Finland, and the USA). During an epidemic, \textit{C. pneumoniae} infection may account for 50 to 60\% of the cases of pneumonia. During these periods, also, more episodes of mixed infections with \textit{S. pneumoniae} have been reported.

Reinfection during adulthood is common; the clinical presentation tends to be milder. Based on population seroprevalence studies, there tends to be increased exposure with age, which is particularly evident among men. Some investigators have speculated that a persistent, asymptomatic \textit{C. pneumoniae} infection state is common.

In adults of middle age or older, \textit{C. pneumoniae} infection may progress to chronic bronchitis and sinusitis. A study in the USA revealed that the incidence of pneumonia caused by \textit{C. pneumoniae} in persons younger than 60 years is 1 case per 1,000 persons per year; but in the elderly, the disease incidence rose three-fold. \textit{C. pneumoniae} infection rarely leads to hospitalization, except in patients with an underlying illness.

Of considerable importance is the association of atherosclerosis and \textit{C. pneumoniae} infection. There are several epidemiological studies showing a correlation of previous infections with \textit{C. pneumoniae} and heart attacks, coronary artery and carotid artery disease (Saikku et al. (1988) Lancet;ii:983; Thom et al. (1992) JAMA 268:68; Linnanmaki et al. (1993), Circulation 87:1030; Saikku et al. (1992)Annals

A number of recent studies have also indicated an association between C. pneumoniae infection and asthma. Infection has been linked to wheezing, asthmatic bronchitis, adult-onset asthma and acute exacerbations of asthma in adults, and small-scale studies have shown that prolonged antibiotic treatment was effective at greatly reducing the severity of the disease in some individuals (Hahn DL, et al. Evidence for Chlamydia pneumoniae infection in steroid-dependent asthma. Ann Allergy Asthma Immunol. 1998 Jan; 80(1): 45-49.; Hahn DL, et al. Association of Chlamydia pneumoniae IgA antibodies with recently symptomatic asthma. Epidemiol Infect. 1996 Dec; 117(3): 513-517; Bjornsson E, et al. Serology of Chlamydia in relation to asthma and bronchial hyperresponsiveness. Scand J Infect Dis. 1996; 28(1): 63-69.; Hahn DL. Treatment of Chlamydia pneumoniae infection in adult asthma: a before-after

In light of these results a protective vaccine against C. pneumoniae infection would be of considerable importance. There is not yet an effective vaccine for any human Chlamydial infection. It is conceivable that an effective vaccine can be developed using physically or chemically inactivated Chlamydiae. However, such a vaccine does not have a high margin of safety. In general, safer vaccines are made by genetically manipulating the organism by attenuation or by recombinant means. Accordingly, a major obstacle in creating an effective and safe vaccine against human Chlamydial infection has been the paucity of genetic information regarding Chlamydia, specifically C. pneumoniae.

Studies with C. trachomatis and C. psittaci indicate that safe and effective vaccine against Chlamydia is an attainable goal. For example, mice which have recovered from a lung infection with C. trachomatis are protected from infertility induced by a subsequent vaginal challenge (Pal et al. (1996) Infection and Immunity. 64:5341). Similarly, sheep immunized with inactivated C. psittaci were protected from subsequent Chlamydial-induced abortions and stillbirths (Jones et al. (1995) Vaccine 13:715). Protection from Chlamydial infections has been associated with Th1 immune responses, particularly the induction of INFg - producing CD4+T-cells (Igietsemes et al. (1993) Immunology 5:317). The adoptive transfer of CD4+ cell lines or clones to nude or SCID mice conferred protection from challenge or cleared chronic disease (Igietseme et al 1993) Regional Immunology 5:317; Magee et al (1993) Regional Immunology 5: 305), and in vivo depletion of

available a better understanding of antigenic variation may be gained.


Immunoblotting of isolates with sera from patients does show variation of blotting patterns between isolates, indicating that serotypes C. pneumoniae may exist (Grayston et al. (1995) Journal of Infectious Diseases 168:1231; Ramirez et al (1996) Annals of Internal Medicine 125:979). However, the results are potentially confounded by the infection status of the patients, since immunoblot profiles of a patient's sera change with time post-infection. An assessment of the number and relative frequency of any serotypes, and the defining antigens, is not yet possible.

Accordingly, a need exists for identifying and isolating polynucleotide sequences of C. pneumoniae for use in preventing and treating Chlamydia infection.

**SUMMARY OF THE INVENTION**

The present invention provides purified and isolated polynucleotide molecules that encode the Chlamydia polypeptide designated 76kDa protein (SEQ ID No: 1) which can be used in methods to prevent, treat, and diagnose Chlamydia infection. In one form of the invention, the polynucleotide molecules are DNA that encode the polypeptide of SEQ ID No: 2.
Another form of the invention provides polypeptides corresponding to the isolated DNA molecules. The amino acid sequence of the corresponding encoded polypeptide is shown as SEQ ID No: 2.

Another form of the invention provides truncated polypeptides corresponding to truncated DNA molecules. In one embodiment, the truncated nucleotide and amino acid sequences are shown as SEQ ID Nos: 3 and 4 respectively. In another embodiment, the truncated nucleotide and amino acid sequences are shown as SEQ ID Nos: 5 and 6 respectively.

Although Melgosa et al. has reported cloning a 76kDa protein from C. pneumoniae, comparison of the gene sequence as reported by Melgosa et al. to the published genome sequence of C. pneumoniae (http://chlamydia-www.berkeley.edu:4231/) reveals that, in fact, the genomic sequence in this region contains at least two open reading frames (ORFs), one in the 5′ portion and one in the 3′ portion. The sequence reported in Melgosa et al. is an in-frame fusion of the 5′ end of the 5′ ORF. Thus, Melgosa’s deduced protein is merely a 76kDa fusion protein and not the 76kDa protein observed by immunoblotting from various C. pneumoniae isolates. By contrast, the 76kDa protein of the present invention is the full-length protein encoded by the 3′ORF in this region of the genome. Notably, further analysis of the genome sequence (http://chlamydia-www.berkeley.edu:4231/) reveals at least one in-frame ATG upstream of the start codon of the 5′ ORF, suggesting that the 5′ ORF may form part of one or more larger ORFs.

Those skilled in the art will readily understand that the invention, having provided the polynucleotide sequences encoding the Chlamydia 76kDa protein, also provides polynucleotides encoding fragments derived from such a polypeptide. Moreover, the invention is understood to provide mutants and derivatives of such polypeptides and fragments derived therefrom, which result from the addition, deletion, or
substitution of non-essential amino acids as described herein. Those skilled in the art would also readily understand that the invention, having provided the polynucleotide sequences encoding Chlamydia polypeptides, further provides monospecific antibodies that specifically bind to such polypeptides.

The present invention has wide application and includes expression cassettes, vectors, and cells transformed or transfected with the polynucleotides of the invention. Accordingly, the present invention further provides (i) a method for producing a polypeptide of the invention in a recombinant host system and related expression cassettes, vectors, and transformed or transfected cells; (ii) a vaccine, or a live vaccine vector such as a pox virus, Salmonella typhimurium, or Vibrio cholerae vector, containing a polynucleotide of the invention, such vaccines and vaccine vectors being useful for, e.g., preventing and treating Chlamydia infection, in combination with a diluent or carrier, and related pharmaceutical compositions and associated therapeutic and/or prophylactic methods; (iii) a therapeutic and/or prophylactic use of an RNA or DNA molecule of the invention, either in a naked form or formulated with a delivery vehicle, a polypeptide or combination of polypeptides, or a monospecific antibody of the invention, and related pharmaceutical compositions; (iv) a method for diagnosing the presence of Chlamydia in a biological sample, which can involve the use of a DNA or RNA molecule, a monospecific antibody, or a polypeptide of the invention; and (v) a method for purifying a polypeptide of the invention by antibody-based affinity chromatography.

BRIEF DESCRIPTION OF THE DRAWINGS
The present invention will be further understood from the following description with reference to embodiments shown in the drawings, in which:

Figure 1 shows the full-length nucleotide sequence of the 76kDa protein gene (SEQ ID No: 1) and the deduced amino acid sequence of the 76kDa protein from Chlamydia pneumoniae (SEQ ID No: 2).

Figure 2 shows the restriction enzyme analysis of the C. pneumoniae 76kDa protein gene.

Figure 3 shows the nucleotide sequence containing a 3′-truncated 76kDa protein gene and its corresponding deduced amino acid sequence from Chlamydia pneumoniae; (note that nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa protein gene).

Figure 4 shows the construction and elements of plasmid pCACPNM555a, containing the full-length 76kDa gene.

Figure 5 shows the construction and elements of plasmid pCAI555, containing a 5′-truncated version of the 76kDa gene.

Figure 6 shows the construction and elements of plasmid pCAD76kDa, containing a 3′-truncated version of the 76kDa gene from Figure 3.

Figure 7 illustrates protection against C. pneumoniae infection by pCACPNM555a following DNA immunization.

Figure 8 illustrates protection against C. pneumoniae infection by pCAI555 following DNA immunization.

Figure 9 illustrates protection against C. pneumoniae infection by pCAD76kDa following DNA immunization. For Figures 7 to 9, individual data points are shown for each animal (hollow diamonds) as well as mean and standard deviations for each group (solid squares).
DETAILED DESCRIPTION OF INVENTION

The invention is described with reference to the following sequences which are embodiments of the invention:

SEQ ID NO: 1 is the full-length sequence of the 76kDa protein gene.

SEQ ID NO: 2 is the deduced full-length amino acid sequence of the 76kDa protein.

SEQ ID NO: 3 is the 5'-truncated nucleotide sequence of the 76kDa protein gene.

SEQ ID NO: 4 is the 5'-truncated amino acid sequence of the 76kDa protein.

SEQ ID NO: 5 is the 3'-truncated nucleotide sequence of the 76kDa protein gene.

SEQ ID NO: 6 is the 3'-truncated amino acid sequence of the 76kDa protein, which forms the basis for immunoprotection by pCAD76kDa in Figure 9.

SEQ ID NO: 7 is the sequence encoding a polypeptide containing a truncated 76kDa protein. Using this sequence as a template, a fragment was amplified by PCR to form part of construct pCAD76kDa.

SEQ ID NO: 8 is the deduced amino acid sequence of a truncated 76kDa protein, as expressed from pCAD76kDa.

SEQ ID NO: 9 is the 5' primer used to clone the full-length 76kDa protein gene and to amplify the full-length 76kDa protein gene for pCACPNM555a.

SEQ ID NO: 10 is the 3' primer used to clone the full-length 76kDa protein gene and to amplify the full-length 76kDa protein gene for pCACPNM555a.

SEQ ID NO: 11 is the 5' primer used to amplify the 5'-truncated 76kDa protein gene fragment for pCAI555.

SEQ ID NO: 12 is the 3' primer used to amplify the 5'-truncated 76kDa protein gene fragment for pCAI555.
SEQ ID NO: 13 is the 5′ primer used to amplify the 3′-truncated 76kDa protein gene fragment for pCAD76kDa.

SEQ ID NO: 14 is the 3′ primer used to amplify the truncated 76kDa protein gene fragment for pCAD76kDa.

An open reading frame (ORF) encoding the Chlamydia 76kDa protein has been identified from the C. pneumoniae genome. The gene encoding this protein and its fragments have been inserted into expression plasmids and shown to confer immune protection against Chlamydial infection. Accordingly, this 76kDa protein and related polypeptides can be used to prevent and treat Chlamydia infection.

According to a first aspect of the invention, isolated polynucleotides are provided which encode Chlamydia polypeptides, whose amino acid sequences are shown in SEQ ID Nos: 2, 4 and 6.

The term "isolated polynucleotide" is defined as a polynucleotide removed from the environment in which it naturally occurs. For example, a naturally-occurring DNA molecule present in the genome of a living bacteria or as part of a gene bank is not isolated, but the same molecule separated from the remaining part of the bacterial genome, as a result of, e.g., a cloning event (amplification), is isolated. Typically, an isolated DNA molecule is free from DNA regions (e.g., coding regions) with which it is immediately contiguous at the 5′ or 3′ end, in the naturally occurring genome. Such isolated polynucleotides may be part of a vector or a composition and still be defined as isolated in that such a vector or composition is not part of the natural environment of such polynucleotide.

The polynucleotide of the invention is either RNA or DNA (cDNA, genomic DNA, or synthetic DNA), or modifications, variants, homologs or fragments thereof. The DNA is either double-stranded or single-stranded, and, if single-stranded, is either the coding strand or the non-coding (anti-sense) strand.
Any one of the sequences that encode the polypeptides of the invention as shown in SEQ ID No: 1, 3 or 5 is (a) a coding sequence, (b) a ribonucleotide sequence derived from transcription of (a), or (c) a coding sequence which uses the redundancy or degeneracy of the genetic code to encode the same polypeptides. By "polypeptide" or "protein" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Both terms are used interchangeably in the present application.

Consistent with the first aspect of the invention, amino acid sequences are provided which are homologous to SEQ ID No: 2, 4 or 6. As used herein, "homologous amino acid sequence" is any polypeptide which is encoded, in whole or in part, by a nucleic acid sequence which hybridizes at 25-35°C below critical melting temperature (Tm), to any portion of the nucleic acid sequence of SEQ ID No: 1, 3 or 5. A homologous amino acid sequence is one that differs from an amino acid sequence shown in SEQ ID No: 2, 4 or 6 by one or more conservative amino acid substitutions. Such a sequence also encompass serotypic variants (defined below) as well as sequences containing deletions or insertions which retain inherent characteristics of the polypeptide such as immunogenicity. Preferably, such a sequence is at least 75%, more preferably 80%, and most preferably 90% identical to SEQ ID No: 2, 4 or 6.

Homologous amino acid sequences include sequences that are identical or substantially identical to SEQ ID No: 2, 4 or 6. By "amino acid sequence substantially identical" is meant a sequence that is at least 90%, preferably 95%, more preferably 97%, and most preferably 99% identical to an amino acid sequence of reference and that preferably differs from the sequence of reference by a majority of conservative amino acid substitutions.
Conservative amino acid substitutions are substitutions among amino acids of the same class. These classes include, for example, amino acids having uncharged polar side chains, such as asparagine, glutamine, serine, threonine, and tyrosine; amino acids having basic side chains, such as lysine, arginine, and histidine; amino acids having acidic side chains, such as aspartic acid and glutamic acid; and amino acids having nonpolar side chains, such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and cysteine.

Homology is measured using sequence analysis software such as Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705. Amino acid sequences are aligned to maximize identity. Gaps may be artificially introduced into the sequence to attain proper alignment. Once the optimal alignment has been set up, the degree of homology is established by recording all of the positions in which the amino acids of both sequences are identical, relative to the total number of positions.

Homologous polynucleotide sequences are defined in a similar way. Preferably, a homologous sequence is one that is at least 45%, more preferably 60%, and most preferably 85% identical to the coding sequence of SEQ ID No: 1, 3 or 5.

Consistent with the first aspect of the invention, polypeptides having a sequence homologous to SEQ ID No: 2, 4 or 6 include naturally-occurring allelic variants, as well as mutants or any other non-naturally occurring variants that retain the inherent characteristics of the polypeptide of SEQ ID No: 2, 4 or 6.

As is known in the art, an allelic variant is an alternate form of a polypeptide that is characterized as having a substitution, deletion, or addition of one or more amino acids that does not alter the biological function of the
polypeptide. By "biological function" is meant the function of the polypeptide in the cells in which it naturally occurs, even if the function is not necessary for the growth or survival of the cells. For example, the biological function of a porin is to allow the entry into cells of compounds present in the extracellular medium. Biological function is distinct from antigenic property. A polypeptide can have more than one biological function.

Allelic variants are very common in nature. For example, a bacterial species such as C. pneumoniae, is usually represented by a variety of strains that differ from each other by minor allelic variations. Indeed, a polypeptide that fulfills the same biological function in different strains can have an amino acid sequence (and polynucleotide sequence) that is not identical in each of the strains. Despite this variation, an immune response directed generally against many allelic variants has been demonstrated. In studies of the Chlamydial MOMP antigen, cross-strain antibody binding plus neutralization of infectivity occurs despite amino acid sequence variation of MOMP from strain to strain, indicating that the MOMP, when used as an immunogen, is tolerant of amino acid variations.

Polynucleotides encoding homologous polypeptides or allelic variants are retrieved by polymerase chain reaction (PCR) amplification of genomic bacterial DNA extracted by conventional methods. This involves the use of synthetic oligonucleotide primers matching upstream and downstream of the 5' and 3' ends of the encoding domain. Suitable primers are designed according to the nucleotide sequence information provided in SEQ ID No:1, 3 or 5. The procedure is as follows: a primer is selected which consists of 10 to 40, preferably 15 to 25 nucleotides. It is advantageous to select primers containing C and G nucleotides in a proportion sufficient to ensure efficient hybridization; i.e., an amount of C and G
nucleotides of at least 40%, preferably 50% of the total nucleotide content. A standard PCR reaction contains typically
0.5 to 5 Units of Taq DNA polymerase per 100 μL, 20 to 200 μM
deoxyribonucleotide each, preferably at equivalent concentrations,
0.5 to 2.5 mM magnesium over the total deoxyribonucleotide
concentration, 10^5 to 10^6 target molecules, and about 20 pmol
of each primer. About 25 to 50 PCR cycles are performed, with
an annealing temperature 15°C to 5°C below the true Tm of the
primers. A more stringent annealing temperature improves
discrimination against incorrectly annealed primers and reduces
incorporation of incorrect nucleotides at the 3’ end of
primers. A denaturation temperature of 95°C to 97°C is typical,
although higher temperatures may be appropriate for
denaturation of G+C-rich targets. The number of cycles
performed depends on the starting concentration of target
molecules, though typically more than 40 cycles is not
recommended as non-specific background products tend to
accumulate.

An alternative method for retrieving polynucleotides
encoding homologous polypeptides or allelic variants is by
hybridization screening of a DNA or RNA library. Hybridization
procedures are well-known in the art and are described in
Ausubel et al., (Ausubel et al., Current Protocols in Molecular
Biology, John Wiley & Sons Inc., 1994), Silhavy et al. (Silhavy
et al. Experiments with Gene Fusions, Cold Spring Harbor
Laboratory Press, 1984), and Davis et al. (Davis et al. A
Manual for Genetic Engineering: Advanced Bacterial Genetics,
Cold Spring Harbor Laboratory Press, 1980)). Important
parameters for optimizing hybridization conditions are
reflected in a formula used to obtain the critical melting
temperature above which two complementary DNA strands separate
from each other (Casey & Davidson, Nucl. Acid Res. (1977)
4:1539). For polynucleotides of about 600 nucleotides or
larger, this formula is as follows: \( Tm = 81.5 + 0.41 \times (\% \text{ G+C}) + 16.6 \log (\text{cation ion concentration}) - 0.63 \times (\% \text{ formamide}) - 600/\text{base number}. \) Under appropriate stringency conditions, hybridization temperature (\( Th \)) is approximately 20 to 40°C, 20 to 25°C, or, preferably 30 to 40°C below the calculated \( Tm \).

Those skilled in the art will understand that optimal temperature and salt conditions can be readily determined.

For the polynucleotides of the invention, stringent conditions are achieved for both pre-hybridizing and hybridizing incubations (i) within 4-16 hours at 42°C, in 6 x SSC containing 50% formamide, or (ii) within 4-16 hours at 65°C in an aqueous 6 x SSC solution (1 M NaCl, 0.1 M sodium citrate \( \text{pH 7.0} \)). Typically, hybridization experiments are performed at a temperature from 60 to 68°C, e.g. 65°C. At such a temperature, stringent hybridization conditions can be achieved in 6xSSC, preferably in 2xSSC or 1xSSC, more preferably in 0.5xSSC, 0.3xSSC or 0.1xSSC (in the absence of formamide).

1xSSC contains 0.15 M NaCl and 0.015 M sodium citrate.

Useful homologs and fragments thereof that do not occur naturally are designed using known methods for identifying regions of an antigen that are likely to tolerate amino acid sequence changes and/or deletions. As an example, homologous polypeptides from different species are compared; conserved sequences are identified. The more divergent sequences are the most likely to tolerate sequence changes. Homology among sequences may be analyzed using, as an example, the BLAST homology searching algorithm of Altschul et al., Nucleic Acids Res.; 25:3389-3402 (1997). Alternatively, sequences are modified such that they become more reactive to T- and/or B-cells, based on computer-assisted analysis of probable T- or B-cell epitopes. Yet another alternative is to mutate a particular amino acid residue or sequence within the polypeptide in vitro, then screen the mutant polypeptides for
their ability to prevent or treat Chlamydia infection according to the method outlined below.

A person skilled in the art will readily understand that by following the screening process of this invention, it will be determined without undue experimentation whether a particular homolog of SEQ ID No: 2, 4 or 6 may be useful in the prevention or treatment of Chlamydia infection. The screening procedure comprises the steps:

(i) immunizing an animal, preferably mouse, with the test homolog or fragment;

(ii) inoculating the immunized animal with Chlamydia; and

(iii) selecting those homologs or fragments which confer protection against Chlamydia.

By "conferring protection" is meant that there is a reduction in severity of any of the effects of Chlamydia infection, in comparison with a control animal which was not immunized with the test homolog or fragment.

Consistent with the first aspect of the invention, polypeptide derivatives are provided that are partial sequences of SEQ ID No: 2, 4 or 6, partial sequences of polypeptide sequences homologous to SEQ ID No: 2, 4 or 6, polypeptides derived from full-length polypeptides by internal deletion, and fusion proteins.

It is an accepted practice in the field of immunology to use fragments and variants of protein immunogens as vaccines, as all that is required to induce an immune response to a protein is a small (e.g., 8 to 10 amino acid) immunogenic region of the protein. Various short synthetic peptides corresponding to surface-exposed antigens of pathogens other than Chlamydia have been shown to be effective vaccine antigens against their respective pathogens, e.g. an 11 residue peptide of murine mammary tumor virus (Casey & Davidson, Nucl. Acid Res. (1977) 4:1539), a 16-residue peptide of Semliki Forest
virus (Snijders et al., 1991. J. Gen. Virol. 72:557-565), and
two overlapping peptides of 15 residues each from canine
parvovirus (Langeveld et al., Vaccine 12(15):1473-1480, 1994).

Accordingly, it will be readily apparent to one
skilled in the art, having read the present description, that
partial sequences of SEQ ID No: 2, 4 or 6 or their homologous
amino acid sequences are inherent to the full-length sequences
and are taught by the present invention. Such polypeptide
fragments preferably are at least 12 amino acids in length.

Advantageously, they are at least 20 amino acids, preferably at
least 50 amino acids, and more preferably at least 75 amino
acids and most preferably at least 100 amino acids in length.

Polynucleotides of 30 to 600 nucleotides encoding
partial sequences of sequences homologous to SEQ ID No: 2, 4 or
6 are retrieved by PCR amplification using the parameters
outlined above and using primers matching the sequences
upstream and downstream of the 5' and 3' ends of the fragment
to be amplified. The template polynucleotide for such
amplification is either the full length polynucleotide
homologous to SEQ ID No: 1, 3 or 5, or a polynucleotide
contained in a mixture of polynucleotides such as a DNA or RNA
library. As an alternative method for retrieving the partial
sequences, screening hybridization is carried out under
conditions described above and using the formula for
calculating Tm. Where fragments of 30 to 600 nucleotides are
to be retrieved, the calculated Tm is corrected by subtracting
(600/polynucleotide size in base pairs) and the stringency
conditions are defined by a hybridization temperature that is
5 to 10°C below Tm. Where oligonucleotides shorter than 20-30
bases are to be obtained, the formula for calculating the Tm is
as follows: Tm = 4 x (G+C) + 2 (A+T). For example, an
18 nucleotide fragment of 50% G+C would have an approximate Tm
of 54°C. Short peptides that are fragments of SEQ ID No: 2, 4
or 6 or its homologous sequences, are obtained directly by

Useful polypeptide derivatives, e.g., polypeptide fragments, are designed using computer-assisted analysis of amino acid sequences. This would identify probable surface-exposed, antigenic regions (Hughes et al., 1992. Infect. Immun. 60(9):3497). Analysis of 6 amino acid sequences contained in SEQ ID No: 2, 4 or 6, based on the product of flexibility and hydrophobicity propensities using the program SEQSEE (Wishart DS, et al. "SEQSEE: a comprehensive program suite for protein sequence analysis." Comput Appl Biosci. 1994 Apr;10(2):121-32), can reveal potential B- and T-cell epitopes which may be used as a basis for selecting useful immunogenic fragments and variants. This analysis uses a reasonable combination of external surface features that is likely to be recognized by antibodies. Probable T-cell epitopes for HLA-A0201 MHC subclass may be revealed by an algorithms that emulate an approach developed at the NIH (Parker KC, et al. "Peptide binding to MHC class I molecules: implications for antigenic peptide prediction." Immunol Res 1995;14(1):34-57).

Epitopes which induce a protective T cell-dependent immune response are present throughout the length of the polypeptide. However, some epitopes may be masked by secondary and tertiary structures of the polypeptide. To reveal such masked epitopes large internal deletions are created which remove much of the original protein structure and exposes the masked epitopes. Such internal deletions sometimes effect the additional advantage of removing immunodominant regions of high variability among strains.

Polynucleotides encoding polypeptide fragments and polypeptides having large internal deletions are constructed using standard methods (Ausubel et al., Current Protocols in

Components for these methods and instructions for their use are readily available from various commercial sources such as Stratagene. Once the deletion mutants have been constructed, they are tested for their ability to prevent or treat Chlamydia infection as described above.

As used herein, a fusion polypeptide is one that contains a polypeptide or a polypeptide derivative of the invention fused at the N- or C-terminal end to any other polypeptide (hereinafter referred to as a peptide tail). A simple way to obtain such a fusion polypeptide is by translation of an in-frame fusion of the polynucleotide sequences, i.e., a hybrid gene. The hybrid gene encoding the fusion polypeptide is inserted into an expression vector which is used to transform or transfect a host cell. Alternatively, the polynucleotide sequence encoding the polypeptide or polypeptide derivative is inserted into an expression vector in which the polynucleotide encoding the peptide tail is already present. Such vectors and instructions for their use are commercially available, e.g. the pMal-c2 or pMal-p2 system from New England Biolabs, in which the peptide tail is a maltose binding protein, the glutathione-S-transferase system of Pharmacia, or the His-Tag system available from Novagen. These and other expression systems provide convenient means for further purification of polypeptides and derivatives of the invention.

An advantageous example of a fusion polypeptide is one where the polypeptide or homolog or fragment of the invention is fused to a polypeptide having adjuvant activity, such as subunit B of either cholera toxin or E. coli heat-labile toxin. Another advantageous fusion is one where the
polypeptide, homolog or fragment is fused to a strong T-cell epitope or B-cell epitope. Such an epitope may be one known in the art (e.g. the Hepatitis B virus core antigen, D.R. Millich et al., "Antibody production to the nucleocapsid and envelope of the Hepatitis B virus primed by a single synthetic T cell site", Nature. 1987. 329:547-549), or one which has been identified in another polypeptide of the invention based on computer-assisted analysis of probable T- or B-cell epitopes. Consistent with this aspect of the invention is a fusion polypeptide comprising T- or B-cell epitopes from SEQ ID No: 2, 4 or 6 or its homolog or fragment, wherein the epitopes are derived from multiple variants of said polypeptide or homolog or fragment, each variant differing from another in the location and sequence of its epitope within the polypeptide. Such a fusion is effective in the prevention and treatment of Chlamydia infection since it optimizes the T- and B-cell response to the overall polypeptide, homolog or fragment.

To effect fusion, the polypeptide of the invention is fused to the N-, or preferably, to the C-terminal end of the polypeptide having adjuvant activity or T- or B-cell epitope. Alternatively, a polypeptide fragment of the invention is inserted internally within the amino acid sequence of the polypeptide having adjuvant activity. The T- or B-cell epitope may also be inserted internally within the amino acid sequence of the polypeptide of the invention.

Consistent with the first aspect, the polynucleotides of the invention also encode hybrid precursor polypeptides containing heterologous signal peptides, which mature into polypeptides of the invention. By "heterologous signal peptide" is meant a signal peptide that is not found in naturally-occurring precursors of polypeptides of the invention.

Polynucleotide molecules according to the invention, including RNA, DNA, or modifications or combinations thereof,
have various applications. A DNA molecule is used, for example, (i) in a process for producing the encoded polypeptide in a recombinant host system, (ii) in the construction of vaccine vectors such as poxviruses, which are further used in methods and compositions for preventing and/or treating *Chlamydia* infection, (iii) as a vaccine agent (as well as an RNA molecule), in a naked form or formulated with a delivery vehicle and, (iv) in the construction of attenuated *Chlamydia* strains that can over-express a polynucleotide of the invention or express it in a non-toxic, mutated form.

Accordingly, a second aspect of the invention encompasses (i) an expression cassette containing a DNA molecule of the invention placed under the control of the elements required for expression, in particular under the control of an appropriate promoter; (ii) an expression vector containing an expression cassette of the invention; (iii) a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, as well as (iv) a process for producing a polypeptide or polypeptide derivative encoded by a polynucleotide of the invention, which involves culturing a procaryotic or eucaryotic cell transformed or transfected with an expression cassette and/or vector of the invention, under conditions that allow expression of the DNA molecule of the invention and, recovering the encoded polypeptide or polypeptide derivative from the cell culture.

A recombinant expression system is selected from procaryotic and eucaryotic hosts. Eucaryotic hosts include yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris*), mammalian cells (e.g., COS1, NIH3T3, or JEG3 cells), arthropods cells (e.g., *Spodoptera frugiperda* (SP9) cells), and plant cells. A preferred expression system is a procaryotic host such as *E. coli*. Bacterial and eucaryotic cells are available from a number of different sources including commercial sources to those skilled in the art, e.g., the
American Type Culture Collection (ATCC; Rockville, Maryland). Commercial sources of cells used for recombinant protein expression also provide instructions for usage of the cells. The choice of the expression system depends on the features desired for the expressed polypeptide. For example, it may be useful to produce a polypeptide of the invention in a particular lipidated form or any other form.

One skilled in the art would readily understand that not all vectors and expression control sequences and hosts would be expected to express equally well the polynucleotides of this invention. With the guidelines described below, however, a selection of vectors, expression control sequences and hosts may be made without undue experimentation and without departing from the scope of this invention.

In selecting a vector, the host must be chosen that is compatible with the vector which is to exist and possibly replicate in it. Considerations are made with respect to the vector copy number, the ability to control the copy number, expression of other proteins such as antibiotic resistance. In selecting an expression control sequence, a number of variables are considered. Among the important variable are the relative strength of the sequence (e.g. the ability to drive expression under various conditions), the ability to control the sequence's function, compatibility between the polynucleotide to be expressed and the control sequence (e.g. secondary structures are considered to avoid hairpin structures which prevent efficient transcription). In selecting the host, unicellular hosts are selected which are compatible with the selected vector, tolerant of any possible toxic effects of the expressed product, able to secrete the expressed product efficiently if such is desired, to be able to express the product in the desired conformation, to be easily scaled up, and to which ease of purification of the final product.
The choice of the expression cassette depends on the host system selected as well as the features desired for the expressed polypeptide. Typically, an expression cassette includes a promoter that is functional in the selected host system and can be constitutive or inducible; a ribosome binding site; a start codon (ATG) if necessary; a region encoding a signal peptide, e.g., a lipidation signal peptide; a DNA molecule of the invention; a stop codon; and optionally a 3' terminal region (translation and/or transcription terminator).

The signal peptide encoding region is adjacent to the polynucleotide of the invention and placed in proper reading frame. The signal peptide-encoding region is homologous or heterologous to the DNA molecule encoding the mature polypeptide and is compatible with the secretion apparatus of the host used for expression. The open reading frame constituted by the DNA molecule of the invention, solely or together with the signal peptide, is placed under the control of the promoter so that transcription and translation occur in the host system. Promoters and signal peptide encoding regions are widely known and available to those skilled in the art and include, for example, the promoter of Salmonella typhimurium (and derivatives) that is inducible by arabinose (promoter araB) and is functional in Gram-negative bacteria such as E. coli (as described in U.S. Patent No. 5,028,530 and in Cagnon et al., (Cagnon et al., Protein Engineering (1991) 4(7):843)); the promoter of the gene of bacteriophage T7 encoding RNA polymerase, that is functional in a number of E. coli strains expressing T7 polymerase (described in U.S. Patent No. 4,952,496); OspA lipidation signal peptide; and RlpB lipidation signal peptide (Takase et al., J. Bact. (1987) 169:5692).

The expression cassette is typically part of an expression vector, which is selected for its ability to replicate in the chosen expression system. Expression vectors
(e.g., plasmids or viral vectors) can be chosen, for example, from those described in Pouwels et al. (Cloning Vectors: A Laboratory Manual 1985, Supp. 1987). Suitable expression vectors can be purchased from various commercial sources.

Methods for transforming/transflecting host cells with expression vectors are well-known in the art and depend on the host system selected as described in Ausubel et al., (Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons Inc., 1994).

Upon expression, a recombinant polypeptide of the invention (or a polypeptide derivative) is produced and remains in the intracellular compartment, is secreted/excreted in the extracellular medium or in the periplasmic space, or is embedded in the cellular membrane. The polypeptide is recovered in a substantially purified form from the cell extract or from the supernatant after centrifugation of the recombinant cell culture. Typically, the recombinant polypeptide is purified by antibody-based affinity purification or by other well-known methods that can be readily adapted by a person skilled in the art, such as fusion of the polynucleotide encoding the polypeptide or its derivative to a small affinity binding domain. Antibodies useful for purifying by immunoaffinity the polypeptides of the invention are obtained as described below.

A polynucleotide of the invention can also be useful as a vaccine. There are two major routes, either using a viral or bacterial host as gene delivery vehicle (live vaccine vector) or administering the gene in a free form, e.g., inserted into a plasmid. Therapeutic or prophylactic efficacy of a polynucleotide of the invention is evaluated as described below.

Accordingly, a third aspect of the invention provides (i) a vaccine vector such as a poxvirus, containing a DNA molecule of the invention, placed under the control of elements
required for expression; (ii) a composition of matter comprising a vaccine vector of the invention, together with a diluent or carrier; specifically (iii) a pharmaceutical composition containing a therapeutically or prophylactically effective amount of a vaccine vector of the invention; (iv) a method for inducing an immune response against Chlamydia in a mammal (e.g., a human; alternatively, the method can be used in veterinary applications for treating or preventing Chlamydia infection of animals, e.g., cats or birds), which involves administering to the mammal an immunogenically effective amount of a vaccine vector of the invention to elicit a protective or therapeutic immune response to Chlamydia; and particularly, (v) a method for preventing and/or treating a Chlamydia (e.g., C. trachomatis, C. psittaci, C. pneumonia, C. pecorum) infection, which involves administering a prophylactic or therapeutic amount of a vaccine vector of the invention to an infected individual. Additionally, the third aspect of the invention encompasses the use of a vaccine vector of the invention in the preparation of a medicament for preventing and/or treating Chlamydia infection.

As used herein, a vaccine vector expresses one or several polypeptides or derivatives of the invention. The vaccine vector may express additionally a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12), that enhances the immune response (adjuvant effect). It is understood that each of the components to be expressed is placed under the control of elements required for expression in a mammalian cell.

Consistent with the third aspect of the invention is a composition comprising several vaccine vectors, each of them capable of expressing a polypeptide or derivative of the invention. A composition may also comprise a vaccine vector capable of expressing an additional Chlamydia antigen, or a
subunit, fragment, homolog, mutant, or derivative thereof; optionally together with or a cytokine such as IL-2 or IL-12.

Vaccination methods for treating or preventing infection in a mammal comprises use of a vaccine vector of the invention to be administered by any conventional route, particularly to a mucosal (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) surface or via the parenteral (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal) route. Preferred routes depend upon the choice of the vaccine vector. Treatment may be effected in a single dose or repeated at intervals. The appropriate dosage depends on various parameters understood by skilled artisans such as the vaccine vector itself, the route of administration or the condition of the mammal to be vaccinated (weight, age and the like).

Live vaccine vectors available in the art include viral vectors such as adenoviruses and poxviruses as well as bacterial vectors, e.g., Shigella, Salmonella, Vibrio cholerae, Lactobacillus, Bacille bilié de Calmette-Guérin (BCG), and Streptococcus.

An example of an adenovirus vector, as well as a method for constructing an adenovirus vector capable of expressing a DNA molecule of the invention, are described in U.S. Patent No. 4,920,209. Poxvirus vectors include vaccinia and canary pox virus, described in U.S. Patent No. 4,722,848 and U.S. Patent No. 5,364,773, respectively. (Also see, e.g., Tartaglia et al., Virology (1992) 188:217 for a description of a vaccinia virus vector and Taylor et al, Vaccine (1995) 13:539 for a reference of a canary pox.) Poxvirus vectors capable of expressing a polynucleotide of the invention are obtained by homologous recombination as described in Kieny et al., Nature (1984) 312:163 so that the polynucleotide of the invention is inserted in the viral genome under appropriate conditions for expression in mammalian cells. Generally, the dose of vaccine
viral vector, for therapeutic or prophylactic use, can be of
from about 1x10^6 to about 1x10^11, advantageously from about 1x10^7
to about 1x10^10, preferably of from about 1x10^7 to about 1x10^9
plaque-forming units per kilogram. Preferably, viral vectors
are administered parenterally; for example, in 3 doses, 4 weeks
apart. It is preferable to avoid adding a chemical adjuvant to
a composition containing a viral vector of the invention and
thereby minimizing the immune response to the viral vector
itself.

Non-toxicogenic Vibrio cholerae mutant strains that
are useful as a live oral vaccine are known. Mekalanos et al.,
strains which have a substantial amount of the coding sequence
of each of the two ctxA alleles deleted so that no functional
cholerae toxin is produced. WO 92/11354 describes a strain in
which the irgA locus is inactivated by mutation; this mutation
can be combined in a single strain with ctxA mutations. WO
94/01533 describes a deletion mutant lacking functional ctxA
and attRSI DNA sequences. These mutant strains are genetically
engineered to express heterologous antigens, as described in
WO 94/19482. An effective vaccine dose of a Vibrio cholerae
strain capable of expressing a polypeptide or polypeptide
derivative encoded by a DNA molecule of the invention contains
about 1x10^6 to about 1x10^9, preferably about 1x10^6 to about
1x10^8, viable bacteria in a volume appropriate for the selected
route of administration. Preferred routes of administration
include all mucosal routes; most preferably, these vectors are
administered intranasally or orally.

Attenuated Salmonella typhimurium strains,
genetically engineered for recombinant expression of
heterologous antigens or not, and their use as oral vaccines
are described in Nakayama et al. (Bio/Technology (1988) 6:693)
and WO 92/11361. Preferred routes of administration include
all mucosal routes; most preferably, these vectors are administered intranasally or orally.


In bacterial vectors, the polynucleotide of the invention is inserted into the bacterial genome or remains in a free state as part of a plasmid.

The composition comprising a vaccine bacterial vector of the present invention may further contain an adjuvant. A number of adjuvants are known to those skilled in the art. Preferred adjuvants are selected as provided below.

Accordingly, a fourth aspect of the invention provides (i) a composition of matter comprising a polynucleotide of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a polynucleotide of the invention; (iii) a method for inducing an immune response against *Chlamydia* in a mammal by administration of an immunogenically effective amount of a polynucleotide of the invention to elicit a protective immune response to *Chlamydia*; and particularly, (iv) a method for preventing and/or treating a *Chlamydia* (e.g., *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, or *C. pecorum*) infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to an infected individual. Additionally, the fourth aspect of the invention encompasses the use of a polynucleotide of the invention in the preparation of a medicament for preventing and/or treating *Chlamydia* infection. A preferred
use includes the use of a DNA molecule placed under conditions for expression in a mammalian cell, especially in a plasmid that is unable to replicate in mammalian cells and to substantially integrate in a mammalian genome.

Use of the polynucleotides of the invention include their administration to a mammal as a vaccine, for therapeutic or prophylactic purposes. Such polynucleotides are used in the form of DNA as part of a plasmid that is unable to replicate in a mammalian cell and unable to integrate into the mammalian genome. Typically, such a DNA molecule is placed under the control of a promoter suitable for expression in a mammalian cell. The promoter functions either ubiquitously or tissue-specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (described in U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter (described in Norton & Coffin, Molec. Cell Biol. (1985) 5:281). An example of a tissue-specific promoter is the desmin promoter which drives expression in muscle cells (Li et al., Gene (1989) 78:243, Li & Paulin, J. Biol. Chem. (1991) 266:6562 and Li & Paulin, J. Biol. Chem. (1993) 268:10403). Use of promoters is well-known to those skilled in the art. Useful vectors are described in numerous publications, specifically WO 94/21797 and Hartikka et al., Human Gene Therapy (1996) 7:1205.

Polynucleotides of the invention which are used as vaccines encode either a precursor or a mature form of the corresponding polypeptide. In the precursor form, the signal peptide is either homologous or heterologous. In the latter case, a eucaryotic leader sequence such as the leader sequence of the tissue-type plasminogen factor (tPA) is preferred.

As used herein, a composition of the invention contains one or several polynucleotides with optionally at least one additional polynucleotide encoding another Chlamydia antigen such as urease subunit A, B, or both, or a fragment, derivative, mutant, or analog thereof. The composition may
also contain an additional polynucleotide encoding a cytokine, such as interleukin-2 (IL-2) or interleukin-12 (IL-12) so that the immune response is enhanced. These additional polynucleotides are placed under appropriate control for expression. Advantageously, DNA molecules of the invention and/or additional DNA molecules to be included in the same composition, are present in the same plasmid.

Standard techniques of molecular biology for preparing and purifying polynucleotides are used in the preparation of polynucleotide therapeutics of the invention. For use as a vaccine, a polynucleotide of the invention is formulated according to various methods outlined below.

One method utilizes the polynucleotide in a naked form, free of any delivery vehicles. Such a polynucleotide is simply diluted in a physiologically acceptable solution such as sterile saline or sterile buffered saline, with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength, such as provided by a sucrose solution, e.g., a solution containing 20% sucrose.

An alternative method utilizes the polynucleotide in association with agents that assist in cellular uptake. Examples of such agents are (i) chemicals that modify cellular permeability, such as bupivacaine (see, e.g., WO 94/16737), (ii) liposomes for encapsulation of the polynucleotide, or (iii) cationic lipids or silica, gold, or tungsten microparticles which associate themselves with the polynucleotides.

Anionic and neutral liposomes are well-known in the art (see, e.g., Liposomes: A Practical Approach, RPC New Ed, IRL press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including polynucleotides.
Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin™ also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1,2-bis(oleyloxy)-3-(trimethylammonio)propane), DDAB (dimethyldioctadecylammonium bromide), DOGS (dioctadecylamidoglycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N' -dimethyl aminomethane)-carbamoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine), as described in WO 90/11092 as an example.

Formulation containing cationic liposomes may optionally contain other transfection-facilitating compounds. A number of them are described in WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/02397. They include spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine S, and cationic bile salts (see, for example, WO 93/19768).

Gold or tungsten microparticles are used for gene delivery, as described in WO 91/00359, WO 93/17706, and Tang et al. Nature (1992) 356:152. The microparticle-coated polynucleotide is injected via intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described in U.S. Patent No. 4,945,050, U.S. Patent No. 5,015,580, and WO 94/24263.

The amount of DNA to be used in a vaccine recipient depends, e.g., on the strength of the promoter used in the DNA construct, the immunogenicity of the expressed gene product, the condition of the mammal intended for administration (e.g., the weight, age, and general health of the mammal), the mode of
administration, and the type of formulation. In general, a therapeutically or prophylactically effective dose from about 1 μg to about 1 mg, preferably, from about 10 μg to about 800 μg and, more preferably, from about 25 μg to about 250 μg, can be administered to human adults. The administration can be achieved in a single dose or repeated at intervals.

The route of administration is any conventional route used in the vaccine field. As general guidance, a polynucleotide of the invention is administered via a mucosal surface, e.g., an ocular, intranasal, pulmonary, oral, intestinal, rectal, vaginal, and urinary tract surface; or via a parenteral route, e.g., by an intravenous, subcutaneous, intraperitoneal, intradermal, intraepidermal, or intramuscular route. The choice of administration route depends on the formulation that is selected. A polynucleotide formulated in association with bupivacaine is advantageously administered into muscles. When a neutral or anionic liposome or a cationic lipid, such as DOTMA or DC-Chol, is used, the formulation can be advantageously injected via intravenous, intranasal (aerosolization), intramuscular, intradermal, and subcutaneous routes. A polynucleotide in a naked form can advantageously be administered via the intramuscular, intradermal, or subcutaneous routes.

Although not absolutely required, such a composition can also contain an adjuvant. If so, a systemic adjuvant that does not require concomitant administration in order to exhibit an adjuvant effect is preferable such as, e.g., QS21, which is described in U.S. Patent No. 5,057,546.

The sequence information provided in the present application enables the design of specific nucleotide probes and primers that are used for diagnostic purposes. Accordingly, a fifth aspect of the invention provides a nucleotide probe or primer having a sequence found in or
derived by degeneracy of the genetic code from a sequence shown in SEQ ID No: 1, 3 or 5

The term "probe" as used in the present application refers to DNA (preferably single stranded) or RNA molecules (or modifications or combinations thereof) that hybridize under the stringent conditions, as defined above, to nucleic acid molecules having SEQ ID No: 1, 3 or 5 or to sequences homologous to SEQ ID No:1, 3 or 5, or to its complementary or anti-sense sequence. Generally, probes are significantly shorter than full-length sequences. Such probes contain from about 5 to about 100, preferably from about 10 to about 80, nucleotides. In particular, probes have sequences that are at least 75%, preferably at least 85%, more preferably 95% homologous to a portion of SEQ ID No:1, 3 or 5 or that are complementary to such sequences. Probes may contain modified bases such as inosine, methyl-5-deoxycytidine, deoxyuridine, dimethylamino-5-deoxyuridine, or diamino-2, 6-purine. Sugar or phosphate residues may also be modified or substituted. For example, a deoxyribose residue may be replaced by a polyamide (Nielsen et al., Science (1991) 254:1497) and phosphate residues may be replaced by ester groups such as diphosphate, alkyl, arylphosphonate and phosphorothioate esters. In addition, the 2'-hydroxyl group on ribonucleotides may be modified by including such groups as alkyl groups.

Probes of the invention are used in diagnostic tests, as capture or detection probes. Such capture probes are conventionally immobilized on a solid support, directly or indirectly, by covalent means or by passive adsorption. A detection probe is labeled by a detection marker selected from: radioactive isotopes, enzymes such as peroxidase, alkaline phosphatase, and enzymes able to hydrolyze a chromogenic, fluorogenic, or luminescent substrate, compounds that are chromogenic, fluorogenic, or luminescent, nucleotide base analogs, and biotin.
Probes of the invention are used in any conventional hybridization technique, such as dot blot (Maniatis et al., Molecular Cloning: A Laboratory Manual (1982) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), Southern blot (Southern, J. Mol. Biol. (1975) 98:503), northern blot (identical to Southern blot with the exception that RNA is used as a target), or the sandwich technique (Dunn et al., Cell (1977) 12:23). The latter technique involves the use of a specific capture probe and/or a specific detection probe with nucleotide sequences that at least partially differ from each other.

A primer is a probe of usually about 10 to about 40 nucleotides that is used to initiate enzymatic polymerization of DNA in an amplification process (e.g., PCR), in an elongation process, or in a reverse transcription method. Primers used in diagnostic methods involving PCR are labeled by methods known in the art.

As described herein, the invention also encompasses (i) a reagent comprising a probe of the invention for detecting and/or identifying the presence of Chlamydia in a biological material; (ii) a method for detecting and/or identifying the presence of Chlamydia in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA or RNA is extracted from the material and denatured, and (c) exposed to a probe of the invention, for example, a capture, detection probe or both, under stringent hybridization conditions, such that hybridization is detected; and (iii) a method for detecting and/or identifying the presence of Chlamydia in a biological material, in which (a) a sample is recovered or derived from the biological material, (b) DNA is extracted therefrom, (c) the extracted DNA is primed with at least one, and preferably two, primers of the invention and amplified by polymerase chain reaction, and (d) the amplified DNA fragment is produced.
It is apparent that disclosure of polynucleotide sequences of SEQ ID No: 1, 3 or 5, its homologs and partial sequences enable their corresponding amino acid sequences. Accordingly, a sixth aspect of the invention features a substantially purified polypeptide or polypeptide derivative having an amino acid sequence encoded by a polynucleotide of the invention.

A "substantially purified polypeptide" as used herein is defined as a polypeptide that is separated from the environment in which it naturally occurs and/or that is free of the majority of the polypeptides that are present in the environment in which it was synthesized. For example, a substantially purified polypeptide is free from cytoplasmic polypeptides. Those skilled in the art would readily understand that the polypeptides of the invention may be purified from a natural source, i.e., a Chlamydia strain, or produced by recombinant means.

Consistent with the sixth aspect of the invention are polypeptides, homologs or fragments which are modified or treated to enhance their immunogenicity in the target animal, in whom the polypeptide, homolog or fragments are intended to confer protection against Chlamydia. Such modifications or treatments include: amino acid substitutions with an amino acid derivative such as 3-methylhistidine, 4-hydroxyproline, 5-hydroxylysine etc., modifications or deletions which are carried out after preparation of the polypeptide, homolog or fragment, such as the modification of free amino, carboxyl or hydroxyl side groups of the amino acids.

Identification of homologous polypeptides or polypeptide derivatives encoded by polynucleotides of the invention which have specific antigenicity is achieved by screening for cross-reactivity with an antiserum raised against the polypeptide of reference having an amino acid sequence of SEQ ID No: 1, 3 or 5. The procedure is as follows: a
monospecific hyperimmune antiserum is raised against a purified reference polypeptide, a fusion polypeptide (for example, an expression product of MBP, GST, or His-tag systems, the description and instructions for use of which are contained in Invitrogen product manuals for pcDNA3.1/Myc-His(+) A, B, and C and for the Xpress™ System Protein Purification), or a synthetic peptide predicted to be antigenic. Where an antiserum is raised against a fusion polypeptide, two different fusion systems are employed. Specific antigenicity can be determined according to a number of methods, including Western blot (Towbin et al., Proc. Natl. Acad. Sci. USA (1979) 76:4350), dot blot, and ELISA, as described below.

In a Western blot assay, the product to be screened, either as a purified preparation or a total E. coli extract, is submitted to SDS-PAGE electrophoresis as described by Laemmli (Nature (1970) 227:680). After transfer to a nitrocellulose membrane, the material is further incubated with the monospecific hyperimmune antiserum diluted in the range of dilutions from about 1:5 to about 1:5000, preferably from about 1:100 to about 1:500. Specific antigenicity is shown once a band corresponding to the product exhibits reactivity at any of the dilutions in the above range.

In an ELISA assay, the product to be screened is preferably used as the coating antigen. A purified preparation is preferred, although a whole cell extract can also be used. Briefly, about 100 μl of a preparation at about 10 μg protein/ml are distributed into wells of a 96-well polycarbonate ELISA plate. The plate is incubated for 2 hours at 37°C then overnight at 4°C. The plate is washed with phosphate buffer saline (PBS) containing 0.05% Tween 20 (PBS/Tween buffer). The wells are saturated with 250 μl PBS containing 1% bovine serum albumin (BSA) to prevent non-specific antibody binding. After 1 hour incubation at 37°C, the plate is washed with PBS/Tween buffer. The antiserum is
serially diluted in PBS/Tween buffer containing 0.5% BSA. 100 µl of dilutions are added per well. The plate is incubated for 90 minutes at 37°C, washed and evaluated according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when specific antibodies were raised in rabbits. Incubation is carried out for 90 minutes at 37°C and the plate is washed. The reaction is developed with the appropriate substrate and the reaction is measured by colorimetry (absorbance measured spectrophotometrically).

Under the above experimental conditions, a positive reaction is shown by O.D. values greater than a non immune control serum.

In a dot blot assay, a purified product is preferred, although a whole cell extract can also be used. Briefly, a solution of the product at about 100 µg/ml is serially two-fold diluted in 50 mM Tris-HCl (pH 7.5). 100 µl of each dilution are applied to a nitrocellulose membrane 0.45 µm set in a 96-well dot blot apparatus (Biorad). The buffer is removed by applying vacuum to the system. Wells are washed by addition of 50 mM Tris-HCl (pH 7.5) and the membrane is air-dried. The membrane is saturated in blocking buffer (50 mM Tris-HCl (pH 7.5) 0.15 M NaCl, 10 g/L skim milk) and incubated with an antiserum dilution from about 1:50 to about 1:5000, preferably about 1:500. The reaction is revealed according to standard procedures. For example, a goat anti-rabbit peroxidase conjugate is added to the wells when rabbit antibodies are used. Incubation is carried out 90 minutes at 37°C and the blot is washed. The reaction is developed with the appropriate substrate and stopped. The reaction is measured visually by the appearance of a colored spot, e.g., by colorimetry. Under the above experimental conditions, a positive reaction is shown once a colored spot is associated with a dilution of at least about 1:5, preferably of at least about 1:500.

Therapeutic or prophylactic efficacy of a polypeptide or derivative of the invention can be evaluated as described
below. A seventh aspect of the invention provides (i) a
composition of matter comprising a polypeptide of the invention
together with a diluent or carrier; specifically (ii) a
pharmaceutical composition containing a therapeutically or
prophylactically effective amount of a polypeptide of the
invention; (iii) a method for inducing an immune response
against Chlamydia in a mammal, by administering to the mammal
an immunogenically effective amount of a polypeptide of the
invention to elicit a protective immune response to Chlamydia;
and particularly, (iv) a method for preventing and/or treating
a Chlamydia (e.g., C. trachomatis, C. psittaci, C. pneumoniae,
or C. pecorum) infection, by administering a prophylactic or
therapeutic amount of a polypeptide of the invention to an
infected individual. Additionally, the seventh aspect of the
invention encompasses the use of a polypeptide of the invention
in the preparation of a medicament for preventing and/or
treating Chlamydia infection.

As used herein, the immunogenic compositions of the
invention are administered by conventional routes known the
vaccine field, in particular to a mucosal (e.g., ocular,
intranasal, pulmonary, oral, gastric, intestinal, rectal,
vaginal, or urinary tract) surface or via the parenteral (e.g.,
subcutaneous, intradermal, intramuscular, intravenous, or
intraperitoneal) route. The choice of administration route
depends upon a number of parameters, such as the adjuvant
associated with the polypeptide. If a mucosal adjuvant is
used, the intranasal or oral route is preferred. If a lipid
formulation or an aluminum compound is used, the parenteral
route is preferred with the sub-cutaneous or intramuscular
route being most preferred. The choice also depends upon the
nature of the vaccine agent. For example, a polypeptide of the
invention fused to CTB or LTB is best administered to a mucosal
surface.
As used herein, the composition of the invention contains one or several polypeptides or derivatives of the invention. The composition optionally contains at least one additional Chlamydia antigen, or a subunit, fragment, homolog, mutant, or derivative thereof.

For use in a composition of the invention, a polypeptide or derivative thereof is formulated into or with liposomes, preferably neutral or anionic liposomes, microspheres, ISCOMS, or virus-like-particles (VLPs) to facilitate delivery and/or enhance the immune response. These compounds are readily available to one skilled in the art; for example, see Liposomes: A Practical Approach, RCP New Ed, IRL press (1990).

Adjuvants other than liposomes and the like are also used and are known in the art. Adjuvants may protect the antigen from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. An appropriate selection can conventionally be made by those skilled in the art, for example, from those described below (under the eleventh aspect of the invention).

Treatment is achieved in a single dose or repeated as necessary at intervals, as can be determined readily by one skilled in the art. For example, a priming dose is followed by three booster doses at weekly or monthly intervals. An appropriate dose depends on various parameters including the recipient (e.g., adult or infant), the particular vaccine antigen, the route and frequency of administration, the presence/absence or type of adjuvant, and the desired effect (e.g., protection and/or treatment), as can be determined by one skilled in the art. In general, a vaccine antigen of the invention is administered by a mucosal route in an amount from about 10 μg to about 500 mg, preferably from about 1 mg to
about 200 mg. For the parenteral route of administration, the dose usually does not exceed about 1 mg, preferably about 100 µg.

When used as vaccine agents, polynucleotides and polypeptides of the invention may be used sequentially as part of a multistep immunization process. For example, a mammal is initially primed with a vaccine vector of the invention such as a pox virus, e.g., via the parenteral route, and then boosted twice with the polypeptide encoded by the vaccine vector, e.g., via the mucosal route. In another example, liposomes associated with a polypeptide or derivative of the invention is also used for priming, with boosting being carried out mucosally using a soluble polypeptide or derivative of the invention in combination with a mucosal adjuvant (e.g., LT).

A polypeptide derivative of the invention is also used in accordance with the seventh aspect as a diagnostic reagent for detecting the presence of anti-Chlamydia antibodies, e.g., in a blood sample. Such polypeptides are about 5 to about 80, preferably about 10 to about 50 amino acids in length. They are either labeled or unlabeled, depending upon the diagnostic method. Diagnostic methods involving such a reagent are described below.

Upon expression of a DNA molecule of the invention, a polypeptide or polypeptide derivative is produced and purified using known laboratory techniques. As described above, the polypeptide or polypeptide derivative may be produced as a fusion protein containing a fused tail that facilitates purification. The fusion product is used to immunize a small mammal, e.g., a mouse or a rabbit, in order to raise antibodies against the polypeptide or polypeptide derivative (monospecific antibodies). Accordingly, an eighth aspect of the invention provides a monospecific antibody that binds to a polypeptide or polypeptide derivative of the invention.
By "monospecific antibody" is meant an antibody that is capable of reacting with a unique naturally-occurring Chlamydia polypeptide. An antibody of the invention is either polyclonal or monoclonal. Monospecific antibodies may be recombinant, e.g., chimeric (e.g., constituted by a variable region of murine origin associated with a human constant region), humanized (a human immunoglobulin constant backbone together with hypervariable region of animal, e.g., murine, origin), and/or single chain. Both polyclonal and monospecific antibodies may also be in the form of immunoglobulin fragments, e.g., F(ab)′2 or Fab fragments. The antibodies of the invention are of any isotype, e.g., IgG or IgA, and polyclonal antibodies are of a single isotype or a mixture of isotypes.

Antibodies against the polypeptides, homologs or fragments of the present invention are generated by immunization of a mammal with a composition comprising said polypeptide, homolog or fragment. Such antibodies may be polyclonal or monoclonal. Methods to produce polyclonal or monoclonal antibodies are well known in the art. For a review, see "Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Eds. E. Harlow and D. Lane (1988), and D.E. Yelton et al., 1981. Ann. Rev. Biochem. 50:657-680. For monoclonal antibodies, see Kohler & Milstein (1975) Nature 256:495-497.

The antibodies of the invention, which are raised to a polypeptide or polypeptide derivative of the invention, are produced and identified using standard immunological assays, e.g., Western blot analysis, dot blot assay, or ELISA (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY). The antibodies are used in diagnostic methods to detect the presence of a Chlamydia antigen in a sample, such as a biological sample. The antibodies are also used in affinity chromatography for purifying a polypeptide or polypeptide derivative of the invention. As is discussed further below, such antibodies may
be used in prophylactic and therapeutic passive immunization methods.

Accordingly, a ninth aspect of the invention provides (i) a reagent for detecting the presence of Chlamydia in a biological sample that contains an antibody, polypeptide, or polypeptide derivative of the invention; and (ii) a diagnostic method for detecting the presence of Chlamydia in a biological sample, by contacting the biological sample with an antibody, a polypeptide, or a polypeptide derivative of the invention, such that an immune complex is formed, and by detecting such complex to indicate the presence of Chlamydia in the sample or the organism from which the sample is derived.

Those skilled in the art will readily understand that the immune complex is formed between a component of the sample and the antibody, polypeptide, or polypeptide derivative, whichever is used, and that any unbound material is removed prior to detecting the complex. It is understood that a polypeptide reagent is useful for detecting the presence of anti-Chlamydia antibodies in a sample, e.g., a blood sample, while an antibody of the invention is used for screening a sample, such as a gastric extract or biopsy, for the presence of Chlamydia polypeptides.

For diagnostic applications, the reagent (i.e., the antibody, polypeptide, or polypeptide derivative of the invention) is either in a free state or immobilized on a solid support, such as a tube, a bead, or any other conventional support used in the field. Immobilization is achieved using direct or indirect means. Direct means include passive adsorption (non-covalent binding) or covalent binding between the support and the reagent. By "indirect means" is meant that an anti-reagent compound that interacts with a reagent is first attached to the solid support. For example, if a polypeptide reagent is used, an antibody that binds to it can serve as an anti-reagent, provided that it binds to an epitope that is not
involved in the recognition of antibodies in biological samples. Indirect means may also employ a ligand-receptor system, for example, where a molecule such as a vitamin is grafted onto the polypeptide reagent and the corresponding receptor immobilized on the solid phase. This is illustrated by the biotin-streptavidin system. Alternatively, a peptide tail is added chemically or by genetic engineering to the reagent and the grafted or fused product immobilized by passive adsorption or covalent linkage of the peptide tail.

Such diagnostic agents may be included in a kit which also comprises instructions for use. The reagent is labeled with a detection means which allows for the detection of the reagent when it is bound to its target. The detection means may be a fluorescent agent such as fluorescein isocyanate or fluorescein isothiocyanate, or an enzyme such as horse radish peroxidase or luciferase or alkaline phosphatase, or a radioactive element such as $^{125}$I or $^{51}$Cr.

Accordingly, a tenth aspect of the invention provides a process for purifying, from a biological sample, a polypeptide or polypeptide derivative of the invention, which involves carrying out antibody-based affinity chromatography with the biological sample, wherein the antibody is a monospecific antibody of the invention.

For use in a purification process of the invention, the antibody is either polyclonal or monospecific, and preferably is of the IgG type. Purified IgGs is prepared from an antiserum using standard methods (see, e.g., Coligan et al., Current Protocols in Immunology (1994) John Wiley & Sons, Inc., New York, NY.). Conventional chromatography supports, as well as standard methods for grafting antibodies, are described in, e.g., Antibodies: A Laboratory Manual, D. Lane, E. Harlow, Eds. (1988) and outlined below.

Briefly, a biological sample, such as an $C.\ pneumoniae$ extract preferably in a buffer solution, is applied
to a chromatography material, preferably equilibrated with the buffer used to dilute the biological sample so that the polypeptide or polypeptide derivative of the invention (i.e., the antigen) is allowed to adsorb onto the material. The chromatography material, such as a gel or a resin coupled to an antibody of the invention, is in either a batch form or a column. The unbound components are washed off and the antigen is then eluted with an appropriate elution buffer, such as a glycine buffer or a buffer containing a chaotropic agent, e.g., guanidine HCl, or high salt concentration (e.g., 3 M MgCl₂). Eluted fractions are recovered and the presence of the antigen is detected, e.g., by measuring the absorbance at 280 nm.

An eleventh aspect of the invention provides (i) a composition of matter comprising a monospecific antibody of the invention, together with a diluent or carrier; (ii) a pharmaceutical composition comprising a therapeutically or prophylactically effective amount of a monospecific antibody of the invention, and (iii) a method for treating or preventing a Chlamydia (e.g., C. trachomatis, C. psittaci, C. pneumoniae or C. pecorum) infection, by administering a therapeutic or prophylactic amount of a monospecific antibody of the invention to an infected individual. Additionally, the eleventh aspect of the invention encompasses the use of a monospecific antibody of the invention in the preparation of a medicament for treating or preventing Chlamydia infection.

The monospecific antibody is either polyclonal or monoclonal, preferably of the IgA isotype (predominantly). In passive immunization, the antibody is administered to a mucosal surface of a mammal, e.g., the gastric mucosa, e.g., orally or intragastrically, advantageously, in the presence of a bicarbonate buffer. Alternatively, systemic administration, not requiring a bicarbonate buffer, is carried out. A monospecific antibody of the invention is administered as a single active component or as a mixture with at least one
monospecific antibody specific for a different Chlamydia polypeptide. The amount of antibody and the particular regimen used are readily determined by one skilled in the art. For example, daily administration of about 100 to 1,000 mg of antibodies over one week, or three doses per day of about 100 to 1,000 mg of antibodies over two or three days, are effective regimens for most purposes.

Therapeutic or prophylactic efficacy are evaluated using standard methods in the art, e.g., by measuring induction of a mucosal immune response or induction of protective and/or therapeutic immunity, using, e.g., the C. pneumoniae mouse model. Those skilled in the art will readily recognize that the C. pneumoniae strain of the model may be replaced with another Chlamydia strain. For example, the efficacy of DNA molecules and polypeptides from C. pneumoniae is preferably evaluated in a mouse model using C. pneumoniae strain. Protection is determined by comparing the degree of Chlamydia infection to that of a control group. Protection is shown when infection is reduced by comparison to the control group. Such an evaluation is made for polynucleotides, vaccine vectors, polypeptides and derivatives thereof, as well as antibodies of the invention.

Adjuvants useful in any of the vaccine compositions described above are as follows.

Adjuvants for parenteral administration include aluminum compounds, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate. The antigen is precipitated with, or adsorbed onto, the aluminum compound according to standard protocols. Other adjuvants, such as RIBI (ImmunoChem, Hamilton, MT), are used in parenteral administration.

Adjuvants for mucosal administration include bacterial toxins, e.g., the cholera toxin (CT), the E. coli heat-labile toxin (LT), the Clostridium difficile toxin A and
the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof such as a purified preparation of native cholera toxin subunit B (CTB). Fragments, homologs, derivatives, and fusions to any of these toxins are also suitable, provided that they retain adjuvant activity. Preferably, a mutant having reduced toxicity is used. Suitable mutants are described, e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/06627 (Arg-192-Gly LT mutant), and WO 95/34323 (Arg-9-Lys and Glu-129-Gly PT mutant). Additional LT mutants that are used in the methods and compositions of the invention include, e.g., Ser-63-Lys, Ala-69Gly, Glu-110-Asp, and Glu-112-Asp mutants. Other adjuvants, such as a bacterial monophosphoryl lipid A (MPLA) of, e.g., E. coli, Salmonella minnesota, Salmonella typhimurium, or Shigella flexneri; saponins, or polylactide glycolide (PLGA) microspheres, is also be used in mucosal administration.

Adjuvants useful for both mucosal and parenteral administrations include polyphosphazene (WO 95/02415), DC-chol (3 b-(N-(N',N' -dimethyl aminomethane)-carbamoyl) cholesterol; U.S. Patent No. 5,283,185 and WO 96/14831) and QS-21 (WO 88/09336).

Any pharmaceutical composition of the invention containing a polynucleotide, a polypeptide, a polypeptide derivative, or an antibody of the invention, is manufactured in a conventional manner. In particular, it is formulated with a pharmaceutically acceptable diluent or carrier, e.g., water or a saline solution such as phosphate buffer saline. In general, a diluent or carrier is selected on the basis of the mode and route of administration, and standard pharmaceutical practice.

Suitable pharmaceutical carriers or diluents, as well as pharmaceutical necessities for their use in pharmaceutical formulations, are described in Remington’s Pharmaceutical Sciences, a standard reference text in this field and in the USP/NF.
The invention also includes methods in which Chlamydia infection are treated by oral administration of a Chlamydia polypeptide of the invention and a mucosal adjuvant, in combination with an antibiotic, an antacid, sucralfate, or a combination thereof. Examples of such compounds that can be administered with the vaccine antigen and the adjuvant are antibiotics, including, e.g., macrolides, tetracyclines, and derivatives thereof (specific examples of antibiotics that can be used include azithromycin or doxicyclin or immunomodulators such as cytokines or steroids). In addition, compounds containing more than one of the above-listed components coupled together, are used. The invention also includes compositions for carrying out these methods, i.e., compositions containing a Chlamydia antigen (or antigens) of the invention, an adjuvant, and one or more of the above-listed compounds, in a pharmaceutically acceptable carrier or diluent.

It has recently been shown that the 9kDa cysteine rich membrane protein contains a sequence cross-reactive with the murine alpha-myosin heavy chain epitope M7A-alpha, an epitope conserved in humans (Bachmaier et al., Science (1999) 283:1335). This cross-reactivity is proposed to contribute to the development of cardiovascular disease, so it may be beneficial to remove this epitope, and any other epitopes cross-reactive with human antigens, from the protein if it is to be used as a vaccine. Accordingly, a further embodiment of the present invention includes the modification of the coding sequence, for example, by deletion or substitution of the nucleotides encoding the epitope from polynucleotides encoding the protein, as to improve the efficacy and safety of the protein as a vaccine. A similar approach may be appropriate for any protective antigen found to have unwanted homologies or cross-reactivities with human antigens.

Amounts of the above-listed compounds used in the methods and compositions of the invention are readily
determined by one skilled in the art. Treatment/immunization
schedules are also known and readily designed by one skilled in
the art. For example, the non-vaccine components can be
administered on days 1-14, and the vaccine antigen + adjuvant
can be administered on days 7, 14, 21, and 28.

EXAMPLES

The above disclosure generally describes the present
invention. A more complete understanding can be obtained by
reference to the following specific examples. These examples
are described solely for purposes of illustration and are not
intended to limit the scope of the invention. Changes in form
and substitution of equivalents are contemplated as
circumstances may suggest or render expedient. Although
specific terms have been employed herein, such terms are
intended in a descriptive sense and not for purposes of
limitation.

Example 1:

This example illustrates the preparation of a plasmid
vector pCACPNM555a containing the full length 76kDa protein
gene.

The full-length 76kDa protein gene was amplified from
Chlamydia pneumoniae genomic DNA by polymerase chain reaction
(PCR) using a 5' primer (5'
ATAAGAATGCGCGGACCACATGGTTAAATCTATTGGTCCAGG 3') (SEQ ID No:9)
and a 3' primer (5' GCGCGGATCCCCTGGGAGATACAACCAGAATATAGAG 3')
(SEQ ID No:10). The 5' primer contains a Not I restriction
site, a ribosome binding site, an initiation codon and a
sequence close to the 5' end of the full-length 76kDa protein
coding sequence. The 3' primer includes the sequence encoding
the C-terminal sequence of the 76kDa protein and a Bam HI
restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.

After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 2 (Fig. 4) with transcription under control of the human CMV promoter.

Example 2:

This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the full-length 76kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCACPNM555a (Fig 4).

The resulting plasmid, pCACPNM555a, was transferred by electroporation into E. coli XL-1 blue (Stratagene) which was grown in LB broth containing 50 μg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.
Example 3:

This example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*.

It has been previously demonstrated (Yang et al., 1993) that mice are susceptible to intranasal infection with different isolates of *C. pneumoniae*. Strain AR-39 (Grayston, 1989) was used in Balb/c mice as a challenge infection model to examine the capacity of Chlamydia gene products delivered as naked DNA to elicit a protective response against a sublethal *C. pneumoniae* lung infection. Protective immunity is defined as an accelerated clearance of pulmonary infection.

Groups of 7 to 9 week old male Balb/c mice (7 to 10 per group) were immunized intramuscularly (i.m.) plus intranasally (i.n.) with plasmid DNA containing the coding sequence of *C. pneumoniae* full-length 76kDa protein as described in Examples 1 and 2. Saline or the plasmid vector lacking an inserted Chlamydial gene was given to groups of control animals.

For i.m. immunization alternate left and right quadriceps were injected with 100μg of DNA in 50μl of PBS on three occasions at 0, 3 and 6 weeks. For i.n. immunization, anaesthetized mice aspirated 50μl of PBS containing 50 μg DNA on three occasions at 0, 3 and 6 weeks. At week 8, immunized mice were inoculated i.n. with $5 \times 10^5$ IFU of *C. pneumoniae*, strain AR39 in 100μl of SPG buffer to test their ability to limit the growth of a sublethal *C. pneumoniae* challenge.

Lungs were taken from mice at days 5 and 9 post-challenge and immediately homogenised in SPG buffer (7.5% sucrose, 5mM glutamate, 12.5mM phosphate pH7.5). The homogenate was stored frozen at -70°C until assay. Dilutions of the homogenate were assayed for the presence of infectious
Chlamydia by inoculation onto monolayers of susceptible cells. The inoculum was centrifuged onto the cells at 3000 rpm for 1 hour, then the cells were incubated for three days at 35°C in the presence of 1 μg/ml cycloheximide. After incubation the monolayers were fixed with formalin and methanol then immunoperoxidase stained for the presence of Chlamydial inclusions using convalescent sera from rabbits infected with C. pneumoniae and metal-enhanced DAB as a peroxidase substrate.

Figure 7 and Table 1 show that mice immunized i.n. and i.m. with pCACPNM555a had Chlamydial lung titers less than 30,000 IFU/lung (mean 23,550) in 5 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 20,800 to 323,300 IFU/lung (mean 206,375) for (Table 1). DNA immunisation per se was not responsible for the observed protective effect since two other plasmid DNA constructs, pCACPNM806 and pCACPNM760, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCACPNM806 and pCACPNM760 are identical to pCACPNM555a except that the nucleotide sequence encoding the full-length 76kDa protein is replaced with C. pneumoniae nucleotide sequences encoding an unrelated sequence.
Table 1

<table>
<thead>
<tr>
<th>MOUSE</th>
<th>BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IMMUNIZING CONSTRUCT</td>
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<tr>
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<td>Saline</td>
</tr>
<tr>
<td></td>
<td>Day 9</td>
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<tr>
<td>Wilcoxon p</td>
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</tbody>
</table>

5 Example 4:

This example illustrates the preparation of a plasmid vector pCAI555 containing a 5'-truncated 76kDa protein gene.

The 5' truncated 76kDa protein gene was amplified from Chlamydia pneumoniae genomic DNA by polymerase chain reaction (PCR) using a 5' primer (5'
ATAAGAATGCGCGCCGACCATTGAGTCTGGCAGATAAAGCTGGG 3') (SEQ ID No:11) and a 3' primer (5' GCGCGGATCCCTTGGAGATAACCAAGATATA 3') (SEQ ID No:12). The 5' primer contains a Not I restriction site, a ribosome binding site, an initiation codon and a sequence at the second Met codon of the 76kDa protein coding sequence. The 3' primer includes the sequence encoding the C-terminal sequence of the 3' 76kDa protein and a Bam HI restriction site. The stop codon was excluded and an additional nucleotide was inserted to obtain an in-frame fusion with the Histidine tag.
After amplification, the PCR fragment was purified using QIAquick™ PCR purification kit (Qiagen) and then digested with Not I and Bam HI and cloned into the pCA-Myc-His eukaryotic expression vector describe in Example 5 (Fig. 5) with transcription under control of the human CMV promoter.

Example 5:

This example illustrates the preparation of the eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was restricted with Spe I and Bam HI to remove the CMV promoter and the remaining vector fragment was isolated. The CMV promoter and intron A from plasmid VR-1012 (Vical) was isolated on a Spe I / Bam HI fragment. The fragments were ligated together to produce plasmid pCA/Myc-His. The Not I/Bam HI restricted PCR fragment containing the 5′ truncated 76kDa protein gene was ligated into the Not I and Bam HI restricted plasmid pCA/Myc-His to produce plasmid pCAI555 (Fig 5).

The resulting plasmid, pCAI555, was transferred by electroporation into E. coli XL-1 blue (Stratagene) which was grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5′ and 3′ ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.
Example 6:

This Example illustrates the immunization of mice to achieve protection against an intranasal challenge of *C. pneumoniae*. The procedures are described in Example 3 above, except that the DNA plasmid used for immunization contains the coding sequence of *C. pneumoniae* 5′-truncated 76kDa protein, as described in Examples 4 and 5.

Figure 8 and Table 2 show that mice immunized i.n. and i.m. with pCAI555 had Chlamydial lung titers less than 13000 IFU/lung (mean 6050) in 6 of 6 cases at day 9 whereas the range of values for control mice sham immunized with saline were 106,100 IFU/lung (mean 39,625) for (Table 2). DNA immunisation *per se* was not responsible for the observed protective effect since two other plasmid DNA constructs, pCAI116 and pCAI178, failed to protect, with lung titers in immunised mice similar to those obtained for saline-immunized control mice. The constructs pCAI116 and pCAI178 are identical to pCAI555 except that the nucleotide sequence encoding the 5′-truncated 76kDa protein is replaced with a *C. pneumoniae* nucleotide sequence encoding an unprotective sequence and the nucleoside 5′-diphosphate phosphotransferase protein.
### Table 2

<table>
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<tr>
<th>MOUSE</th>
<th>BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS</th>
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#### Example 7:

This example illustrates the preparation of a plasmid vector pCAD76kDa containing a 3'-truncated 76kDa protein gene. The 3'-truncated 76kDa protein gene was amplified from *Chlamydia pneumoniae* genomic DNA by polymerase chain
reaction (PCR) using a 5’ primer (5’
GCTCTAGACCGCATGACAAAAAACATTATGCTTTGGG 3’) (SEQ ID No:13) and a
3’ primer (5’ CGGGATCCATAGAACTTGGCTGCACCGGG 3’) (SEQ ID No:14). The 5’ primer contains a Xba I restriction site, a ribosome
5 binding site, an initiation codon and a sequence 765bp upstream of the 5’ end of the 76kDa protein coding sequence. The 3’
primer includes a 21bp the sequence downstream of codon 452 of
the 76kDa protein and a Bam HI restriction site. An additional
nucleotide was inserted to obtain an in-frame fusion with the
10 Histidine tag. Note that inclusion of the 765bp 5’ region and
the 21bp 3’ regions were inadvertent. These sequences are not
part of the 76kDa protein gene. Nevertheless, immunoprotection
was achieved using this sequence (Example 6).

After amplification, the PCR fragment was purified
15 using QIAquick™ PCR purification kit (Qiagen) and then digested
with Xba I and Bam HI and cloned into the pCA-Myc-His
eukaryotic expression vector describe in Example 8 (Fig. 6)
with transcription under control of the human CMV promoter.

Example 8:

This Example illustrates the preparation of the
eukaryotic expression vector pCA/Myc-His.

Plasmid pcDNA3.1(-)Myc-His C (Invitrogen) was
25 restricted with Spe I and Bam HI to remove the CMV promoter and
the remaining vector fragment was isolated. The CMV promoter
and intron A from plasmid VR-1012 (Vical) was isolated on a Spe
I / Bam HI fragment. The fragments were ligated together to
produce plasmid pCA/Myc-His. The Xba I/Bam HI restricted PCR
fragment containing a 3’-truncated 76kDa protein gene was
ligated into the Xba I and Bam HI restricted plasmid pCA/Myc-
His to produce plasmid pCAD76kDa (Fig. 6).

The resulting plasmid, pCAD76kDa, was transferred by
electroporation into E. coli XL-1 blue (Stratagene) which was
grown in LB broth containing 50 µg/ml of carbenicillin. The plasmid was isolated by Endo Free Plasmid Giga Kit™ (Qiagen) large scale DNA purification system. DNA concentration was determined by absorbance at 260 nm and the plasmid was verified after gel electrophoresis and Ethidium bromide staining and comparison to molecular weight standards. The 5' and 3' ends of the gene were verified by sequencing using a LiCor model 4000 L DNA sequencer and IRD-800 labelled primers.

Example 9:

This example illustrates the immunization of mice to achieve protection against an intranasal challenge of C. pneumoniae. The procedures are as described in Example 3 above, except that the DNA plasmid used for immunization contains the coding sequence of C. pneumoniae 3'-truncated 76kDa protein, as described in Examples 7 and 8.

Figure 9 and Table 3 show that mice immunized i.n. and i.m. with pCAD76kDa had Chlamydial lung titers less than 2400 in 5 of 5 cases whereas the range of values for control mice were 1800-23100 IFU/lung (mean 11811) and 16600-26100 IFU/lung (mean 22100) for sham immunized with saline or immunized with the unmodified vector respectively (Table 2). The lack of protection with the unmodified vector confirms that DNA per se was not responsible for the observed protective effect. This is further supported by the results obtained for one additional plasmid DNA construct, pdagA, that failed to protect, and for which the mean lung titers were similar to those obtained for saline-immunized control mice. The construct pdagA is identical to pCAD76kDa except that the nucleotide sequence encoding the 3'-truncated 76kDa protein is replaced with a C. pneumoniae nucleotide sequence encoding the protein dagA.
Table 3

<table>
<thead>
<tr>
<th>MOUSE</th>
<th>BACTERIAL LOAD (INCLUSION FORMING UNITS PER LUNG) IN THE LUNGS OF BALB/C MICE IMMUNIZED WITH VARIOUS DNA IMMUNIZATION CONSTRUCTS</th>
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<tbody>
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</tbody>
</table>
CLAIMS:

1. A nucleic acid molecule comprising a nucleic acid sequence which encodes a polypeptide selected from any of:
   (a) SEQ ID No: 2;
   (b) SEQ ID No: 4;
   (c) SEQ ID No: 6;
   (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
   (e) a polypeptide of any one of (a) to (d) which has been modified to improve its immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).

2. A nucleic acid molecule comprising a nucleic acid sequence selected from any of:
   (a) SEQ ID No: 1;
   (b) SEQ ID No: 3;
   (c) SEQ ID No: 5;
   (d) a sequence which encodes a polypeptide encoded by any one of SEQ ID Nos: 1, 3 and 5;
   (e) a sequence comprising at least 38 consecutive nucleotides from any one of the nucleic acid sequences of (a) to (d); and
   (f) a sequence which encodes a polypeptide which is at least 75% identical in amino acid sequence to the polypeptides encoded by any one of SEQ ID Nos: 1, 3 and 5.

3. A nucleic acid molecule comprising a nucleic acid sequence which is anti-sense to the nucleic acid molecule of claim 1.

4. A nucleic acid molecule comprising a nucleic acid sequence which encodes a fusion protein, said fusion protein comprising a polypeptide encoded by a nucleic acid molecule according to claim 1 and an additional polypeptide.
5. The nucleic acid molecule of claim 4 wherein the additional polypeptide is a heterologous signal peptide.

6. The nucleic acid molecule of claim 4 wherein the additional polypeptide has adjuvant activity.

7. A nucleic acid molecule according to any one of claims 1 to 6, operatively linked to one or more expression control sequences.

8. A vaccine comprising at least one first nucleic acid according to any one of claims 1, 2, and 4 to 7 and a vaccine vector wherein each first nucleic acid is expressed as a polypeptide, the vaccine optionally comprising a second nucleic acid encoding an additional polypeptide which enhances the immune response to the polypeptide expressed by said first nucleic acid.

9. The vaccine of claim 8 wherein the second nucleic acid encodes an additional *Chlamydia* polypeptide.

10. A pharmaceutical composition comprising a nucleic acid according to any one of claims 1 to 7 and a pharmaceutically acceptable carrier.

11. A pharmaceutical composition comprising a vaccine according to claim 8 or 9 and a pharmaceutically acceptable carrier.

12. A unicellular host transformed with the nucleic acid molecule of claim 7.

13. A nucleic acid probe of 5 to 100 nucleotides which hybridizes under stringent conditions to the nucleic acid molecule of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.

14. A primer of 10 to 40 nucleotides which hybridizes under stringent conditions to the nucleic acid molecules of SEQ ID No: 1, or to a homolog or complementary or anti-sense sequence of said nucleic acid molecule.

15. A polypeptide encoded by a nucleic acid sequence according to any one of claims 1, 2 and 4 to 7.
16. A polypeptide comprising an amino acid sequence selected from any of:
   (a) SEQ ID No: 2;
   (b) SEQ ID No: 4;
   (c) SEQ ID No: 6;
   (d) an immunogenic fragment comprising at least 12 consecutive amino acids from a polypeptide of (a); and
   (e) a polypeptide of any one of (a) to (d) which has been modified to improve its immunogenicity, wherein said modified polypeptide is at least 75% identical in amino acid sequence to the corresponding polypeptide of any one of (a) to (d).
17. A fusion polypeptide comprising a polypeptide of claim 15 or 16 and an additional polypeptide.
18. The fusion polypeptide of claim 17 wherein the additional polypeptide is a heterologous signal peptide.
19. The fusion protein of claim 17 wherein the additional polypeptide has adjuvant activity.
20. A method for producing a polypeptide of claim 15 or 16, comprising the step of culturing a unicellular host according to claim 12.
21. An antibody against the polypeptide of any one of claims 15 to 19.
22. A vaccine comprising at least one first polypeptide according to any one of claims 15 to 19 and a pharmaceutically acceptable carrier, optionally comprising a second polypeptide which enhances the immune response to the first polypeptide.
23. The vaccine of claim 22 wherein the second polypeptide comprises an additional Chlamydia polypeptide.
24. A pharmaceutical composition comprising a polypeptide according to any one of claims 15 to 19 and a pharmaceutically acceptable carrier.
25. A pharmaceutical composition comprising a vaccine according to claim 22 or 23 and a pharmaceutically acceptable carrier.
26. A pharmaceutical composition comprising an antibody according to claim 21 and a pharmaceutically acceptable carrier.

27. A method for preventing or treating Chlamydia infection using:
   (a) the nucleic acid of any one of claims 1 to 7;
   (b) the vaccine of any one of claims 8, 9, 22 and 23;
   (c) the pharmaceutical composition of any one of claims 10, 11, 24 to 26;
   (d) the polypeptide of any one of claims 15 to 19; or
   (e) the antibody of claim 21.

28. A method of detecting Chlamydia infection comprising the step of assaying a body fluid of a mammal to be tested, with a component selected from any one of:
   (a) the nucleic acid of any one of claims 1 to 7;
   (b) the polypeptide of any one of claims 15 to 19; and
   (c) the antibody of claim 21.

29. A diagnostic kit comprising instructions for use and a component selected from any one of:
   (a) the nucleic acid of any one of claims 1 to 7;
   (b) the polypeptide of any one of claims 15 to 19; and
   (c) the antibody of claim 21.

30. A method for identifying a polypeptide of claims 15 to 19 which induces an immune response effective to prevent or lessen the severity of Chlamydia infection in a mammal previously immunized with polypeptide, comprising the steps of:
   (a) immunizing a mouse with the polypeptide; and
   (b) inoculating the immunized mouse with Chlamydia; wherein the polypeptide which prevents or lessens the severity of Chlamydia infection in the immunized mouse compared to a non-immunized control mouse is identified.

31. An expression plasmid selected from the group consisting of pCACPNM555a, pCAI555 and pCAD76kDa.
32. A nucleic acid molecule selected from the group consisting of SEQ ID Nos: 1, 3, 5 and 7.
33. A polypeptide selected from the group consisting of SEQ ID Nos: 2, 4, 6 and 8.
34. An isolated 76kDa protein from Chlamydia.
35. An isolated 76kDa protein from Chlamydia pneumoniae
Figure 1: Full-length Sequence of C. pneumoniae 76kDa Gene.

```
ataaatcgt taaaacaggt ctcgcatataa ttattagtga gacgtttttt ttatatttttt 60
ataataaac taaaagatttt ttattatatata ttggatttttt atgtt aat cct att
   Met Val Asn Pro Ile
      1     5
      115
Gly Pro Gly Pro Ile Asp Glu Thr Glu Arg Thr Pro Pro Ala Asp Leu
      10     15  20
      163
ggt cca ggt cct ata gac gaa aca gaa cgc aca cct ccc gcc gat ctt
      Ser Ala Glu Leu Glu Ala Ser Ala Ala Asn Ser Ala Glu Ala
      25     30  35
      211
tct gct cca gga ttg gag gcg agt gcc gca aat aag agt gcg gaa gct
      Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Ser Ala Glu Ala
      40     45  50
      259
caa aga ata gca ggt gcg gaa gct aag cct aaa gaa tct aag acc gat
      Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Ser Lys Thr Asp
      60     65
      307
tct gta gag cga tgt agc atc tgt tgt gca gtt aat gct ctc atg
      Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu Met
      75     80  85
      355
agt ctg gcc gat aag ctc ggt att gct tct aag acc aag ctc tct tct
      Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser Ser
      90     95 100
      403
cct cct cca ccc acg ttg gat gat tat aag act cca gcg cca aca gct
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      451
tac gat act atc ttg acc tca aca tca tga gct gac ata cag gct ggt
      Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Glu Ala Ala
     120    125 130
      499
ttg ggt gcc ctc cag gat gct gtc act aat ata aag gat cca gcg gct
      Leu Val Ser Leu Glu Asp Ala Val Thr Asn Ile Lys Asp Thr Ala Ala
     135    140 145
      547
act gat gag gaa acc cca atc gct gcg gct tgg gaa act aag aat gcc
      Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn Ala
     150    155 160 165
      595
gat gca gtt aaa gtt gcg gcg cca att aca gaa tta gcg aaa tat gct
      Asp Ala Val Lys Val Gly Ala Glu Ile Thr Glu Leu Ala Lys Tyr Ala
     170    175 180
      643
tcg gat aac cca gcg att ctt gac tct tta ggt aaa ctg act tcc tcc
      Ser Asp Asn Glu Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser Phe
     185    190 195
      691
```
Figure 1 (continued)

2/22

gac ctc tta cag gct gct ctt ctc caa tct gta gca aac aat aac aaa
Asp Leu Leu Gln Ala Ala Leu Leu Gln Ser Val Ala Asn Asn Asn Lys
200 205 210

839

gca gct gag ctt ctt aaa gag atg cca gat aac cca gta gtc cca ggg
Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly
215 220 225

877

aaa acc cct gca att gct cca tct tta gtt gat cag cca gat gct cca
Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala Thr
230 235 240 245

835

ggc cag atg gag aaat gga aat ggc att agg gat gca tat ttt
Ala Thr Glu Ile Gln Asp Gly Asn Ala Ile Arg Asp Ala Tyr Phe
250 255 260

883

gca gga cag aac gct aag gca gct gta gaa aat gct aac tct aat aac
Ala Gly Glu Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn Asn
265 270 275

931

agt ata agc aac ata gat tca gct aag gca gca atc gct act gct aag
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280 285 290

979

aca caa ata gct gaa gct cag aca aag ttc ccc gac tct cca att ctt
Thr Glu Ala Ala Glu Glu Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn
295 300 305

1027

caa gaa gcg gaa caa atg gta ata cag gct gag aaa gat ctt aaa aat
Gln Glu Ala Glu Glu Met Val Ile Gln Ala Glu Lys Asp Leu Lys Asn
310 315 320 325

1075

att aca cct gca gat ggt tct gat gtt cca aat cca gga act aca gtt
Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asp Gly Thr Thr Val
330 335 340

1123

GGY Gly Ser Lys Gln Glu Gly Ser Ser Ile Gly Ser Ile Arg Val Ser
345 350 355

1171

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Met Leu Leu Asp Ala Ala Glu Ala Ala Gln Leu Ala Ala Arg Ala Ala Lys Ala
360 365 370

1219

99

GGY Gly Phe Arg Glu Ile His Met Phe Asn Thr Glu Asn Pro Asp Ser
375 380 385

1267

caa gct gcc caa cag gct ctc gca gca gaa aat cct gat tcc
Gln Ala Ala Glu Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys Ala
390 395 400 405

1315

gct gga gat gac aag gct gtc gca gcg ctg gca gat gct cag aag gct
Ala Gly Asp Asp Ser Ala Ala Ala Ala Ala Leu Ala Asp Ala Glu Lys Ala
410 415 420

1363
Figure 1 (continued)

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Tyr Ser Gly Tyr Leu Gln
650

ttattatgtg ctttggtaag gctttttgttg aggcctttacc aacacactag aacgatcttc 2143

aataaataaa aga 2156
Figure 2: Restriction enzyme map of *C. pneumoniae* 76kDa gene.

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Figure 2 (continued)

```
TspRI
BstAPI
BtsI
PstI

HinfI
 Hin4I

TfII
 Hin4I

TaqII
 SfaNI

BccI
 HaeIV

SfiI
 CviRI

MwoI

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241
ATTTCTTAGATTCTGGCTAAGACACATCTCCTGCTACCTGTTGAAGAAGACAGGTCACTTAAG

300

HinfI
 NlaIII

Hpy178III
 RcaI

AluI
 CviJI

BbsI

MboII

BfaI
	AceIII

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360
AGAGTACTCAGACCGCTCTATTGCAACCATAACGAAAGATCTATTGCGAGCACAGAAAGATGATC

HinfI

Hin4I

BsrI

MaeII

PaeI

PstI

CviRI

SfiI

DpnI

BglII

BstYI

Sau3AI

Bce83I

MnlI

MaeII

BseRI

AccI

BseEI

BsaXI

MnlI

CAGATCGTCGACGCTGAGCTCAACGACACGAGCCACCTCGACTCCCTCACCCCACCTTT

361
GTCTAGAGCTCGACCTGAGTTGCCTGCCTGGCTGCGCTGAGTGAGGCAGGAGGTGGTGCA

420

SmaI

HinfI

AluI

CviJI

PaeI

MwoI

TaqII

HhaI

Tth1111I

BfaI

MnlI

MnlI

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421
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480
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Figure 2 (continued)

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8/22

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SfaNI
AluI
CviJI
Bpu102I
DdeI
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CviJI
MspAI
PvuII
Tth111I
Fnu4HI
BseMII
TseI

BsmFI
CviRI
BsrI

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721 +--------------------------------------------------------------+ 780
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ScrlI
MunI
DpnI
BsaJI
Tsp509I
BclI
BsaJI
CviRI
Sau3AI
EcoRII
Cac8I
SfaNI
Sfcl

Hpy188IIX

CCCAGGGAGAACGCTGCATGCTCAATCTTTAGGTTGATCCAGACAGATGCTACAGCGAC
781 +--------------------------------------------------------------+ 840
GGGTCCCTTTTTGCGGAGCTTAACGAGTTAGAAATCAACTAGTCTGTCTACGATGTGCCTG

CviRI
FokI
CjePI
BstAPI
NsiI
BccI
SfaNI

841 +--------------------------------------------------------------+ 900
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GTGTCTATCTTTTCTACCCCTACGCTATGTAATAAAAACGCTCTGTCTTAGGAC

Sfcl
AluI
CviJI
MwoI
CjePI
TaaI
HinfI
AluI
MwoI

901 +--------------------------------------------------------------+ 960
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ACCTCGACATCTTTTACGATTAGTTATTGCTATATTTCGCTTGTATACAGATCTATCGATTTCG
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Figure 2 (continued)
Figure 3: Sequence Containing Truncated Version of *C. pneumoniae* 76kDa Gene; (nucleotides 1 to 665 and 2122 to 2238 are unrelated to the 76kDa Gene).

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acagaaaaag ctcctacaaa cgctatgaa tacaatact gtggttggca gtggctgtgc 180

ggaaagccata gtcaggttcc ttggatcaaat ggacagaaaa agccctctata tctttatgga 240

gctttcttattgagaacccattt agcaagtggct acaagagcta cgctaatgag aaaaagaaac 300

cattgcgtttt ttatggagg aacctttaag ggactcagaa aagtggagaga cttgcctgccc 360

acatgtcgtt attgtatgt gaagaccttg tcggctccag aatataggtg ttcagggatt 420

ggcgctggtta attatcttaa gttttgttgc gcctaaagcaaa tcgtgctaa ctatgcatct 480

aaagagctctc atggtttttc aaattatatt tcctatatag tcatggtcatc 540

acagatttttc tattttccca cttcctcttc ccctcctc tccctcctca 600

agtcaggtt ttggcttcatg agatttttgt gcnaagtttag ctagtgtataa ttcagccttt 660

atatctttaaa ccagcttggcc attatatttt atgtgagagcct ttttttttttt 720

atatatatt caacttaaatt gatttttttt tccttttttgag atgtatatct 777

Met Val Asn Pro

```

```plaintext
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Leu Ser Ala Gln Gly Leu Glu Ala Ser Ala Ala Asn Lys Ser Ala Glu
25 30 35

gct cga aga ata gca ggt gcg gaa gct aag cct aaa gaa tgt act acc 921
Ala Gin Arg Ile Ala Gln Glu Ala Lys Pro Lyt Glu Ser Lys Thr
40 45 50

gat tct gta gag cga tgg aag ctc tct gcg tct gcc gat aat gcg ctc 969
Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala Val Asn Ala Leu
55 60 65

atg aat ctc gca gat aag ctc ggt att gct tct aag acc agc tcc 1017
Met Ser Leu Ala Asp Lys Leu Gly Ile Ala Ser Ser Asn Ser Ser Ser
70 75 80

tct act aag ta ttc gca gac gtt gac tca aag aca gcg acc cct 1065
Ser Thr Ser Arg Ala Asp Val Asp Thr Thr Ala Thr Ala Pro
85 90 95 100
```
Figure 3 (continued)

```
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Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Gln Ala Gln Thr
  105  110  115

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Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala
  120  125  130

agt ttg agc ctc cag gat gct gtc act aat ata aag gat aca gcg
Ala Leu Val Ser Leu Gln Asp Ala Val Thr Asn Ile Lys Asp Thr Ala
  135  140  145

agt act gat gac gaa acc gca atc gct gcc gac tgg gaa act aag aat
Ala Thr Asp Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn
  150  155  160

ggc gat gca gtt aaa gtt gcc ggc cgg cca att aca gaa tta ggc aag tta
Ala Asp Val Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr
  165  170  175  180

gtt ctc gat aac cca ggc att ctt gac tct tta gtt aat ctg act tcc
Ala Ser Asp Ala Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser
  190  195

ttc gac ctc tta cag gct gct ctc cca tta gta gca aac aat aac
Phe Asp Leu Leu Glu Ala Ala Leu Gln Ser Val Ala Asn Asn Asn
  200  205  210

aaa gca gct gag ctt ctt aaa gag atg caa gat aac cca gta gtc cca
Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro
  220  225

ggg aaa acg cct gca att gct cta tta gtt gat cag aca gat gct
Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala
  230  235  240

aca ggc aca cag ata gag aaa gat gga aat agg gat gca tat
Thr Ala Thr Gln Ile Glu Asp Gly Asn Ala Ile Arg Asp Ala Tyr
  245  250  255  260

agt gca gca cag acg gtt gta gca aat gct aat ctt ctc ctc gac ttt gca gaa aag gat aca cag cgg cca atc gct aat ctc gct cgc
Phe Ala Gly Glu Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn
  265  270  275

asa aat aag cag ata atcg tca gct aag gca gca atc gct aat ctc gct
Asp Ser Ile Ser Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala
  280  285  290

aag aca cag cgg aac cgg gat aca gct cag aag cgg aag cgg aac ttc ccc gac tct cca gac
Lys Thr Glu Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile
  295  300  305
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Figure 3 (continued)

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Asn Ile Lys Pro Ala Asp Gly Ser Asp Val Pro Asn Pro Gly Thr Thr
325     330     335     340

gtt gga ggc tcc aag caa cca gga ggt aat gtt ccc aat gtt ccc
Val Gly Gly Ser Lys Gln Gln Gly Ser Ser Thr Gly Ser Thr Arg Val
345     350     355

tcc atg ctg tta gat gat gct gaa aat gag acc gtt ccc att ttg atg
Ser Met Leu Leu Asp Asp Ala Glu Asn Thr Ala Ser Ile Leu Met
360     365     370

tct ggg ttt cct cag atg att cac atg ttc aat acg gaa aat cct gat
Ser Gly Phe Arg Gln Met Ile His Met Phe Asn Thr Glu Asn Pro Asp
375     380     385

tct caa gct gcc caa cag gac ctc gca gca caa gct aga gca gcc gaa
Ser Gln Ala Ala Glu Glu Leu Ala Ala Gln Ala Arg Ala Ala Lys
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Ala Ala Gly Asp Ser Ala Ala Ala Leu Ala Asp Ala Gln Lys
405     410     415     420

gct tta gaa ggc gct cta ggt aaa gct ggg caa caa cag ggc ata ctc
Ala Leu Glu Ala Leu Gly Lys Ala Gln Gln Gln Gly Ile Leu
425     430     435

aat gct tta gga cag atc gct tct gct gct gtt agc gcg gca gga gta
Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val Ser Ala Gly Val
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cct cgg cct gag cta tgg atc cga gct cgg tac ctt gct gcc
Leu Pro Leu Gln Val Leu Trp Ile Arg Ala Arg Tyr Gln Ala Tyr
455     460     465

gta gaa caa aaa ctc atc tca gaa gag gat cgg aat agc gcc gtc gac
Val Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp
470     475     480

cat cat cat cat cat cat tga
His His His His His His
485     490
FIG. 4

Construction of pCACPNM555a
Figure 7: pCACPNM555a Confers Protection Against C. pneumoniae Infection.
Figure 8: pCAI555 Confers Protection Against C. pneumoniacae Infection.
Figure 9: pCAD76kDa Confers Protection against C. pneumoniae Infection.
Aventis Pasteur Limited

Chlamydia antigens and corresponding DNA fragments and uses thereof

US 60/132,270

US 60/141,276

1999-05-03

1999-06-30

14

PatentIn Ver. 2.0

CDS

(101)...(2053)

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atg gtt aat cc tattttttttttttttttttt

Met Val Asn Pro Ile

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25 30 35

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Gln Arg Ile Ala Gly Ala Glu Ala Lys Pro Lys Glu Ser Lys Thr Asp

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Tyr Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Gln Ala Ala
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135 140 145
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Ser Asp Asn Glu Ala Ile Leu Val Asp Ser Leu Gly Lys Leu Thr Ser Phe
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Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Asn Pro Val Val Pro Gly
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Ser Ile Ser Asn Ile Asp Ser Ala Lys Ala Ala Ile Ala Thr Ala Lys
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Thr Glu Ile Ala Glu Ala Gln Lys Lys Phe Pro Asp Ser Pro Ile Leu
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4/22

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Glu Ser Lys Thr Asp Ser Val Glu Arg Trp Ser Ile Leu Arg Ser Ala
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Glu Asn Pro Asp Ser Gln Ala Ala Gln Glu Leu Ala Ala Ala Gln Ala 385 390 395 400
Arg Ala Ala Lys Ala Ala Gly Asp Asp Ser Ala Ala Ala Leu Ala 405 410 415
Asp Ala Gln Lys Ala Leu Glu Ala Ala Leu Gly Lys Ala Gly Gln Gln 420 425 430
Gln Gly Ile Leu Asn Ala Leu Gly Gln Ile Ala Ser Ala Ala Val Val 435 440 445
Ser Ala Gly Val Pro Pro Ala Ala Leu Leu Ser Ser Ile Gly Ser Ser Val 450 455 460
Lys Gln Leu Tyr Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr 465 470 475 480
Gln Ile Ser Ala Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr 485 490 495
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Thr Pro Ala Leu Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg 515 520 525
Gly Pro Glu Lys Thr Asp Gln Ala Leu Ala Arg Val Ile Ser Gly Asn 530 535 540
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Arg Gln Lys Leu Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr 580 585 590
Pro Tyr Val Gln Leu Ser Asn Ser Thr Gln Lys Phe Ile Ala Lys 595 600 605
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| 645 | 650 |

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Ser Thr Ser Arg Ser Ala Asp Val Asp Ser Thr Ala Thr Ala Pro
20   25   30

acg cct cct cca ccc acc cag ctt gat tat aag act caa ggc caa aca
Thr Pro Pro Pro Pro Thr Phe Asp Asp Tyr Lys Thr Glu Ala Glu Thr
35   40   45

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Ala Tyr Asp Thr Ile Phe Thr Ser Thr Ser Leu Ala Asp Ile Glu Ala
50   55   60

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Ala Leu Val Ser Leu Glu Asp Ala Val Thr Asn Ile Lys Asp Thr Ala
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Ala Thr Asp Glu Glu Thr Ala Ile Ala Ala Glu Trp Glu Thr Lys Asn
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Ala Asp Ala Val Lys Val Gly Ala Gln Ile Thr Glu Leu Ala Lys Tyr
100  105  110

gct tcg gat aac cca gcg att ctt gac tct tta gtt aac cgc act tcc
Ala Ser Asp Gln Glu Ala Ile Leu Asp Ser Leu Gly Lys Leu Thr Ser
115  120  125

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Phe Asp Leu Leu Glu Ala Ala Leu Leu Glu Ser Val Ala Asn Asn Asn
130  135  140

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Lys Ala Ala Glu Leu Leu Lys Glu Met Gln Asp Pro Val Val Pro
145  150  155  160

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Gly Lys Thr Pro Ala Ile Ala Gln Ser Leu Val Asp Gln Thr Asp Ala
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Thr Ala Thr Gln Ile Glu Lys Asp Gly Asn Ala Ile Arg Asp Ala Tyr
180 190 195

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Phe Ala Gly Gln Asn Ala Ser Gly Ala Val Glu Asn Ala Lys Ser Asn
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Val Gly Ser Lys Glu Glu Ser Lys Ser Ser Ile Gly Ser Ile Arg Val
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tcc atg ctg tta gat gat gct gaa aat gag acc gct tcc att ttg atg
Ser Met Leu Leu Asp Ala Glu Asn Glu Thr Ala Ser Ile Leu Met
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tct ggg ttt cgt cag atg att cac atg ttc aat acg gaa aat cct gat
Ser Gly Phe Arg Glu Met Ile His Met Phe Asn Thr Glu Asn Pro Asp
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tct cca gct gcc cca cag gag ctc gca gca cca gct aga gca ggc aaa
Ser Glu Ala Ala Glu Glu Leu Ala Ala Glu Ala Arg Ala Ala Lys
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gcc gct gga gat gac agt gct gca ggc gct gca gat gct cag aas
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gct tta gaa ggc gct cta ggt aaa gct ggg caa cag ggc ata ctc
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Cct ccc gct gca agt tct ata ggg tca tct gta aac cag ctt tac
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390 395 400 405
aag acc tca aaa tct aca ggt tct gat tat aaa aca cag ata tca gca 1248
Lys Thr Ser Lys Ser Thr Gly Ser Asp Tyr Lys Thr Gln Ile Ser Ala
410 415 420

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Gly Tyr Asp Ala Tyr Lys Ser Ile Asn Asp Ala Tyr Gly Arg Ala Arg
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Thr Arg Ser Val Pro Arg Ala Arg Thr Glu Ala Arg Gly Pro Glu Lys
455 460 465

aca gat cca gcc ctc gct agg gtg att tct gcc aat agc aga act ctt 1440
Thr Asp Glu Ala Leu Ala Arg Val Ile Ser Gly Asn Ser Arg Thr Leu
470 475 480 485

Gly Val Tyr Ser Gln Val Ser Ala Leu Gln Ser Val Met Gln Ile Ile
490 495 500

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Ile Gln Ser Asn Pro Gln Ala Asn Asn Glu Ile Arg Glu Lys Leu
505 510 515

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Thr Ser Ala Val Thr Lys Pro Pro Gln Phe Gly Tyr Pro Tyr Val Gln
520 525 530

ctt tct aat gac tct aca cag aag ttc ata gct aat aat gaa aat gtc 1632
Leu Ser Asn Ser Ser Thr Glu Lys Phe Ile Ala Lys Leu Glu Ser Leu
535 540 545

Glu Thr Asn Ser Leu Phe Ile Gln Gln Val Leu Val Asn Ile Gly Ser
560 565

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Leu Tyr Ser Gly Tyr Leu Gln
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tttatgtg ctttggttaag gcctttggtg aggcottacc aacacaactag aacgatcttc 1829
Leu Tyr Ser Gly Tyr Leu Gln
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