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(54) Titre : ACIDES NUCLEIQUES CODANT DES ANTIGENES DE 56 ET DE 82 KDA RECOMBINES PROVENANT DE
GAMETOCYTES D'EIMERIA MAXIMA, ET LEURS UTILISATIONS

(54) Title: NUCLEIC ACIDS ENCODING RECOMBINANT 56 AND 82 KDA ANTIGENS FROM GAMETOCYTES OF
EIMERIA MAXIMA AND THEIR USES

(57) **Abrégé/Abstract:**

The present invention is drawn to recombinant 86 kD, 56 kD, 30k D and 14 kD antigens, expressed in expression system using the plasmid pTrcHis. Further, the claimed invention provides a vaccine and method of immunizing a subject against infection by various species of Eimeria including E.maxima and E.tenella.

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(54) Title: NUCLEIC ACIDS ENCODING RECOMBINANT 56 AND 82 KDA ANTIGENS FROM GAMETOCYTES OF EIMERIA MAXIMA AND THEIR USES

(57) Abstract: The present invention is drawn to recombinant 86 kD, 56 kD, 30k D and 14 kD antigens, expressed in expression system using the plasmid pTrcHis. Further, the claimed invention provides a vaccine and method of immunizing a subject against infection by various species of Eimeria including E.maxima and E.tenella.



WO 2003/004683 A3

**NUCLEIC ACIDS ENCODING RECOMBINANT 56 AND 82 kDa
ANTIGENS FROM GAMETOCYTES OF *Eimeria maxima* AND THEIR USES**

5 This application claims the benefit of U.S. Provisional Application No. 60/303,699, filed July 6, 2001, the contents of which are hereby incorporated by reference into this application.

10 Throughout this application various publications are referenced in parenthesis. Full citations for these publications may be found listed in alphabetical order at the end of the specification immediately preceding the claims.

15 The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

20 **BACKGROUND OF THE INVENTION**

The organisms which cause the disease known as "coccidiosis" in chickens belong to the phylum Apicomplexa, class Sporozoa, subclass Coccidia, order Eucoccidia, suborder Eimeriorina,

25 family Eimeriidae, genus *Eimeria*. Within the Eimerian genus there are many species, several of which are pathogenic in chickens. The species of major concern to the chicken industry are *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix* and *Eimeria brunetti*.

30 Coccidiosis has become a major economic problem in the chicken industry over the past several decades, mainly due to the overcrowding of chicken houses and the development of drug resistance by the parasite. The rearing of chickens under

35 crowded conditions on a litter floor provides optimal conditions for the growth and spread of *Eimeria* parasites. Under such circumstances, sanitary control is impossible and the farmer must rely on the effectiveness of coccidiostat drugs. However, drugs must be kept in the feed at all times,

shuttle programs must be used to avoid the appearance of drug resistance strains of *Eimeria*, and certain drugs have costly side effects. Furthermore, these coccidiostats also have antibacterial effects and therefore are considered to be in-feed antibiotics. Recently the European Union has decided to ban the use of all in-feed antibiotics in the chicken industry including anticoccidial drugs. Thus, the only viable approach to the control of coccidiosis in the future is by vaccine development.

10

The *Eimeria* parasite undergoes a complex life cycle in the mucosa of the intestinal tract. This life cycle is very similar to that of the other hemosporidian parasites (i.e. plasmodium, babesia, etc.) except for the lack of an arthropod vector. Oocysts sporulate on the litter floor producing four sporocysts, each containing two sporozoites (thus belonging to the class sporozoa). The oocysts are ingested by the chicken, and the sporocysts are released by the mechanical grinding of the gizzard. The sporozoites are then released from the sporocysts due to the digestion of the sporocyst wall by proteolytic enzymes in the intestine. Mobile sporozoites then invade lymphocytes and go on to invade epithelial cells where the asexual cycle begins. The parasite goes through 2-4 cycles of replication and division (each species having a defined number of divisions) leading to the production of large numbers of daughter merozoites. After the final cycle of merozoite production the sexual cycle begins with the production of the macrogametocyte (female) and microgametocyte (male). The macrogametocyte is characterized by the production of wall forming bodies, while microgametocytes contain the components involved in the formation of microgametes, which bud off from the surface of the intracellular parasite. Microgametes are flagellated and are responsible for the fertilization of the macrogamete. A zygote is formed which matures into the oocyst by fusion of the wall forming bodies and condensation of the nucleus. Oocysts are secreted in the

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feces, thus completing the cycle.

Over the past several years, native antigens from the sexual (gametocyte) stages of *Eimeria maxima* have been used to
5 immunize laying hens. Offspring chicks were consequently vaccinated via maternal immunity (protective maternal antibody). Three major protective antigens have previously been identified in *E. maxima* gametocytes having molecular weights of 250, 82 and 56 kDa (EP Patent No. 0 256 536, U.S.
10 Patent No. 5,496,550, and U.S. Patent No. 5,932,225). EP Patent No. 0 256 536, U.S. Patent No. 5,496,550, and U.S. Patent No. 5,932,225 are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. It was
15 shown that these antigens are well conserved amongst *Eimeria* species (Wallach 1995) and can cross protect against the 3 major species that cause coccidiosis in broiler chickens, *E. maxima*, *E. tenella* and *E. acervulina*. More recently, it was shown that in floor pen trials, chicks from hens vaccinated
20 with these native gametocyte antigens were protected against *Eimeria* under field conditions (Wallach 1996). This protection acts to lower the peak in oocyst shedding to a level which does not cause any damaging effect on the performance of the broiler chicken. Based on the above results it was concluded
25 that these antigens are effective against coccidiosis in chickens and also have the potential for use against coccidiosis in other domestic animals including turkeys, geese, sheep, cattle, pigs and fish.

30 These three antigens were also characterized at the molecular level. Cell free translation experiments were carried out to identify the RNA molecules that encode them (Mencher et al.). cDNA molecules that encode these antigens were cloned by immunoscreening of a cDNA library made in the expression
35 vector lambda zap (4, US Patent No. 5,932,225). By this

approach, the gene encoding the 250 kDa antigen was cloned and sequenced. The clone pEM 250/14 was partially sequenced in US Patent Nos. 5,932,225 and 5,496,550. Figure 13a of the subject application reproduces Figure 11 of USA Patent Nos. 5,932,225 and 5,496,550, which portrays the DNA sequence of the first 293 nucleotides of clone pEM 250/14. Figure 13b of the subject application reproduces Figure 12 of US Patent Nos. 5,932,225 and 5,496,550, which shows the DNA sequence of the last 196 nucleotides of clone pEM 250/14. Also, in in USA Patent Nos. 5,932,225 and 5,496,550, the putative genes encoding the 56 and 82 kDa antigens were cloned and sequenced.

Subsequently, Fried et al. sequenced the entire pEM 250/14 clone and found that the antigen had a molecular weight of 230 kDa rather than 250 kDa as had been previously thought. Fried et al. found that the 230 kDa gene contains highly repetitive motifs and that these repeats are contained throughout the entire gene (Fried et al.). This clone was expressed in bacteria using the pATH plasmid vector and it was shown that it is recognized by convalescent chicken sera taken 14 days post infection with *E. maxima*. Finally, it was shown that this gene is expressed only in the macrogametocyte stage and by immunofluorescence was found to be located in the wall forming bodies of the macrogamete (Fried et al.).

cDNA clones encoding the 56 and 82 kDa antigens were also obtained by screening the library with polyclonal antibodies as well as a monoclonal antibody against the 56 kDa antigen. This monoclonal antibody was previously shown to provide passive immunity to naive chicks (Wallach 1990). A few clones were obtained and analyzed. One of the clones was found to encode a small 10 kDa antigen and therefore was not the desired clone. Another clone was found to contain only a small part of the open reading frame (ORF) and by northern blotting was shown to hybridize with two mRNAs of about the expected

size for the 56 and 82 kDa antigens. It was therefore concluded that this was the desired clone. Genomic libraries were then screened to obtain the full length clone. However, due to the highly repetitive GCA motifs in this clone, it was not possible to specifically isolate the full length clone. Attempts to clone the full length cDNA molecule were also not successful due to these repeats. Finally, attempts to express the partial cDNA clones in bacteria failed as well probably due to their unusual sequences and a reasonable level of gene expression was not obtained. It has previously been shown that the 56 and 82 kDa antigens are glycosylated (US Patent No. 5,932,225). This is based on their strong reactivity with Soybean lectin. Therefore, glycosylation may be required in order to obtain good expression of these genes and for proper conformation of the gene products.

In addition to the 56, 82 and 230 kDa antigens, a 14 kDa antigen obtained from highly purified fractions of oocyst walls has been proposed as a possible candidate for vaccines against coccidiosis (Eschenbacher et al.). However, this hypothesis has not been explored.

Several laboratories have been working on a subunit vaccine against coccidiosis. Most of these researchers have focused their efforts on the extracellular asexual stages of the life cycle, in particular the sporozoite and merozoite stages which are considered to be the most vulnerable to immune attack. In a previous study it was found that sporozoite extracts from *E. tenella* could induce in broilers protection against challenge infections against this parasite for up to 7 weeks of age (Karkhanis et al.). Work carried out using monoclonal antibodies against antigens from sporozoites of *E. tenella* led to the identification of a 25,000 molecular weight antigen which was cloned and sequenced (Eur. Patent publication No. 0 164 176, Dec. 11, 1985). Several other sporozoite genes were

identified and their recombinant antigens or the transformed bacteria themselves were tested for protective immunity (Danforth et al.). The results indicated that these recombinants were only able to provide a relatively low level
5 of protection against challenge infection with *Eimeria* and did not always prevent the appearance of significant lesions.

A vaccine using antigens from the merozoite stage has also been tested (European patent publication No. 0 135 073). Using
10 these antigens to immunize young broiler chicks, it was once again found that the protection afforded was relatively low (Danforth et al.).

In 1993, it was found that there was a correlation between
15 protective maternal immunity with the appearance of maternal antibodies against a 230 kDa merozoite (as opposed to gametocyte) antigen of *Eimeria maxima* (Smith et al.). This protection was often over 90% and was found to occur even when the maternal antibody level was relatively low (although
20 reactivity with the 230 kDa protein remained strong). It was also found that a very small quantity of the native 230 kDa merozoite antigen cut out of an SDS-PAGE gel could induce a significant (60%) level of protective maternal immunity against infection with *E. maxima* in offspring chicks.
25 Furthermore, Western blotting showed that this protein was expressed in both merozoites and sporozoites of *E. maxima* and is also well conserved between *Eimeria* species.

SUMMARY OF THE INVENTION

The present invention provides the nucleic acid encoding two of the major *Eimeria maxima* gametocyte antigens having
5 molecular weights of 56 and 82 kDa.

The subject invention also provides a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown by SEQ. ID NO. 35.

10

The subject invention also provides a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown by SEQ. ID NO. 42.

15 The subject invention also provides a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown by SEQ. ID NO. 37.

20 The subject invention also provides a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown by SEQ. ID NO. 39.

25 The subject invention also provides a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown by SEQ. ID NO. 41.

The subject invention also provides a vaccine against coccidiosis comprising the recombinant 56 kDa antigen alone or in combination with any of the aforementioned proteins.

30

The subject invention also provides a vaccine against coccidiosis comprising the recombinant 82 kDa antigen alone or in combination with any of the aforementioned proteins.

35 The subject invention also provides a method of immunizing an

subject against infection by *Eimeria tenella*, *Eimeria maxima*,
Eimeria acervulina, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria*
mitis or *Eimeria brunetti*, or a microorganism expressing an
immunologically cross-reactive antigen, comprising the step
5 of administering to the subject any of the aforementioned
vaccines.

10

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts a Coomassie stained SDS PAGE gel of affinity purified native gametocyte antigens. Arrows point to the 56 and 82 kDa antigens. Molecular weight marker proteins are indicated.

Figure 2 depicts a two-dimensional (2D) SDS-PAGE gel of affinity purified native gametocyte antigens after immunoblotting and silver staining. Molecular weight marker proteins are indicated.

Figure 3 depicts a Coomassie stained PVDF filter from a two-dimensional SDS PAGE gel and the identification of the spots that were cut out for sequence analysis. Arrows point to the 56 and 82 kDa native antigens.

Figure 4 depicts the complete DNA sequence of the 56 kDa gametocyte antigen. The amino terminus as well as internal tryptic peptide fragments are designated. In addition, the predicted initiator methionine and signal peptide cleavage site are shown. The coding sequence, its complement and amino acid sequences are shown (SEQ. ID. NOs. 1-3).

Figure 5 depicts the complete DNA sequence of the 82 kDa gametocyte antigen. The amino terminus as well as internal tryptic peptide fragments are designated. In addition, the predicted initiator methionine and signal peptide cleavage site are shown. The coding sequence, its complement and amino acid sequences are shown (SEQ. ID. NOs. 4-6).

Figure 6 depicts a Southern blot of gametocyte and control chicken DNA probed with the cDNA clone for the 56 kDa antigen. The restriction enzymes used for digestion of the DNA and the marker band sizes in kilobases are indicated.

Figure 7 depicts a Southern blot of gametocyte and control chicken DNA probed with the cDNA clone for the 82 kDa antigen. The restriction enzymes used for digestion of the DNA and the marker band sizes are indicated.

5

Figure 8 depicts a northern blot of gametocyte (G) and control (C) chicken total RNA probed with the 82 kDa cDNA clone. The sizes of the marker bands in kilobases are indicated on the left.

10

Figure 9 depicts an immunoblot showing reactivity of the anti polyhistidine antibody and chicken anti-APGA with proteins expressed by IPTG induced and non-induced (control) bacteria containing the 56 kDa cDNA clone in pTrcHisB. As a further negative control, bacteria that were transformed with the pTrcHisB plasmid containing no insert were tested. Finally, native APGA was used as a positive control for the blot with the anti APGA antiserum. The sizes of the protein marker bands are indicated. Arrows show the positions of the 41 kDa recombinant and 56 and 82 kDa native proteins.

20

Figure 10 depicts a Coomassie stained gel and immunoblot of proteins from bacteria containing pTrcHisB-82 kDa cDNA cloned plasmids. The immunoblot shows reactivity of the anti polyhistidine, chicken anti-APGA as well as uninfected chicken (negative control) sera with the 82 kDa recombinant protein under IPTG induced and non-induced conditions at various times after induction. As a negative control, the experiment was also performed using bacteria transformed with the same plasmid without an insert. As a positive control, native APGA is also run. The arrow shows the position of the 82 kDa recombinant protein. The sizes of the protein marker bands are indicated on the left.

25

30

Figure 11 depicts an immunoblot of a whole lysate of

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unsporulated *E. maxima* oocysts separated by 2 dimensional SDS PAGE. The gel was blotted onto a membrane filter and probed with an antiserum raised against APGA. The strongly reacting 30 kDa spot is shown with an arrow. This was the spot that was cut out of the gel and used for sequence analysis.

Figure 12 depicts DNA sequence alignment of the 230 kDa cDNA *E. maxima* clone with a homologous DNA sequence from patent WO 90/00403 showing 60% homology (SEQ. ID. NOS. 26-27).

10

Figure 13a depicts the DNA sequence of the first 293 nucleotides of clone pEM 250/14. The coding sequence and its amino acid sequences are shown (SEQ. ID. NOS. 28-29). **Figure 13b** depicts the DNA sequence of the last 196 nucleotides of clone pEM 250/14. The coding sequence and its amino acid sequences are shown (SEQ. ID. NOS. 30-31).

Figure 14 A & B ELISA results for chicken immunogenicity trial of the recombinant form of the 56kDa and 82 kDa gametocyte antigen. All serum samples were tested at 1:1000 dilution. A) Coating antigen: APGA to test sera against APGA; r56 purified to test sera taken from chickens immunized with PBS, FIA and the two doses of r56. B) Coating antigen: APGA to test sera against APGA; r82 purified protein to test sera taken from chickens immunized with PBS, FIA and the two doses of r82.

Figure 15 DNA and encoded amino acid sequence of the expressed protein fragment from the 250 kDa asexual stage protein (SEQ. ID NOS. 32-33).

Figure 16 Mouse immunogenicity trial of the recombinant fragment of the 250kDa asexual stage protein. The average of each group for the three consecutive bleeds is shown, with standard error bars indicated. All serum samples were tested

at 1:1000 dilution. Coating antigen was 100ng of APGA for sera from the positive control APGA group, or 100ng of the recombinant protein for the negative control PBS and PBS/FIA groups and the two recombinant protein doses (r0.5µg and
5 r5µg)

Figure 17 Chicken immunogenicity trial of the recombinant fragment of the 250kDa asexual stage protein. The average of each group for the three consecutive bleeds is shown, with
10 standard error bars indicated. All serum samples were tested at 1:1000 dilution. Coating antigen was 100ng of APGA for sera from the positive control APGA group, or 100ng of the recombinant protein for the negative control PBS and PBS/FIA groups and the two recombinant protein doses (1µg and r10µg).

15

Figure 18 Anti-r56 recognition of gametocyte and wall antigens in *Eimeria maxima*.

Figure 19 Anti-r82 recognition of gametocyte and wall antigens
20 in *Eimeria maxima*.

25

Figure 20 Alignment of the N-terminus sequence of the oocyst wall proteins to the 56 kDa and 82 kDa gametocyte antigens (SEQ. ID NOS. 34-42).

DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide
5 from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

In one embodiment, the polypeptide has the amino acid sequence shown in Figure 4 (SEQ. ID. NO. 3).

10

In another embodiment, the homolog of the polypeptide has at least 50% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

15 In an additional embodiment, the homolog of the polypeptide has at least 60% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

In a further embodiment, the homolog of the polypeptide has
20 at least 70% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

In an added embodiment, the homolog of the polypeptide has at least 75% identity to the polypeptide having the sequence
25 shown as SEQ. ID. NO. 3.

In yet another embodiment, the homolog of the polypeptide has at least 80% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

30

In a further embodiment, the homolog of the polypeptide has at least 85% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

35 In one embodiment, the homolog of the polypeptide has at least

90% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

5 In another embodiment, the homolog of the polypeptide has at least 93% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

10 In an additional embodiment, the homolog of the polypeptide has at least 95% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

15 In a further embodiment, the homolog of the polypeptide has at least 97% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

In yet another embodiment, the homolog of the polypeptide has at least 99% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.

20 In an additional embodiment, the nucleotide sequence has at least 50% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown in Figure 4 (SEQ. ID. NO. 1.)

25 In another embodiment, the nucleotide sequence has at least 60% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

30 In a further embodiment, the nucleotide sequence has at least 70% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

35 In one embodiment, the nucleotide sequence has at least 75% identity to the nucleotide sequence starting at nucleotide No.

103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In another embodiment, the nucleotide sequence has at least
5 80% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In an added embodiment, the nucleotide sequence has at least
10 85% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In one embodiment, the nucleotide sequence has at least 90%
15 identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In a further embodiment, the nucleotide sequence has at least
20 93% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In another embodiment, the nucleotide sequence has at least
25 95% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In an added embodiment, the nucleotide sequence has at least
30 97% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.

In one embodiment, the nucleotide sequence has at least 99%
35 identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO.

1.

In a further embodiment, the nucleic acid is a DNA molecule.

5 In yet another embodiment, the DNA molecule is a cDNA molecule.

In an added embodiment, the nucleic acid has the nucleotide sequence starting at nucleotide No. 103 and ending at
10 nucleotide No. 1529 shown in Figure 4 (SEQ. ID. NO. 1).

In another embodiment, the nucleic acid is an RNA molecule.

In one embodiment, the isolated nucleic acid is operatively
15 linked to a promoter of RNA transcription.

The subject invention also includes a vector comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria*
20 *maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

In one embodiment, the vector comprises the nucleic acid having the nucleotide sequence starting at nucleotide No. 103
25 and ending at nucleotide No. 1529 shown in Figure 4 (SEQ. ID. NO. 1).

In another embodiment, the vector is a plasmid.

30 In a further embodiment, the plasmid comprises the nucleic acid having the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown in Figure 4 (SEQ. ID. NO. 1).

35 In an additional embodiment, the plasmid comprises an isolated

nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

5

In yet another embodiment, the plasmid is the plasmid designated 56TRCHisb1 plasmid (Australian Government Analytical Laboratories Accession No. NM01/22400).

10 The subject invention also encompasses a host cell comprising a vector which comprises an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

15

In one embodiment, the host cell comprises a vector comprising a nucleic acid having the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown in Figure 4 (SEQ. ID. NO. 1).

20

In another embodiment, the host cell is selected from the group consisting of a bacterial cell; a plant cell; an insect cell; and a mammalian cell.

25 The subject invention additionally presents a transformed cell comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

30

In one embodiment, the transformed cell is the transformed cell designated clone 56TRCHisb1 in bacteria (Australian Government Analytical Laboratories Accession No. NM01/22401).

35 A plasmid encoding the 56 kDa antigen was deposited with the

Australian Government Analytical Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22400. The bacterial cell transformed with the 56 kDa antigen was deposited with the Australian Government Analytical
5 Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22401. Both deposits were made according to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

10

In an added embodiment, the transformed cell further comprises a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or a homolog of the polypeptide.

15 The subject invention further contains a method of producing a recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima* comprising culturing a transformed cell comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria*
20 *maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid and isolating the recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima*. The recombinant polypeptide produced by this method is also encompassed by the subject invention.

25

The subject invention also provides a vaccine against *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix* or *Eimeria brunetti*, *Eimeria praecox*, *Eimeria mitis* or a microorganism expressing an immunologically cross-reactive
30 antigen, comprising the isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

35 In one embodiment of the vaccine, the isolated nucleic acid

is a plasmid.

In addition, the subject invention presents a vaccine against *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria*
5 *necatrix* or *Eimeria brunetti*, *Eimeria praecox*, *Eimeria mitis*
or a microorganism expressing an immunologically cross-reactive antigen, comprising a recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima* produced by culturing a transformed cell comprising an isolated nucleic acid
10 comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid and isolating the recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima*.

15

In another embodiment, the vaccine is comprised of a mixture of the isolated nucleic acid of the subject invention and the recombinant polypeptide of the subject invention.

20 In another embodiment, the vaccine is comprised of a mixture of the isolated nucleic acid of the subject invention, the recombinant polypeptide of the subject invention and a plasmid comprising the isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of
25 *Eimeria maxima*.

In an added embodiment, the vaccine further comprises a second antigen.

30 In one embodiment, the second antigen is selected from the group consisting of a nucleic acid coding for an antigen from *Eimeria maxima*, a plasmid comprising such a nucleic acid, and a polypeptide coded by such a nucleic acid.

35 In another embodiment, the second antigen is selected from the

group consisting of a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35, or SEQ. ID NO. 42 or a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 37, SEQ. ID NO. 39 or SEQ. ID NO. 41.

In yet another embodiment, the second antigen is a nucleic acid having the nucleotide sequence shown in Seq. ID. No. 4, a plasmid comprising the nucleic acid or a polypeptide coded by the nucleic acid.

In a further embodiment, the vaccine further comprises a third antigen.

15

The subject invention also provides a vaccine wherein the third antigen is a 230 kDa sporozoite/merozoite antigen from *E. maxima*.

20 The 230 kDa antigen was isolated from purified *E. maxima* sporozoites which are present in sporulated oocysts (see life cycle above). The isolation procedure involved extraction of proteins from the sporulated oocysts and separation of the extracted proteins on a DEAE-sephacel anion-exchange column. 25 This was followed by SDS-PAGE of the peak fractions and Western blotting to identify the 230kDa antigen. Furthermore, protective maternal antisera both from vaccinated hens and offspring chicks were used to confirm the identity of the purified antigen. Finally, the 230 kDa protein was isolated 30 from a PVDF membrane filter for carrying out protein sequencing and cloning.

The amino terminal and tryptic peptide digest products of the 230 kDa antigen were sequenced. The sequences from the tryptic digest were used to design degenerate PCR oligonucleotide

35

primers. The primers were used in RACE (rapid amplification of cDNA ends) PCR to amplify partial gene products. From the sequences of these products, gene specific primers were designed and used in RACE PCR to define the 3' and 5' ends of the mRNA. A full length 7 kilobase cDNA clone encoding the antigen was then amplified by PCR using gene specific primers designed to the 5' and 3' ends. This clone was fully sequenced and shown to contain the correct DNA sequence at its 5' end when compared to the amino acid sequence of the N-terminus of the native protein. Thus, this nucleic acid sequence encoded the protective 230 kDa sporozoite/merozoite antigen and could now be used to produce recombinant antigen for vaccination of chickens against coccidiosis.

A plasmid encoding the 230 kDa antigen was deposited with the Australian Government Analytical Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22396. The bacterial cell transformed with the 230 kDa antigen was deposited with the Australian Government Analytical Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22397. Both deposits were made according to the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

It was previously thought that the antigen from the sporozoites/merozoites of *E. maxima* was a 230 kDa antigen. However, our subsequent studies have revealed that the antigen actually is a 250 kDa antigen of the sporozoites/merozoites of *E. maxima*.

In an additional embodiment, the third antigen is a nucleic acid having the nucleotide sequence shown in Figure 12 (SEQ. ID. NO. 26), a plasmid comprising the nucleic acid, or a polypeptide coded by the nucleic acid.

In still another embodiment, the vaccine further comprises a fourth antigen.

In one embodiment, the fourth antigen is a polypeptide from
5 Gametocytes of *Eimeria maxima* having a molecular weight from 230 kDa to 270 kDa, a nucleotide sequence encoding the polypeptide, or a plasmid comprising the nucleotide sequence.

In a further embodiment, the antigen comprises a polypeptide
10 having the amino acid sequence shown in Figure 13a (SEQ. ID. NO. 29) at its 5' end or the amino acid sequence shown in Figure 13b (SEQ. ID. NO. 31) at its 3' end.

The subject invention also provides a method of immunizing a
15 subject against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of administering to the subject the vaccine of the subject
20 invention.

In one embodiment, the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.

25 In another embodiment, the subject is an avian species.

In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

30

In an additional embodiment, the avian species is chickens.

In one embodiment, the administering step comprises spraying the vaccine into the nostrils of the subject.

35

In a further embodiment, the administration comprises intravenous, intramuscular or intraperitoneal injection.

In another embodiment, the administration is performed *in ovo*.

5

In a further embodiment, the administration is to the air sac of an egg, thus contacting an embryo with the vaccine.

The subject invention also contains a fertilized egg from an
10 avian species having an air sac which is inoculated with the vaccine of the subject invention, which vaccine is capable of inducing before or immediately after hatching an immune response in the embryo against a virulent form of *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*,
15 *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen.

In one embodiment, the avian species is selected from the
20 group consisting of chickens, ducks, turkeys, geese, bantams, quail and pigeons.

In another embodiment, the avian species is chickens.

25 The subject invention additionally provides an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

30

In one embodiment, the polypeptide has the amino acid sequence shown in Figure 5 (SEQ. ID. NO. 6).

In another embodiment, the homolog of the polypeptide has at
35 least 50% identity to the polypeptide having the sequence

shown as SEQ. ID. NO. 6.

In an additional embodiment, the homolog of the polypeptide has at least 60% identity to the polypeptide having the
5 sequence shown as SEQ. ID. NO. 6.

In a further embodiment, the homolog of the polypeptide has at least 70% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

10

In another embodiment, the homolog of the polypeptide has at least 75% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

15 In yet another embodiment, the homolog of the polypeptide has at least 80% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

In an added embodiment, the homolog of the polypeptide has at
20 least 85% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

In one embodiment, the homolog of the polypeptide has at least 90% identity to the polypeptide having the sequence shown as
25 SEQ. ID. NO. 6.

In a further embodiment, the homolog of the polypeptide has at least 93% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

30

In yet another embodiment, the homolog of the polypeptide has at least 95% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.

35 In one embodiment, the homolog of the polypeptide has at least 97% identity to the polypeptide having the sequence shown as

SEQ. ID. NO. 6.

In an additional embodiment, the homolog of the polypeptide has at least 99% identity to the polypeptide having the
5 sequence shown as SEQ. ID. NO. 6.

In an additional embodiment, the nucleotide sequence has greater than 50% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown
10 in Figure 5 (SEQ. ID. NO. 4).

In another embodiment, the nucleotide sequence has greater than 60% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as
15 SEQ. ID. NO. 4.

In a further embodiment, the nucleotide sequence has at least 70% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID.
20 NO. 4.

In an additional embodiment, the nucleotide sequence has at least 75% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as
25 SEQ. ID. NO. 4.

In another embodiment, the nucleotide sequence has at least 80% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID.
30 NO. 4.

In yet another embodiment, the nucleotide sequence has at least 85% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as
35 SEQ. ID. NO. 4.

In one embodiment, the nucleotide sequence has at least 90% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

5

In an additional embodiment, the nucleotide sequence has at least 93% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

10

In another embodiment, the nucleotide sequence has at least 95% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

15

In a further embodiment, the nucleotide sequence has at least 97% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

20

In one embodiment, the nucleotide sequence has at least 99% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

25

In a further embodiment, the nucleic acid is a DNA molecule.

In yet another embodiment, the DNA molecule is a cDNA molecule.

30

In an added embodiment, the nucleic acid has the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.

35 In another embodiment, the nucleic acid is an RNA molecule.

In one embodiment, the isolated nucleic acid is operatively linked to a promoter of RNA transcription.

The subject invention also includes a vector comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

10 In one embodiment, the vector comprises the nucleic acid having the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown in Figure 5 (SEQ. ID. NO. 4).

15 In another embodiment, the vector is a plasmid.

In a further embodiment, the plasmid comprises the nucleic acid having the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown in Figure 5 (SEQ. ID. NO. 4).

In an additional embodiment, the plasmid comprises an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

In yet another embodiment, the plasmid is the plasmid designated 82TRCHisb8 plasmid (Australian Government Analytical Laboratories Accession No. NM01/22398).

The subject invention also encompasses a host cell comprising a vector which comprises an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the

polypeptide, or a complement of the nucleic acid.

In one embodiment, the host cell comprises a vector comprising a nucleic acid having the nucleotide sequence starting at
5 nucleotide No. 100 and ending at nucleotide No. 1886 shown in Figure 5 (SEQ. ID. NO. 4).

In another embodiment, the host cell is selected from the group consisting of a bacterial cell; a plant cell; an insect
10 cell; and a mammalian cell.

The subject invention additionally presents a transformed cell comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of
15 *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

In one embodiment, the transformed cell is the transformed cell designated clone 82TRCHisb8 in bacteria (Australian
20 Government Analytical Laboratories Accession No. NM01/22399).

A plasmid encoding the 82 kDa antigen was deposited with the Australian Government Analytical Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22398.
25 The bacterial cell transformed with the 82 kDa antigen was deposited with the Australian Government Analytical Laboratories, Pymble, Australia, on June 26, 2001, under Accession No. NM01/22399. Both deposits were made according to the Budapest Treaty on the International Recognition of the
30 Deposit of Microorganisms for the Purposes of Patent Procedure.

In an added embodiment, the transformed cell further comprises a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or
35 a homolog of the polypeptide.

The subject invention further contains a method of producing a recombinant 82 kDa polypeptide from Gametocytes of *Eimeria maxima* comprising culturing a transformed cell comprising an isolated nucleic acid comprising a nucleotide sequence
5 encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid and isolating the recombinant 82 kDa polypeptide from Gametocytes of *Eimeria maxima*. The recombinant polypeptide produced by this method is also
10 encompassed by the subject invention.

The subject invention also provides a vaccine against *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a
15 microorganism expressing an immunologically cross-reactive antigen, comprising the isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.
20

In one embodiment of the vaccine, the isolated nucleic acid is a plasmid.

In addition, the subject invention presents a vaccine against
25 *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising a recombinant 82 kDa polypeptide from Gametocytes of *Eimeria maxima* produced by culturing a
30 transformed cell comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid and isolating the recombinant 82 kDa polypeptide from Gametocytes
35 of *Eimeria maxima*.

In another embodiment, the vaccine is comprised of a mixture of the isolated nucleic acid of the subject invention and the recombinant polypeptide of the subject invention.

- 5 In another embodiment, the vaccine is comprised of a mixture of the isolated nucleic acid of the subject invention, the recombinant polypeptide of the subject invention and a plasmid comprising the isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of
10 *Eimeria maxima*.

In an added embodiment, the vaccine further comprises a second antigen.

- 15 In one embodiment, the second antigen is selected from the group consisting of a nucleic acid coding for an antigen from *Eimeria maxima*, a plasmid comprising such a nucleic acid, and a polypeptide coded by such a nucleic acid.

- 20 In another embodiment, the second antigen is selected from the group consisting of a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35, or SEQ. ID NO. 42 or a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-
25 terminal end the amino acid sequence shown as SEQ. ID NO. 37, SEQ. ID NO. 39 or SEQ. ID NO. 41.

- The subject invention also provides a method of immunizing a subject against infection by *Eimeria tenella*, *Eimeria maxima*,
30 *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of administering to the subject the vaccine of the subject invention.

35

In one embodiment, the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.

In another embodiment, the subject is an avian species.

5

In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

10 In an additional embodiment, the avian species is chickens.

In a further embodiment, the administration comprises intravenous, intramuscular or intraperitoneal injection.

15 In one embodiment, the administering step comprises spraying the vaccine into the nostrils of the subject.

In another embodiment, the administration is performed *in ovo*.

20 In a further embodiment, the administration is to the air sac of an egg, thus contacting an embryo with the vaccine.

The subject invention also contains a fertilized egg from an avian species having an air sac which is inoculated with the vaccine of the subject invention, which vaccine is capable of inducing before or immediately after hatching an immune response in the embryo against a virulent form of *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen.

35 In one embodiment, the avian species is selected from the group consisting of chickens, ducks, turkeys, geese, bantams, quail and pigeons.

In another embodiment, the avian species is chickens.

The subject invention also provides a recombinant polypeptide, wherein the amino acid sequence is shown as SEQ. ID NO. 3.

5

The subject invention also provides a recombinant polypeptide, wherein the amino acid sequence is shown as SEQ. ID NO. 6.

The subject invention also provides a 30 kDa protein from
10 *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35

The subject invention also provides a 30 kDa protein from
15 *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 42.

The subject invention also provides a 14 kDa protein from
20 *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 37.

The subject invention also provides a 14 kDa protein from
Eimeria maxima gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 39.

25 The subject invention also provides a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 41.

The aforementioned proteins and their corresponding nucleotide
30 sequences can be used in the same manner as described above for the 56 kDa and 82 kDa proteins, including being used to immunize a subject, and to incorporate a plasmid containing a nucleotide sequence encoding the protein into a host cell.

35 The subject invention also provides a method of conferring

upon a newborn subject of an avian species maternal immunity (antibodies) against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism
5 expressing an immunologically cross-reactive antigen, comprising the step of administering to the mother of the subject at a suitable time prior to the laying of a fertilized egg the vaccine of the subject invention in order to thereby confer protection via maternal immunity against infection by
10 *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, in the newborn subject.

15 In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

In a further embodiment, the administration comprises
20 intravenous, intramuscular or intraperitoneal injection.

The subject invention also provides a method of reducing the output of *Eimeria* oocysts in feces from a newborn subject of an avian species which comprises the step of administering to
25 the mother of the subject at a suitable time prior to the laying of a fertilized egg the vaccine of the subject invention in order induce an immune response and transmit maternal antibodies to the newborn so that the output of oocysts from the newborn is reduced.

30

In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

35 In a further embodiment, the administration comprises

intravenous, intramuscular or intraperitoneal injection.

The subject invention also provides a method of immunizing a subject against infection by *Eimeria tenella*, *Eimeria maxima*,
5 *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of administering to the subject a live vaccine comprising a living non-virulent micro-organism or live virus that
10 expresses a 56 kDa or 82 kDa polypeptide from the gametocytes of *Eimeria maxima*.

In one embodiment, the live virus is the pox virus.

15 In one embodiment, the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.

In another embodiment, the subject is an avian species.

20 In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

In a further embodiment, the administration comprises
25 intravenous, intramuscular or intraperitoneal injection.

The subject invention also provides a method of immunizing a subject against infection by *Eimeria tenella*, *Eimeria maxima*,
Eimeria acervulina, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria*
30 *mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of feeding to the subject a plant whose cells express a 56 kDa or 82 kDa polypeptide from the gametocytes of *Eimeria maxima*.

35 In one embodiment, the plant is wheat.

In another embodiment, the plant is corn.

In one embodiment, the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.

In another embodiment, the subject is an avian species.

5

In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

10 The subject invention also provides a method of immunizing a subject against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step
15 of administering to the subject a plasmid comprising an isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa or 82 kDa polypeptide from the gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

20

In one embodiment, the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.

In another embodiment, the subject is an avian species.

25

In a further embodiment, the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.

30 In a further embodiment, the administration comprises intravenous, intramuscular or intraperitoneal injection.

A homolog of the *nucleic acid* of the invention is a nucleic acid that codes for a polypeptide which has substantially the
35 same biological activity as the polypeptide encoded by the

nucleic acid. The term "homology", as used herein, refers to a degree of complementarity. There may be partial homology or complete homology (i.e., identity). A partially complementary sequence is one that at least partially inhibits
5 an identical sequence from hybridizing to a target nucleic acid; it is referred to using the functional term "substantially homologous." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern
10 or northern blot, solution hybridization and the like) under conditions of low stringency. A substantially homologous sequence or probe will compete for and inhibit the binding (i.e., the hybridization) of a completely homologous sequence or probe to the target sequence under conditions of low
15 stringency. This is not to say that conditions of low stringency are such that non-specific binding is permitted; low stringency conditions require that the binding of two sequences to one another be a specific (i.e., selective) interaction. The absence of non-specific binding may be
20 tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% identity); in the absence of non-specific binding, the probe will not hybridize to the second non-complementary target sequence.

25

As known in the art, numerous equivalent conditions may be employed to comprise either low or high stringency conditions. Factors such as the length and nature (DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA,
30 base composition, presence in solution or immobilization, etc.), and the concentration of the salts and other components (e.g., the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization solution may be varied to generate conditions
35 of either low or high stringency different from, but equivalent to, the above listed conditions.

It is an object of the present invention to provide nucleotide sequences encoding the 56 and 82 kDa antigens from Gametocytes of *Eimeria maxima* and the deduced amino acid sequence therefor. Specifically exemplified coding sequences are given
5 in Figures 4 and 5, together with the deduced amino acid sequence. All synonymous coding sequences for the exemplified amino acid sequences are within the scope of the present invention.

10 It is a further object of the present invention to provide functionally equivalent coding and protein sequences, including equivalent sequences from other *Eimeria* species. Functionally equivalent 56 and 82 kDa antigens from Gametocytes of *Eimeria maxima* coding sequences are desirably
15 from about 50% to about 80% nucleotide sequence homology (identity) to the specifically identified coding sequence, from about 80% to about 95%, and desirably from about 95% to about 100% identical in coding sequence to the specifically exemplified coding sequence.

20

Hybridization conditions of particular stringency provide for the identification of homologs of the coding sequence from other species and the identification of variant sequences, where those homologs and/or variant sequences have at least
25 (inclusively) 50 to 85%, 85 to 100% nucleotide sequence identity, 90 to 100%, or 95 to 100% nucleotide sequence identity. Each integer and each subset of each specified range is intended within the context of the present invention.

30 The coding sequence and methods of the present invention include the homologous coding sequences in species other than *Eimeria maxima*. Methods can be employed to isolate the corresponding coding sequences (for example, from cDNA) from other organisms, including but not limited to other species
35 such as *Eimeria tenella*, *Eimeria acervulina*, *Eimeria necatrix*,

Eimeria praecox, *Eimeria mitis* and *Eimeria brunetti* useful in the methods of this invention using the sequences disclosed herein and experimental techniques well known to the art.

5 Specifically included in this invention are sequences from other species than those exemplified herein, which sequences hybridize to the sequences disclosed under stringent conditions. Stringent conditions refer to conditions understood in the art for a given probe length and nucleotide
10 composition and capable of hybridizing under stringent conditions means annealing to a subject nucleotide sequence, or its complementary strand, under standard conditions (i.e., high temperature and/or low salt content) which tend to disfavor annealing of unrelated sequences.

15 "Conditions of high stringency" means hybridization and wash conditions of 65°-68° C, 0.1 x SSC and 0.1% SDS (indicating about 95-100% nucleotide sequence identity/similarity). Hybridization assays and conditions are further described in
20 Sambrook et al. (1989) Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y. As used herein, conditions of moderate (medium) stringency are those with hybridization and wash conditions of 50-65° C., 1 x SSC and 0.1% SDS (where a positive hybridization result reflects about
25 80-95% nucleotide sequence identity). Conditions of low stringency are typically those with hybridization and wash conditions of 40-50° C, 6 x .SSC and 0.1% SDS (reflecting about 50-80% nucleotide sequence identity).

30 A homolog of the *polypeptide* of the invention is a polypeptide which has substantially the same amino acid sequence and biological activity as the polypeptide. Thus, a homolog may differ from the polypeptide of the invention by the addition, deletion, or substitution of one or more non-essential amino
35 acid residues, provided that the resulting polypeptide retains

the biological activity of the polypeptide. Persons skilled in the art can readily determine which amino acids residues may be added, deleted, or substituted (including with which amino acids such substitutions may be made) using established and well known procedures, including, for example, conventional methods for the design and manufacture of DNA sequences coding for bacterial expression of polypeptide homologs of the subject polypeptide, the modification of cDNA and genomic sequences by site-directed mutagenesis techniques, the construction of recombinant polypeptides and expression vectors, the bacterial expression of the polypeptides, and the measurement of the biochemical activity of the polypeptides by means of conventional biochemical assays.

Examples of homologs are deletion homologs containing less than all the residues specified in the subject polypeptide, substitution homologs wherein one or more residues specified are replaced by other residues, and addition homologs wherein one or more amino acids residues are added to the polypeptide. All such homologs share the biological activity of the polypeptide of the invention.

"Substantially the same polypeptide" is herein defined as encompassing the deletion, addition or substitution of fewer than four amino acids at the N-terminus of the amino acid sequence of the polypeptide. Furthermore, there may be deletions, additions or substitutions in the sequence which do not eliminate the biological activity of the polypeptide. Such modifications are known to those skilled in the art. For example, substitutions may encompass up to 10 residues in accordance with the homologous or equivalent groups described by e.g. Lehninger, Biochemistry, 2nd ed. Worth Pub., New York. (1975); Creighton, Protein Structure, a Practical Approach, IRL Press at Oxford Univ. Press, Oxford, England (1989); and Dayhoff, Atlas of Protein Sequence and Structure 1972, National Biomedical Research Foundation, Maryland (1972).

The term "biologically active", as used herein, refers to a polypeptide having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise,
5 "immunologically active" refers to the capability of the natural, recombinant, or synthetic polypeptide, or any oligopeptide portion thereof, to induce a specific immune response in an animal or cells and to bind with specific antibodies.

10

"Substantially the same biological activity" refers to biological activity the same as that of the naturally occurring molecule possibly differing slightly in degree or level which would still be known by the skilled artisan to be
15 the same biological activity.

The term "portion", as used herein, in connection with a polypeptide (as in "a portion of a given polypeptide") refers to fragments of that polypeptide. The fragments may range in
20 size from four (4) amino acid residues to the entire amino acid sequence minus one amino acid. The term "portion", as used herein, in connection with a nucleic acid (as in "a portion of a given nucleic acid") refers to fragments of that nucleic acid. The fragments may range in size from twelve (12)
25 nucleotide residues to the entire nucleic acid sequence minus one nucleotide.

A "deletion", as used herein, refers to a change in either amino acid or nucleotide sequence in which one or more amino
30 acid or nucleotide residues, respectively, are absent.

An "insertion" or "addition", as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues,
35 respectively, as compared to the naturally occurring molecule.

A "substitution", as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

- 5 The present invention provides the recombinant cloning and sequencing of two of the major *Eimeria maxima* gametocyte antigens having molecular weights of 56 and 82 kDa.

10 The present invention also provides the expression of these recombinant antigens in an *E. coli* expression system using the plasmid pTrcHis.

The subject invention also provides a vaccine against coccidiosis comprising the recombinant 56 kDa antigen. In
15 addition, the present invention provides a vaccine against coccidiosis comprising the recombinant 82 kDa antigen.

The present invention provides the cloning and sequencing of two of the major *Eimeria maxima* gametocyte antigens having
20 molecular weights of 56 and 82 kDa.

The production of gametocytes was scaled-up in order to isolate enough gametocyte antigen to carry out amino acid sequencing (i.e. milligram quantities of the specific
25 antigens) on the 56 and 82 kDa glycoproteins themselves. This scale up production was in itself a very difficult task, and required infecting several thousand chickens in order to provide enough material for carrying out sequence analyses. After achieving this goal, it was possible to produce enough
30 affinity purified gametocyte antigen (APGA) to start isolating the two glycoproteins on a large scale.

The purified gametocyte antigenic glycoproteins were separated by two-dimensional, SDS polyacrylamide gel electrophoresis.
35 After analysis of the two-dimensional gels by staining, the

position of the 56 and 82 kDa antigens was determined by transfer to a PVDF membrane filter and immunodetection using antisera to APGA. After identification and removal of the 56 and 82 kDa antigens from the filter, amino acid sequencing of both their N-termini as well as internal protein sequences obtained from tryptic peptides was performed. These peptide sequences were used to predict the DNA sequences, based on which small, specific oligonucleotide probes were synthesized.

10 The specific oligonucleotide probes were used in RACE PCR (rapid amplification of cDNA ends) to prepare cDNA molecules from the gametocyte RNA that encodes the 56 and 82 kDa antigens. This method allowed for the production of full length cDNA molecules that are specifically amplified from mRNA molecules that contain within them the RNA sequences that encode the desired peptides. This cDNA product was then fully sequenced and the presence of the various peptides sequenced above was confirmed. Surprisingly, we found that the cDNA clones we obtained were not related to those described in Wallach et al., US Patent No. 5,932,225. Therefore, it appears that in Wallach et al., artifacts occurred when screening the cDNA library with antibodies and the clones thought to encode the 56 and 82 kDa antigens which were isolated did not in fact encode these antigens.

25 Finally the two new cDNA clones were used as a probe in Southern and northern blotting experiments to identify the specific gene(s) and mRNA molecule(s) that encode for the 56 and 82 kDa antigens. Whereas previously no clear banding patterns could be obtained on blots (US Patent No. 5,932,225), the number and size of gene fragments and mRNA transcripts that encode for the two antigens were clearly discerned.

30 The present invention further provides a method for cloning the 56 and 82 kDa antigens into a bacterial expression vector, pTrcHis, containing a poly his tag (to aid in the purification

of the recombinant antigens). The two genes are then expressed in *E. coli* by adding a specific inducer molecule (isopropyl- α -D-thiogalactopyranoside), followed by the identification of the recombinant 56 and 82 kDa antigenic proteins by western blotting. The results of these blots showed that the 56 and 82 kDa recombinant antigens had the correct size based on measurements by mass spectrometry, and were recognized by antibodies to the his tag as well as protective antisera raised against the native 56 and 82 kDa gametocyte antigens. These results confirmed the identity of the clones and showed that these recombinant antigens can be used to replace the native antigens in the maternally based vaccine against coccidiosis in chickens.

The present invention further describes the relationship between the 56 kDa gametocyte antigen with a 30 kDa oocyst protein. This oocyst protein was shown, by immunoblotting, to strongly react with antiserum against the 56 and 82 kDa gametocyte antigens. By sequencing the amino terminus of the 30 kDa oocyst protein, we found that there was a precise match with the amino terminus of the 56 kDa antigen. It was therefore concluded that the 56 kDa antigen is processed during the development of oocysts from gametocytes into the 30 kDa protein.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. One skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

EXPERIMENTAL DETAILS

EXAMPLE 1

Purification of *Eimeria maxima* Gametocytes on a Large Scale

In order to produce very large quantities of gametocytes, 4,000 heavy breed chickens were infected with 10,000 sporulated *E. maxima* oocysts, and were then sacrificed on day
5 six (about 134 hours) post infection. The chicken intestines were removed, washed with PBS and cut open longitudinally. They were then cut into 1 cm long pieces and placed in a SAC buffered solution (170 mM NaCl, 10 mM Tris pH 7, 10 mM glucose, 5 mM CaCl₂, 1% powdered milk) containing 0.5 mg/ml
10 hyaluronidase (Type III from Sigma, 700 units/mg). The intestinal pieces were incubated at 37°C for 20 minutes after which they were placed on top of a gauze filter. The pieces were rinsed with large quantities of SAC buffer and the resulting filtrate was collected. This was then filtered
15 through a 17 micron polymon filter (Swiss Silk Bolting Cloth Mfg. Co. Ltd., Zurich, Switzerland) and the resulting filtrate was then filtered through a 10 micron filter. The gametocytes were collected from the top of the 10 micron filter, examined and counted microscopically, and placed in centrifuge bottles,
20 which were spun at 800xg for 10 minutes. The gametocytes were then washed twice with SAC buffer, and frozen at -70°C.

EXAMPLE 2

Purification of the 56, 82 and 230 kDa gametocyte antigens

25

The frozen gametocytes were thawed at room temperature and the proteins were extracted as described previously (Wallach 1995). The 56 and 82 kDa gametocyte antigens were isolated from the protein extract by running it over a Sepharose 4B
30 column containing the monoclonal antibody 1E11-11 raised against the 56 kDa antigen. A complex of the gametocyte antigens were allowed to bind to the monoclonal antibody attached to the resin, the non-specific material was washed off using buffer, and the affinity purified gametocyte
35 antigens (APGA) were eluted from the column. The purified APGA

was lyophilized. A small sample of APGA was analyzed by SDS-PAGE where the 56 and 82 kDa native antigens were clearly visualized (Figure 1).

5 EXAMPLE 3

Two Dimensional Gel Electrophoresis of
APGA and Isolation of the major 56 and 82 kDa antigens

The 56 and 82 kDa gametocyte antigens were isolated from APGA.
10 Lyophilized APGA was prepared as described in Example 2, and was solubilized in water. The proteins were then separated by two-dimensional SDS-PAGE (Figure 2), and identified by immunoblotting using a polyclonal chicken anti-APGA antibody, which recognizes both the 56 and 82 kDa proteins. Once
15 identified and their location established on two-dimensional SDS-PAGE gels, the proteins were then transferred to a PVDF membrane filter, and stained with Coomassie Blue (Figure 3). Immunoblotting was carried out at the same time, and the two blots were compared to clearly identify the 56 and 82 kDa
20 proteins. The spots corresponding to the 56 and 82 kDa gametocyte antigens were cut out of the membranes and the amino-terminus of each antigen was sequenced.

EXAMPLE 4

25 Amino acid sequencing of the amino- terminus as well
as internal tryptic peptides from the 56 and 82 kDa antigens

The amino-termini of the 56 and 82 kDa proteins were sequenced:

30

amino-terminus of the 56 kDa protein: VPSTTPVENQVHPY-EM
(SEQ. ID. NO. 7)

amino-terminus of the 82 kDa protein: -PTVLDTTTG-QVEDT

35

(SEQ. ID. NO. 8)

In order to determine the protein sequence of internal tryptic

fragments of the 56 and 82 kDa proteins, the APGA preparation was first separated by one dimensional SDS-PAGE and stained with Coomassie Blue. The proteins were then excised from gels and digested with trypsin and sequenced.

5

Several tryptic peptide sequences were obtained from both proteins and the results are summarized in Table 1.

Table 1: Amino acid sequences of tryptic peptides isolated from the 56 and 82 kDa antigens.

	<u>Peptide</u>	<u>56 kDa Antigen</u>	<u>82 kDa antigen</u>	<u>SEQ. ID. NO.</u>
	A	VQDV(L/I)VDA(L/I)WAS(L/I)R	ATGFSEEEVMR	9, 10
	B	VTEMMDM(L/I)SNR	TGGLFDQACNDAPPSR	11, 12
15	C	Q(L/I)Q(L/I)QDQMMR	TGP(L/I)STTGATGATTGPVAA(L/I)R	13, 14
	D	AAEEF(L/I)HR	P(L/I)THVE	15, 16
	E		R(L/I)AAVPGTTAGT	17
	F	D(L/I)QEY(L/I)STAFNWA- ENQSTAYTR	(L/I)AEGAEPRPVMPPAAATAAANLR	18, 19
20	G	RQTAAWMDRTA(L/I)EQEETT		20
	H	MNAAMDSSNE(L/I)MTT		21
	I	KFPET(L/I)F		22

The amino acid sequences obtained did not show any homology to any other known protein.

25

EXAMPLE 5

RACE PCR cloning and Sequencing of the genes encoding the 56 and 82 kDa antigens

30

The genes for the 56 and 82 kDa proteins were amplified from gametocyte cDNA using SMART RACE PCR technology (Clontech). RNA was isolated from *E. maxima* gametocytes and mRNA was purified using Dynal beads (Dynal). SMART ready cDNA was synthesized following the protocols according to the manufacturer's instructions using the reverse transcriptase Powerscript (Clontech). Amplifications of both the 5' and

35

3' ends were carried out using the protocols described in the SMART RACE PCR manual, and the DNA polymerase, Advantage *Taq* (Clonetech), a high fidelity enzyme mixture.

5 The gametocytes had been isolated from chicken intestines, filtered a number of times and washed thoroughly as described in Example 1. There was a concern that residual chicken intestinal material was still present in this preparation. Consequently, PCRs carried out using degenerate primers
10 designed to the amino-terminus of the 56 and 82 kDa genes and degenerate primers designed to internal tryptic peptide fragments gave rise to bands in both cDNA samples prepared from purified gametocytes and uninfected chicken cells. In this situation PCR bands, which stained intensely with
15 ethidium bromide on agarose gels, were purified, cloned into pGEMT-Easy (Promega) and sequenced (SUPAMAC sequencing service, Sydney, Australia). In some cases, when rearrangements were observed or the cloned fragment was difficult to sequence, sequence was obtained directly from the
20 PCR product. If the DNA sequence data from the PCR product translated to any of the amino acid sequences of the tryptic peptides, the PCR product was of parasitic origin and sequencing continued.

25 The full length sequence of the 56 and 82 kDa proteins are shown in Figures 4 and 5, respectively. The full length sequence of the 230 kDa protein is presented in Figure 12.

Amino acid sequence of the tryptic peptides and N-terminus of
30 the 56 gametocyte antigen matched the deduced amino acid sequence arising from the corresponding cloned DNA.

Nine tryptic peptides were sequenced for the 56 kDa protein (Table 1). All peptides but one, *sb56i*, could be mapped to
35 the cloned gene corresponding to the 56 kDa protein (Figure

4). This tryptic fragment may correspond to a contaminating band present in the sample. In detail:

-Tryptic peptides *sb56a*, *sb56b*, *sb56c*, *sb56d*, *sb56f* and *sb56h* matched precisely to the deduced amino acid sequence predicted by the cloned DNA.

-Tryptic peptide *sb56g* did not match precisely to the deduced amino acid sequence predicted by the cloned DNA. The sequence of the tryptic fragment was reanalysed, and the new sequence matched more closely with the predicted sequence derived from the cloned DNA.

Tryptic fragment *sb56g* original sequence:

RQ--TAAWMDR-TA[L/I]EQEETT (SEQ. ID. NO. 23)

15 Reanalysed *sb56g* sequence:

RGVQTAAWM**D**GVTA I EKEETT (SEQ. ID. NO. 24)

Deduced aa sequence from DNA:

RGVQTAAWM**N**GVTA I EKEETT (SEQ. ID. NO. 25)

A discrepancy still remains in this peptide at amino acid 10, where the protein sequence reveals a D and the DNA sequence predicts a N. This segment of DNA was sequenced 4 times, and each time predicted an N.

Amino acid sequence of the tryptic peptides and N-terminus of the 82 gametocyte antigen match the deduced amino acid sequence arising from the corresponding cloned DNA.

30 Seven tryptic peptides were sequenced for the 82 kDa protein (Table 1). All peptides but two, *sb82d* and *sb82e* could be mapped to the cloned gene corresponding to the 82 kDa protein. This tryptic fragment may correspond to a contaminating band present in the sample. In detail:

35 -Tryptic peptides *sb82a*, *sb82b*, *sb82c*, *sb56d* and *sb82f*

matched precisely to the deduced amino acid sequence predicted by the cloned DNA.

-Tryptic peptides *sb82d* and *sb82e* did not match to the deduced amino acid sequence predicted by the cloned DNA.

5

In addition to the sequence information described above:

1) The predicted size of the ORF encoding the mature form of the 82 kDa protein is 64,275 Da, which corresponded to the true size of the native protein of 62,236 Da, as determined by mass spectrometry.

2) The predicted size of the ORF encoding the mature form of the 56 kDa protein is 51,407 Da which corresponded to the true size of the native protein of 52,450 Da, as determined by mass spectrometry.

Finally, the two protein and DNA sequences did not show any homology to any other known gene or protein.

20

EXAMPLE 6

Southern and Northern blotting
using the 56 and 82 kDa cDNA cloned probes.

25 Southern blotting using *E. maxima* and chicken DNA was carried out by first cutting the DNA with a variety of restriction enzymes and separating the resulting DNA fragments on an agarose gel. This is followed by transferring the DNA to nitrocellulose paper, probing with a P³² labeled cDNA probe for the 56 (Figure 6) or 82 (Figure 7) kDa antigens and performing autoradiography. The results showed that for both the 56 and 82 kDa antigens there appear to be two different, single copy genes, which encodes the two proteins.

35 Northern blotting using *E. maxima* and chicken RNA was carried out by separating the RNA molecules on an agarose gel,

transferring it to a nitrocellulose filter and probing with the P³² labelled 56 and 82 cDNA clones. The results showed that the 56 kDa mRNA has a molecular weight of about 1.9 KB and the 82 kDa mRNA had a molecular weight of about 2.4 KB (Figure 8). These sizes are very similar to those predicted from the DNA sequences.

EXAMPLE 7

10 Expression of the recombinant 56 and 82 kDa antigens using the pTrcHis vector in *E. coli* and their analysis using sera against native APGA.

The full length gene encoding the 82 kDa protein was amplified from *E. maxima* gametocyte cDNA using gene specific primers carrying terminal restriction sites to facilitate directional cloning into the expression vector pTRCHisb (Invitrogen). The full length gene included the coding region of the amino-terminus of the mature protein and sequence up to, but not including, the stop codon (575 aa). A partial fragment of the gene encoding the 56 kDa protein was amplified from *E. maxima* gametocyte cDNA using gene specific primers carrying terminal restriction sites. This included the amino-terminus of the protein and a further 323 amino acids of sequence, 133 amino acids shorter than the full length mature protein. Both genes were cloned into the commercially available vector pTrcHisb (Invitrogen).

1) Expression of the 56 kDa gene in pTrcHis B

Transformed bacteria were induced with 1 mM IPTG, and bacterial lysates were analyzed by 1D-SDS PAGE and immunoblotting (Figure 9). A commercially available anti-His antibody to the His fusion tag of the recombinant protein recognized a band of the predicted size of 40 kDa (this clone lacks the coding region for 133 amino acids) under inducing

conditions. Under non-induced conditions there was also a low level of reactivity with this band indicating that there is some degree of leakiness of the gene expression. Recognition of the recombinant 56 kDa protein was then assessed by immunoblotting with the chicken polyclonal anti-APGA antibody. The immunoblot showed that the anti-APGA antibody recognized the native form of the protein by one dimensional SDS-PAGE, as well as the recombinant protein, clearly demonstrating that the cloned gene product indeed codes for the 56 kDa protein.

10

2) *Expression of the 82 kDa gene in pTrcHis B*

Transformed bacteria were induced with 1 mM IPTG, and bacterial lysates were analysed by one dimensional SDS PAGE and immunoblotting (Figure 10). A commercially available anti-His antibody to the His fusion tag of the recombinant protein recognized a band of the predicted size of 82 kDa under inducing and non-inducing conditions. Recognition of the recombinant 82 kDa protein was then assessed by immunoblotting with the chicken polyclonal anti-APGA antibody. This antibody was produced by immunizing chickens with native APGA isolated from purified gametocytes. The immunoblot showed that the anti-APGA antibody recognized the native form of the protein by 1D SDS-PAGE, as well as the recombinant protein, clearly demonstrating that the cloned gene product indeed codes for the 82 kDa protein.

Based on the above results together with the sequence analyses described in Example 5, we concluded that the two cDNA clones described above are the authentic genes encoding for the 56 and 82 kDa antigens. In addition, the strong reactivity with the antisera raised against the native antigens shows that these recombinant proteins can now be used to replace APGA for the immunization of chickens against coccidiosis.

35

EXAMPLE 8

Homology of the 56 kDa antigen with
a 30 kDa antigen from *E. maxima* oocysts.

5 Antibodies to APGA were used to detect homologous proteins on
 a two-dimensional blot of oocyst antigens. We found that there
 was very strong reactivity with a protein of 30 kDa (Figure
 11). This spot was cut out of the membrane filter and the N-
 terminus of the protein was sequenced. The resulting amino
 10 acid sequence corresponded precisely to the N-terminal
 sequence of the gametocyte 56 kDa antigen. Based on this
 finding we concluded that the gametocyte 56 kDa antigen is
 processed into the 30 kDa protein of the oocyst stage of
 development.

15

EXAMPLE 9

Expression of *gam* 56, a 56 kDa
gametocyte antigen from *Eimeria maxima*

20 Native protein: Mr 56,000 (size determined by molecular sizing
 (MS): 52,450 Da)

Source: Parasitic: *Eimeria maxima*

Life cycle stage: macrogametocyte

Gene: 1,754 base pairs sequenced presented over 5
 25 polymerase chain reaction (PCR) fragments, all of
 which are cloned into pGEMT-Easy, except for the
 last ~600 bp of the gene, which includes ~400 bp of
 the coding region.

30 5'UTR(1-102 bp)

ORF (103-1,533 bp)

3'UTR (1,534-1,731 bp)

polyA tail(1,732-1,754 bp)

pI: 4.8 predicted from sequence (by 2D SDS-PAGE, the protein
 35 migrates towards the acidic end of the gel)

Expression constructs

Expression vectors used: pTRCHisb, pET25b

Expression construct: given p56TRCHisb1

Gene fragment that was cloned into the expression vector:

5 *gam* 56 was amplified from cDNA using the following primer
pairs: SB74/SB75 (172-1137 bp) for directional cloning into
the BamHI/EcoRI site of TRCHisb. The amplified region contains
the sequence encoding the amino terminus of the mature
protein, excluding the initiator methionine and leader
sequence. It contains a tyrosine-serine rich region and
10 excludes a proline-methionine rich region.

Amino acid composition of cloned *gam* 56 fragment:

2 cysteines present

amino acid composition of gene fragment cloned into pTRCHisb:

15

S(12.7%)	Y(11.5%)	A(8.7%)	T(8.4%)	P(7.2%)
R(6.6%)	E(6.0%)	M(5.5%)	L(5.5%)	V(4.6%)
Q(4.3%)	N(4.3%)	G(3.8%)	D(3.5%)	W(1.7%)
F(1.4%)	K(1.4%)	I(1.4%)	H(0.9%)	C(0.6%)

20

Predicted protein size: 41 kDa

Yield: 0.9 - 1.4 µg/ml (nickel agarose purified protein/ml
culture) Difficult to see induced protein in crude bacteria
25 lysate on a Commassie Blue stained gel.

Expression conditions:

The promoter is leaky, therefore we can get expression in the
absence of IPTG.

30 Used baffled flasks, 37°C, 4 h induction, with 1 mM IPTG, 0.1
mg/ml ampicillin in 0.01 M Mg²⁺+SOB(SOB better than LB).

Normally, one predominate band at ~42 kDa is obtained after
purification and detection with silver staining. Often some
35 higher molecular weight bands, which may be aggregates, are

obtained after purification as well as the main ~42 kDa band. The protein seems to aggregate at -20 °C and 4°C; after purification we desalt and add stabilisers (3% lactose, 1% monosodium glutamate).

5

EXAMPLE 10

Immunization and challenge trial of the recombinant 56 kDa (r56) and 82 kDa (r82) gametocyte antigens, and the 250 kDa (r250) asexual stage antigen in chickens

10

IMMUNIZATION

Animals

- Chickens: - 84 day old (~12 weeks) Australorp cockerels
- 15 - kept on medicated (robenidene) food
- all chickens were individually tagged and recorded

Antigens

- Recombinant proteins in the pTRCHisb expression system were
- 20 grown at 37° C in 0.1 mg/ml ampicillin in 0.01M Mg²⁺SOB and induced for 4 hours with 1 mM IPTG. Proteins were purified on a Ni-agarose column, concentrated, desalted, and lyophilized with stabilizers (3% lactose, 1% monosodium glutamate). Protein concentrations used for all antigens were
- 25 measured using the Bradford assay. Affinity Purified Gametocyte Antigen (APGA) preparations provided by M. Wallach was used as a positive control for the trial.

Groups and Doses

- 9 chickens used per group; 9 groups in total; 81 chickens
- 30 used in total.
- Chickens were immunized with 0.5 ml antigen/Freunds Incomplete Antigen (FIA) cocktail (0.25 ml antigen/0.25 ml FIA) per bird, intra-muscularly, on one side only of the

chicken, with the following antigens:

- Group 1 PBS only
- Group 2 Adjuvant (FIA)/PBS
- Group 3 APGA (2.5 g)
- 5 Group 4 r250 protein (1.0 g)
- Group 5 r250 protein (10.0 g)
- Group 6 r56 protein (0.5 g)
- Group 7 r56 protein (5.0 g)
- Group 8 r82 protein (0.5 g)
- 10 Group 9 r82 protein (5.0 g)

Immunization Schedule

- Immunization 1: week 1
- Immunization 2: week 3
- Bleed: week 6
- 15 Bleed: week 8
- Bleed/Kill: week 9

Analyzes

- Bleeds were taken (~1.5-2 ml/ bird), sera separated and tested by ELISA and immunoblotting
- 20

Results

Results of the bleeds are shown in Figure 14.

CHALLENGE

25 Animals and parasites

- 5 chickens (148 days old; ~4.5 months) from each group which had the highest antibody titre based on the ELISA results of bleed 1 were used; in the case of the PBS and FIA controls, chickens with the lowest antibody titres were used
- 30 - *E. maxima* (strain Houghton);

-robenidene was removed from the feed one week prior to challenge

Groups

The following groups and chickens were taken from the immunization trial described above, and used in the challenge experiments

Group 1	PBS only	chicken numbers 2, 3, 4, 6, 8
Group 2	Adjuvant (FIA)/PBS	chicken numbers 12-16
10 Group 3	APGA (2.5 g)	chicken numbers 20, 22, 23, 25, 27
Group 5	r250 protein (10.0 g)	chicken numbers 37, 39, 41, 44, 45
15 Group 7	r56 protein (5.0 g)	chicken numbers 57, 59, 60, 61, 63
Group 9	r82 protein (5.0 g)	chicken numbers 74, 75, 76, 79, 80

Challenge Schedule

Robenidene removed

20 Challenged with 100 sporulated oocysts per bird Day 6

Oocyst harvest and count schedule

Day 0 post-infection

Day 1 post-infection

Day 2 post-infection

25 Day 3 post-infection

Day 4 post-infection

Checked oocyst output for contamination of another species

Replaced plastic sheet to start collections.

Day 5 post-infection Feces collected, and oocysts counted

Day 6 post-infection Feces collected, and oocysts counted

Day 7 post-infection Feces collected, and oocysts counted

5 Day 8 post-infection Feces collected, and oocysts counted

Day 9 post-infection Feces collected, and oocysts counted

Day 10 post-infection Feces collected, and oocysts counted

Table 2 Immunization and Challenge Trial I

Groups/ Day p.i.	Cumulative oocyst counts (x10 ⁶)					Output (%)					% inhibition				
	6	7	8	9	10	6	7	8	9	10	6	7	8	9	10
1. PBS only	6.67	17.0	26.4	27.3	27.4	100	100	100	100	100	0	0	0	0	0
15 2. FIA only	3.20	14.4	17.3	17.5	17.5	48	85	66	64	64	52	15	34	36	36
		0	0	0	0	(100)	(100)	(100)	(100)	(100)	(0)	(0)	(0)	(0)	(0)
3. APGA (2.5 µg)	2.77	9.35	13.4	13.5	13.6	42	55	51	50	50	58	45	49	50	50
			8	8	1	(87)	(65)	(78)	(78)	(78)	(13)	(35)	(22)	(22)	(22)
5. r250 (10 µg)	0.83	8.35	13.7	14.7	14.7	12	49	52	54	54	88	51	48	46	46
			2	2	2	(26)	(58)	(79)	(84)	(84)	(74)	(42)	(21)	(16)	(16)
20 7. r56 (5 µg)	0.33	4.53	7.20	8.16	8.53	5	27	27	30	31	95	73	73	70	69
						(10)	(32)	(42)	(47)	(49)	(90)	(68)	(58)	(53)	(51)
9. r82 (5 µg)	4.23	10.3	14.7	14.9	15.0	63	61	56	55	55	37	39	44	45	45
		3	3	3	6	(132)	(72)	(85)	(85)	(86)	(0)	(28)	(15)	(15)	(14)

EXAMPLE 11

25 Expression of a recombinant fragment of the 250kDa asexual stage protein

The region of the 250kDa protein encoding the predicted transmembrane domain/cytosolic tail and upstream hydrophilic domain was selected for expression studies (Figure 15). The area was chosen for a number of reasons and are as follows: 1) similar 3' hydrophilic tail regions have been identified in a number of apicomplexan microneme proteins and appear unique to this family of proteins; 2) such regions have been identified in other microneme proteins also recognised as

immunodominant, primarily *Eimeria tenella* microneme protein 1 (EtMIC1) and surface antigen 5401 (EtMIC4); 3) a similar region was expressed from the *E. tenella* 5401 antigen (EtMIC4) and was found to afford significant protection against
5 challenge with *E. tenella* (Danforth et al, 1988); 4) other regions of the protein consist primarily of the EGF-like and TSP-1-like domains. These domain types are found highly conserved within eukaryotes and therefore the possibility of their inducing auto-immunity must be considered. Furthermore
10 because of the prevalence of such domain types it seems unlikely that they would be responsible for inducing a strong immune response.

PCR primers EP006 (5'-TTGGATCCCGAATTGCACCCCA TTCC-3') and
15 EP007 (5'-TTGAATTCTGAATGTCGCCGCTGTCG-3') were designed to amplify the selected DNA region from a cDNA clone encoding the 250kDa protein. The primers incorporated *Bam*HI (EP006) and *Eco*RI (EP007) restriction sites to facilitate cloning into the selected expression vector. The PCR product subsequently
20 generated using the primers was gel-purified and its identity confirmed by sequencing.

The bacterial expression vector pTrcHisB (Invitrogen) was selected for expression studies. Plasmid vector DNA and gel
25 purified cDNA insert were digested with the restriction enzymes *Bam*HI and *Eco*RI, and the digested DNA fragments gel purified and ligated. The ligation mixture was transformed into *E. coli* strain DH5-a and following plating and incubation, resulting colonies were selected, cultured and
30 used for plasmid preparation. The identity of the selected recombinants was confirmed by DNA sequencing.

In preparation for expression, plasmid DNA containing the expression construct was transformed into the *E. coli* host
35 expression strain TOP10. Following plating and incubation, a

single bacterial colony was selected and used to establish an O/N culture in LB media. A vector only negative control culture was also established. Aliquots of each culture were then transferred to fresh LB media and incubated until the
5 cells reached mid-log phase, at which stage expression was induced with the addition of 1mM IPTG. Samples from the expression culture and negative control culture were taken at 0, 1, 2, 5 and 24hrs post induction, and centrifuged to pellet the bacterial cells. All pellets were subsequently
10 resuspended in TE buffer, sonicated and centrifuged to separate the aqueous soluble fraction (supernatant) from the insoluble fraction (pellet). All fractions were analysed under reducing conditions on SDS-PAGE gels and subsequently stained with Coomassie Blue. When compared to the negative
15 control samples, an over-expressed protein was detected in the soluble fractions, migrating at just below the 45kDa marker. Western analysis of the soluble fractions using an antibody reactive with the 6xHistidine tag of pTrcHis expression products, detected a protein band of the same apparent
20 molecular weight. The predicted size of the expressed protein is approximately 30kDa, somewhat less than that observed on SDS-PAGE gels. The size difference might be explained by the high frequency of proline residues in the expressed protein, known to cause proteins to migrate with apparently high
25 molecular weight.

In preparation for immunogenicity trials, the expressed protein was purified using Ni-NTA Agarose nickel-charged resin (QIAGEN), with minor modifications to the manufacturer's
30 recommended protocol. Expressed proteins containing the 6xHis tag bind to the resin and are displaced by an increased concentration of imidazole in the elution buffer. Briefly, cell pellets were resuspended in Lysis buffer (50mM NaH₂PO₄, 300mM NaCl, 10mM imidazole, pH 8.0), containing 1 mg/ml
35 lysozyme. The suspension was sonicated on ice and centrifuged to pellet insoluble material. The supernatant containing the

soluble expressed protein was then mixed with Ni-NTA resin and added to a disposable elution column. The slurry was allowed to settle then washed with Wash buffer (50mM NaH₂PO₄, 300mM NaCl, 20mM imidazole, pH 8.0), before elution with Elution
5 buffer (50mM NaH₂PO₄, 300mM NaCl, 250mM imidazole, pH 8.0). The purity of eluted fractions was analysed by reducing SDS-PAGE and Coomassie Blue staining.

Details for the immunogenicity trials are as for the 56kDa and
10 82kDa trials. For the mouse trial, 0.5µg and 5µg doses of the recombinant protein per mouse were used (6 mice/group). For the chicken trial, 1µg and 10µg doses per bird were used (9 chickens/group). ELISA results for the collected serum samples from the mouse and chicken trials are presented in Figures 16
15 and 17 respectively.

EXAMPLE 12

The oocyst wall of *Eimeria* is derived from precursor proteins found in the sexual stage of the parasite (macrogametocyte)
20 which undergo processing and di-tyrosine crosslinking to form the hardened, protective barrier of the excreted form of the parasite

The genes encoding the 56 kDa and 82 kDa sexual stage,
25 macrogametocyte antigens have been cloned and sequenced. Both genes show an unusual amino acid composition, and in particular, both have tyrosine-rich regions; the 56 kDa protein possesses one tyrosine-rich region and the 82 kDa protein possesses two tyrosine-rich regions. Proteins rich in
30 tyrosine have been previously implicated in oocyst wall formation in *E. acervulina* and *E. tenella*. (Eschenbacher et al.) Thus, the role of the tyrosine rich region in the 56 kDa and 82 kDa sexual stage antigens in oocyst wall formation was explored in *Eimeria maxima*.

35

Antibodies to the recombinant form of the 56 kDa protein (anti-r56) and antibodies to the recombinant form of the 82 kDa protein (anti-r82) recognize a ~30 kDa protein in unsporulated and sporulated oocysts, and a ~30 kDa protein in purified wall fragments (see Figs. 18 and 19). They also recognize their native form counterparts in gametocyte extracts. The ~30 kDa protein recognized in purified oocyst wall fragments by the anti-r82 kDa antibody is not the same as the ~30 kDa protein recognized by the anti-r56; it is slightly smaller. The ~30 kDa protein recognized by the anti-r56 antibody was purified and the N-terminus sequenced. The N-terminus of the ~30 kDa protein corresponds exactly to the N-terminus of the 56 kDa gametocyte antigen (see Fig. 20a).

Others have shown by SDS-PAGE and coomassie blue staining that the oocyst wall of *Eimeria* is composed of two predominant proteins of 14 kDa and 30 kDa. Using better SDS-PAGE separation techniques, we have resolved the 14 kDa protein into 3 components of ~10-14 kDa, and named them 14.1, 14.2 and 14.3, where 14.1 represents the protein which has migrated the slowest on SDS-PAGE gels, and 14.3 the fastest (see Fig. 18c). We have sequenced the N-terminus of all four proteins and the results are presented in Fig. 20. In summary, the 30 kDa protein is a novel protein which does not show any similarity to any other previously characterized protein as determined through a BLAST protein search (see Fig. 20c). The N-terminus of protein 14.3 corresponds to the beginning of the tyrosine rich region in domain 1 of the 82 kDa protein (see Fig. 20b), the N-terminus of protein 14.2 corresponds to the beginning of the tyrosine rich region in domain 2 of the 82 kDa protein (see Fig. 20b), and the N-terminus of protein 14.1 corresponds to the beginning of the tyrosine rich region in the 56 kDa protein (see Fig. 20a).

Together these results show that the oocyst wall of *Eimeria* is derived from precursor proteins found in the wall forming bodies of the sexual stage (macrogametocyte) of the parasite. Through some signaling mechanism, they are proteolytically
5 processed into several shorter proteins of ~30 kDa and ~14 kDa. Contrary to previous findings, our data indicates that the oocyst wall is composed of more than two proteins. Our findings suggest that the oocyst wall is composed of several proteins present at different levels in the parasite, some of
10 which are in high abundance that they are recognized by coomassie blue staining of SDS-PAGE gels, and others that are present at low levels, only detected through the more sensitive technique of immunoblotting. The ~30 kDa protein seen in coomassie blue stained SDS-PAGE gels is not related to
15 the 56 kDa and 82 kDa gametocyte antigens, however, the smaller ~10-14 kDa proteins are. Our most recent finding that di-tyrosine is present at detectable levels in the order of 0.00338 mmol/mol in oocysts, indicates that the small tyrosine rich proteins are probably held in the wall through a
20 mechanism involving di-tyrosine crosslinks. However, we believe that not all the proteins are held in the wall in this way and are currently investigating this.

References

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What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.
2. The isolated nucleic acid of claim 1, wherein the polypeptide has the amino acid sequence shown as SEQ. ID. NO. 3.
3. The isolated nucleic acid of claim 1, wherein the homolog of the polypeptide has at least 50% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 3.
4. The isolated nucleic acid of claim 1, wherein the nucleotide sequence has at least 50% identity to the nucleotide sequence starting at nucleotide No. 103 and ending at nucleotide No. 1529 shown as SEQ. ID. NO. 1.
5. The isolated nucleic acid of claim 1, wherein the nucleic acid is a DNA molecule.
6. The isolated nucleic acid of claim 5, wherein the DNA molecule is a cDNA molecule.
7. The isolated nucleic acid of claim 1, wherein the nucleic acid has the nucleotide sequence shown as SEQ. ID. NO. 1.
8. The isolated nucleic acid of claim 1, wherein the nucleic acid is an RNA molecule.
9. The isolated nucleic acid of claim 1 operatively linked to a promoter of RNA transcription.

10. A vector comprising the nucleic acid of claim 1.
11. A vector comprising the nucleic acid of claim 7.
12. The vector of claim 10, wherein the vector is a plasmid.
13. The vector of claim 11, wherein the vector is a plasmid.
14. A plasmid comprising the nucleic acid of claim 1.
15. The plasmid of claim 14, designated 56TRCHisb1 plasmid deposited under Australian Government Analytical Laboratories Accession No. NM01/22400.
16. A host cell comprising the vector of claim 10.
17. A host cell comprising the vector of claim 12.
18. The host cell of claim 17, wherein the cell is selected from the group consisting of a bacterial cell; a plant cell; an insect cell; and a mammalian cell.
19. A transformed cell comprising the nucleic acid of claim 1.
20. The transformed cell of claim 19, designated clone 56TRCHisb1 bacteria deposited under Australian Government Analytical Laboratories Accession No. NM01/22401.
21. The transformed cell of claim 19, further comprising a 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, or a homolog of the polypeptide.
22. A method of producing a recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima*, the method comprising

culturing the transformed cell of claim 19 and isolating the recombinant 56 kDa polypeptide from Gametocytes of *Eimeria maxima*.

23. A recombinant polypeptide produced by the method of claim 22.
24. A 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35.
25. A 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 42.
26. A 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 37.
27. A 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 39.
28. A 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 41.
29. A vaccine against *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the nucleic acid of claim 1 or the plasmid of claim 14, or the recombinant polypeptide of claim 23.

30. The vaccine of claim 29, further comprising a second antigen.
31. The vaccine of claim 30, wherein the second antigen is selected from the group consisting of a nucleic acid coding for an antigen from *Eimeria maxima*, a plasmid comprising such a nucleic acid, and a polypeptide coded by such a nucleic acid.
32. The vaccine of claim 31, wherein the second antigen is a nucleic acid having the nucleotide sequence shown as SEQ. ID. NO. 4, a plasmid comprising the nucleic acid, or a polypeptide coded by the nucleic acid.
33. The vaccine of claim 30, wherein the second antigen is selected from the group consisting of a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35, or SEQ. ID NO. 42 or a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 37, SEQ. ID NO. 39 or SEQ. ID NO. 41.
34. The vaccine of claim 32, further comprising a third antigen.
35. The vaccine of claim 34, wherein the third antigen is a nucleic acid having the nucleotide sequence shown as SEQ. ID. NO. 26, a plasmid comprising the nucleic acid, or a polypeptide coded by the nucleic acid.
36. The vaccine of claim 32, further comprising a fourth antigen.

37. The vaccine of claim 36, wherein the fourth antigen is a polypeptide from Gametocytes of *Eimeria maxima* having a molecular weight from 230 kDa to 270 kDa, a nucleotide sequence encoding the polypeptide, or a plasmid comprising the nucleotide sequence.
38. The vaccine of claim 37, wherein the antigen comprises a polypeptide having the amino acid sequence shown as SEQ. ID. NO. 29 at its 5' end or the amino acid sequence shown as SEQ. ID. NO. 31 at its 3' end.
39. A method of immunizing a subject against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of administering to the subject the vaccine of any one of claims 29-38.
40. The method of claim 39, wherein the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.
41. The method of claim 39, wherein the subject is an avian species.
42. The method of claim 41, wherein the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail, and pigeons.
43. The method of claim 42 wherein the avian species is chickens.

44. The method of claim 39, wherein said administering step comprises spraying said vaccine into the nostrils of the subject.
45. The method of claim 39, wherein said administration comprises intravenous, intramuscular or intraperitoneal injection.
46. The method of claim 39, wherein the administration is performed *in ovo*.
47. The method of claim 46, wherein the administration is to the air sac of an egg, thus contacting an embryo with the vaccine.
48. A fertilized egg from an avian species having an air sac wherein the air sac is inoculated with the vaccine of any one of claims 29-38, which vaccine is capable of inducing before or immediately after hatching an immune response in an embryo against a virulent form of *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen.
49. The fertilized egg of claim 48, wherein the avian species is selected from the group consisting of chickens, ducks, turkeys, geese, bantams, quail and pigeons.
50. The fertilized egg of claim 49, wherein the avian species is chickens.
51. An isolated nucleic acid comprising a nucleotide sequence encoding a 82 kDa polypeptide from Gametocytes of *Eimeria*

maxima, or encoding a homolog of the polypeptide, or a complement of the nucleic acid.

52. The isolated nucleic acid of claim 51, wherein the polypeptide has the amino acid sequence shown as SEQ. ID. NO. 6.
53. The isolated nucleic acid of claim 51, wherein the homolog of the polypeptide has at least 50% identity to the polypeptide having the sequence shown as SEQ. ID. NO. 6.
54. The isolated nucleic acid of claim 51, wherein the nucleotide sequence has at least 50% identity to the nucleotide sequence starting at nucleotide No. 100 and ending at nucleotide No. 1886 shown as SEQ. ID. NO. 4.
55. The isolated nucleic acid of claim 51, wherein the nucleic acid is a DNA molecule.
56. The isolated nucleic acid of claim 55, wherein the DNA molecule is a cDNA molecule.
57. The isolated nucleic acid of claim 51, wherein the nucleic acid has the nucleotide sequence shown as SEQ. ID. NO. 4.
58. The isolated nucleic acid of claim 51, wherein the nucleic acid is an RNA molecule.
59. The isolated nucleic acid of claim 51 operatively linked to a promoter of RNA transcription.
60. A vector comprising the nucleic acid of claim 51.

61. A vector comprising the nucleic acid of claim 57.
62. The vector of claim 60, wherein the vector is a plasmid.
63. The vector of claim 61, wherein the vector is a plasmid.
64. A plasmid comprising the nucleic acid of claim 51.
65. The plasmid of claim 64, designated 82TRCHisb8 plasmid deposited under Australian Government Analytical Laboratories Accession No. NM01/22398.
66. A host cell comprising the vector of claim 60.
67. A host cell comprising the vector of claim 61.
68. The host cell of claim 66, wherein the cell is selected from the group consisting of a bacterial cell; a plant cell; an insect cell; and a mammalian cell.
69. A transformed cell comprising the nucleic acid of claim 51.
70. The transformed cell of claim 69, designated clone 82TRCHisb8 bacteria deposited under Australian Government Analytical Laboratories Accession No. NM01/22399.
71. The transformed cell of claim 69, further comprising a 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, or a homolog of the polypeptide.
72. A method of producing a recombinant 82 kDa polypeptide from Gametocytes of *Eimeria maxima*, the method comprising culturing the transformed cell of claim 69 and isolating

the recombinant 82 kDa polypeptide from Gametocytes of *Eimeria maxima*.

73. A recombinant polypeptide produced by the method of claim 72.
74. A vaccine against *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the nucleic acid of claim 51 or the plasmid of claim 64, or the recombinant polypeptide of claim 73.
75. The vaccine of claim 74, further comprising a second antigen.
76. The vaccine of claim 75, wherein the second antigen is selected from the group consisting of a nucleic acid coding for an antigen from *Eimeria maxima*, a plasmid comprising such a nucleic acid, and a polypeptide coded by such a nucleic acid.
77. The vaccine of claim 75, wherein the second antigen is selected from the group consisting of a 30 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 35, or SEQ. ID NO. 42 or a 14 kDa protein from *Eimeria maxima* gametocytes having at the N-terminal end the amino acid sequence shown as SEQ. ID NO. 37, SEQ. ID NO. 39 or SEQ. ID NO. 41.
78. A method of immunizing an subject against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an

immunologically cross-reactive antigen, comprising the step of administering to the subject the vaccine of any one of claims 74-77.

79. The method of claim 78, wherein the subject is a species selected from the group consisting of cattle, sheep, pigs and fish.
80. The method of claim 78, wherein the subject is an avian species.
81. The method of claim 80, wherein the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail, and pigeons.
82. The method of claim 81, wherein the avian species is chickens.
83. The method of claim 78, wherein said administering step comprises spraying said vaccine into the nostrils of the subject.
84. The method of claim 78, wherein the administration comprises intravenous, intramuscular or intraperitoneal injection.
85. The method of claim 80, wherein the administration is performed *in ovo*.
86. The method of claim 85, wherein the administration is to the air sac of an egg, thus contacting an embryo with the vaccine.
87. A fertilized egg from an avian species having an air sac wherein the air sac is inoculated with the vaccine of

claim 74, which vaccine is capable of inducing before or immediately after hatching an immune response in an embryo against a virulent form of *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen.

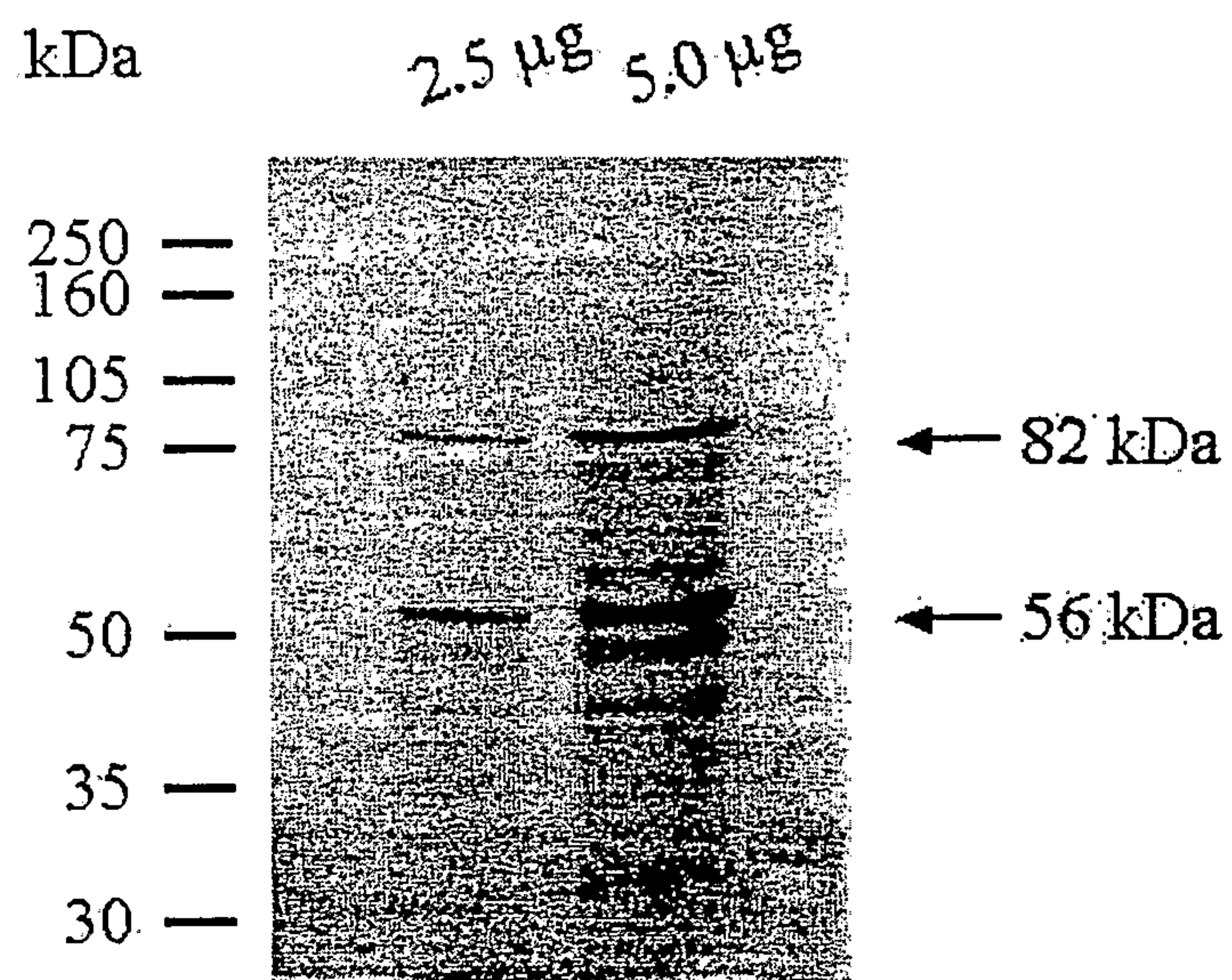
88. The fertilized egg of claim 87, wherein the avian species is selected from the group consisting of chickens, ducks, turkeys, geese, bantams, quail and pigeons.
89. The fertilized egg of claim 88, wherein the avian species is chickens.
90. A recombinant polypeptide, wherein the amino acid sequence is shown by SEQ. ID NO. 3.
91. A recombinant polypeptide, wherein the amino acid sequence is shown by SEQ. ID NO. 6.
92. A method of immunizing an subject against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the step of administering to the subject the protein of any one of claims 24-28.
93. A method of conferring upon a newborn subject of an avian species maternal immunity against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, comprising the

step of administering to the mother of the subject at a suitable time prior to the laying of a fertilized egg the vaccine of any one of claims 29-38 or 74-77 in order to thereby confer protection via maternal immunity against infection by *Eimeria tenella*, *Eimeria maxima*, *Eimeria acervulina*, *Eimeria necatrix*, *Eimeria praecox*, *Eimeria mitis* or *Eimeria brunetti*, or a microorganism expressing an immunologically cross-reactive antigen, in the newborn subject.

94. The method of claim 93, wherein the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.
95. The method of claim 93, wherein the administration comprises intravenous, intramuscular or intraperitoneal injection.
96. A method of reducing the output of *Eimeria* oocysts in feces from a newborn subject of an avian species which comprises the step of administering to the mother of the subject at a suitable time prior to the laying of a fertilized egg the vaccine of claim 29-38 or 74-77 in order induce an immune response and transmit maternal antibodies to the newborn so that the output of oocysts from the newborn is reduced.
97. The method of claim 96, wherein the avian species is selected from the group consisting of chickens, turkeys, geese, ducks, bantams, quail and pigeons.
98. The method of claim 96, wherein the administration comprises intravenous, intramuscular or intraperitoneal injection.

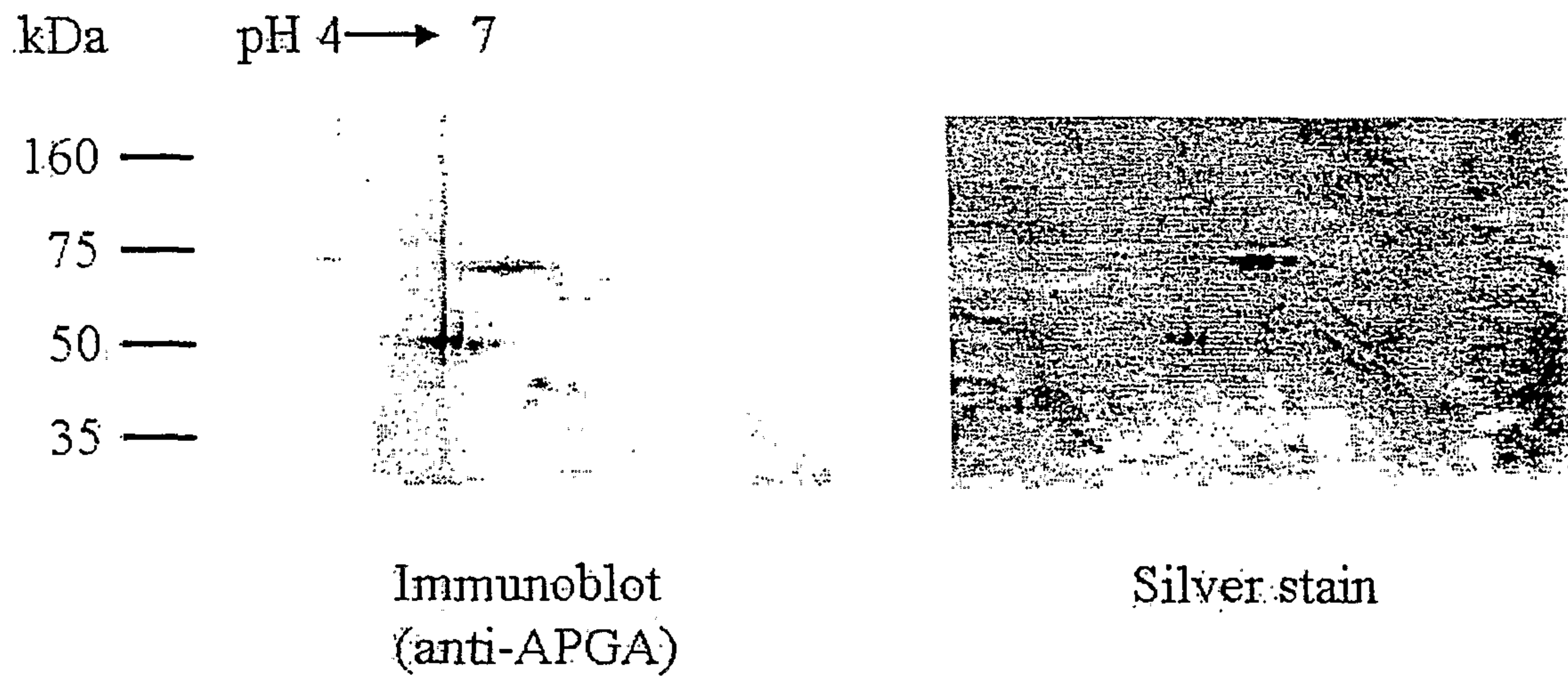
1/36

FIGURE 1

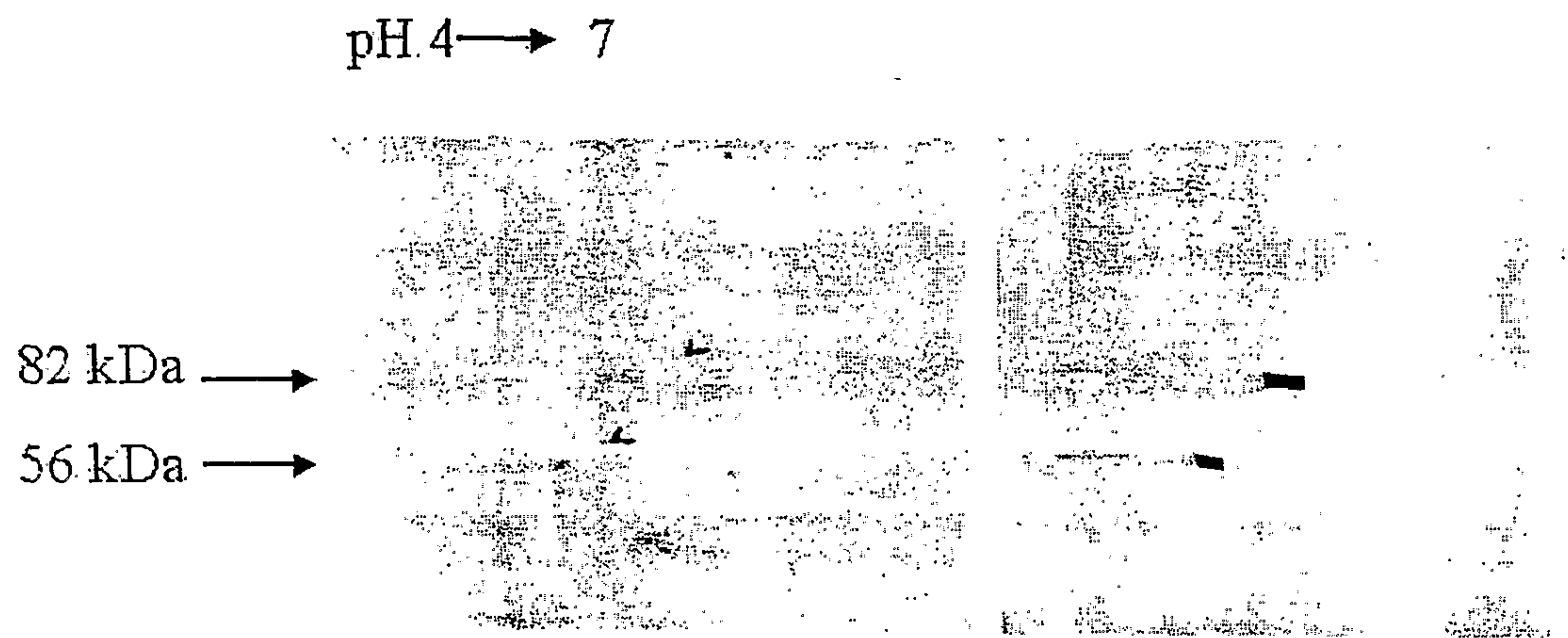


2/36

FIGURE 2



3/36

FIGURE 3

tryptic peptide sb3

5/36

500 510 520 530 540 550 560
TGGACAGCTCAAACGAACTCATGACCCTAGCGACACCACAGACCCCGAGACCCCTCCGCCGTGCAACTCG
ACCTGTCGAGTTTGCTTGAGTACTGCTGATCGCTGTGGTGTCTGSSGCTCTGGGAGGCGGCACGTTGAGC
M D S S M E L M T T S D T T D P E T L R R A T R>

570 580 590 600 610 620 630
CAAGTACATGAAGGAGGTTCGGCTTCAGGACGTCCTGGTAGATGCTCTCTGGGCCTCTCTCCGCCGTGTA
GTTTCATGTACTTCCTCCAAGCGCAAGTCTGTCAGGACCATCTACGAGAGACCCCGAGAGAGGCGCCACAT
K Y M K E V R V Q D V L V D A L W A E L R G V>

tryptic peptide sb56a

640 650 660 670 680 690 700
CAGACAGCTGCCCTGGATGAATGGAGTGACCGCTATTGAGAAGGAGGAGACGACTCCCATGGCTAGCCGCG
GTCTGTGACCGGACCTACTTACCTCACTGGCGATAACTCTTCCTCCTCTGCTGAGGGTACCGATCGGCGC
Q T A A W M M Q V T A I E K E E F T P M A S R>

tryptic peptide sb56g

710 720 730 740 750 760 770
CTGCTGAGGAGTTCTCTCCACCGCATGTACCATAACCTGAGGGCAGCAGGTATGCTCTGAAGAAGATGTTGC
GACGACTCCTCAAGGAGGTGGCGTACATGCTATTGGACTCCCGTCTGCTCCATACAGACTTCTTCTACAACG
A A E E F L E R M Y H N L R A A G M S E E E D V A>
tryptic peptide sb56d

780 790 800 810 820 830 840
CAAGTTCATCCCTAGAGCCGAGTACAACCCCTCCGAGCAGTCAAGAAATATGGGCAGAAAGGGCAGGAGC
GTTCAAGTAGGGATCTCGGCTCATGTTGGGGAGGCTCGTCASTTCTTTATACCGTCTTTCCCGTCTCTCG
K F I P R A E Y N P S E Q S R N M G R K G R S>

850 860 870 880 890 900 910
TTCTACTACGGCGGCTATCCCGAGCTACTACAACCTCCCGCTACTACAGCTACAGCAGCTACCCCGAGCTACT
AAGATGATGCCGCCGATAGGGTCCGATGATGTTGAGGGGGATGATGTCGATGTCGTCGATGGGGTCCGATGA
F Y Y G G Y P S Y Y N S P Y Y S Y S S Y P S Y>

920 930 940 950 960 970 980
ACAACTACAGCTACCCGTCATACAGCTACAGCAGCTACCCCGAGCTACTACCGCTACAGCAGCTACCCCTA
TGTGATGTCGATGGGCGAGTATGTCGATGTCGTCGATGGGGTCCGATGATGGCGATGTCGTCGATGGGGAT
Y N Y S Y P S Y S Y S S Y P S Y Y R Y S S Y P Y>

990 1000 1010 1020 1030 1040 1050
CTACAACCTACAGCTATCCCGAGCTACTACAACCTACGGCAGCTACCCCTACTACAGTTATAGCAGCTACCCC
GATGTTGATGTCGATAGGGTCCGATGATGTTGATGCCGTCGATGGGGATGATGTCATATATCGTCGATGGGG
Y N Y S Y P S Y Y N Y G S Y P Y Y S Y S S Y P>

1060 1070 1080 1090 1100 1110 1120
AGCTGGTACTGGCGCCGCTCTCCGCTCTTTGGCAACAGCAACTTGCCCGAGACTGCCCTCCTCTCACCCTC
TCGACCATGACCGCGGCGAGAGGCGAGAAACCGTTGTCGTTGAACGGGTCTGACCGGAGGAGAGTGGTGAG
S W Y W R R L R S L A T A T C P D C P P L T T>

6/36

1130 1140 1150 1160 1170 1180 1190
CCAGCATGATCCCAACTCCCCCCCCAATGATGAACATGATGAACACCCCCACCCCCCATGGCAAACATGAT
GGTCGTACTAGGGTTGAGGGGGGGGTTACTACTTTGTACTACTTTGTGGGGTGGGGGGTACCGTTTGTACTA
P S M I P T P P P M M N M M N T P P P M A N M M>

1200 1210 1220 1230 1240 1250 1260
GACCAGCATGATGATGAACACTCCCATGGTTCTCTCCCCGACCCCTCGGAAGTGAAGCCATGAGCCTC
CTGGTCGTACTACTACTTTGTGAGGGTACCAAGGAGGAGGGGGCGTGGGAGCCTTGACTTCGGTACTCGGAG
T S M M M N T P M V P P P R T L G T E A M S L>

1270 1280 1290 1300 1310 1320 1330
GGCTTGGCCCCCATCGGTATCACCGCGCGCCCCCATGACAGGTTTCGGTGTTCCTCTCTGAGTTCGGTCCCT
CCGAACCGGGCGTAGCCATAGTGGCCGCGGGGCTACTGTCCAAAGCCACAAGGAGGACTCAAGCCAGGGA
G L A P I G I T G A P M T G F G V P P E F G P>

1340 1350 1360 1370 1380 1390 1400
TTGGAGCCGAAGGTATCGGCCTCCCCACCGATGCCCTCGGCAGCACCCCCGAAATGACACCATTCGACCC
AACCTCGGCTTCATAGCCGGAGGGGTGGCTACGGGAGCCGTCGTGGGGGCTTTACTGTGGTAAGCTGGG
F G A E G I G L P T D A L G S T P E M T P P D P>

Biotech Aus. tryptic peptide

1410 1420 1430 1440 1450 1460 1470
AACTACCCCTACAGAAGTCTCGCCCCCATGGACCTCCCCCCCCATCCCCCTCTCTCTTCCCTGAAACC
TTGATCGGGGATGTCTTGAGAGCGGGGGTACCTGGAGGGGGGGTAGGGGGGAGGACAGAAGGGACTTTGG
T T P Y R T L A P M D L P P I P P P V P P E T>

1480 1490 1500 1510 1520 1530 1540
CCTATGAGGCCACCTACTCCCTTCGGCTTCGGACCTGCACCTGTTCTCTCCCATGCCCTTCTAAACGACCT
GGATACTCCGGTGGATGAGGGAAGCCGAAGCCTGGACCTGGACAAGGAGGGTACGGGAAGATTTCCTGGA
P M R P P T P F G F G P A P V P P M P P *

stop

1550 1560 1570 1580 1590 1600 1610
ACCATCCCTCAATCCATAGCTCACATTTCTGTAGCCTCAAAACAGTTTTTGTTCATTTCACTTCCAGGAC
TCGTAGGGAGTTAGGTATCGAGTGTAAGCATCGGAGTTTTGTCAAAAACAAGTAAAGTGAAGGTCCTG

1620 1630 1640 1650 1660 1670 1680
TCATGCTGCGACATTTGCATTCGTACCTCGAAACCGTCAACCTCAAACCCCAAACCATTTCTGTGACCTCC
AGTACGACGCTGTAAACGTAAGCATGGAGCTTTGGCAGTTGGAGTTTGGGGTTTGGTAAGACACTGGAGG

1690 1700 1710 1720 1730 1740 1750
CCTCGCAAACGCGGAAGGCGGAACATTTTTTCTGAAGTATATTACTACGTTAAAAA
GGAGCGTTTGGCCCTTCCGCCTTGTAAGAAAGACTTCATATAATGATGCAATTTTTTTTTTTTTTTTTT

AAAA

TTTT

7/36

FIGURE 5

Sequence Range: 1 to 2145

10 20 30 40 50 60 70
ATACAAATCCTTTTATCTGGTTCCAAACACGCTCACTCAACCACCACCTGGACACACCCTCCCCATACAT
TATGTTTAGGAAAAATAGACCAAGGTTGTGCCAGTGAGTTGGTGGTGGACCTGTGTGGGAGGGGTATGTA

80 90 100 110 120 130 140
ACAGGAGCAGCAGCAACACCAGCATCAAGATGACCGCTGCCGACGCGCTTGCCGGGGTTTGGCCCTGGC
TGTCTCGTCGTCGTTGTGGTCGTAGTTCTACTGCCGACGCGCTGCCGAACGCCCCAAAACCGGACCG
M T R A A A L A G V L A L A>
Predicted initiator methionine

150 160 170 180 190 200 210
TGCAGCAGGCAGCAGCCTTGCTCTACCTACTGTATTGGACACAACGACTGGCACCCTAAGTGGAGTGGACT
ACGTCGTCGTCGTCGGAACGAGATGGATGACATAACCTGTGTGTGCTGACCGTGGGTTACCTCACCTGA
A A G S S L A L P T V L D T T T G T Q V E W T>
Predicted signal peptide cleavage site *amino terminus*

220 230 240 250 260 270 280
GAGACCCCTTAGACACAACAGAGGTAATACTATGGGGGAGATGGCCAGCACCACCAGCGGCACGACTCCAA
CTCTGGGGGAATCTGTGTGTCTCCATTGATACCCCTCTACCGTCGTCGGTGGTCCCGTGTCTGAGGTT
E T P L D T T E V T M G E M G S T T S G T T P>

290 300 310 320 330 340 350
CCAGCACTGGTGTGCGAATGATGGAGGCTGAAACTACAACCCCATCAACCCCTGAGGCTCCCCAGCAGCA
GGTCGTGACCACACGCTTACTACCTCCGACTTTGATGTYGGGAGTAGTTGGGGACTCCGAGGGGTCTGTCGT
T S T G V R M M E A E T T T P S T P E A P Q Q Q>

360 370 380 390 400 410 420
GCAGCAGATGCCTCAGCCTCAACCTCAGCCACAGCAAACTCCCCTTCTGAGGCCGTATTAGAGGCA
CGTCGTCTACGGAGTCGGAGTTGGAGTCCGTGTCTGTTGAGGGCAAGGACTCCGGCATAATCTCCGT
Q Q M P Q P Q P Q P Q Q T T P V P E A V L E A>

430 440 450 460 470 480 490
ATTATGCAAGAAATGCAAAATATTTTCCGTTCTTCTCTTGTACCAGGTTGGGATACTGTCTGGTACAGCAG
TAATACGTTCTTTACGTTTATAAAAGGCAAGAAGAGAACATGGTCCAACCTATGACAGCCATGTCTGTC
I M Q E M Q N I F R S S L V P G W D T V Q T A>

8/36

500 510 520 530 540 550 560
CAGATGCTGTACGTCAGATTGTAACCGTGTAAAGAGAACGTCCTTACAGGACCATTAAATGATGACAGAGAT
GTCTACGACATGCAGTCTAACATTGGGCACATTCTCTTGCAGAAATGTCCTGGTAATTACTACTGTCTCTA
A D A V R Q I V T R V R E R L T G P L M M T E M>

570 580 590 600 610 620 630
GGTACTGGTCTTGGTAGAACAGGACCTTTATCAACCACAGGTGCAACAGGAGCAACAACAGGTCTCTGTT
CCTATGACCAGAACCATCTTGTCTCTGGAAATAGTTGGTGTCCACGTTGTCTCGTTGTTGTCCAGGACAA
D T G L G R T G P L S T T G A T G A T T G P V>
tryptic peptide 82c

640 650 660 670 680 690 700
GCTGCATTACGCGGTGTAAACAAATGATTTCTTAGGGAAATAATGATTCAAGAAGCAGTACTTGAGACAT
CGACGTAATGCGCCACATTGTTTACTAAAGGAATCCCTTTATTACTAAGTTCTTCGTCATGAACCTCTGTA
A A L R G V T N D F L R E I M I Q E A V L E T>

710 720 730 740 750 760 770
TATGGGCAGTTGTACGTGATGCACAAGAAAGACCATGGCTAGTTAATGAACAGGAAGTATTGCATGCAGT
ATACCCGTCAACATGCACCTACGTGTTCTTCTGTGTACCGATCAATTACTTGTCTTCATAACGTACGTCA
L W A V V R D A Q E R F W L V N E Q E V L H A V>

780 790 800 810 820 830 840
AACAGCAGATGCTGTACAAGGTTTCCTTGGTTCGCATGCATGATCGTCTTCGTGCAACAGGTTTCTCTGAG
TTGTCTGTCTACGACATGTTCCAAAGGAACCAGCCTACGTACTAGCAGAAGCACGTTGTCCAAAGAGACTC
T A D A V Q G F L G R M H D R L R A T G F S E>
tryptic peptide 82a

850 860 870 880 890 900 910
GAAGAAGTCATGAGACTTCTACCTAGGTACGTAATGGTGGTTGTACCCGTACAGGGGGCCTCTTTGATC
CTTCTTCAGTACTCTGAAGATGGATCCAGTGCATTACCACCAACATGGGCATGTCCCCCGGAGAACTAG
E E V M R L L P R S R N G G C T R T G G L F D>
tryptic peptide 82b

920 930 940 950 960 970 980
AATGTAACGATGCCCCCTCCCTCTCGTCTTCTTGGTAAGAGGATGTATAGTACTGGATATTATCGTTATGG
TTACATTGCTACGGGGAGGGAGAGCAGAAGAACCATTCTCCTACATATCATGACCTATAATACCAATACC
Q C N D A P P S R L L G K R M Y S T G Y Y G Y G>

990 1000 1010 1020 1030 1040 1050
ATATCCTTCTTATTATAGCTATGGATATAGTTATCCAGCTTATTCACATTATCCTGTTTCTTATCCTTAC
TATAGGAAGAATAATATCGATACCTATATCAATAGGTGGAATAAGTGTAAATAGGACAAAGAATAGGAATG
Y P S Y Y S Y G Y S Y P A Y S H Y P V S Y P Y>

1060 1070 1080 1090 1100 1110 1120
TATGGGTATAGCTGGGGCCCCCTCATACTACTATGGCAGCGGATACTATGGTAAACATGGATATAAGTACG
ATACCCATATCGACCCCGGGGAGTATGATGATACCGTTCGCTATGATACCATTTGTACCTATATTCATGC
Y G Y S W G P S Y Y Y G S G Y Y G K H G Y K Y>

1690 1700 1710 1720 1730 1740 1750
ACTACTGCTGTACCTACAGAAACCATTACTACTCCCTCTACTCGTAATACACACAGCAGCAGCCTAAGAA
TGATGACGACATGGATGTCCTTTGGTAATGATGAGGGAGATGAGCATTATGTGTGTCTCGTCGTCGGATTCTT
T T A V P T E T I T T F S T R N T H S S S L R>

10/36

1760 1770 1780 1790 1800 1810 1820
GAGTAGGAGAAAGATATGAGCCTATTACCCCTACACAAAGAACTTTTATAATAATACAGAAGGCTACTAA
CTCATCCTCTTTCTATACTCGGATAATGGGGATGTGTTTCTTGAAAAATATTATTATGTCCTCCATGATT
R V G E R Y E P I T P T Q R T F Y N N T E Q T N>
Biotech Aus. tryptic peptide

1830 1840 1850 1860 1870 1880 1890
CAACCCTGTCTATACACCCGAAATCTTACAGAAGATGAACCACAACTGTATGGGAAACATACAACCTAA
GTTGGGACAGATATGTGGGCTTTTAGAATGTCCTTCTACTTGGTGTGTTGACATACCCTTTGTATGTTGATT
N P V Y T P E N L T E D E P Q T V W E T Y N *>
stop

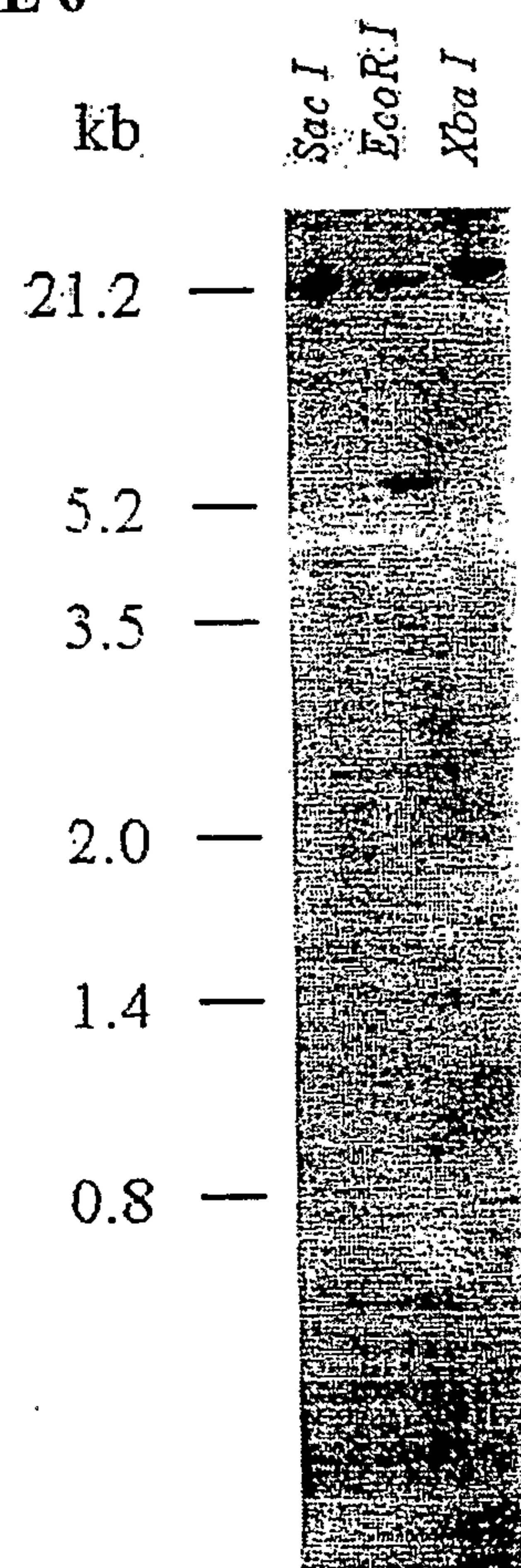
1900 1910 1920 1930 1940 1950 1960
ACCCTAAACCCTAAACCCTAAACCCTCAACCCTAACATTTCTCATTTTTTTATAGAGAAATTTTAGGGAA
TGGGATTTGGGATTTGGGATTTGGGAGTTGGGATTTGTAAAGAGTAAAAAATATCTCTTTAAATCCCTT

1970 1980 1990 2000 2010 2020 2030
CACTAACCTGCCTGCCTTGCCATCGTTTATATATATCCATTTGTTTATTAATAAACAATTTTATTTACC
GTGATTGGACGGACGGAACGGTAGCAAATATATATAGGTAAACAAATAATTATTTGTTAAAAATAAATGC

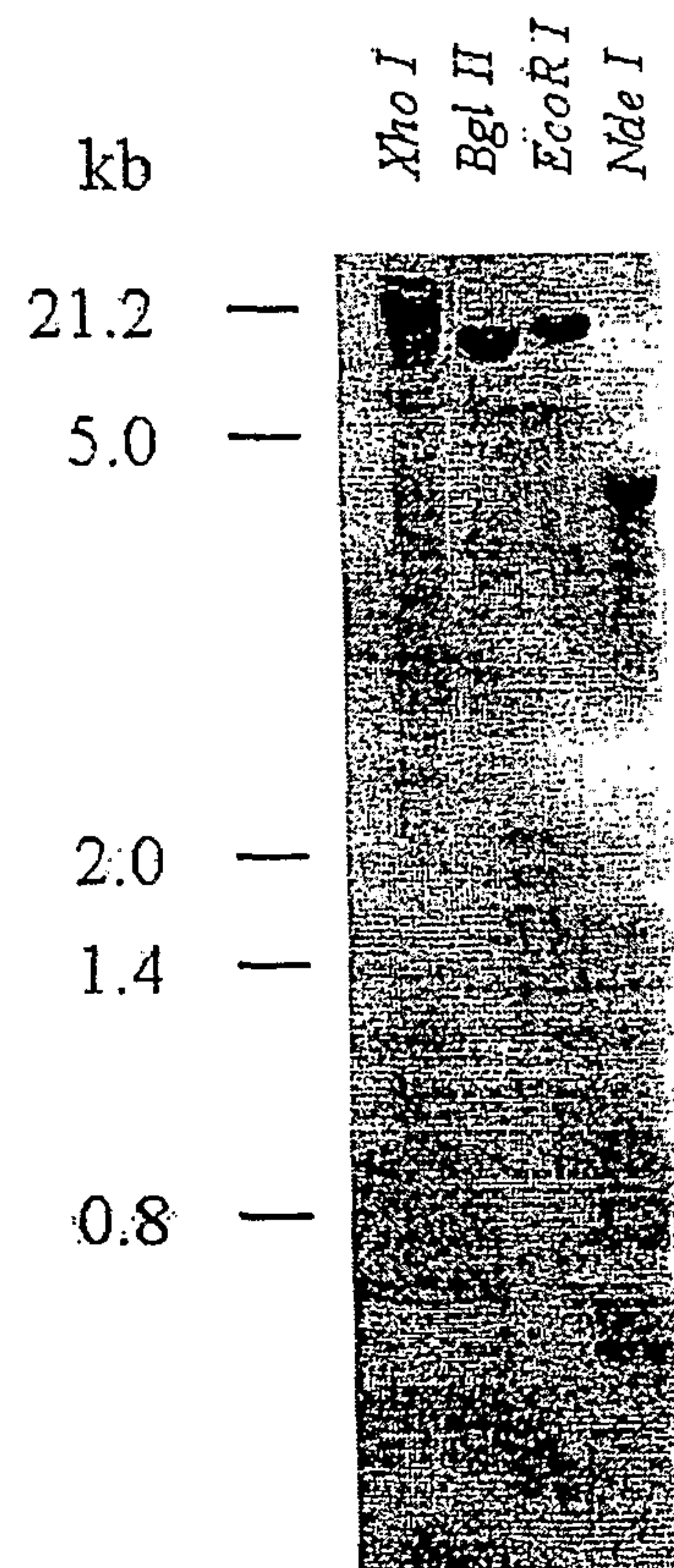
2040 2050 2060 2070 2080 2090 2100
TCTAGTCGTCTTTTATTAAACAGCGCTTATTCGGGTTGTTTATACAACTACTACTATTTTACCCAATA
AGATCAGCAGAAAAATAATTGTCGCGAATAAGCGCAACAAATATGTTTGATGATGATAAAAAATGGGTTAT

2110 2120 2130 2140
ATACTTGTACAGGCATTTTTTAAAAAATAAAAAAAAAAAAAAAAAA
TATGAACATGTCCGTAAAAATTTTFTTTTFTTTTFTTTTFTTTT

11/36

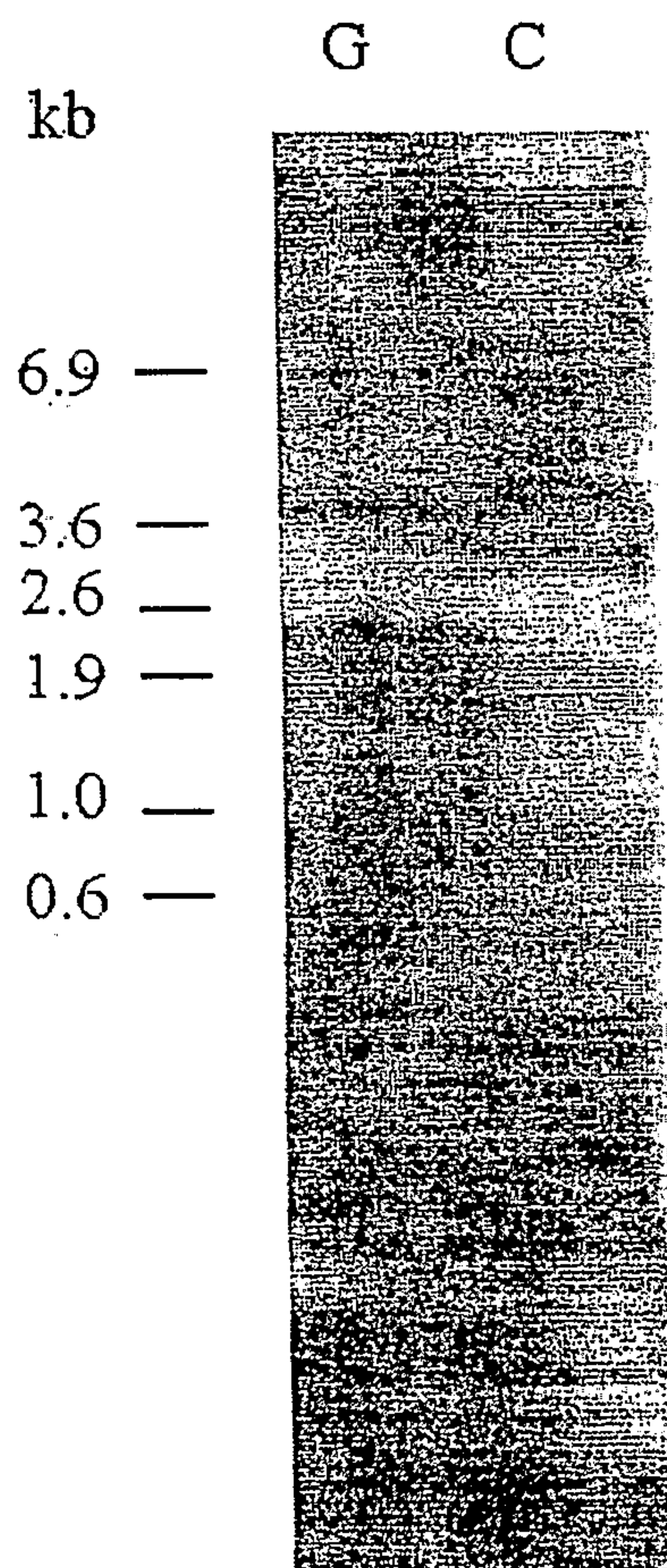
FIGURE 6

12/36

FIGURE 7

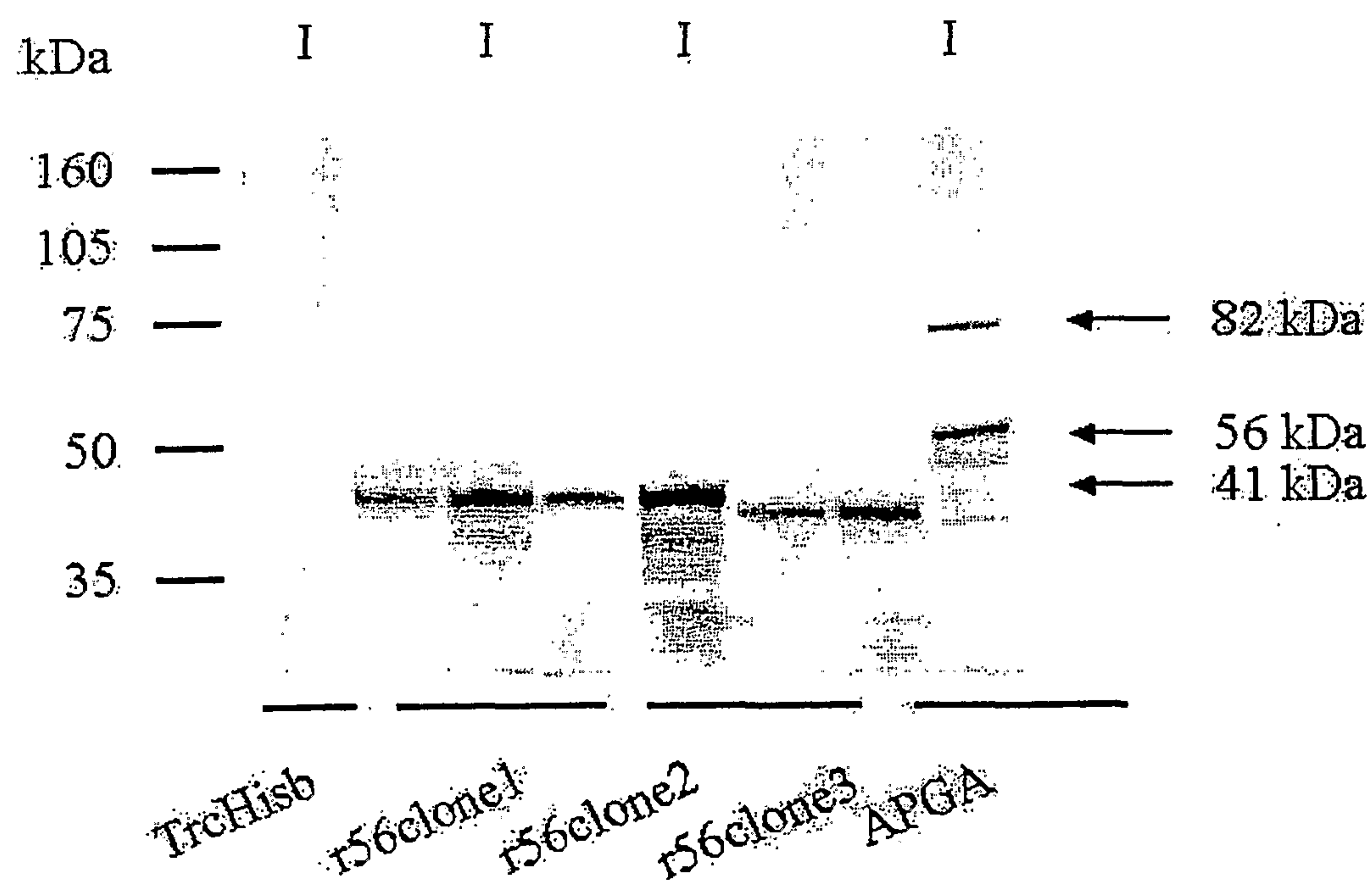
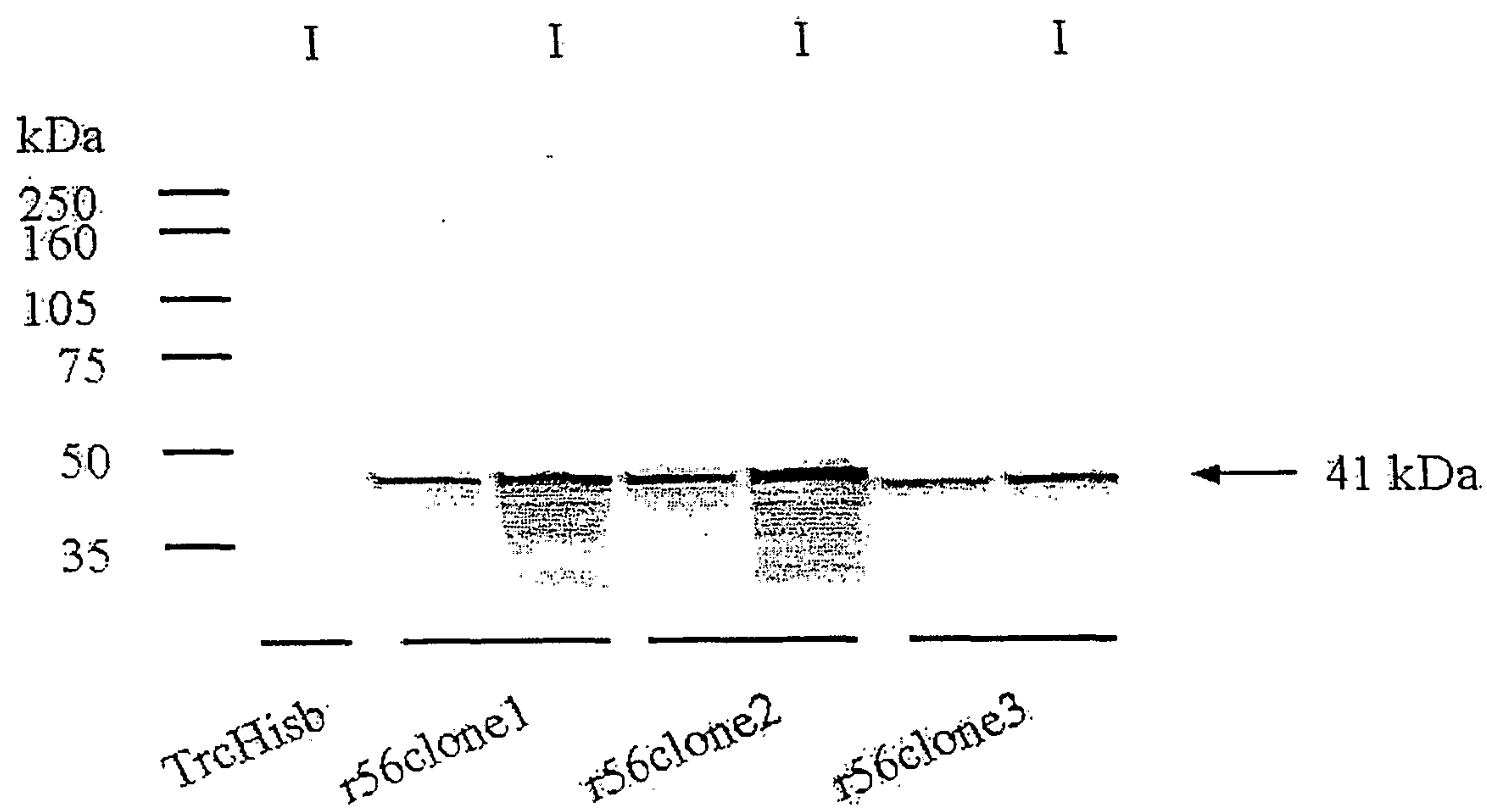
13/36

FIGURE 8



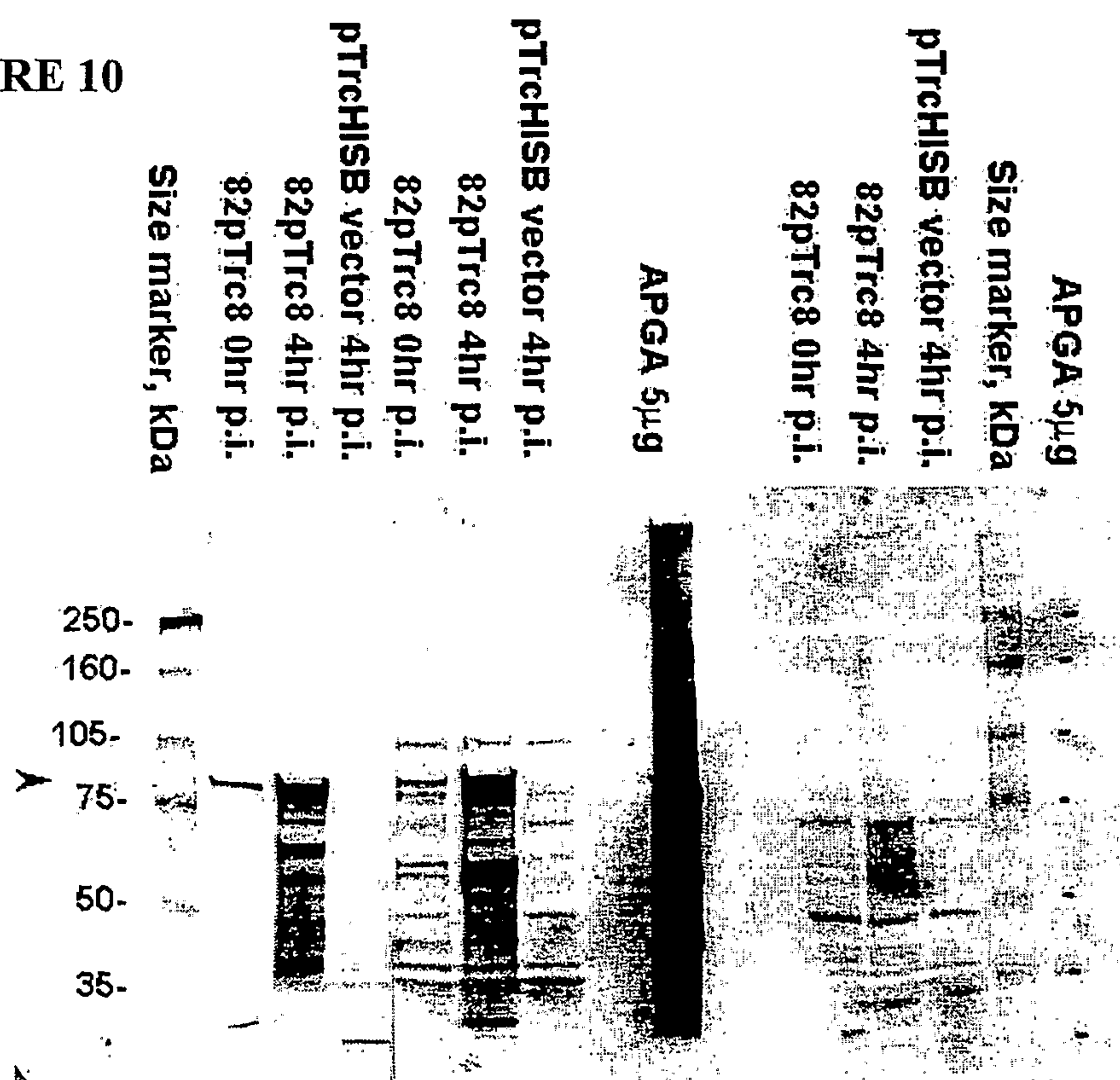
14/36

FIGURE 9



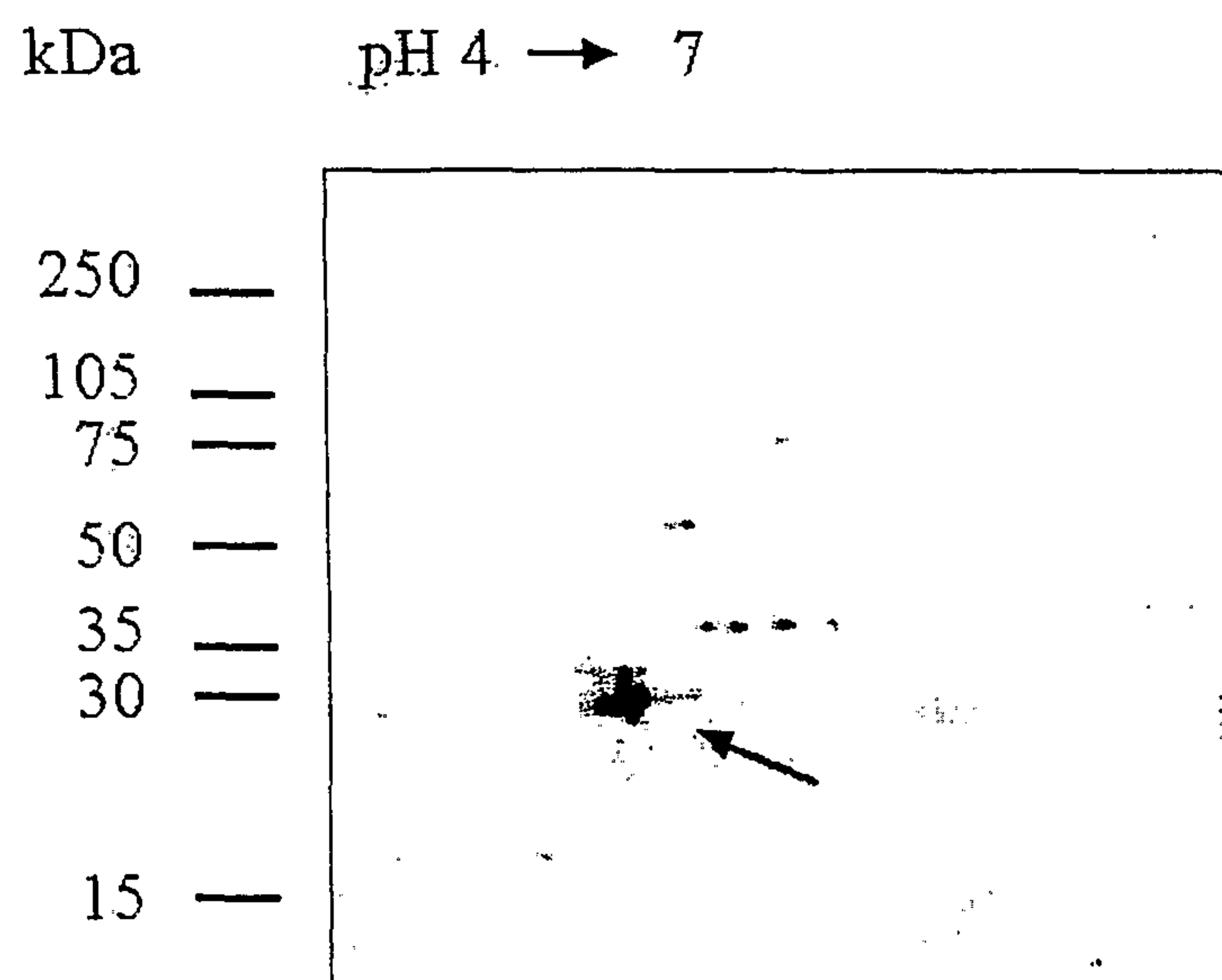
15/36

FIGURE 10



16/36

FIGURE 11



17/36

FIGURE 12

230	1	ATGCTGCATCGCAACCCGCGGTGGGCGCTTTGTGCAGCCCTGGCTGCACT	50
WO 9	1		0
230	51	CTATGGCGGAACAGGAATCGCCAGCGCCGAAGTTAACAATGAATTGAGCA	100
WO 9	1		0
230	101	AGTGGGAATCTGGGTGGACACCCCTGGACTACCTGCAACCCGCAAACTGGT	150
WO 9	1		0
230	151	CTGCGGGAGAGGCACAATGCACAGTGGGAGACATGGGTGGAGGTTGAGGA	200
WO 9	1		0
230	201	ATGCCAGAAGCTGACAGGATGTGGCAACTGGACTCCTTGGTCTCCCGGCG	250
WO 9	1		0
230	251	ATATGTCGTGTGTGGTGGGACAGTTTCAAACCCGCAACAGGGAGGGCTGC	300
WO 9	1		0
230	301	CCAGAGGTGCAGGAAGTGAGGGCATGCAGGCCTGTACTTCTAGAATGCAA	350
WO 9	1		0
230	351	CGATCAATGGACCCCTGGACAATGTGGGACACCAACCGGTTCCAGGAAA	400
WO 9	1		0
230	401	GATACAACTCAAAGTGCGGACCCGTOGAAGTCCCGGAGTGCAACATGGAC	450
WO 9	1	ATG	3

18/36

FIGURE 12 (CONT.)

230 451 GACGCAGAGATCGAGAAATGCCGGCGAGTTCGTGGAATGGGATGCCCTAT 500
WO 9 4 GGTTCCTTCGTCTTCACAGGCGGTGATTTGGGCGACTGGAGCCCCCTCT 53
* ** * * **** ** **** ** *** ***** *

230 501 GAATGGAGACTGCGTACGOGGGGGTACCCACACGCGTTACCGTCAAACT 550
WO 9 54 CGCTGCTGACTGCGTGCCTGSCACTACTCACACACGCCAGAGGGCAAATT 103
*** ***** * ** *** ***** ** * * *** *

230 551 GCCCAGACCGCAAAGAGGTTGCGGGTGTGCGGAGCCTTTGATTGCAGTAGC 600
WO 9 104 GCCCAAACCAACAAGGAGGTGCGGGTTTGGCGGCTTCGATTGTAGCCAG 153
***** *** *** ***** ***** ***** ***** **

230 601 TGCTCTGTAAACGCCACTTGGGATCCCATTTGGTGCATCCTGCGAATGCAA 650
WO 9 154 TGCTCAGTCAACGCTACCTGCGACCCCTCGGAGCCACTTGTCAGTGCAA 203
***** ** ***** ** ***** *** * ** * * ** *

230 651 GCCTGGTTTTCGGCGGCAATGGGAAGACCTGCGAGGCTTCAACCCCTGCG 700
WO 9 204 ACCGGGTTTTCGGAGGCEATGGGACTCAGTGGGAGGCATTCAACCCCTGCG 253
** ***** *** ***** ***** ***** ***** *

230 701 AAGATACCCCTGCACCTTGGGACAGCAAGGCCATCTGCACCCAGACGCA 750
WO 9 254 AAGGGGAGACGGCTCCTTGTGATGCGAAGCGGACCTGCAAGGCTGATGGA 303
*** * ** ***** ** ***** * ***** * ** *

230 751 A-TGACGCCAAATGCCAGTGCAGGGCAGGCTGGGACGCAGATTTCGGAGC 799
WO 9 304 AATGACGCCAAATGCCACTGCACAAGGCTTGGAAAGCAGACAGCAAGGC 353
* ***** ***** ***** ***** * **

230 800 AGGCAGCAGCAAGAAGCCTTGGCTTGAGGTGACGAGTGGGCATCCAACA 849
WO 9 354 AGGTGCCAGCGGTCAAGCATGGGTGGAGGAGGAGCAATGGGCCAACAACA 403
*** ***** * * ***** ***** ***** ***** *****

230 850 CCCACCAGTGCCTCGGCACACTCCACATGCATCAACACCAAGGGCTCTTAT 899
WO 9 404 CGCACGAATGTCCGACGCACTCAACTTGGCTCAACACTGAGGGCTCCTAT 453
* *** * ** *** ***** ** *** ***** ***** *****

230 900 AAGTGGGACTGCAACCAGGGATACCGTCAAGGGAGAGGACGGACAGTGTG 949
WO 9 454 GAATGCAACTGCTTACCGGGTTATCAG-AAGGATCAGGATGGGAAATGCC 502
* *** ***** * *** ** * **** ***** ** * ** *

230 950 ATGACGTGATGAATGCCCAACGGAGAGCACACCTGCCCGCTCACTCC 999
WO 9 503 AGGACATAGACGAGTGGCT--GGGGAACATGGTTGTCCCGCACACTCG 549
* *** * ** ** *** * ** ** ** ** ** ** ***** *****

230 1000 ACTTGTTTGAATACAGCTGGCAGCTACGAGTGGCGCTGGGACACTGGGTA 1049
WO 9 550 ACTTGGCTGAACAAGGCAGGCTTGGAGTGCAGTGGGACGCGGTTT 599
***** ***** ** ** ***** ***** ***** * ** *

230 1050 CAGCGGAATGCAACTGCAGACAGCCCTTGCAAGAACATTGACGAATGCC 1099
WO 9 600 CAGTGGCAGTGCCTACTTCTGAGAGTCTTGGCTGGAATATAGACGAGTGGC 649
*** ** * *** ** * ** ** ***** ** ** ***** ***

230 1100 CCAACCCCAACGCTGCTGGGCCAAGCTATCTGCACAGACACCGACGGC 1149
WO 9 650 AAGACCCGGATGCTGCTCAGCCAAGCAATCTGCGCAGACACTGAGGGC 699
***** * ***** ***** ***** ***** ***** ** ***

230 1150 TCCTTCACCTGCAGCTGCCCGAAGGGTACAGGGCCAGGGAACCCATGA 1199

19/36

FIGURE 12 (CONT.)

WO 9 700 TCCTTCACTTGCAGCTGCCCTGAGGGTTACAGCGGTGGGGGATCACAAGA 749
** ***** ** ** ***** **

230 1200 CTCTCCCTGCTCCAAGATCGACTTCTGCGCATACCCCTCACTCAATACAT 1249
WO 9 750 CTCTCCCTGCTCGAAGATAGATTACTGCGCGGACCCCACTGAACACCT 799
***** ** * ***** **

230 1250 GCGGAGCCCACTCCACTTGC---AACACCCCTCACATCTTTCAAGTGCATC 1296
WO 9 800 GCGGGGCCCACTCGACTTGTGTGAACACACTAAGGACGTTCAAGTGCCTT 849
**** ***** ** ** * ***** *

230 1297 TCGGATGCGGGATATGAAGGCGCGGCACTGCGGAGAGCCCGTGGGTGGA 1346
WO 9 850 TCGGATGCGCGGTATGACGGCGCGGGAACGCACGAGAGCCCTTGTGTGGA 899
***** ** ***** ** ** * ***** **

230 1347 CGTGAACGAGTGTCTCGAAGGAGAAGCCCAACAACACTGCAACAGAAAG 1396
WO 9 900 TATCGACGAGTGTCTCAAGGAGAAACCATCCAATGACTGCAACCGAAGC 949
* ***** ** ***** **

230 1397 CAAACTGCACCAACACCGAGGGATCCTACACTTGGGAATGCAAGCCCGGT 1446
WO 9 950 CCGTTTGCACAAATACTGAGGGATCGTACACCTGCGCATGCAAGGAAGGC 999
* ***** ** ***** **

230 1447 TTCTCTGGGACGGCATGGGTCCCAACGGGTGTACCGACATCGACGAGTG 1496
WO 9 1000 TTCTCTGGGAGGGGTTTCGGAGCTGCAGGGTGTGCAGATGTGATGAGTG 1049
***** ** * ** * ***** **

230 1497 CGCGGCGGAGCAGTCCCTCTGCGACCTCACGCTCTCTGCGCAACACTG 1546
WO 9 1050 CGCGA-----ATTGCGCTCTGCGACGCGCCACGCTCTTGTGCGCAACACG 1093
**** * ** ***** ** ***** *

230 1547 AGGGCTCGTATGTATGCACCTGCAACACCGGCTACGAGCCAGCTTCAACC 1596
WO 9 1094 AGGGTTCCTACGTTTGCACCTGCAACCCCTGGCTATGAACCGAGCTCAAGC 1143
**** ** ** * ***** ** ***** **

230 1597 GACGGGCATGCATGCAAGATATCGACGAGTGGGCCACCGGTGCAGCTGG 1646
WO 9 1144 GACGGACATGCATGCAAGGACGTTGACGAGTGTGCAGCGGGGACGGGGA 1193
***** ***** ** * ***** **

230 1647 GTGCCACGTGTTCAGCACAGTGTCTGAACACGGACGGCAGCTACGAGTGCA 1696
WO 9 1194 ATGCCACGTCTCCGCACAGTGTGTGAACGTGGATGGCAGCTATGAATGCC 1243
***** ** ***** ** ** ***** **

230 1697 AGTGTCTTGAGGGCTTCTGCGGCGACGGAAGACCTGCAACGACGTGAT 1746
WO 9 1244 ACTGCTTGGAAGGTTTCATTGCGGACGGAAGGTGTGCAGTGACGTGAC 1293
* ** * ** * ** * ***** ***** **

230 1747 GAGTGGCTGCGGCGACATCTCCTTGGGTGACAACACTCACTGCCAGAA 1796
WO 9 1294 GAGTGTGCGGCTGAGGCTTCCCTGTGCGGCAACACCGCATTGCCCTGAA 1343
***** ** * ** * ** * ***** **

230 1797 CACAATTGGCAGCTACGAGTGGAGTGAAGGCTGGCTATGGCAACATGC 1846
WO 9 1344 CACCATGGCAGCTACGAGTGGAGTGAAGGACGGATATGGCCACATGC 1393
*** ** ***** ***** **

230 1847 AAGACAACGCATGCAGCGACATTGACGAGTGCAGGATGCGAACACCAAG 1896
WO 9 1394 AGGGCAACGGGTGCAGCGACATCGATGAGTGTCTCAGAGGGGTCTACAGAG 1443

20/36

FIGURE 12 (CONT.)

* *

230 1897 ATCCCTGACAACTGTCTTTGCGTGAACAATGATGGCAGCTACTCCCTTGA 1946
WO 9 1444 ATCCCAGAGAACTGCAACTGTGTCAACACCGAGGGGAGCTTTCTCCCTTGA 1493
***** ** ***** ** ** ***** ** ** ***** *****

230 1947 GGCGAAGCCTTGGATACGAATTGGTGAAACGGGAGTGCATCAAGATCGACT 1996
WO 9 1494 GGCAAAGCCTGGGTACGAGCTCTCTGACGGCAAGTGGTCAAGATCGACT 1543
*** ** ***** * ** ***** ***** *****

230 1997 TCTGCGCCCGCGGCGCATGCAACTCGCTGGCCTCCTGCAAGGAGAATGAA 2046
WO 9 1544 TCTGCGCCCGTGGTGCATGCAACTCGCTGGCSCACTGCAAGGAGAATCCC 1593
***** ** ***** ***** *****

230 2047 GAAGGCACAGCGGCGATCTGCACTGCTGCCAGGCTACAGCGGCGACGG 2096
WO 9 1594 GAGGCAACGCGCGGATCTGCACTGCTGCTAGCTGGCTATTGAGGTGACGG 1643
** ***** ***** ** * ***** ** *****

230 2097 CACTGCTGAAGGCCACTGCAACGACATTGACGAGTGTGCAGGTGAGAATG 2146
WO 9 1644 CACAGCTCAGGGCCACTGCGATGACATCGATGAGTGTGCTGGCGGAGAATG 1693
*** ** * ***** * ***** ** ***** * *****

230 2147 ACTGTGCTCTCTGCGAGCAGGGAGGCACTCTGCGAGAACACTGTGGCTCG 2196
WO 9 1694 ACTGCACCCCTGCGGATCAAGGAGGGATTCTGCGAGAACACTGTGGCTCT 1743
***** * ***** ** ***** ** ***** *****

230 2197 TACACCTGCAAGTGCAAAAGAGGGGTACAGGCAAGATGGAACTCATGCAC 2246
WO 9 1744 TACACCTGCAAAATGCGCAGCTGGGTACCAGCAAGACGGCAACTCATGCAC 1793
***** ** * ***** ***** ** *****

230 2247 TGAGATCGACGAGTGGCTGAGGGAAACCCACAACCTGCCACCCCTTCGCGCA 2296
WO 9 1794 TGACATTGACGAGTGGCGCAACGGCACTCACAACCTGCCATGCTCCGCGA 1843
*** ** ***** * ** * ***** * ***** *

230 2297 CCTGCAGCAACACCCCGGAAGCTTCACCTGCCAATGCAACAGTGGATTTC 2346
WO 9 1844 CATGCACGAACACGCAAGGCTCTTTGAGTGGCGCTGCAACGCGAGCTTC 1893
* ***** ***** * ** * ***** ** ***** ** *****

230 2347 ACTGGCAGCGGTGTGGAGTGGGAAGACATTGACGAGTGTCTCAACTGAGGC 2396
WO 9 1894 AGCGGCAACGGSETTGAATGCAACGACGTGACGAGTGTCTGACTGACGC 1943
* ***** ** * ***** * ***** ***** *****

230 2397 AGATGATTGTGGTGCAAAACACCATCTGCAGCAACACCATTTGGTCTTTTCG 2446
WO 9 1944 TGACGATTGCGGAGAGAAACACTGTGCAACAAACACAGTTGGCAGCTTCG 1993
** ***** ** * ***** * ***** ***** *****

230 2447 AGTGCAACTGCGGTGAAGGCTATGAACGCGCAGACGCAAGACGTTGCTC 2496
WO 9 1994 AGTGCAACTGCGGTGCGCTGCGTTGAGGCGCGGACGCGAAGACCTGCAA 2043
***** ** * ***** ** * ***** ***** *****

230 2497 GACATGACGAATGCGCGACAGGCACACACACTTGTCTGAAACCAAGCCAC 2546
WO 9 2044 GACATGACGAATGTGCAAGCGGGACCCACACTTGTCTGCAACCAAGCCAC 2093
***** ***** ** * ***** ***** ***** *****

230 2547 CTGCACCAATACCGATGGGTGATTCACATGCCAGTGCACACCCCGGCTTCG 2596
WO 9 2094 ATGCACCAACACTGCTGGGTGCTTCACATGTGAGTGCACACCCAGGCTTCG 2143
***** ** * ***** ***** ***** *****

21/36

FIGURE 12 (CONT.)

230 2597 AAGGTGACGGCCACAAGTGGGAGGACATCGACTTCTGCGGTGCTGGACAG 2646
WO 9 2144 ACGGTGACGGCCACAAGTGGGAGGACGTTGACTTCTGCGGCCAGGGGCTG 2193
* ***** * ***** ** *

230 2647 CACGACTGCAATGTGCAATGCCGAGTGCTCTGAGAGCGAGGACAACACCAC 2696
WO 9 2194 CACGACTGCAACGTGCAATGCAGAGTGCTCGGAAAGCGACGACAACACCAC 2243
***** ***** ** *****

230 2697 TTTCAAGTGCACCTGTATAACAGGGTACGCTGGAGACGGCCATGGCGAGG 2746
WO 9 2244 CTTCAAGTGCACCTGCGGCATTGGGTACAGCGGGGAAGGCCAOGGGGAGA 2293
***** * ***** ** ** ***** ** *

230 2747 CAGGCTGCCAAGACATTGATGAGTGCGCAGAGAAACATCTGCGGAAGC 2796
WO 9 2294 ATGGTTGCCAAGACATTGATGAGTGCGCCCAAGATGCCATCTGTGCGGAG 2343
** ***** **** ***** **

230 2797 AACGCTGTCTGCACAAACACCGCAGGAAGCTACCAATGCGCATGCGGTGA 2846
WO 9 2344 AACACAGTGTGTACCAACACACCGAGGTAGCTTTGAATGTGCGTGTGTGA 2393
*** * ** ** ** ***** **** ***** ** *

230 2847 GGGCTTCGTTGCATCAGCTGAACAGCAGCAGGGAACCCAGCACTGG 2896
WO 9 2394 AGGGTTCGTTGG---CTGTGGGAGCGAAGCTCAAGGGAGCAACTTCATTGA 2440
** ***** * * * * * **** * * ** *

230 2897 TTTGCGTGGACGTCGACGAGTGCGAGCGAGCTTCGAAGAACACATGTGCC 2946
WO 9 2441 CCGCATAGACATCGATGAATGCAACGAGCGCTCGAAAAACACTTGGCGC 2490
*** * *** ***** ** ***** ***** ***** ** ***

230 2947 AAGCCAGCTGACGGAGGCTTTGCACAAACACTGAAGGCAGCTACGAATG 2996
WO 9 2491 ACGTCAGCTGACGGAGGCTCTTGCAGAACACCGCAGGCAGCTATGAGTG 2540
* * ***** ***** ***** * ***** ** **

230 2997 CGCTTGCAAGCCAGGCTACCAAGGTGACGGCCACAGCTGCGCAGACATCA 3046
WO 9 2541 CTGCTGTCTTGCCTGGGTTCAGGGGACCGGCCACAGCTGCACAGATATTG 2590
* * ** *** ** * ** ** ***** ***** ** *

230 3047 ACGAATGCACTGCACAGGGCACCTGCGGCGAACACACAACCTTGCAAGAAC 3096
WO 9 2591 ATGAGTGCSCCACCACAGGCGTATGCGGGGAACATGCGACCTGCGAAAC 2640
* ** *** * * ** ** ***** ***** * ** *** * **

230 3097 ACACCCGGATCCTTCCAGTGGGACTGCGTTGAGGGATTG---AGCGCGC 3143
WO 9 2641 ACTGCGGGTTCGTACAAATTGCACCTGCGAGGGCGGTTCACACTCAGCAAGA 2690
** * ** ** * * * ** ***** * *** * * *** *

230 3144 TGATGAACGCACCTGCGGTGACATCAACGAGTCCGAGACAGGAGCAGTGG 3193
WO 9 2691 TGGGGCGGTGCGCTGCATTGATATTGATGAGTGTGCGCCTCCACAGCAG 2740
** * * **** ** ** * ***** * * *** *

230 3194 TGCTGCCACCGAAGCTCCACCTGCGTCAACACTGAAGGCAGCTACGACTTC 3243
WO 9 2741 TGTACCGCGCAACGCGCACTTGCGTGAACACTGAAGGCAGCTATACATTTC 2790
** * ** * *** ***** ***** *****

230 3244 GACTGCGTTGCTGGGTACCGCGGCACTGATGGAGCTTGTGTGAAGATCGA 3293
WO 9 2791 GAATGCGTGGCGGCTACCGCCATACGGAGAATGGCTGTACCAAGATTGA 2840
** ***** * ** ***** ** ** * *** ***** **

22/36

FIGURE 12 (CONT.)

230 3294 CTTCCTGCAAGGAGAAGGGATGCAACGCAACGCCACATGCCGGGAAAADG 3343
WO 9 2841 TTTCTGCAGCGAAAAGGGATGCAATGCGAATGCCAGCTGCAAGGAGAAAG 2890
***** ** ***** ** ** ***** ** ** *****

230 3344 ATGCCGGCACCGAGGCCATCTGCACTTGCAAGGAAGGCTATGAAGGCAGC 3393
WO 9 2891 ATGCCGGCACCGAAGGCATCTGCACCTGCCACAGCGGGTACGAGGGCAAT 2940
***** ***** ***** ** * ** ** ** *****

230 3394 GGAGAAGGCGAAGATGGTTGCCAGAACATCAATGAGTGGAGAGAGGGCGA 3443
WO 9 2941 GGCGAAGGAGAGAAGAAGGGTGCAAAAACATTGAAGAGTGTCTCGTGGGAGA 2990
** ***** ** ** * ***** * ***** ** **

230 3444 ACCCTGCAAGGACTTCGGGGAAGGGGGTGTTCGCTCGACACACCAGGAT 3493
WO 9 2991 GCCATGCAAGACTTCGGGAGAGGGGGGCTCTGTGTCTGATTTCTCGGGAT 3040
** ***** ***** ***** ** ** ***** * ** *****

230 3494 CATTCACTTGGAGTGGGCTGCTGGATTCAATCAACGGCGCTCCGTTTGC 3543
WO 9 3041 CCTTCAGCTGCTCTTTCGCCACCGGTTTTCATCAAGAGGCGATCTACTTGC 3090
* ***** ** ***** * ** ** * * * ** ** *****

230 3544 CAAGATGTTGAOGAATGTCTCGACGGAAAGCTGAACACCTGGGCTGCCAC 3593
WO 9 3091 CAGGACATAGATGAGTGGCTCGAAGGAAAGATGAACACTTGGCCCCCGT 3140
** ** * ** ** ** ***** ***** ***** **

230 3594 CGGAGGCGTCTGCTCCAACACCGTCCGTTCTTTCACCTGCTCGTGGGCCA 3643
WO 9 3141 CGGGGGTATCTGCACGAACACCGTCCGCTCCTTCACCTGCTCTTGGGCTG 3190
*** ** ***** * ***** ***** ***** *****

230 3644 GCGGCTTCGAAGGCGATGGCCACACCTGCAATGATGTGACGAATGGCGA 3693
WO 9 3191 CTGGCTTCACGGGTGACGGCCTTACTTGGAGGACATCGACGAATGTGCT 3240
***** ** ** ***** ** ** * ** ***** **

230 3694 ACAGCACAGCACACCTGTGACCCGAATGCCACTTGGCTCAACACCGAAGG 3743
WO 9 3241 ACGGCGGCACACACGTTGGACCCCAAGGCCACTGTGTCAACACTGTGCG 3290
** ** ***** ** ***** ** ***** ** ***** * **

230 3744 CAGCTTCGAGTGGCGCTGCAATGCCGATTCGAGGGGAGCGGACACACTT 3793
WO 9 3291 CAGCTTCGAATGGGATGCAAGGAGGGATTCTCTGGTGAAGGOCACACAT 3340
***** ** * ***** * ***** ** ***** ***** *

230 3794 GCGCAGACATCGACGAATGGCGAGACCCAGCCAAAACACATGGGATACA 3843
WO 9 3341 GCACCGATATCGACGAATGGCGTGACCTTAACCTTAACAAATGGGACACA 3390
** * ** ***** ***** * ***** ***** **

230 3844 CACAAGGGTGTATGOCAAAACACCAAGGGTCTTACACTTGGGGCTGCAA 3893
WO 9 3391 CACAAGGGCATCTGCCAGAACGGCACTGGATCTTACACTTGGGGATGCAG 3440
***** * ***** ** ** ** ***** ***** *****

230 3894 GACCGGATTCACTCTTTCAGCTGACGGAAGCACATGCGAAAACGTCGACG 3943
WO 9 3441 GCGTGGATACAGTCTGGGGGGGACGGCTTCACTTGGGACAAATGTGATG 3490
* * ***** ***** ** ** ***** ** ***** ** ***** *

230 3944 AGTGGCGGGGGGAACTGCCAACTGCCAAGAGCGAAGCTTCTGTAAAGGAC 3993
WO 9 3491 AGTGGCGTGGGGGACGGGACCTTGGCGAGAGCGAGCTTCTGGGTGGAC 3540
***** ***** ** ** * ** ***** ***** *****

230 3994 ACAGAGGGTTCCTACCAATGCGAGTGCAAGAACGGCTACAAGGCTGCAGG 4043

23/36

FIGURE 12 (CONT.)

WO 9 3541 ACGCAAGGGTCATACAAGTGGGAGTGCAGAACGGCTACCGCCAGTCTGG 3590
** * ** * ** * ***** * **

230 4044 AGAGGACTGTGTGGACGTTGACGAGTGGGAGGCTGGCGTGCAATGGATGCA 4093
WO 9 3591 GGAGGACTGGGTGGACGTTGACGAGTGGGAGGCTGATGTGCACACATGCA 3640
***** ***** *****

230 4094 GCGAGCAAGCAATCTGCACAAATACAGACGGCAGCTACTCCTGCGAATGC 4143
WO 9 3641 GCGAGCAAGCTAAGTGCACGAACACTGAGGGGAGCCACACCTGCACCTGC 3690
***** * ***** ** ** ** ** ** ***** **

230 4144 ATGGAGGGATACCAGGGAGACGGCAAGGCTTGCGAGAAGACAGTGGGCGT 4193
WO 9 3691 AATGAAGGGTAACAGGGAGACGGAAAGAAGTGGGAGAAGACAGTGGGCGC 3740
* ** * ***** * ***** * **

230 4194 CTGCGACTCCGCTCCCTGCGGTGCCCAAGCCACCTGGGAGCCTGCAGGGG 4243
WO 9 3741 TTGCGACAACTCGCCATGCGGCAACAACGCCATGTGTGAAGCTACTGCCG 3790
***** * * ** ***** * ***** ** ** * * *

230 4244 ACAACTACACTTGCACATGCCACCCAGGCTACGAGATGCGCGAAGGAGCC 4293
WO 9 3791 ATAGCTACAACTGCACCTGCCAAAGCTGGCTACGAGATGAAGGACGGGGCC 3840
* * ***** ***** ** * * ***** ** ** **

230 4294 TGCGTTGACATCGATGAGTGCACAGCAGGCAGCCTCAACTGCGACCCCTCA 4343
WO 9 3841 TGTGTGACATCGATGAGTGCAGTCCGAGTCCGGCACCCACAAGTGCAGACCCGCA 3890
** ** ***** * **** ** ***** **

230 4344 TGCCATTTCACACAAACACGACGGCTCCTTCACTTGGGTCTGTGGCAGCG 4393
WO 9 3891 TGCTGACTGCAGCAACACCGATGGATCCTTCAAGTGCACGTGCGGTTCTG 3940
*** ***** ** ***** ** ** *

230 4394 GCTATACCGGCCTTGGCACATCCTGCGAAGACATCGACGAGTGGCGGGGT 4443
WO 9 3941 GCTACACTGGTGTGGGTACCTTTGCGAGGATGTGGACGAGTGGCGGGGC 3990
**** ** ** * ** ** ***** ** * *****

230 4444 AACGCAGCAGGCTGGGACATCCACGCCGCTCTGCACGAACACTCCCGGATC 4493
WO 9 3991 AACCATGCGGGCTGTGACATCAACGCTGTTTGCACTAACGTCCCTGGCTC 4040
*** ** ***** ***** **** ** ***** ** ** **

230 4494 GTTCAAGTGGGAGTGCAGAAGAGCGGCTTCGAAGGCGATGGCAAGCAATGCA 4543
WO 9 4041 GTTCACTTGGGAGTGCAGAAGAGTGGCTTCGAAGGCGATGGGCAAGAGTGTGTA 4090
***** ***** ***** * ** *

230 4544 CGGAGAAGGTGTGTGCTCCCGGACAGATTCACTGCGAAGCCTGGACTGCA 4593
WO 9 4091 CGGAGAAAGTGTGTGCTCCCTGGCCAGATTCACTGCGATTCTGTGGACTGCA 4140
***** *** ***** ** ***** * *****

230 4594 TGGACAGAGTGTACCGACGGCGCCAAAACAGCACACGACGCTGCCTTGC 4643
WO 9 4141 TGGACCGAATGTACAGCTGAAACTAAGCAGAGCACCCGCAAGTGGGTGGC 4190
***** ** ***** * * * ***** ***** . *** * **

230 4644 ACTGCCGCTTAAGAAGGAGATGCCCGCCTGCCCTGCAGCTGACTTCTCCC 4693
WO 9 4191 TCTTCCTCTCAAGGTGAGGTGAAGCTTTGCCCGGATGCTGACATTTCAG 4240
** ** * ** * ** * ***** * ***** * **

230 4694 AGTGGCGAGAGTTCACTGAATGGACTGCCCTGCCCTGGAAACCAACAATAAC 4743
WO 9 4241 CCTGCCGTGAAGTCCGGGAGTGGTCAATCATGCCCGAGGAGTTGACAAAC 4290

24/36

FIGURE 12 (CONT.)

```
***** ** ** ** * * ***** ** * * * *  
230 4744 CTGTCTCATAGGCGCACTGAAAGATTGGGAGAACCGGATGCGAAGATGC 4793  
WO 9 4291 CTGTCCGACCGCAGAGCAGAGAAGTTCCGGGAGCCGGGCTGTGAGCACGC 4340  
***** ** * * * * * ***** ** * * * * *  
230 4794 AGAGGAAGTCCGCGAATGCCCAGATGAAGAGACCGAGCAGAAATGCGGCG 4843  
WO 9 4341 TGAGGAGGTCAGGCGAGTGCCTCAGATGAAGAAGTTGAGGAGCGCTGTGGTG 4390  
***** ** * * * ***** ** * * * *  
230 4844 CCTGGGGTGAGTGGACCGCTGCGGCGACCCATCCCTTGGCTGAGAACT 4893  
WO 9 4391 CCTTTGGCGAGTGGACTGCTATCGCGCGATCCTTCTGAGGGCTTGAGGACC 4440  
*** ** ***** ** ***** ** * * * * *  
230 4894 CGCGCACGCGAGAACTGCCCCGATGTGTGTAGAGTTCSAGCGTTGCACTAT 4943  
WO 9 4441 AGGACGCGCCAGAACTGCCCCAGAAGAGGCAGAAATTCAGCACTGCACAAT 4490  
* * * * * ***** ** * * * * *  
230 4944 GCCCAGTGAGCCTGAGGCTGGCGAAGTGACTGAGCCTCACACAGAAGGAG 4993  
WO 9 4491 GCCCTCTGCACCATCCGTTCCCGAGGGCGGCAGCAGCTGCACAGAGTTGG 4540  
***** ** * * * * * ***** *  
230 4994 GAGCCGGAGTTGGTGGCGAAGTGACTGAGCCTGACACCGAAGAAGGAGCC 5043  
WO 9 4541 GGGCCTGGAGTGAATGCG---TGGCTGACGCT---CATGGGATCAAGATGC 4585  
* * * * * ** * * * * * * * * * * *  
230 5044 GGAGTTGGTGGTGAAGTGCAGCCCGGTACAGAAGAAGGAGCAGGAGTTGG 5093  
WO 9 4586 AGCACAGAAGCTGC---GTACACAATGAAGCTGTGCAGGAACACAGAATCTG 4634  
* * * * * * * * * * * * * * * *  
230 5094 TGGTGAAGTGCAGCCCGGTACAGAAGAAGGAGCCGGAGTTGGTGGTGAAG 5143  
WO 9 4635 CACCGTGGAGA---TCCACAACAGTGGCGGGAGTGGTTCGCACTGGTCAGAG 4683  
* * * * * * * * * * * * * * * *  
230 5144 TGCA-GCCCGGTACAGAAGAAGGAGCCGGAGTTGGTGGTGAAGTGCAGCC 5192  
WO 9 4684 TGCAAGAATGGCAAGCAGTACAGAGGCGCCCGCGG-----ATGCGCGTCT 4728  
***** * * * * * * * * * * * * * * *  
230 5193 CGGTACGGAAGAAGGAGCCGGCATTTGGTGGCGAAGTGACTGAGCCTGACA 5242  
WO 9 4729 GTGTACG-AAGTCAGAGCCTGCAGCGCGG-----CTAGCG 4762  
***** ** ***** ** * * * *  
230 5243 CCGAAGGAGGAGCCGGAGTTAGTG-GCGAACCAGCCGAAGAAGAGGGCAC 5291  
WO 9 4763 ATGCGAAGAATGCTCTTTTGGTGGTGGAGGGCTGCGTGGTGGAGTTT 4812  
* * * * * * * * * * * * * * * *  
230 5292 CGAAAGCACCGGTCCATGCAAAGAGTTCCGACCCCTGGACGGCCTGCAAGG 5341  
WO 9 4813 GCGGCTCAC-----ACTTACAAAGTCCGAAACTCAATCGAC-TGCGAGC 4855  
* * * * * * * * * * * * * * * *  
230 5342 AGGACGAGAACGGAGTCGGCATCCAAAGCCGTTATGTGCGCCGCGCAGAGAA 5391  
WO 9 4856 TCAGTGAGCT---GCAGGCTTGCAA-GCC---GAGCGCCGCGCCAGAG 4896  
*** * * * * * * * * * * * * * * *  
230 5392 GACATCATCGAATCCAGAATTTGCACTGTACCGGATGACTGCGGAGAATG 5441  
WO 9 4897 GCGGAGGGCAAGTGCGCTGCTTGGAGCCCTGGAAGATCTGCAGGGA--C 4944  
* * * * * * * * * * * * * * * *
```

25/36

FIGURE 12 (CONT.)

230 5442 GACCCCTGGTCAACTTGCACIAACGGCAGCCAGGCCAGAAACAAACGCT 5491
 WO 9 4945 GGCATGCAGACTCGCGACTGCAAAAGCCTGGGTGTTTCAGGAGTCC-CGCC 4993
 * * * * * * * * * * * * * * * * * *

230 5492 TCTGCACCAACGTTAGGGAAGTCCGTCTCTGCGGAGCTGACATTCCAGTT 5541
 WO 9 4994 CATGCTCAGCTGAAGGAGAGACCGATTCTTTCGGAGCCCTTGGACCC-TT 5042
 *** * * * * * * * * * * * * * * * * *

230 5542 ACAGACGGATGCACGTGGAGCGAGTGGACTTCTTGCAGTCTAGTCAATGA 5591
 WO 9 5043 CGAGCCGGCAGCTTGCAGGGCTGGGAGATGGTCACGA---GGACGGGG 5089
 ** *** ** * * * * * * * * * * * *

230 5592 GGAGGGCGGCTACTTCCGCAAGCGCACATCCTCTGACTGCAACATGAATG 5641
 WO 9 5090 AGTGCACCGGTGCT-CAGCAGAAGGAAACC---AGACTGTGCAATCC-TG 5134
 * * * * * * * * * * * * * * * * *

230 5642 AAGTGCAGGCCTGCTCTCCAGCAGCAGCACAAACCGCAGACAGCGAAACA 5691
 WO 9 5135 AGGGCAATGACAACTGCAACAACCTGGGGTGCTTGGACAGAGTGCTCGCTA 5184
 * * * * * * * * * * * * * * * * *

230 5692 GAAGGCACCTGCTCTGCAATGGAACCCCTGGACGGAGTGCTCGAAGCGCCA 5741
 WO 9 5185 ATTGTGGCGGGCTCTGCCCTGCGGTCTCGCGAGSAGTCCACTTGCGGCTA 5234
 * * * * * * * * * * * * * * * * *

230 5742 CCAGACACGCAAGTGTGCCACAATGGAAGCAGAAGAATCGCGCACTTGGC 5791
 WO 9 5235 TGTGGA--GTTAGAGGAGTGCAGTGGCAGCAGCAGCAGCGCGAOCAGAC 5282
 + * * * * * * * * * * * * * * * *

230 5792 GAGAGACTCCAGAGAACTGCGGAGAAATTCGGCCCCCTTCGAACCCGCAAAC 5841
 WO 9 5283 CGTCCACTGCGGC-AGCTGGTTCGGAGT---GCTCCATGAGAAAAACGGAG 5328
 *** * * * * * * * * * * * * * * *

230 5842 TGCACGGCCGGCCAAATGGTCAACAGGACGCGCACCTGCGGAGAAACCGA 5891
 WO 9 5329 CGCACCTGTGATGTCTCTCTGACGGATCCACACACAGCGTCACTGAAGT 5378
 **** * . * * * * * * * * * *

230 5892 GCAGAAGGAACCAAACTGTGCGAGCTCAGCTCCACCGAAGAGGAAAC 5941
 WO 9 5379 GCTCACCTGCGACGACGTGCTGCTGACTCTTTCGGGTGAATTTGGCGAGT 5428
 ** * * * * * * * * * * * * * *

230 5942 AATGCGGTCAAGTGGGGCCCATGGAGCGAATGCAACATCCACCTGGGCTCA 5991
 WO 9 5429 GGTCCGAAT-GTAGCGCTGACGGCTTGCACCTCGAGGTCCCTGTCAGGCTG 5477
 * ** * * * * * * * * * * * *

230 5992 GAGGACAATGTGCGTGTTCGTGAGG-ACACCGCTTTCGGCGGTGACGGAGT 6040
 WO 9 5478 COCAGACGTAAGTGAAGTGATGACTTGGCGCAGCGAAACTGCCCCGGCTT 5527
 * * * * * * * * * * * * * *

230 6041 ACGAGGAGTGCAGCAAGC'-CGGCGAACAACGCCCTTTGTCTGCACACCTTG 6089
 WO 9 5528 TCGGCGAGTGGAGCGAGTGGCGCAGCCAGAGGACGGCCCTACGGTGGCGT 5577
 ** ***** ** * * * * * * * *

230 6090 GAGTGAATGCTCGGACAAGAAGGAGCGGAGAAAGTGCACCATCCGCAAAA 6139
 WO 9 5578 CAGCGAAGCAACTGCGAAGAGGGATCCGGCTGCATTTCG---TCCGAGACA 5625
 ** *** * * * * * * * * * * * *

26/36

FIGURE 12 (CONT.)

230 6140 ACGGTCT-TGTTTCAGACACGTCAAGAATTCAGAACATGCAGTGTAGACAT 6188
WO 9 5626 GAAGCCTGTGTTAACACTGAGCTCCACCCCATCCCATTTGCCAGTTCTCTGG 5675
* ** ***** * ** * * ** *** **

230 6189 CGCCACAACCTTGCGGCGATTTCGGCGCA--TGGTCTGAATGCCAACGCTGA 6236
WO 9 5676 CGCGCGCGAGGGCAGCGAGAAACGGCGAGGGTGGCCAAACCGGAGAGGAGG 5725
** * ** ***** ***** *** * * * * *

230 6237 GGGCTTGCATCAGCGCAGTCT-CGAGAAATGCCCGGACGTCTATCGAGGTC 6285
WO 9 5726 GAACGGAGGGAGGCGCAGGCGGTGCTGGAGGATCCGGTGGTGTCTGAGGA- 5774
* * ***** * * * * *** * ****

230 6286 GCAACTTGCGGCAGTGAGGATTGCCCGCCATTTCGGCGAGTGGACTGAATG 6335
WO 9 5775 GCTGCC--CGGAGAAGAGGGGTGGCGCAGGTGCCGGCGGAGAAGGAGGCTC 5822
** * *** ***** * ** * **** * *

230 6336 CGGCGTTCCAGAGGAGGGCATGCGTTCTCGCCAAACGCATTGACTGCGTTG 6385
WO 9 5823 TGGCGTAATGCTGAGGAGCTGC---CGGAGAAGGGGGTGTCTGGOG--- 5866
***** * * ***** *** * * * * ** ***

230 6386 AATCTGCAGCCTGCCAGTGCCACAGAAGTGGAGAGCTGCTTCGACACCGAA 6435
WO 9 5867 AAGCTGGAGGCT-CTGGCG-GTAGTGTGAGGAGCTGC-----CCGGA 5907
** *** ** * * * * ** ** ***** *** *

230 6436 TTGCACCCCATTTCCAGCCCCCGGTACGGAAACAGGCGAAGGAGAGGGAGA 6485
WO 9 5908 GAAGAGGGCGGCGCAGGTGCCGGCGGAGGAGGAGGCTCTGGCGGTAGTGC 5957
* * *** ***** * * ***** ** * *

230 6486 GACCGAGACAGGCGAAGGCGAAACTGGTGAAGCAGGTGGCGAGGAAGGCG 6535
WO 9 5958 TGAGGAGCTGCCTGGAGAAGAGGGCGCGCAGGTGCCCGCGGAGAAGGAG 6007
*** * ** ** ** * * * * ***** *

230 6536 AGCAACAGGAGAAGGCGAAGTGCAGCCCCCAGAAGAAGAGCTTCTTGGG 6585
WO 9 6008 GCTCTGGCGGCAATGCTGAGGAGCTGCCCGGAGAAGAGGGCGGCGCAGGT 6057
** * * ** * ** ***** ***** * **

230 6586 GAGAGTGTAACTGAGCCTTAG--GAGAAGCCTGAGGAGGAGCTACCTGA 6632
WO 9 6058 GCTGGAGGAGCCGAAGGCGAGACAGGGAACCTGGCGGCGAAGAGGGTGG 6107
* * * * * ** *** * **** ***** * **

230 6633 GGAGGAGGTTACTGAGCCTGAGGAGAAGCCTGAGGAGGGTGTGACTCAGC 6682
WO 9 6108 CGCAGGCGGCGCTGGTGAGGGTGTCTGGCGGTGAAGGTGGTGAAGTCCAGC 6157
* * * *** * * *** * ***** * ****

230 6683 CTGAGGAGACACCTGAGCAGCCTGTGTGAGGGTACCGAAGAAGAGGGCAAG 6732
WO 9 6158 CTGGAGAGGGAGAAGCGGCGAGTGAAGGAGGCGAGCAAGTCCCGGAACC 6207
*** *** * * * * * ** * ** *** ** *

230 6733 CAGGAG--TCTGAGGCTG---CCCCGAAACT-----CTTGGCGTCCA 6770
WO 9 6208 CCTGAGACACCCGAACCGGAACACCTGAAGCTGAGAGACCTGAAGAGCA 6257
* *** * ** * * * * * **** * **

230 6771 GCCAAAACCAAGAGGA---GGGTACGAACGCCCCAGAACCCGAAGAGGAGG 6817
WO 9 6258 ACCCTGACCGGAACCTCCAGCAGAGGAGCCACCGAAGGCGGTGCAGAAG 6307
** * ** * * * * * **** ** * ** *

230 6818 AGGAGAAGAAGCAAGAAGGCGGCGGCTTCCCAACAGCTGCAGTGGCAGGA 6867

27/36

FIGURE 12 (CONT.)

WO 9 6308 AACAGGAGAAGGAGGAGGSCAGCGGCTTCCCCACGGCAGCTGTTGCCGGA 6357
* *** ***** ** *** ***** ** ** ** **

230 6868 GGTTGTTGGTGGTGTGTTGCTCATAGCTGCTGTAGGTGGTGGTGTTCAGC 6917
WO 9 6358 GGTGTAGGTGGTGTACTACTGCTGGCAGCAGTGGGTGGTGGCGTTGCCGC 6407
***** ***** * ** * ** ** ***** ***** **

230 6918 CTTCACTAGCGGCGGAGGTGGCGCTGGCGCACAGGAGGCAGAACAGGTGG 6967
WO 9 6408 GTACTCCGGTGGTGGTGGAGGTGGCGGTGCCGAGGAGGCTGAGCAAGTTG 6457
* * * * ** ** ** * ** ** ***** ** ** ** *

230 6968 AGTTGGAAGGAGAAGATACCGGAGCAGCAACTGCCGAGACAOTGGAAGCC 7017
WO 9 6458 AGTTTGAAGGTGAAGAGTCCGGTGGTGGCTCTGCCGAAACAOTGAGGCT 6507
***** ***** ***** * ** * ** ***** ***** **

230 7018 GATACAGTTATCGACATCACAGACGAAGACGACTACTGGGCCGACAGCGG 7067
WO 9 6508 GATACTGTGATTGACATCACTGACGAAGACGACTACTGGGCCAGACAGTGG 6557
***** ** ** ***** ***** ***** ***** **

230 7068 CGACATTCAG 7077
WO 9 6558 TGACATCCAG 6567
***** ***

FIGURE 13A

28/36

5' GTG GTG ATT GAA TCT GCT CCA GCC AAG ATG GCT CAC CCT CCT GTG GTG ATT GAG TCT GCT Val Val Ile Glu Ser Ala Pro Ala Lys Met Ala His Pro Pro Val Val Ile Glu Ser Ala	10 30 50
CCG GTC GAG GTG GTC CAT CCT CCT ATG GTG ATT GAA TCT GCT CCA CCC AAG ATG GCT CAA Pro Val Glu Val Val His Pro Pro Met Val Ile Glu Ser Ala Pro Pro Lys Met Ala Gln	70 90 110
CCT CCG ATG GTG ATT GAG TCT GCT CCA CCC AAG ATG GCT CAA CCT ATG GTG ATT GAG Pro Pro Met Val Ile Glu Ser Ala Pro Pro Lys Met Ala Gln Pro Pro Met Val Ile Glu	130 150 170
TCG GCT CCC GTC GAG GTG GTC CAT CCT CCT ATG GTG ATG GAA GCC GCT CCC ACC GTG AAG Ser Ala Pro Val Glu Val Val His Pro Pro Met Val Met Glu Ala Ala Pro Thr Val Lys	190 210 230
GGA AGA TAC CTC GCT GCT GAG GAT GAG GTG GAA GAG CAG TTT GAA TCG AAC AG 3' Gly Arg Tyr Leu Ala Ala Glu Asp Glu Val Glu Glu Phe Glu Ser Asn	

Nucleic acid sequence of the first 293 nucleotides of clone pEM 250/14.
Note the presence of a 14 amino acid repetitive sequence in the single translated open reading frame.

FIGURE 13B

10 30 50
 5' C CTG CAG GTT GTA CTA AGA GCG CTT TAT GAC TAT CGG GAG CTC AAA TGC GGC TCA GCA TGC
 Leu Gln Val Val Leu Arg Ala Leu Tyr Asp Tyr Arg Glu Leu Lys Cys Gly Ser Ala Cys

70 90 110
 CGG AAC GTG GGC ATT TTG GTA CAC GGA GGT ATC ACC TCG AGC GAA TGG GCG GGG GTC TTT
 Arg Asn Val Gly Ile Leu Val Hys Gly Gly Ile Thr Ser Ser Glu Trp Ala Gly Val Phe

130 150 170
 CCG CAA ACA AGC GTT CCA CCA AAA CCT AAG GTG GAA AAC TGT TCA GTT GCA TTT AAT TAC
 Pro Gln Thr Ser Val Pro Pro Lys Pro Lys Val Glu Asn Cys Ser Val Ala Phe Asn Tyr

190
 GCT TTT GTA AAT ACC 3'
 Ala Phe Val Asn Thr

Nucleic Acid sequence of the last 196 nucleotides of clone PEM 250/14.
 The single open reading frame is translated into the amino acid sequence shown below the
 nucleotide sequence. A potential N-linked glycosylation site (Asn-Cys-Ser) is
 underlined.

30/36

FIGURE 14A

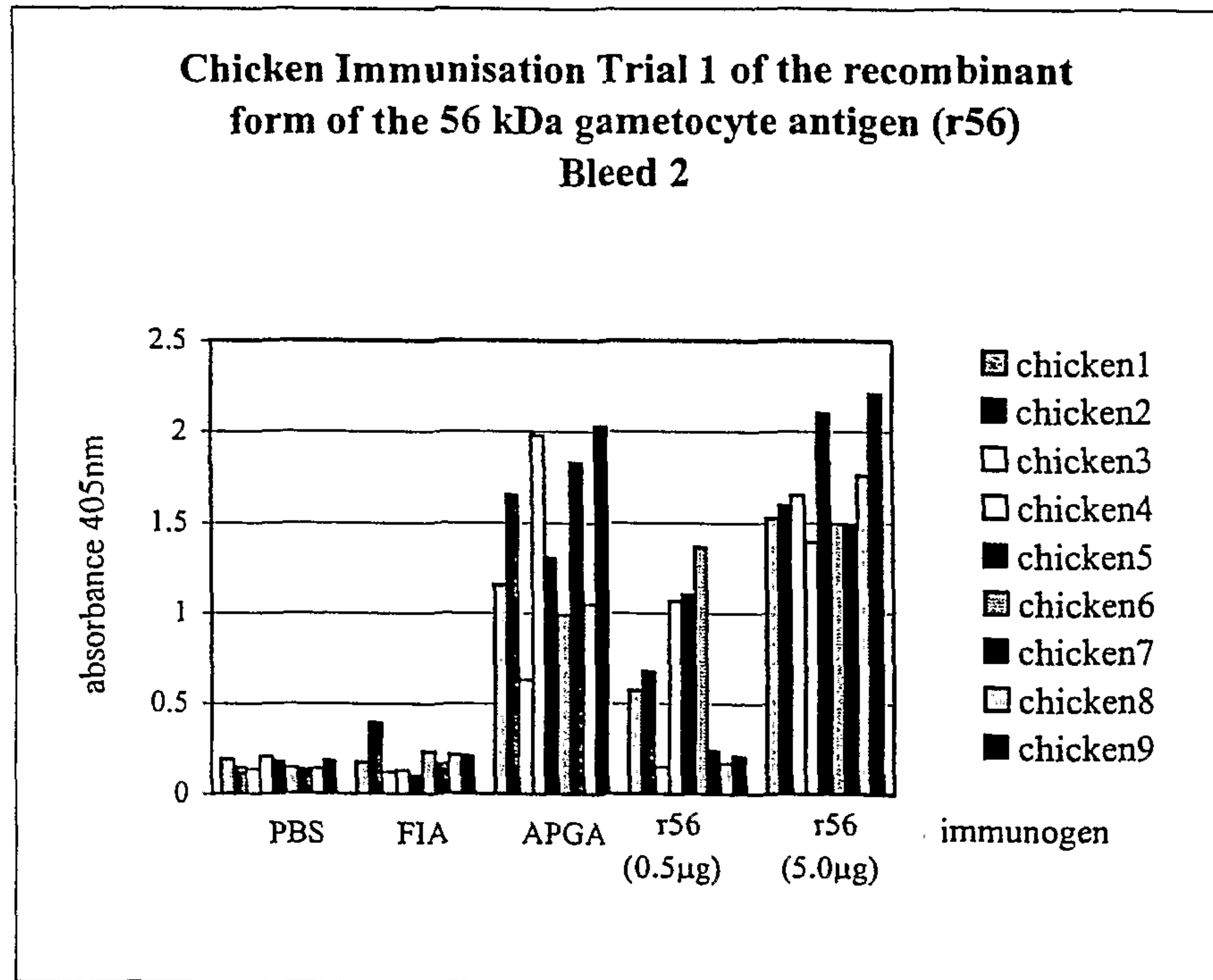
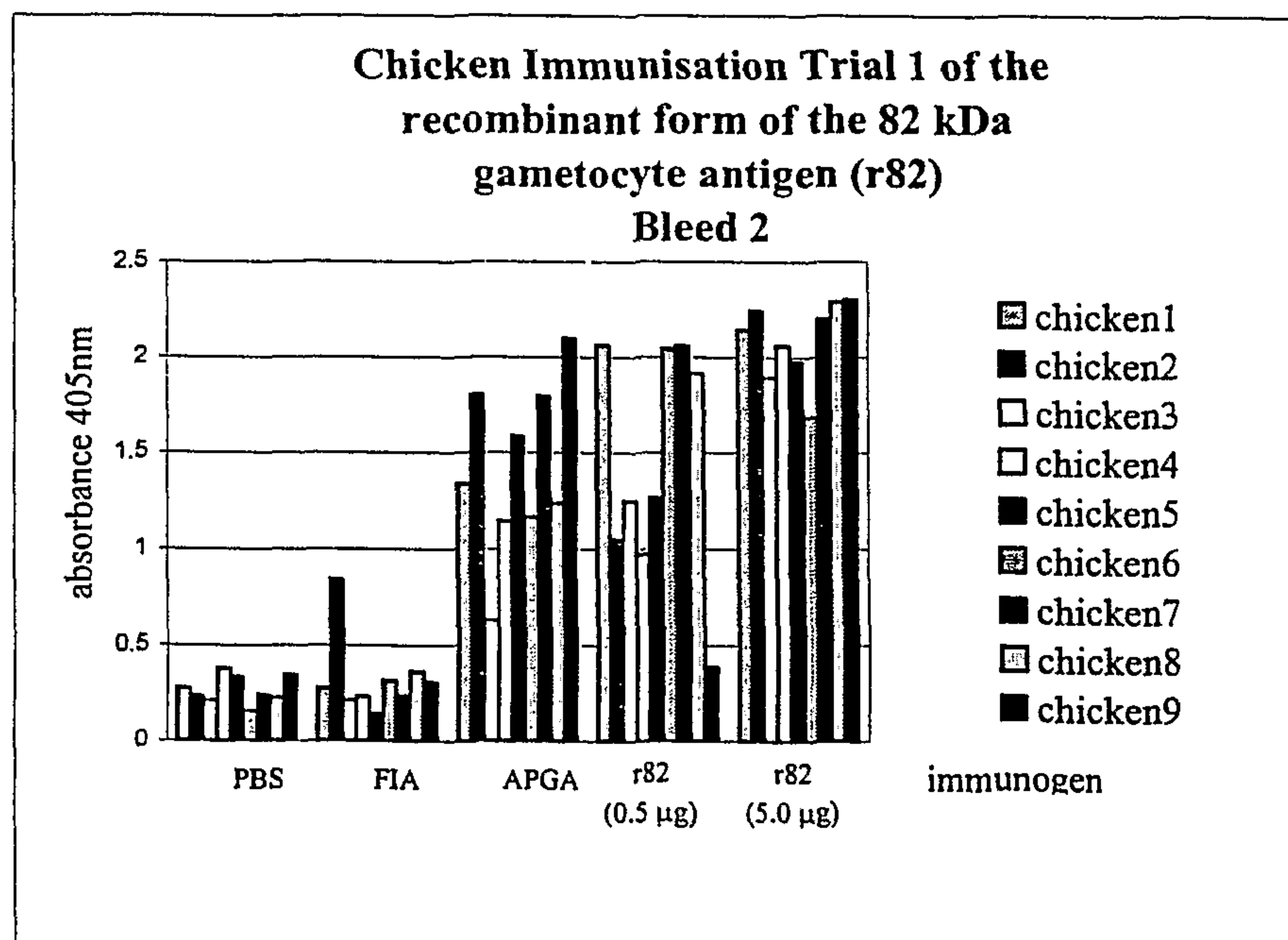


FIGURE 14B



31/36

FIGURE 15

CGAATTGCACCCCATTCAGCCCCCGGTACGGAAACAGGCGAAGGAGAGGGAGAGACCGAGACAGGCGAAGGCGAAACTGGTGAAG 6750
E L H P I P A P G T E T G E G E G E T E T G E G E T G E 2173

CAGGTGGCGAGGAAGGCGAGCAAACAGGAGAAGGCGAAGTGCAGCCCCAGAAGAAGAGCTTCCTGGGGAGAGTGTAAGTGAAGCCTGAGG 6840
A G G E E G E Q T G E G E V Q P P E E E L P G E S V T E P E 2203

AGAAGCCTGAGGAGGAGCTACCTGAGGAGGAGGTTACTGAGCCTGAGGAGAAGCCTGAGGAGGGTGTGACTCAGCCTGAGGAGACACCTG 6930
E K P E E E L P E E E V T E P E E K P E E G V T Q P E E T P 2233

AGCAGCCTGTTGAGGGTACCGAAGAAGAGGGCAAGCAGGAGTCTGAGGCTGCCCCGAAACTCCTGCCGTCCAGCCAAAACCAGAGGAGG 7020
E Q P V E G T E E E G K Q E S E A A P E T P A V Q P K P E E 2263

GTCACGAACGCCCAGAACCCGAAGAGGAGGAGGAGAAGAAGGAAGAAGGCGGCGCTTCCCAACAGCTGCAGTGGCAGGAGGTGTTGGTG 7110
G H E R P E P E E E E E K K E E G G G F P T A A V A G G V G 2293

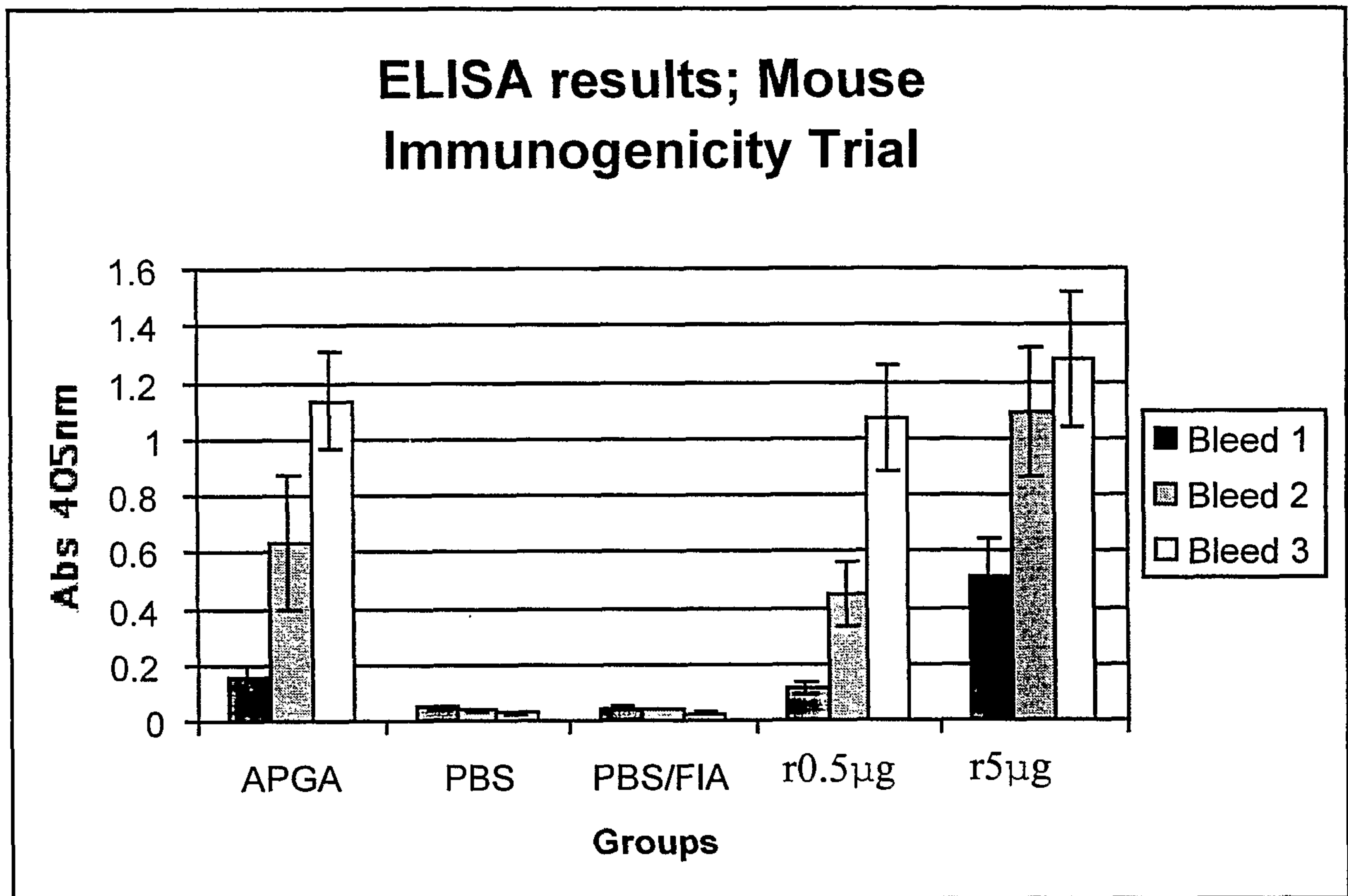
GTGTGTTGCTCATAGCTGCTGTAGGTGGTGGTGTGTCAGCCTTCACTAGCGGCGGAGGTGGCGCTGGCGCACAGGAGGCAGAACAGGTCCG 7200
G V L L I A A V G G G V A A F T S G G G G A G A Q E A E Q V 2323

AGTTCGAAGGAGAAGATACCGGAGCAGCAACTGCCGAGACACCTGAAGCCGATACAGTTATCGACATCACAGACGAAGACGACTACTGGG 7290
E F E G E D T G A A T A E T P E A D T V I D I T D E D D Y W 2353

CCGACAGCGGCGACATTCAG
A D S G D I Q

32/36

FIGURE 16



33/36

FIGURE 17

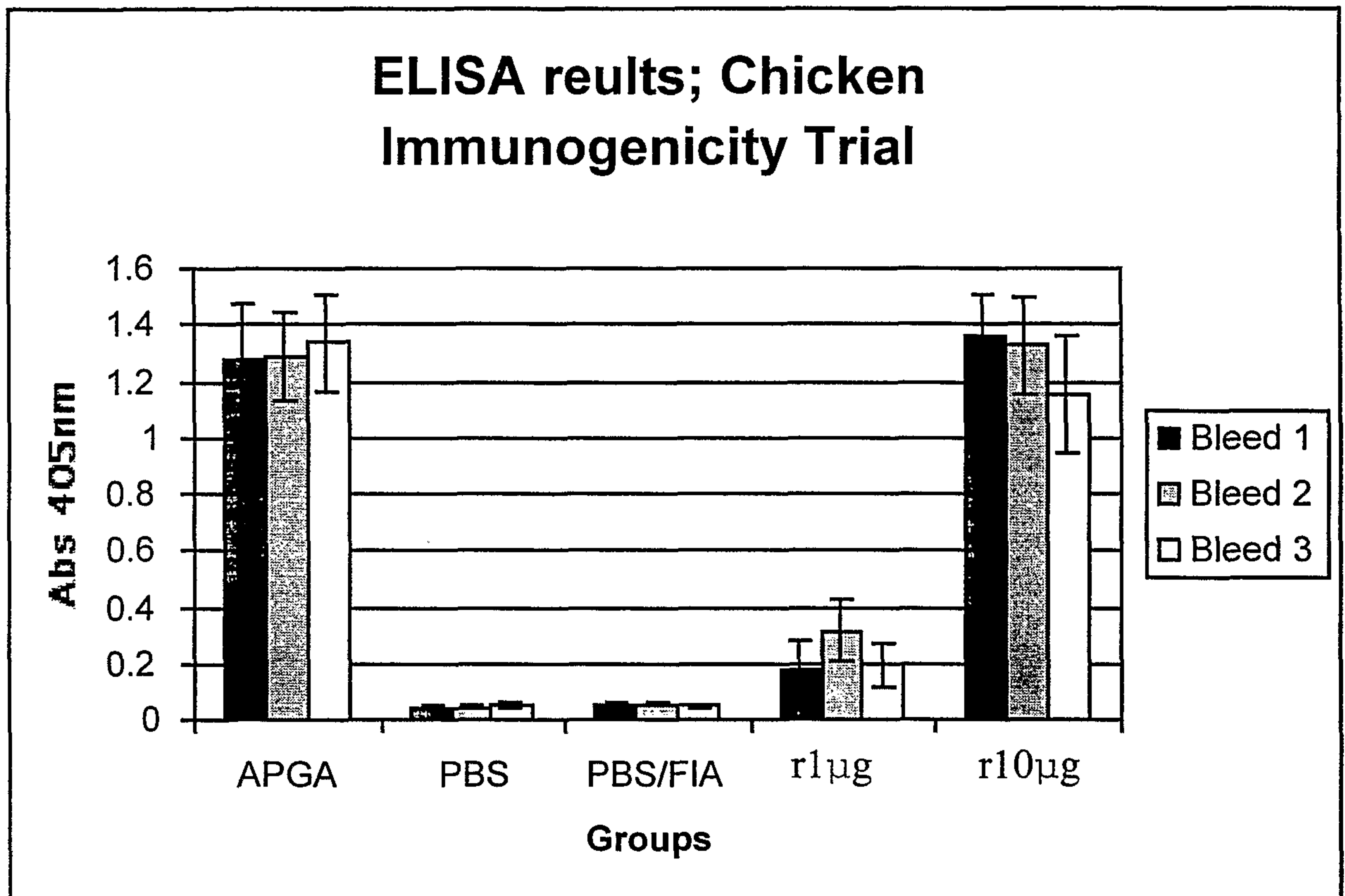
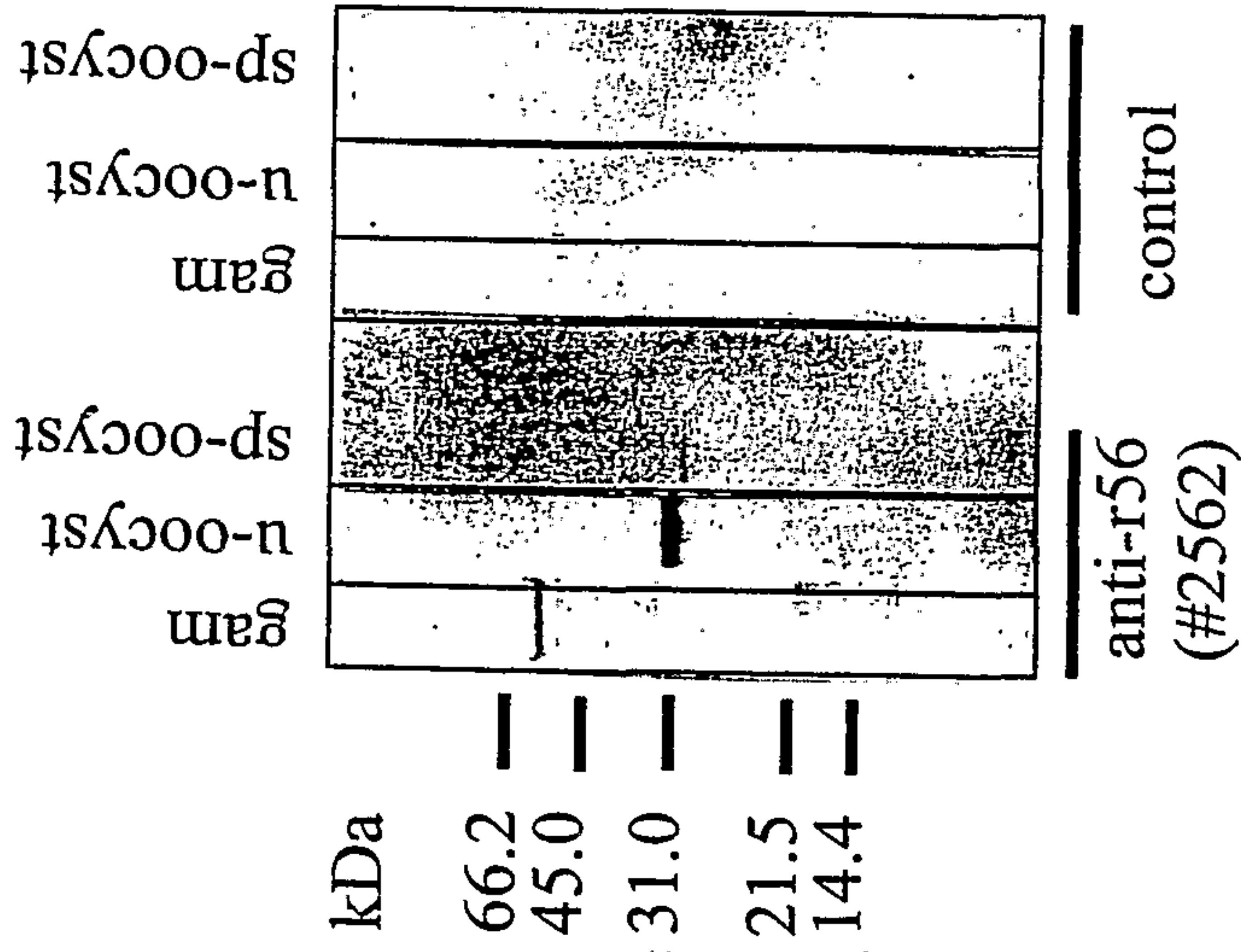
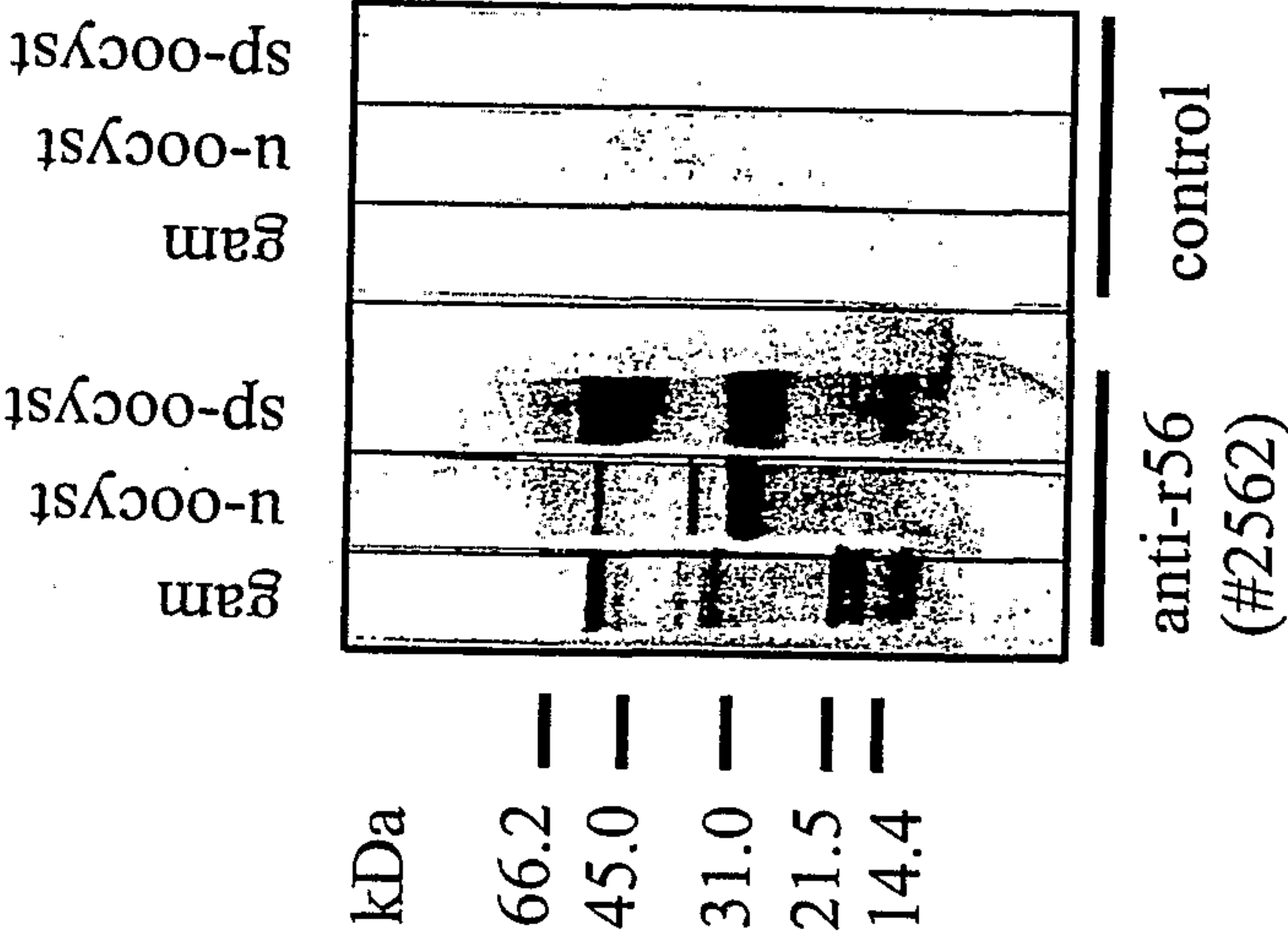


FIGURE 18A



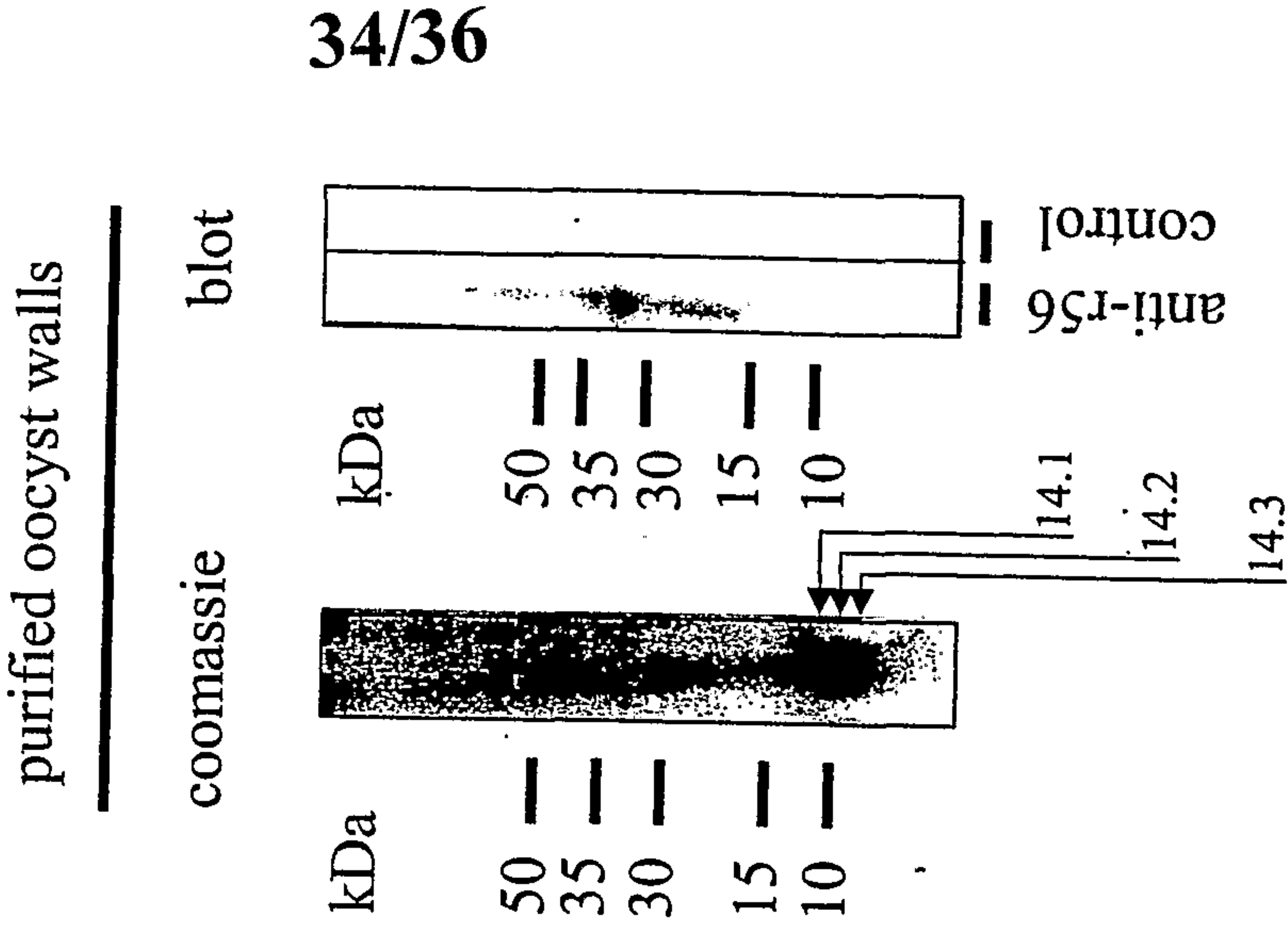
equal parasite equivalents

FIGURE 18B



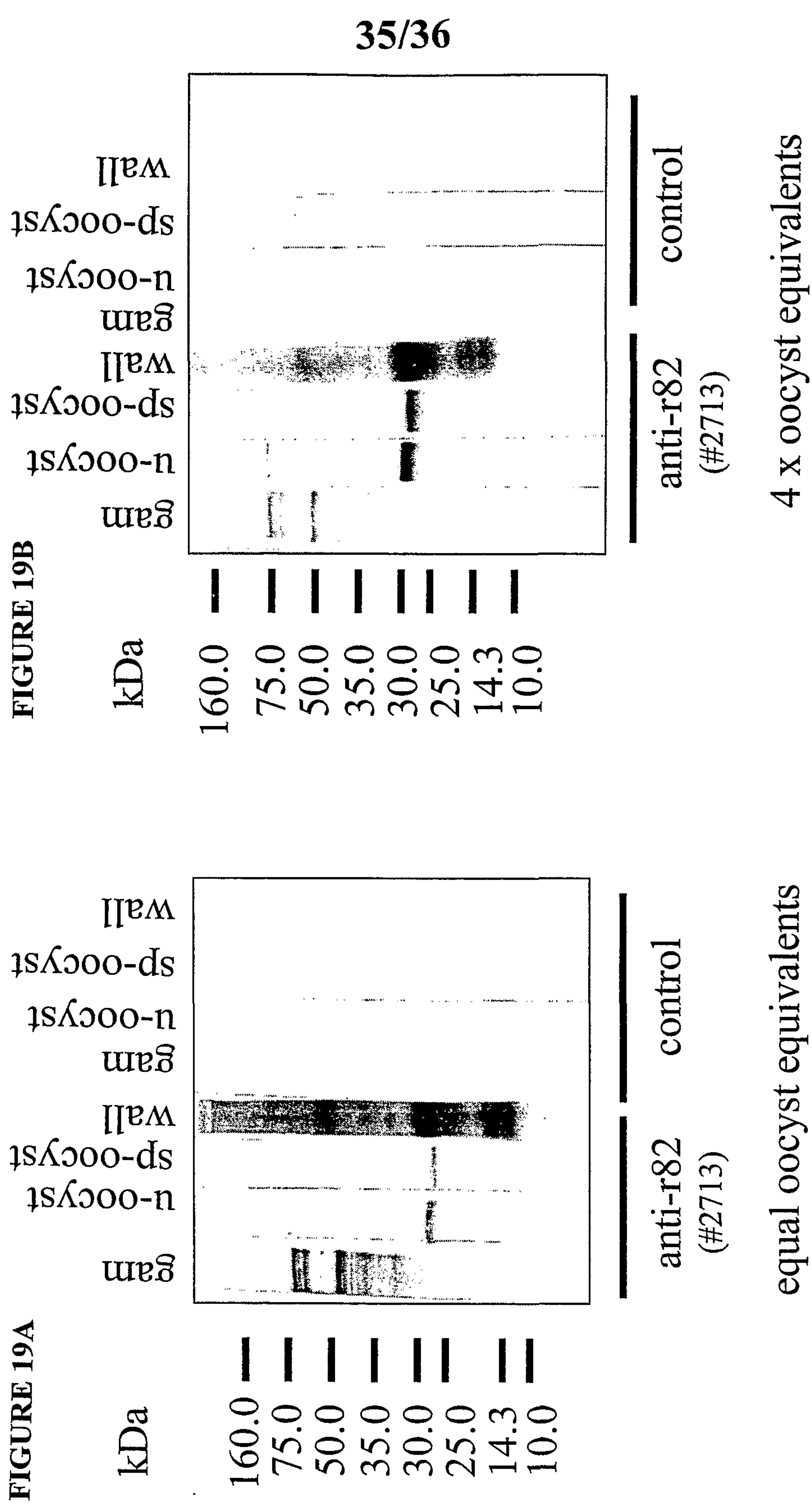
4 x parasite equivalents

FIGURE 18C



34/36

Figure 19



36/36

FIGURE 20A

	21.....35
56 kDa gametocyte protein (N-terminus)	VPSTTPVENQHVHPYS
~30 kDa oocyst wall protein	VPSTTPVENQH-HPYS
<i>(this wall protein represents the low abundance protein recognized by anti-r56 kDa antibody)</i>	

	240.....254
56 kDa gametocyte protein (domain 1)	MGRKGRSFYYGGYPS
14.1 oocyst wall protein	YGRKGRSFYYGGYPS
	G

FIGURE 20B

	418.....431
82 kDa gametocyte protein (domain 2)	YPSYSWSYPAYTRV
14.2 oocyst wall protein	YPSYS-SYPAYTRV

	282.....296
82 kDa gametocyte protein (domain 1)	GKRMYSTGYGYGYGP
14.3 kDa oocyst wall protein	-KRMYSTGY-G---P

FIGURE 20C

30 kDa oocyst wall protein	-SFSPVAPQELF-- (L)
<i>(this protein represents the 30 kDa protein detected by coomassie blue staining of purified oocyst wall fragments)</i>	