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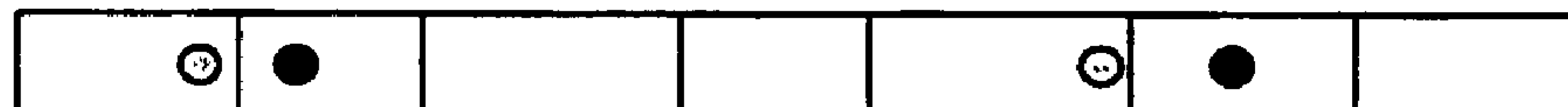
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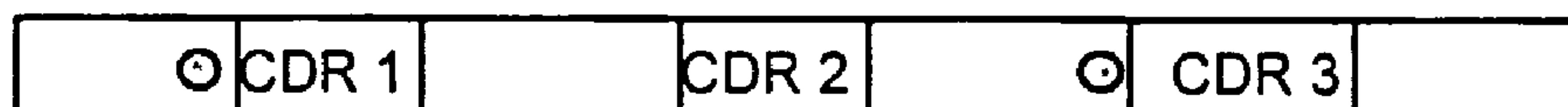
IgNAR type I



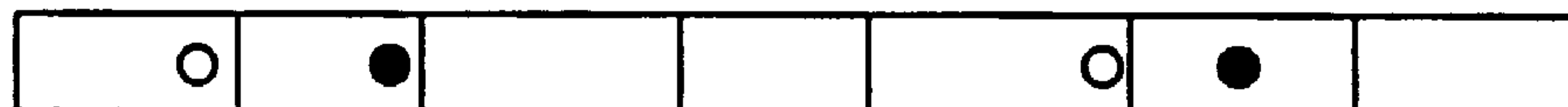
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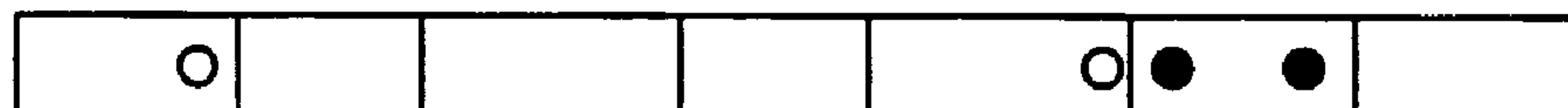
Human VH



Camel VHH



Cattle VH



(57) Abrégé/Abstract:

A process for the production of an antigen specific antigen binding domain using a transformed host containing an expressible DNA sequence encoding the antigen specific antigen binding domain, wherein the antigen specific antigen binding domain is derived from a variable region of the immunoglobulin isotype NAR found in fish.



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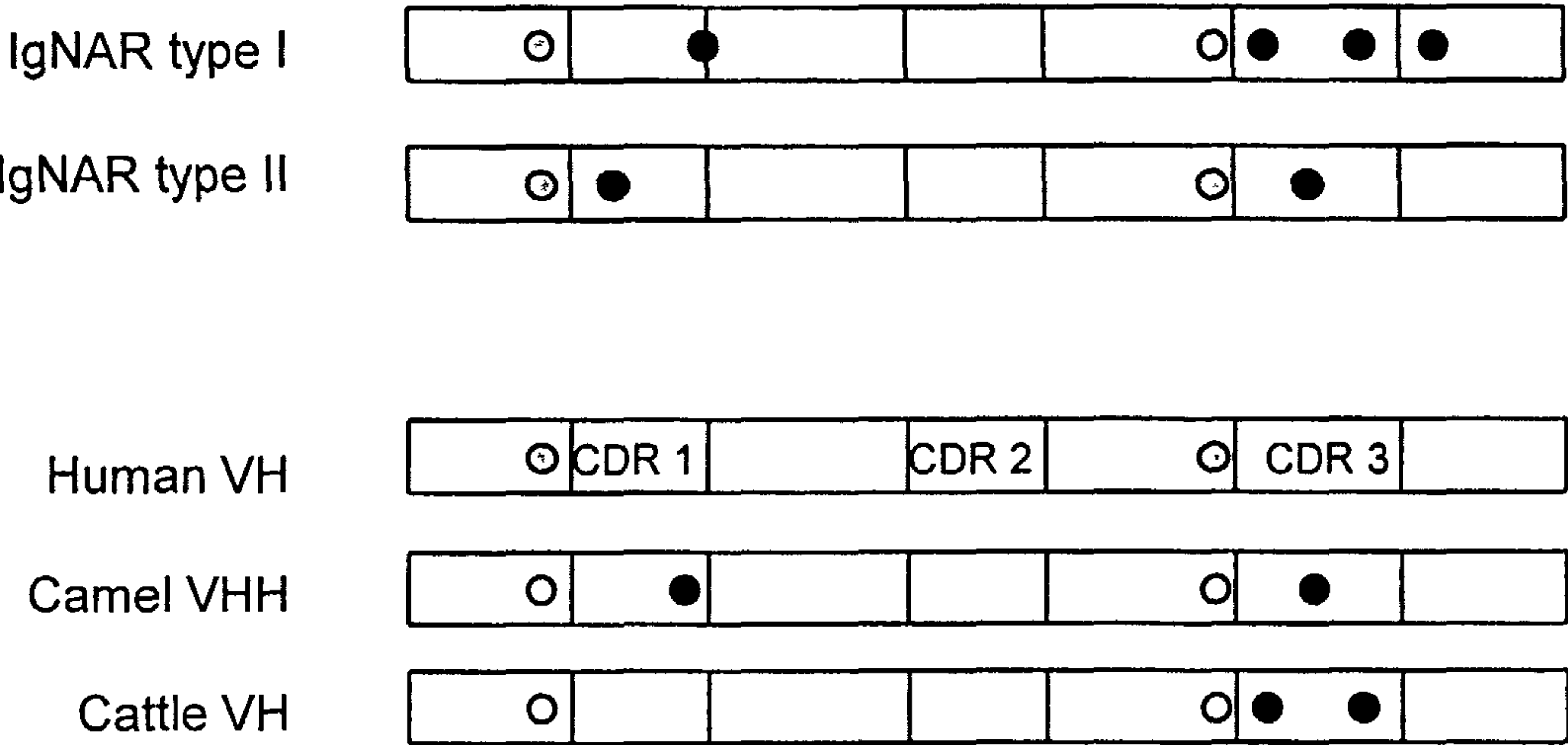
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(54) Title: ANTIGEN BINDING DOMAINS FROM FISH



(57) Abstract: A process for the production of an antigen specific antigen binding domain using a transformed host containing an expressible DNA sequence encoding the antigen specific antigen binding domain, wherein the antigen specific antigen binding domain is derived from a variable region of the immunoglobulin isotype NAR found in fish.

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Antigen Binding Domains

The present invention relates to the production of antigen specific antigen binding domains (single domain antibodies) from fish, where the term fish encompasses both cartilaginous (subclass Elasmobranchii) and bony fish(class Osteichthyes). By antigen specific antigen binding domains we mean the variable region of a Novel Antigen Receptor (NAR).

10 Antibodies, especially monoclonal antibodies, are useful in, among other things, molecular diagnostics and therapeutics because of their high affinity binding and specificity. However, although it is now relatively simple to produce monoclonal antibodies using animal models the production of 15 human monoclonal antibodies remains difficult. As will be appreciated, when monoclonal antibodies from non-human models are introduced into humans, the body mounts an immune response because the monoclonal antibody is foreign to the human system.

20

Recently, it has been appreciated that the activity of the monoclonal antibody can be retained while reducing the rejection thereof in humans by producing single domain antibodies (sda) from the variable chain of the relevant 25 antibody. European patent number 0368684 discloses such single domain antibodies and methods for the production thereof in mice.

Single domain antibodies are also important as they can 30 penetrate tissues taking with them any linked compounds. In addition, they can bind within cavities on the surface of proteins, for example within enzyme binding sites thus disrupting function.

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Single domain antibodies produced from *Camelidae* have been shown to recognize protein cavities and as such have the ability to inhibit enzymes (Lauwereys et al., *EMBO* 17 pp3512-5 3520 1998).

Although the small size of single domain antibodies produced from *Camelidae* has allowed the recognition of protein cavities and inhibition of enzyme activity, the range of possible
10 targets may still be relatively low, since many protein cavities may still be too small to be penetrable by single domain antibodies derived from *Camelidae*.

WIPO publication application No. WO1994/025591 and European
15 patent application number 0954978 relate to the production of single domain antibodies from *Camelidae* heavy chain antibodies. Single domain antibodies produced from *Camelidae* heavy chain antibodies are more stable than mouse single domain antibodies and can be produced in larger quantities.
20 However, as will be appreciated if, even the smaller members of the *Camelidae* family, for example llama, are to be kept in humane conditions they require significant areas of land to live upon.

25 An object of the present invention is to provide a process for the production of antigen specific antigen binding domains which seeks to alleviate the above problems.

A further object of the present invention is to provide a
30 composition comprising antigen specific antigen binding domains for the inhibition of protein activity which seeks to alleviate the above problems.

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According to an aspect of the present invention there is provided a process for the production of an antigen specific antigen binding domain using a transformed host containing an expressible DNA sequence encoding the antigen specific antigen
5 binding domain, wherein the antigen specific antigen binding domain is derived from a variable region of the immunoglobulin isotype NAR found in a species of *Elasmobranchii* subclass wherein the specificity of the antigen specific antigen binding domain is determined by an antigen which is introduced
10 into a member of the *Elasmobranchii* subclass.

It has been found that antigen specific antigen binding domains produced from the variable region of NAR found in fish are as stable as single domain antibodies produced from
15 members of the *Camelidae* family.

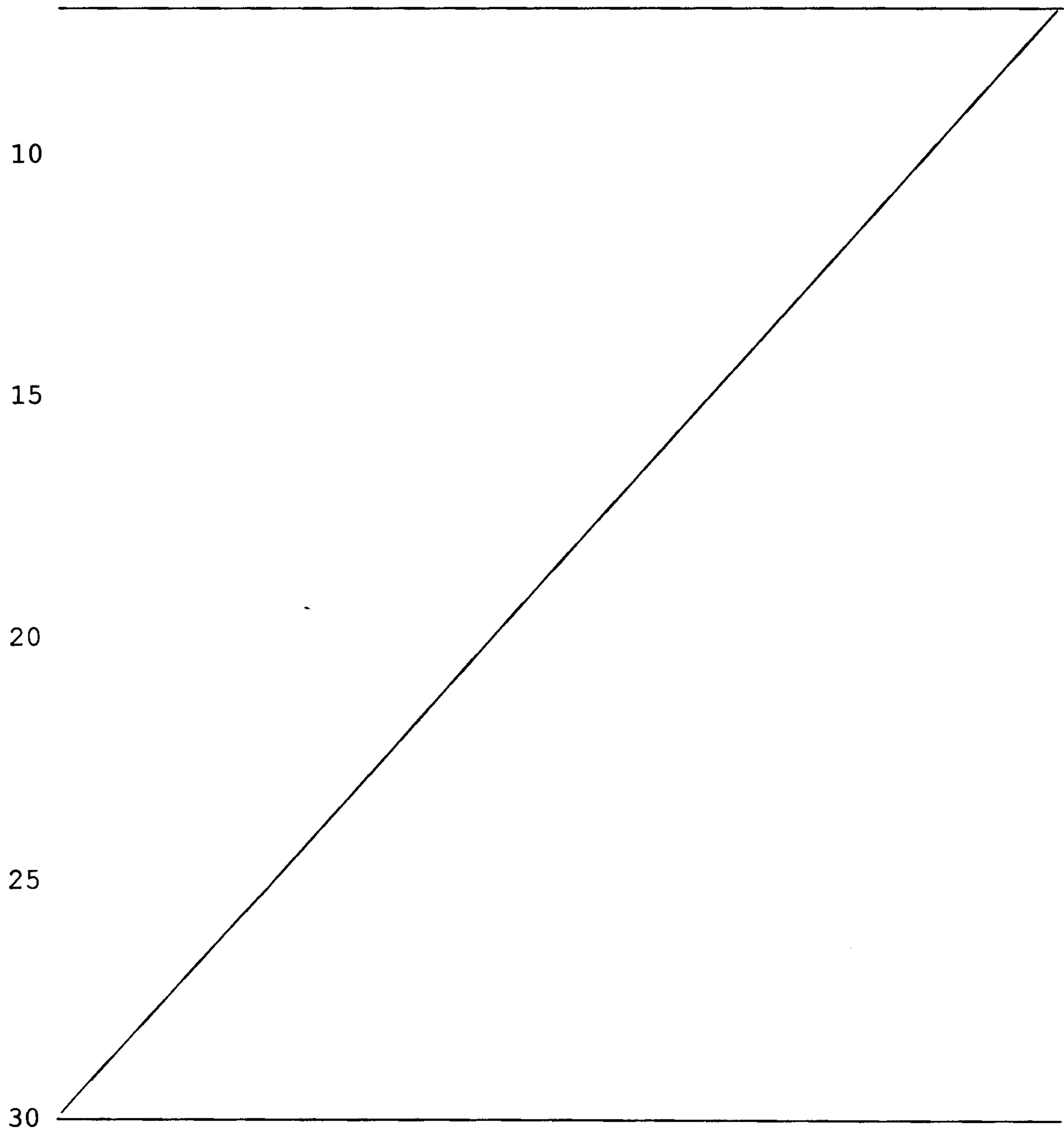
Further many more fish can be kept per unit area than members of the *Camelidae* family.

20 The immunoglobulin isotype now known as NAR (Novel Antigen Receptor), was discovered in the serum of the nurse shark (*Ginglymostoma cirratum*) as a homodimeric heavy chain complex, naturally lacking light chains (Greenberg et al., *Nature* 374 pp168-173 1995). However, before the present work by the
25 inventors identification of NAR as an antigen binding domain was not fully appreciated neither was its ability to be raised against a specific antigen.

Only mammals (humans, mice, rabbits, sheep, camels, llamas,
30 etc.) and some birds (chickens) were believed to be capable of something approaching a secondary immune response such as affinity maturation, antibody class switching etc. as a response to the presence of foreign antigen. For example,

- 3A -

teleost fish (bony), which are much more advanced evolutionary than sharks, appear to rely solely upon the production of a low affinity, non-specific IgM type response (Watts et al., Aust Vet J 79 pp570-574 2001). A defining characteristic of



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teleost IgM is their low affinity and ability to non-specifically bind multiple antigens. IgM neutralisation is through non-specific multiple binding, resulting mainly in agglutination, etc.. Neutralisation without complement is usually associated with specific, high affinity binding and had not until this invention been seen in fish species. The antigen specific antigen binding domains of the present invention have been shown to neutralise activity of an enzyme immunogen directly without calling upon other components of the immune system.

The NAR variable (V) region conforms to the model of typical Ig superfamily domains with the predicted canonical, intradomain disulphide bond. However, whilst camelid VHH regions have up to 75% sequence identity with other mammalian VH regions, the identity between NAR V and conventional VH domains is as low as 25% (Roux et al., *Proceedings of the National Academy of Sciences. USA* 95 pp11804-11809 1998).

Due to this low identity and lack of NAR sequences in the Kabat database, the amino acids of NAR V regions have previously been numbered sequentially (Roux et al., *Proceedings of the National Academy of Sciences. USA* 95 pp11804-11809 1998). To enable easy comparison of residues in different NAR V molecules, or NAR V region sequences with those of other species during this work, a numbering system was derived for NAR V region based upon that of Kabat et al., (1991) (*Sequences of Proteins of immunological Interest, 5th Edition. National Institutes of Health, Bethesda, USA*). (Note: this numbering system is used in the Figures attached hereto).

- 5 -

Immediately apparent from the alignment is the deletion of a large portion of CDR2 (residues 54-65) giving the NAR V region its characteristically small size (see, for example, Figure 2A).

5

Initial sequence analysis allowed the classification of NAR V domains into two closely related classes (type I or II), both being constructed from one V, three D and one J segment. Type I regions have non-canonical cys residues in Fr2 (C35) and Fr4 (C103), which likely form a domain-stabilising disulphide bond. In longer NAR CDR3 loops additional cysteine residue pairs have been observed and almost certainly form disulphide bridges within the CDR, as is found in some cattle VH domains with an unusually long CDR3.

15

Type II regions are very similar in overall structure to type I but instead have non-canonical cysteine residues located in Fr1 (C29) and CDR3, which are proposed to form a constraining disulphide bond like that observed in camelid VHH domains. The presence of cysteines within each NAR type is shown in schematic form in Figure 1.

Recently, an additional NAR type has been identified as the predominant expressed form in nurse shark pups (Type III) but due to its germline joined state displays no junctional diversity.

In type I and II NAR the DNA encoding the V region is generated by the physical joining of DNA segments which are spatially separate in the genome. This joining process occurs in B-cells and helps generate the diversity of sequence seen for these NAR types. For type III these DNA segments are already physically joined in the DNA of all cells, hence the

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term germline joined.

NAR possesses the cluster type genomic organisation usually observed for Ig receptors in cartilaginous fish, but less than 5 five NAR loci are thought to exist, with only two or three being capable of functional rearrangement and expression. The diversity observed in the primary repertoire is generated through recombination mechanisms and, although extensive (due to the presence of three D segments), is localised to CDR3. 10 On encounter with antigen this repertoire is rapidly expanded by extensive mutation. The pattern of mutation in NAR is unlike that observed in shark IgM, which shows low levels of mutation and poor clustering to CDRs, but rather bears the hallmarks of mammalian-like somatic mutation.

15

It has recently been found that NAR V is similar to VH, VL and TCR V but distinct from all three, hence its "unique domain architecture". The VH name has been used in the past because the constant portion of NAR is a heavy chain but the V region 20 is actually more like VL/TCR V than VH (i.e. groups closer on a phylogenetic tree). NAR V is not like the camel VHH domains which are derived from bona fide heavy chain V regions. The antigen binding domain of the NAR is closer to a VL domain naturally lacking VH rather than the other way round.

25

The sequence alignment of NAR V and camel VHH clearly shows the huge difference in sequence. If NAR V and camel VHH have the same physical structure (which has been implied but not proven) they achieve this using completely different amino 30 acid sequences, and one would not be able to amplify a NAR V region library using camel VHH library primers. In addition, the ways in which the NAR V and camel VHH gene repertoires are created during VDJ joining are different due to the

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organisation of the immunoglobulin genes (Schluter et al Immunol Today 18 pp543-549 1997).

Preferably the transformed host is a prokaryote or a lower
5 eukaryote.

There are many established prokaryote and lower eukaryote hosts. These hosts are known to correctly express foreign proteins.

10

Conveniently the prokaryote host is *Escherichia coli*.

In preferred embodiments the expressible DNA sequence is in the form of a phagemid vector.

15

Phagemid expression has advantages over phage genome expression in that it results in greater genetic stability and the bacterial transformation efficiency is higher thus enabling the construction of potentially larger and more
20 diverse libraries.

To display antibody fragments on phage the gene encoding the variable region of the antibody can be fused to that of a phage surface protein, usually gene III or VIII. Gene III
25 fusion is favoured due to its limited copy number (3-5 copies) on the tip of each phage, minimising possible avidity effects which are undesirable when trying to isolate binders of high affinity. The antibody fragment genes can be cloned directly into the phage genome or fused to gene segments present within
30 phagemid plasmids.

Preferably the species of the *Elasmobranchii* subclass comprises for example, a shark or a dogfish.

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A greater number of smaller members of the *Elasmobranchii* subclass can be kept in tanks which are smaller in unit area than the grazing area required for the same number of members of the *Camelidae* family. As the members of the *Elasmobranchii* subclass are kept in tanks they can easily be caught for extraction of their blood.

Conveniently the shark is a nurse shark, *Ginglymostoma cirratum*.

10

Preferably the antigen specific antigen binding domain has a specific specificity. Accordingly, the antigen specific antigen binding domain can be targeted to a specific antigen(s).

15

Conveniently the antigen specific antigen binding domain is monoclonal. In this connection, the antigen specific antigen binding domain is raised to a single antigen.

20 In preferred embodiments the specificity of the antigen specific antigen binding domain is determined by an antigen which is introduced into the chosen fish.

According to a further aspect of the present invention there is provided a process for the production of an antigen specific antigen binding domain comprising the steps of:

- a) immunising a member of the *Elasmobranchii* subclass with an antigen;
- b) isolating lymphocytes from the member;
- 30 c) isolating RNA from the lymphocytes;
- d) amplifying DNA sequences encoding the antigen specific antigen binding domain by PCR;

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- e) cloning the amplified DNA into a display vector;
- f) transforming a host to produce a library;
- g) selecting the desired clones from the library;
- h) isolating and purifying the antigen specific antigen
5 binding domain from these clones;
- i) cloning the DNA sequences encoding the antigen specific antigen binding domain into an expression vector;
- j) transforming a host to allow expression of the expression vector.

10

Screening of displayed libraries for specific binding sites involves repeated cycles of selection with the desired antigen in the process of biopanning. Generally during selection, the library of phage displayed antigen binding domains is
15 incubated with immobilised antigen, unbound phage are washed out and bound phage eluted. This selected population is expanded by bacterial infection and put through further rounds of selection. As each phage encapsulates the DNA encoding the V region it displays, there is a functional linking of
20 genotype and phenotype, reminiscent of membrane bound immunoglobulin on the surface of B-cells. Such cyclic panning has thus proven able to enrich for clones of high affinity, much like *in vivo* antibody selection.

25 Preferably before step d) the cDNA of the antigen binding domains is generated.

Conveniently restriction enzymes are used to digest the amplified DNA sequences encoding the antigen specific antigen
30 binding domain. The restrictions enzymes can be chosen depending upon, for example, the handle of the primers used in the above process.

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In preferred embodiments the restriction enzymes are *NcoI* and *NotI*.

Conveniently the display vector is any phagemid vector, for
5 example, pHEN2.

Preferably the expression vector is a soluble expression vector such as pIMS100.

10 The above vectors are merely examples of the vectors which can be used. It is common general knowledge to those skilled in the art which vectors can be used.

An antigen specific antigen binding domain produced by the
15 process as defined above is described.

A composition for the inhibition of protein activity comprising antigen specific antigen binding domains derived from a variable region of the immunoglobulin isotype NAR found
20 in fish is also described.

Despite the fact that the NAR V region is 12 kDa which is 20% smaller than any 15 kDa single domain antibody derived from *Camelidae*, it was still possible to alter protein activity
25 therewith. Size is a significant factor in the therapeutic applications of antigen specific antigen binding domains and other single domain antibodies, with therapeutic benefits of increased tissue penetration, better access to protein clefts for neutralisation via steric hindrance and reduced
30 immunogenicity, resulting from the use of antigen specific antigen binding domains of the present invention.

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Antigen specific antigen binding domains derived from NAR therefore have a wider target population than single domain antibodies derived from *Camelidae* by virtue of their smaller size. The potential for immunogenicity is also reduced since
5 in general the smaller the size of a protein the less the immunogenicity.

Furthermore, although NAR sequences have, in work previous to that of the inventors, been identified at the DNA level, there
10 has been no clue from the DNA evidence that a somatically maturable repertoire, capable of selecting high affinity, specific binders could be a characteristic of the NAR response. Hence, it is unexpected to be able to generate an NAR library of antigen binding domains derived from sharks and
15 the selection from this of specific and functional antigen specific antigen binding domains and their corresponding receptor genes. Sequencing of these genes confirms that an atypical (for fish and organisms of this evolutionary lineage) somatically-maturable (showing mutation from the germ line
20 repertoire) response occurs within the NAR repertoire, driven by the immunisation process. This has resulted in the selection of highly specific, high affinity antigen binding domains capable of antigen neutralisation in isolation and not the expected non-specific, low affinity IgM like response
25 typically found in fish and sharks.

Further still, the inventors have been able to isolate NAR antigen specific antigen binding domains and demonstrate for the first time that the NAR V is able to fold and function in
30 isolation from the rest of the molecule (and in a non-shark environment), that the antigen specific antigen binding domain matures from the germ line genes to become specific for antigen (only possible with a library derived from mRNA and

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not DNA) and that the antigen specific antigen binding domain is able to bind specifically to the immunising antigen.

In summary, as described below, the inventors have been able
5 to immunize a shark and derive from this immunization a specific, somatically matured antigen specific antigen binding domain that is of high affinity and specific for the immunogen. In addition, the antigen specific antigen binding domain is able to neutralise the activity of the immunogen
10 directly, without calling upon other components of the immune system. According to previous understandings, this should not have been possible for a primitive species such as sharks.

Conveniently, a composition is provided wherein the antigen
15 specific antigen binding domain is a product of the process as defined above.

Preferably, inhibition of protein activity is in a concentration dependent manner.

20

Preferably, the composition further comprises a pharmaceutical carrier or diluent therefor.

Such pharmaceutical carriers are well known in the art.

25 An antigen specific antigen binding domain produced from a variable region of NAR is also described.

An embodiment of the invention will now be described, by way
30 of illustration only, with reference to the following examples and the accompanying figures.

Figure 1 shows the presence of cysteine amino acid residues

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within each NAR type, and human, cattle and camel variable regions for comparison. Canonical cysteines are shown by $\odot$ and non-canonical cysteines are shown by $\bullet$.

5 Figures 2A, 2B and 2C show the amino acid translations of the sequences obtained in the Examples (SEQ ID. 1 to 51). The sequences are aligned against a typical type I and type II clone sequence (top of each Figure with CDR's highlighted in bold) dashes indicate identity to the type I clone and *
10 indicates an in-frame stop codon.

Figure 3 shows NAR type I and II variable region amino acid sequence alignment (SEQ 1 and 2). Germline sequence is given for type I, whilst that given for type II is typical of those
15 observed from somatically mutated cDNA sequences (Roux *et al.*, *Proceedings of the National Academy of Sciences. USA* 95 pp11804-11809 1998). Sequence identity is indicated by a dash and the CDR's of both sequences are in bold. The numbering above the sequences was generated by comparison of
20 conserved residues (underlined) with those of other species and is used to enable comparison of NAR V region sequences.

Figure 4 shows a variability plot for the 29 immune library sequences identified in the Example (pre-selection and
25 functional). Variability at each position was calculated according to the method of Wu & Kabat (1970) (*Journal of Experimental Medicine* **132** pp211-250). The canonical cysteine residues, C22 and 92, are marked by an asterisk.

30 Figures 5A and B show polyclonal and monoclonal phage ELISA results for selection on Hen egg white lysozyme (HEL) (Figure 5A) and Chicken ovalbumin (Ova) (Figure 5B). Phage numbers were normalised for each pan prior to polyclonal analysis.

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Data presented is a mean of triplicate wells and representative of at least three assays. Monoclonal results are percentages obtained from 96 clones for each pan.

5 Figure 6 shows the DNA (SEQ ID. 53 & 54) and encoded amino acid sequence (SEQ ID. 52) of the α -HEL 5A7 clone. CDRs are highlighted in bold.

Figure 7 shows the DNA (SEQ ID. 56 & 57) and encoded amino
10 acid sequence (SEQ ID. 55) of the α -HEL 4F11 clone. CDRs are highlighted in bold.

Figure 8 shows the amino acid alignment of the two α -HEL clones, 5A7 (SEQ ID. 52) and 4F11 (SEQ ID. 55), with a typical
15 type I clone (SEQ ID. 1). Sequences are numbered according to Figure 3 for ease of comparison, differences between the selected clones are highlighted in underlined and CDR's are highlighted in bold, * conserved residues in all sequences, : conserved substitutions, . semi-conserved substitutions.

20

Figure 9 shows the DNA (SEQ ID. 59 & 60) and encoded amino acid sequence (SEQ ID. 58) of the α -Ova 4H11 clone. CDRs are highlighted in bold.

25 Figure 10 shows the DNA (SEQ ID. 62 & 63) and encoded amino acid sequence (SEQ ID. 61) of the α -Ova 3E4 clone. CDRs are highlighted in bold.

Figure 11 shows amino acid alignment of the two α -Ova clones,
30 4H11 (SEQ ID. 58) and 3E4 (SEQ ID. 61), with a typical type I clone (SEQ ID. 1). Sequences are numbered according to Figure 3 for ease of comparison, differences between the selected clones are underlined and the CDR's are highlighted

- 15 -

in bold. * conserved residues in all sequences, : conserved substitutions, . semi-conserved substitutions.

Figure 12 shows binding analysis of α -HEL clone 5A7. Serial dilutions of crude periplasmic release solution were applied to an ELISA plate coated with each of the test proteins at 10 μ g/ml and blocked with Marvel. Data presented is a mean of triplicate wells and representative of at least three repeat assays.

10

Figure 13 shows binding analysis of α -HEL clone 4F11. Serial dilutions of crude periplasmic release solution were applied to an ELISA plate coated with each of the test proteins at 10 μ g/ml and blocked with Marvel. Data presented is a mean of triplicate wells and representative of at least three repeat assays.

Figure 14 shows binding analysis of α -Ova clone 4H11. Serial dilutions of crude periplasmic release solution were applied to an ELISA plate coated with each of the test proteins at 10 μ g/ml and blocked with Marvel. Data presented is a mean of triplicate wells and representative of at least three repeat assays.

Figure 15 shows a comparison of the stability of the anti-HEL clones 5A7 and 4F11 to irreversible thermal denaturation. Data presented is a mean of triplicate wells and representative of at least three repeat assays.

Figure 16 shows a lysozyme enzymatic inhibition assay. Purified HEL-5A7 NAR V region protein at a final concentration of 2500 nM (filled circle), 250 nM (open triangle) or 25 nM (filled square) were pre-incubated with HEL prior to the

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introduction of *M. lysodeikticus* bacterium. The control wells (open diamond) contained buffer in place of HEL-5A7 protein. The data presented is an average of 3 replicates and a typical data set from three repeat experiments.

5

Example

Bacterial strains

The electroporation-competent strain *E. coli* XL1-Blue {*recA1*
10 *endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F' *proAB lacI^q*
ZΔM15 Tn10 (Tet^r)]} (Stratagene Ltd.) was used to prepare and
pan the NAR V region phage display libraries.

PCR materials

15 All custom oligonucleotides used throughout this work were
ordered from Sigma-Genosys Ltd., and were desalted and/or HPLC
purified. Library primer sequences were as follows (all 5'
to 3'):

20 NAR F4 For1 ATA ATC AAG CTT GCG GCC GCA TTC ACA GTC ACG
 ACA GTG CCA CCT C (SEQ ID. 64)
NAR F4 For2 ATA ATC AAG CTT GCG GCC GCA TTC ACA GTC ACG
 GCA GTG CCA TCT C (SEQ ID. 65)
NAR F1 Rev ATA ATA AGG AAT TCC ATG GCT CGA GTG GAC CAA
25 ACA CCG (SEQ ID. 66)

All PCR reactions were performed on a Hybaid PCR sprint block
in Hybaid 0.2 ml thin-walled omnitubes.

Construction of NAR V region libraries for phage display

30

RNA preparation

To enable production of the immune library, three nurse sharks
were immunised five times with Hen egg-white lysozyme (HEL)

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(over a period of approximately 8 months). Blood samples were taken from each shark following each immunisation, peripheral blood lymphocytes isolated, and total RNA prepared for each bleed. The RNA from bleeds 4 and 5 for each of the three sharks was pooled and stored at -80 °C until required for cDNA synthesis.

cDNA synthesis and PCR amplification

For cDNA synthesis, ready-to-go[™] RT-PCR beads (200 µM each dNTP, 10 mM Tris-HCl buffer, 60 mM KCl, 1.5 mM MgCl₂, M-MuLV reverse transcriptase, RNAGuard⁷, RNase/DNase free BSA and 2 U Taq DNA polymerase) (APB Ltd.) were reconstituted in 45 µl of DEPC treated H₂O by incubating on ice for 5 min or until the beads were completely dissolved. To each tube 2 µl of nurse shark tRNA at 2 µg/µl and 2 µl of NAR F4 For primer or F4 For2 primer at 25 pM/µl were added. Both of these primers are specific for NAR framework region 4 and have a NotI site incorporated in the handle to allow subsequent cloning into the phagemid vector. Tubes were flicked gently to mix contents and incubated on a PCR block pre-warmed to 46 °C for 30 min. Following cDNA synthesis, tubes were incubated at 95 °C for 7 min to inactivate the reverse transcriptase and denature the template.

To each tube 2 µl of the common primer NAR F1 Rev at 25 pM/µl, containing a NcoI site in its handle was added, tubes were pre-heated to 95°C and 1 µl of Taq DNA polymerase at 1 U/µl added to each prior to cycling 32 times at 95 °C for 2 min, 55 °C for 1 min and 72 °C for 1 min 30 s.

30

Following PCR amplification type I and type II, products were PAGE purified on a 1.5% gel a strong band was visualised at approximately 400 bp for both primer sets indicating

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successful amplification of the NAR V region.

Cloning of NAR V region into the phagemid vector pHEN2

PAGE-purified PCR product was digested with *NcoI* and *NotI* 5 restriction enzymes, at the sites incorporated by the handled primers used for amplification, to allow cloning into the phagemid vector pHEN2. Restricted DNA was purified on a 1.5% agarose gel and the DNA excised and cleaned.

10 Plasmid DNA, harvested from an overnight culture of *E. coli* XL1-Blue and phenol:chloroform treated, was similarly cut with *NcoI* and *NotI* restriction enzymes. Double-cut vector was purified on a 0.7% agarose gel and DNA extracted. For library construction digested vector was not treated with calf 15 alkaline phosphatase.

To enable quantification, 2 μ l of suitably digested PCR product and pHEN2 vector were run on a 1% agarose gel against 2 μ l of DNA marker VI (Boehringer Ltd.) and band intensities 20 evaluated by eye to judge relative amounts of DNA present. Ligations were performed with equal amounts of vector and insert DNA in the presence of 2.5 μ l of 10 x ligase buffer and 1 μ l of T4 ligase. The final volume was made up to 25 μ l with H₂O and incubated overnight at 15 °C. For library 25 construction 30-40 such ligations were performed.

Following incubation overnight, ligation products were pooled, phenol:chloroform cleaned and the resultant DNA pellet reconstituted in approximately 100 μ l of 1:10 dilution of 10 30 mM Tris-HCl, pH 8.5. DNA was then ready for transformation into electroporation-competent cells.

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Transformation of electroporation-competent cells and evaluation of the resultant library

Ligated DNA was aliquotted into chilled electroporation cuvettes and to each 40 μ l of freshly thawed electroporation-competent XL1-Blue cells was added. Cells were electroporated and resuspended in 100 μ l ice-cold 2xTY media with 1% glucose (w/v) added. Dilutions at 10^{-2} , 10^{-4} and 10^{-6} were performed for each transformation and plated on TYE agar containing 100 μ g/ml ampicillin and 1-2% glucose (w/v). The remaining bacterial suspension was plated straight onto 140 mm petri-dishes containing TYE with ampicillin and glucose (as above). All plates were grown overnight at 37 °C.

Following incubation overnight, colonies from the dilution plates were counted to give an estimate of the final library size, approximately 5×10^6 members. Approximately 100 individual colonies were PCR screened using 1 μ l each of the primers LMB3 (5' CAGGAAACAGCTATGAC 3') (SEQ ID.69) and pHEN seq (5' CTATGCGGCCCCATTCA 3') (SEQ ID. 70) at 25 pM/ μ l, 1 μ l of dNTPs at 25 pM each, 2 μ l of 50 mM $MgCl_2$, 5 μ l of 10 x Taq polymerase buffer, 1 μ l Taq polymerase (at 1 U/ μ l) and 39 μ l Steripak H_2O . PCR was undertaken as follows; 1 cycle at 95 °C for 3 minutes (to lyse bacteria) and 20 cycles of 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. PCR product was run on a 1.5% agarose gel containing EtBr against molecular weight marker VI (Boehringer Ltd.) to evaluate the percentage of the library carrying NAR V region insert. Using this method, 75% of the library was observed to be carrying an insert approximating that expected for the NAR V region, giving a functional library size of 3.75×10^6 members. Fifty clones, established in this way to be carrying correctly sized inserts, were then sequenced to evaluate library diversity.

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The encoded amino acid translations of the sequences obtained are shown in Figures 2A, B and C.

Of the 50 clones sequenced, 6 were found to harbour one or 5 more stop codons encoded by an in-frame TGA codon within CDR3. In the case of clones 13 and 19 the stop codon is probably a consequence of the D3 and D2 segments (respectively) being utilised in a non-preferred reading frame (Roux et al., *Proceedings of the National Academy of Sciences. USA* **95** 10 pp11804-11809 1998). The reason for the stop codons in the other 5 clones is less distinct but is likely due to somatic hypermutation within this region.

A further 15 clones carried frameshift mutations leading to 15 the production of non-sense or truncated proteins. For the majority of these clones the frameshift occurred within CDR3, possibly as a consequence of nucleotide addition or deletion during the recombination process. For clones 14 and 41 the frameshift mutation arose within Fr2 (position 41 according 20 to Figure 3) and Fr3 (position 67) respectively and are more likely due to polymerase errors during library construction (the frameshift in clone 14 occurs immediately after a long poly-A tract in the DNA sequence).

25 The sequence alignment and the variability plot of the 28 clones encoding functional inserts (Figures 2 and 4) show good diversity, with each clone having a unique amino acid sequence. Variability is seen to be focussed across CDR3 which, like clones from a similarly constructed naïve library, 30 varied greatly in both sequence and length. The immune nature of the library is important as NAR V regions which bound to antigen could not be isolated from a naïve library (ie. without prior immunisation).

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Both NAR types were represented, with approximately 80% being type I and 20% type II, however a number of clones proved difficult to assign to an NAR type. For example, clone 33 has a type II Fr1 but type I CDR3 and Fr4, whilst clones 06, 40 5 and 46 have a type I Fr1 and CDR3 but a type II Fr2 and Fr4. This finding suggests the possibility that gene conversion may be occurring between the NAR genes.

A number of other clones also show some atypical features 10 which were not observed with the naïve library pre-selection clones. Clones 24 and 36 are both assigned as type I on the basis of other sequence characteristics but do not carry the pair of cysteine residues normally observed in the type I CDR3. The clones 06, 40, 46 and 48 all encode an uneven 15 number of cysteine residues. As mentioned previously in the case of 06, this may be due to gene conversion. Very few clones bearing an uneven number of cysteines have been observed previously and so it is thought that the V region must be under considerable pressure to maintain an even number 20 of cysteine residues, enabling formation of disulphide bonds. The consequence of unpaired cysteines within the NAR V region is, as yet, unknown but may be detrimental to domain folding. If this is indeed the case then such clones will probably be eliminated from the library during early pans due to their 25 toxicity to the expressing bacteria.

Clone 02 encodes 4 cysteine residues in its CDR3, giving this V region a total of 8 cysteine residues and the potential to form 4 disulphide bonds. Such type I domains carrying 4, or 30 occasionally 6 or more, cysteine residues have been previously encountered. The ability to form these additional disulphide bonds, combined with the small size of the NAR V region, may provide an additional source for highly stable antibody

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fragments.

Colonies, which were not sequenced, were scraped from the library plates with a sterile spreader into a final volume of 5 10 ml 2xTY medium containing 100 μ g/ml ampicillin and 2% glucose. Cells were combined with sterile glycerol to 20% (v/v), and following thorough mixing aliquotted as 500 μ l shots and flash-frozen prior to storage at -80 °C.

10 Panning of NAR V region library against protein antigens

Growth of the library

A single aliquot of library stock was added to 200 ml of pre-warmed 2xTY medium containing ampicillin at 100 μ g/ml and 1-2% glucose (w/v) and grown at 37 °C/ 250 rpm until log phase 15 (OD₆₀₀ of 0.4 - 0.8) was reached. To a 50 ml sample taken from the culture approximately 10¹⁵ of M13K07 helper phage were added and the culture incubated at 37 °C without shaking to allow infection. Following incubation the culture was spun at 3.5K rpm/ 4 °C for 10 min and the cell pellet re-suspended 20 in 100 ml of 2xTY containing 100 μ g/ml ampicillin, 50 μ g/ml kanamycin and 0.1-0.25% glucose and incubated overnight at 30 °C/ 250 rpm to allow library expression and rescue.

The overnight culture was spun at 12K rpm/ 4 °C for 20 min, 80 25 ml of supernatant was removed and added to 20 ml of PEG/NaCl, mixed well and incubated on ice for at least 1 h. The precipitated phage was pelleted at 12K rpm/ 4 °C and re-suspended in 2 ml PBS. The phage suspension was spun at 13K rpm for 10 min to remove any remaining bacterial debris and 30 the phage supernatant stored at 4 °C. The phage stock was titrated by performing serial dilutions in PBS and the addition of 900 μ l of a log phase culture to 100 μ l of each dilution. Following incubation at 37 °C for 30 min, 100 μ l of

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each dilution was plated on TYE plates containing ampicillin at 100 $\mu\text{g/ml}$ and 1% glucose and incubated overnight at 37 °C. The phage titre could be estimated by counting the resulting colonies.

5

Library selection

Nunc Maxisorp Immuno test tubes (Gibco BRL, Life technologies Ltd.) were coated with either HEL or Ova in 4 ml of PBS overnight at 4 °C. The tube was then washed 3 times with PBS
10 before being blocked with 2% Marvel in PBS (MPBS) for 2 h at room temperature, following which it was washed a further 3 times with PBS. Selection was conducted by incubating the coated immunotube for 1 h at room temperature with 1 ml of phage stock in 3 ml of 2% MPBS on an over-and-under tumbler.
15 A further hour of stationary incubation was allowed before the supernatant containing unbound phage was discarded and bound phage eluted as described below.

Elution and rescue of antigen-bound phage20 **Triethylamine elution**

Binding individuals of the antigen specific antigen binding domain library, displayed on the phage strain M13K07, were eluted using the alkali triethylamine.

25 Following incubation with phage the immunotube was washed 20 times with PBST, excess liquid drained off and 1 ml of 100 mM triethylamine added. The tube was then rotated for a maximum of 10 min at room temperature to elute bound phage. Following incubation the phage solution was neutralized by mixing with
30 500 μl of 1 M Tris-HCl. In this state the phage solution was stored at 4 °C for further use (or long-term at -20 °C if glycerol added at 15% v/v).

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To 750 μ l of the triethylamine-eluted phage 10 ml of a log phase bacterial culture was added and the culture incubated at 37 °C without shaking for 30 min. Serial dilutions of the culture were prepared in 2xTY and plated on TYE plates 5 containing 100 μ g/ml ampicillin and 2% glucose to allow the number of rescued phage to be estimated. The remaining infected culture was spun for 10 min at 13K rpm, re-suspended in 100 μ l of 2xTY and plated on a 140 mm petri-dish containing TYE as above. Plates were grown overnight at 37 °C.

10

Rescue of selected phage

After overnight growth, colonies were scraped from the large petri-dishes into 2 ml of 2xTY medium with a sterile scraper and the suspension mixed thoroughly. Following inoculation 15 of 50 ml 2xTY containing 100 μ g/ml ampicillin and 1-2% glucose with 50 μ l of this suspension, 1 ml of the remaining bacteria was mixed with 15% glycerol (v/v) and stored at -80 °C as a stock. The 50 ml culture was incubated at 37 °C/250 rpm until the OD₆₀₀ reached 0.4, whereupon 15 ml was removed, added to 20 approximately 10¹⁰ helper phage and incubated for 30 min at 37 °C. Following incubation the culture was spun at 3.5 K rpm for 10 min and the resultant cell pellet re-suspended in 2xTY containing 100 μ g/ml ampicillin, 50 μ g/ml kanamycin and 0.1-0.25% glucose and incubated overnight at 30 °C/250 rpm.

25

The overnight culture was spun at 12K rpm for 10 min and 40 ml of supernatant added to 10 ml of PEG/NaCl, and mixed well prior to incubation on ice for at least 1 h. The phage pellet was again re-suspended in 2 ml of PBS and spun for 10 min at 30 13K rpm to remove any remaining bacterial debris and the phage stored at 4 °C for the short term.

Further rounds of selection were carried out with phage

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rescued from the previous round of selection, as above, on antigen coated immunotubes.

The immune library was subject to five rounds of panning 5 against the protein antigens Hen egg white lysozyme (HEL) and Chicken ovalbumin (Ova), independently, using M13K07 helper phage and triethylamine elution. A summary of the panning results are given in Table 1.

10 In an attempt to minimize loss of clone diversity in early rounds of selection the antigen coating density was kept constant at 100 $\mu\text{g/ml}$ for pans 1 and 2. Following the first round of panning approximately 10^6 phage were eluted from both the HEL and Ova coated immunotubes, increasing 10-fold 15 following pan 2. For pans 3 and 4 the antigen coating density was reduced for each pan in an attempt to select higher affinity binders. Whilst the number of phage eluted following HEL selection remained constant at $\sim 10^6$ for both pans that for Ova selection dropped to 10^3 in pan 3, rising back to 10^6 20 following pan 4. For pan 5 the antigen coating concentration was further reduced and selection was accompanied by a significant drop in the number of phage eluted. Due to this reduction in the number of phage eluted polyclonal and monoclonal phage ELISAs were conducted to determine if 25 enrichment of HEL or Ova binders was occurring (Figure 5).

The binding of the HEL-selected polyclonal phage showed a small increase in OD_{450} over pans 1 and 2, with a significant increase following pan 3. A further small increase in signal 30 followed pan 4, but afterwards pan 5 dropped back to the level observed for earlier pans. A similar pattern was observed for the Ova-selected polyclonal phage with the highest binding being obtained for phage rescued after pan 4, however in this

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instance the OD₄₅₀ values remain low (below 0.25) for all pans.

Monoclonal phage ELISAs show an increase in the number of positive phage for both sets of selection over pans 1 to 4. In the case of HEL selection this increase was from less than 1% to approximately 80% following pan 4. For Ova selected clones the numbers of positives was slightly lower but regardless increased from less than 1% to approximately 66% after the fourth pan. Following pan 5 the number of HEL-positive clones remained constant at 80% but the number of Ova-positive monoclonals dropped back to the levels observed in earlier pans (~10%).

The drop in the number of clones able to bind Ova after pan 5 indicates that for this pan the protein coating concentration has been reduced such that the selection is too stringent and the majority of clones are no longer able to bind. No such drop is observed for the HEL-selected monoclonal assay, indicating that the affinity of these clones for their antigen is probably higher. This shows that the antigen specific antigen binding domains produced by the sharks are very specific as the sharks were immunised with HEL and only HEL binders could be isolated, Ova data shows no binders. For this reason a selection of clones from pans 3 and 4 were sequenced for Ova but from pans 4 and 5 for HEL.

Table 1

Anti-Hel selection

Pan	phage added (ϕ /ml)	coating density μ g/ml	phage eluted (ϕ /ml)
1	$>10^{12}$	100	105
2	$>10^{12}$	100	106

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3	$>10^{12}$	50	106
4	$>10^{12}$	1	106
5	$>10^{12}$	0.1	103

Anti-Ova selection

5	1	$>10^{12}$	100	105
	2	$>10^{12}$	100	106
	3	$>10^{12}$	50	103
	4	$>10^{12}$	1	105
10	5	$>10^{12}$	0.1	103

Selection analysis**Polyclonal phage ELISA**

A 96-well Immulon 4 ELISA plate (Dynatech Laboratories Ltd.) was coated with 100 μ l of antigen at 10 μ g/ml for 1 h at 37°C. Following three washes with PBST the wells were blocked with 300 μ l of 2% MPBS (PBS with 2% w/v marvel added) for a further hour at room temperature of overnight at 4°C. Wells were washed 3 times with PBST and to individual wells 10 μ l of PEG precipitated phage from each pan, in 100 μ l of 2% MPBS, was added and the plate incubated for 1 h at room temperature. The phage solution was discarded and the plate washed with PBST 3 times. To each well 100 μ l of anti-M13 monoclonal HRP conjugate (APB Ltd.), diluted 1 in 5000 in PBS, was added and incubated at room temperature for 1 h. The plate was washed 5 times with PBST and developed with 100 μ l per well of TMB substrate, the reaction stopped with 50 μ l per well of 1 M H₂SO₄ and the plate read at 450 nm.

Monoclonal phage ELISA

Individual colonies growing on TYE plates were picked into 100 μ l 2xTY medium containing 100 μ g/ml ampicillin and 1-2% glucose on a sterile 96-well ELISA plate, for each of the

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pans, and grown overnight at 37°C/ 250 rpm. Following growth, a 96-well transfer device was used to inoculate a fresh 96-well plate containing 200 µl per well of 2xTY with 100 µg/ml ampicillin and 1-2% glucose. Bacteria were grown for 2 h at 5 37 °C/ 250 rpm. To the original overnight plate glycerol was added to give a final concentration of 15% and the plates stored at -80°C as a bacterial stock.

After the two hour incubation 25 µl of 2xTY containing 100 10 µg/ml ampicillin, 1-2% glucose and 10¹⁰ helper phage were added to each well. The plate was then incubated for a further hour at 37°C/ 250 rpm before being spun at 2K rpm for 10 min to pellet the bacteria. Supernatant was aspirated from the plate and the resultant pellet re-suspended in 200 µl 2xTY 15 containing 100 µg/ml ampicillin, 50 µg/ml kanamycin and glucose at 0.25% (w/v). The plate was then incubated overnight at 30°C/ 250 rpm.

The overnight plate was spun at 2K rpm for 10 min to give a 20 supernatant containing monoclonal phage supernatant. To suitably coated and blocked plates, 50 µl of this phage supernatant in 50 µl of MPBS was added per well and the plate incubated at room temperature for 1 h. Following incubation the plate was incubated with anti-M13 HRP conjugated antibody 25 and developed as normal.

Subcloning and sequencing of positive monoclonal phage clones

Following determination of individual clones giving a positive signal for antigen binding, 5 ml of 2xTY containing 2% glucose 30 and 100 µg/ml ampicillin was inoculated from the appropriate clone source. Taking into account the results of the monoclonal phage ELISAs fifteen HEL-positive clones were picked at random from pans 4 and 5, whilst those for Ova were

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picked from pans 3 and 4. Following overnight incubation of the cultures at 37°C/250 rpm plasmid was prepared as set out above. A 20 µl sample of plasmid was then digested with the restriction enzymes *NcoI* and *NotI* and the ~400 bp fragment corresponding to the NAR V region fragment PAGE purified and recovered. Purified V region fragments were then ligated into similarly cut, alkaline phosphatase treated and cleaned pIMS100 expression vector. Following overnight incubation at 15°C the resultant vector, harbouring the NAR V insert fused upstream of the HuCk domain and 6His tail, was transformed into electroporation-competent *E. coli* XL1-Blue cells. Colonies were picked, grown as overnight cultures in 5 ml TB (containing 2% glucose (v/v), 100 µg/ml ampicillin, 25 µg/ml tetracycline) and glycerol stocks and plasmid prepared.

15

Inserts were sequenced from plasmid using the M13 reverse (5' TTCACACAGGAAACAG 3') (SEQ ID. 67) and HuCk forward (5' GAAGATGAAGACAGATGGTGC 3') (SEQ ID. 68) primer. Once sequence data had been generated the clone was given a unique name to enable identification.

20

On translation only two different sequences were obtained from the 15 HEL-selected clones and two from the 15 Ova-selected clones.

25

The clones 5A7 and 4F11 were chosen to represent the two different amino acid sequences found within the HEL-selected positive clones (Figures 6 and 7). The two clones are both conventional NAR type I, and so are illustrated aligned against a typical type I clone in Figure 8. The two clones differ from one another at only two positions (43 & 44), both lying within Fr2 and carry identical CDR3 regions.

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- 30 -

The clones 4H11 and 3E4 were chosen to represent the two different amino acid sequences found within the Ova-selected positive clones (Figures 9 and 10). Again these clones were both conventional NAR type I and as such are shown aligned against a typical type I clone in Figure 11. These clones differ at 6 amino acids; three within Fr1 (positions 13, 14 & 30), two within Fr2 (positions 46 & 47) and one within CDR3 (position 101).

10 Expression of antigen binding domains in *E. coli*

Large scale expression

A single colony of transformed *E. coli* was used to inoculate 5 ml LB containing 1% glucose (v/v), 12.5 µg/ml tetracycline and 50 µg/ml ampicillin and grown up at 37°C /250 rpm overnight. This culture was used to seed 50 ml TB medium containing 1% glucose (v/v), 12.5 µg/ml tetracycline and 50 µg/ml ampicillin in 250 ml baffled flasks, at 1% v/v. The 50 ml cultures were grown over a period of 24 hours at 25°C /250 rpm, with one change of media after approximately 10 hours growth. Growth of all the cultures was good with the overnight OD₆₀₀ being in the order of 10-20 OD units.

Overnight cultures were pelleted at 4 K rpm /4°C for 20 min. Pellets were resuspended in 50 ml fresh TB containing 50 µg/ml ampicillin and given 1 h at 25°C /250 rpm to recover before induction with 1.5 mM IPTG for 3.5-4 h and release of periplasmic contents.

Periplasmic burst release method

The cell pellet resulting from centrifugation was resuspended in 10% of the original culture volume of fractionation buffer (100 ml 200 mM Tris-HCl, 20% sucrose, pH 7.5, 1 ml 100 mM EDTA/L of culture). The suspension was incubated on ice with

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gentle shaking for 15 min following which an equal volume of ice-cold sterile H₂O was added and incubation continued for a further 15 min (method modified from French *et al.*, *Enzyme & Microbial Technology* 19 pp332-338 1996). The suspension was spun at 13K rpm / 4°C for 20 min, the supernatant containing the periplasmic fraction harvested and passed through a 0.22 µm filter (Sartorius Instruments Ltd.).

None of the cultures showed any sign of bacterial lysis during the 4 h induction period and expression yields in the order of 1 mg crude NAR protein per litre of culture were obtained. In this example the protein expressed from the four selected clones was IMAC purified via the 6His tail.

15 ELISA analysis of antigen binding domains

Antigen binding ELISA

An Immulon 4 96-well flat bottomed ELISA plate was coated with a suitable concentration of the desired antigen at 100 µl per well and the plate incubated at 37°C for 1 h. The plate was washed 3 times with PBST prior to blocking with 200 µl per well of PBS containing 2% Marvel (w/v) for 1 h at 37°C. Wells were washed a further three times with PBST before addition of samples.

A 1 in 5 dilution of crude periplasmic release solution was prepared, added to the top wells of the plate at 200 µl per well and doubling dilutions in PBS performed. Plates were then incubated at 4°C for 1 h. Each plate was washed a further 5 times with PBST. Goat anti-HuCK peroxidase conjugate antibody was diluted 1:1000 in PBS and 100 µl added to wells containing antigen binding domains. Plates were incubated for 1 h at 4°C and following 6 washes with PBST the ELISA was developed as described previously and the plate read at 450 nm.

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The HEL-selected clone 5A7 (Figure 12) shows good binding to HEL at the top dilution applied and as the sample is serially diluted binding reduces accordingly. Limited binding to the highly related protein turkey egg-white lysozyme (TEL) is observed at the highest dilution but no binding is observed to the proteins Chicken ovalbumin (Ova), Bovine serum albumin (BSA), Keyhole limpet haemocyanin (KLH) or the blocking agent Marvel. An identical pattern of protein binding is also observed for the HEL-selected clone 4F11 (Figure 13), which is not surprising considering the high degree of amino acid sequence similarity between these two clones (111/113 aa identical). The OD₄₅₀ signals obtained for 3F11 are slightly higher than those for 5A7, but this may simply be due to small differences in the amount of protein present in the samples.

15

The Ova-selected clone 4H11 (Figure 14) showed no binding to any of the proteins tested, including Ova, the antigen it was selected against. To ensure that this was not simply a consequence of there being too little protein present in the assay, a binding assay was performed with undiluted periplasmic release solution. In this instance some binding to all of the proteins was observed for the wells containing the top dilutions of 4H11 protein. This binding was immediately lost once the sample was diluted and so is likely to be non-specific, no doubt resulting from very high concentrations of protein being present. This data supported the initial finding that the 4H11 clone does not bind significantly to Ova. The 3E4 clone, like 4H11, does not show binding to the proteins HEL, BSA, KLH, TEL or the blocking agent Marvel, however low level binding is observed for this clone to the selection antigen Ova. The pattern of binding by this clone to Ova is unusual in that binding at the highest protein concentration is low and shows no significant drop on

30

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dilution of the sample. When the protein concentration was increased by repeating the assay with undiluted periplasmic solution a similar pattern of binding was observed, thus negating the possibility that the protein concentration was initially too low. The reason for this unusual binding is as yet unknown, but may be due to 3E4 binding only with low affinity to Ova.

The distinct lack of NAR clones capable of binding antigen in a library previously constructed from material from a naïve animal and the isolation of HEL-binding, but not Ova-binding clones, from the library constructed from the HEL immunised animals illustrates the highly specific nature of the NAR response following antigen challenge. In other words, antigen specific antigen binding domains with a specific specificity are produced.

Stability analysis of selected clones

As clones 5A7 and 4F11 were shown to be capable of binding HEL in the antigen binding ELISA it was possible to test the stability of these clones to thermal denaturation. Sub-saturating dilutions of both of the clones, ascertained from the antigen binding curves, were prepared and incubated at a range of temperatures for 3 h prior to their addition to a HEL coated ELISA plate. The samples were then incubated on the ELISA plate for an hour at 4 °C and binding detected with an anti-HuCk HRP conjugated antibody. Stability of the antigen binding domains was plotted as a percentage of that obtained for a control sample which had not been heat treated (Figure 15).

Both clone 5A7 and clone 4F11 show considerable resistance to irreversible denaturation losing 50% functionality at

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approximately 85°C and retaining approximately 30% functionality after 3 h at 95°C. This high stability is probably a consequence of the additional, non-canonical cysteine residues found within the NAR V domain. Both clones 5 encode 6 cysteine residues and therefore are capable of forming 3 intradomain disulphide bonds, which (if formed) would contribute greatly to the high stability of these domains. The shape of the stability curves for both of the clones is almost identical and the minor difference in 10 stability between the clones may be simply due to assay variability.

Repetition of this assay utilising an anti-His HRP conjugated antibody to detect binding generated values which were not 15 significantly different to those obtained with the anti-HuCh secondary antibody, indicating the drop in signal is caused by reduced binding of the NAR V domains, due to denaturation, and not simply reduced detection via the HuCh tag.

20 **Inhibition of protein activity**

The ability of HEL-5A7 to inhibit the enzymatic activity of HEL was tested by mixing 12.5 µl of HEL with 12.5 µl of purified HEL-5A7 protein in a sterile 96 well tissue culture plate, to give a final HEL concentration of 10 µg/ml and HEL- 25 5A7 concentrations of 2500 nM, 250 nM and 25 nM. The control well was set up with buffer replacing HEL-5A7. A sample of freeze dried *Micrococcus lysodeikticus* was reconstituted in 0.1 M phosphate/citrate buffer (pH 5.8) containing 0.09% NaCl, mixed thoroughly and 175 µl added to the prepared wells. The 30 plate was read over a period of 30 min (at 1 min intervals) at 450 nm. Enzymatic activity was plotted as percentage initial absorbance against time for each sample.

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The introduction of HEL-5A7 protein to the assay reduced the rate of cell lysis in a concentration dependent manner with respect to the control (Figure 16). With HEL-5A7 protein at a final concentration of 2500 nM the rate of cell lysis 5 (9.3×10^{-3} OD units/min) is almost halved when compared to the control (17×10^{-3} OD units/min) indicating that the HEL-5A7 region binds within or adjacent to the lysozyme active site cavity. A similarly prepared antigen specific antigen binding domain raised against an unrelated antigen showed no effect 10 upon the rate of cell lysis when introduced to the assay at the same concentrations.

It will be understood that the embodiment illustrated shows one application of the invention only for the purposes of 15 illustration. In practice the invention may be applied to many different configurations, the detailed embodiments being straightforward for those skilled in the art to implement.

SEQUENCE LISTING

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<150> GB 0210508.8

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<170> PatentIn version 3.0

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		35					40					45			

Ile	Ser	Lys	Gly	Gly	Arg	Tyr	Val	Glu	Thr	Val	Asn	Ser	Gly	Ser	Lys
	50					55					60				

Ser	Phe	Ser	Leu	Arg	Ile	Asn	Asp	Leu	Thr	Val	Glu	Asp	Gly	Gly	Thr
65					70					75					80

Tyr	Arg	Cys	Gly	Val	Ser	Pro	Trp	Gly	Trp	Gly	Arg	Ser	Cys	Asp	Tyr
				85					90					95	

Pro	Ser	Cys	Ala	Gln	Arg	Pro	Tyr	Ala	Ala	Cys	Gly	Asp	Gly	Thr	Ala
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Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Gly Ser Trp Glu Pro Val Thr Gly Cys Ala Val Asn
85 90 95

Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val Asn
100 105

<210> 5

<211> 127

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 5

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Asn Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Val Cys Thr Val Met Ser Leu Ile Phe His Leu Asp
 85 90 95

Arg Ile Leu Ser Asn Leu Leu Ser Asn Thr Asp Asp Leu Ile Asp Cys
 100 105 110

Asp Asn Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val Asn
 115 120 125

<210> 6

<211> 111

<212> PRT

<213> Ginglymostoma cirratum

<400> 6

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
 20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
 65 70 75 80

Tyr Arg Cys Gly Glu Pro Leu Val Trp Ser Glu Leu His Ala Cys Ser
 85 90 95

Ser Pro Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val Asn
 100 105 110

<210> 7

<211> 112

<212> PRT

<213> Ginglymostoma cirratum

<400> 7

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
 20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60
 Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
 65 70 75 80
 Tyr Arg Cys Gly Leu Asn Pro Thr Leu Leu Leu Cys Ser Cys Gly
 85 90 95
 Ser Ser Ile Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val Asn
 100 105 110

<210> 8

<211> 114

<212> PRT

<213> Ginglymostoma cirratum

<400> 8

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

Val Asn

<210> 9

<211> 103

<212> PRT

<213> Ginglymostoma cirratum

<400> 9

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15
 Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ser Asn Cys Val Phe Ser

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

<210> 10

<211> 111

<212> PRT

<213> Ginglymostoma cirratum

<400> 10

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

<210> 11

<211> 105

<212> PRT

<213> Ginglymostoma cirratum

<400> 11

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu	1	5	10	15
---	---	---	----	----

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

<210> 12

<211> 116

<212> PRT

<213> Ginglymostoma cirratum

<400> 12

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

<210> 13

<211> 105

<212> PRT

<213> Ginglymostoma cirratum

<400> 13

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

<210> 14

<211> 114

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 14

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

Pro Leu

<210> 15

<211> 104

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 15

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

<210> 16

<211> 109

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 16

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

<210> 17

<211> 112

<212> PRT

<213> Ginglymostoma cirratum

<400> 17

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

<210> 18

<211> 105

<212> PRT

<213> Ginglymostoma cirratum

<400> 18

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

<210> 19

<211> 108

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 19

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

<210> 20

<211> 109

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 20

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

Ala Leu Asp Arg Ala Ala Cys Gly Asp Gly Thr Ala Leu
 100 105

<210> 21

<211> 108

<212> PRT

<213> Ginglymostoma cirratum

<400> 21

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

<210> 22

<211> 102

<212> PRT

<213> Ginglymostoma cirratum

<400> 22

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

Tyr Arg Cys Lys Val Gly Tyr Ile Gly Gly Leu Gly Val Met Tyr Thr
 85 90 95

Glu Val Ala Leu Ser Leu
 100

<210> 23

<211> 111

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 23

Ala Arg Val Asp Gln Thr Pro Gln Thr Ile Thr Lys Glu Thr Gly Glu
 1 5 10 15

Ser Leu Thr Ile Tyr Cys Val Leu Gln Asp Ser Ile Cys Gly Leu Ser
 20 25 30

Ser Thr Tyr Trp Tyr Arg Lys Arg Ser Gly Ser Pro Asn Glu Leu Ser
 35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60

Ser Phe Ser Leu Arg Ile Asn Gly Leu Thr Val Leu Asp Ser Ala Gly
 65 70 75 80

Gly Thr Pro Leu Cys Lys Leu Val Pro Asn Gln Leu Ala Pro Asp Leu
 85 90 95

Thr Phe Arg Thr Thr Leu Met Tyr Thr Glu Met Ala Leu Pro Leu
 100 105 110

<210> 24

<211> 108

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 24

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
 20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ile Gly Leu Asn Lys Arg Gly Glu
 35 40 45

His Ile Glu Arg Trp Thr Ile Cys Asn Ser Gln Arg Ile Lys Val Val
 50 55 60

Leu Phe Phe Glu Asn Ser Asn Ser Arg Arg Trp His Val Ser Leu Arg
 65 70 75 80
 Cys Leu Asp Arg Leu Gly Ala Val Thr Thr Tyr Arg Cys Ala Leu Pro
 85 90 95
 Arg Gly Met Leu His Ala Glu Met Ala Leu Pro Leu
 100 105

<210> 25

<211> 109

<212> PRT

<213> Ginglymostoma cirratum

<400> 25

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

<210> 26

<211> 113

<212> PRT

<213> Ginglymostoma cirratum

<400> 26

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15
 Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
 20 25 30
 Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60
Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80
Tyr Arg Cys Gly Val Pro Asp Ser Trp Trp Arg Phe Ala Val Val Cys
85 90 95
Ala Leu Glu Pro Asp Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val
100 105 110

Asn

<210> 27

<211> 111

<212> PRT

<213> Ginglymostoma cirratum

<400> 27

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

<210> 28

<211> 117

<212> PRT

<213> Ginglymostoma cirratum

<400> 28

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

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

<210> 29

<211> 109

<212> PRT

<213> Ginglymostoma cirratum

<400> 29

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

<210> 30

<211> 115

<212> PRT

<213> Ginglymostoma cirratum

<400> 30

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

<210> 31

<211> 113

<212> PRT

<213> Ginglymostoma cirratum

<400> 31

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

<210> 32

<211> 117

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 32

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

<210> 33

<211> 111

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 33

Ala	Arg	Val	Asp	Gln	Thr	Pro	Arg	Ser	Val	Thr	Lys	Glu	Thr	Gly	Glu
1				5					10					15	
Ser	Leu	Thr	Ile	Asn	Cys	Val	Leu	Arg	Asp	Ala	Ser	Tyr	Ala	Leu	Gly
			20					25					30		
Ser	Thr	Cys	Trp	Tyr	Arg	Lys	Lys	Ser	Gly	Ser	Thr	Asn	Glu	Glu	Ser
		35					40					45			
Ile	Ser	Lys	Gly	Gly	Arg	Tyr	Val	Glu	Thr	Val	Asn	Ser	Gly	Ser	Lys
	50					55					60				

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Val Ser Trp Ala Tyr Ser Cys Asp Tyr Leu Cys Ser
85 90 95

Asp Glu Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val Asn
100 105 110

<210> 34

<211> 114

<212> PRT

<213> Ginglymostoma cirratum

<400> 34

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Val Ser Leu Gly Ala Arg Tyr Ser Cys Asp Tyr Asn
85 90 95

Pro Cys Ser Ser Gly Tyr Ala Ala Cys Gly Gly Gly Thr Val Val Thr
100 105 110

Val Asn

<210> 35

<211> 113

<212> PRT

<213> Ginglymostoma cirratum

<400> 35

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Pro Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45
 Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60
 Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
 65 70 75 80
 Tyr Arg Cys Gly Val Arg Ile Phe Leu Tyr Ser Cys Asp Tyr Ala Cys
 85 90 95
 Ala Leu Asp Gly Tyr Ala Ala Cys Gly Asp Gly Thr Ala Val Thr Val
 100 105 110

Asn

<210> 36
 <211> 113
 <212> PRT
 <213> *Ginglymostoma cirratum*
 <400> 36

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

Asn

<210> 37
 <211> 115
 <212> PRT
 <213> *Ginglymostoma cirratum*

<400> 37

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

<210> 38

<211> 115

<212> PRT

<213> Ginglymostoma cirratum

<400> 38

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

<210> 39

<211> 114

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 39

Ala	Arg	Val	Asp	Gln	Thr	Pro	Arg	Ser	Val	Thr	Lys	Glu	Thr	Gly	Glu
1				5					10					15	

Ser	Leu	Thr	Ile	Asn	Cys	Val	Leu	Arg	Asp	Ala	Ser	Tyr	Ala	Leu	Gly
			20					25					30		

Ser	Thr	Cys	Trp	Tyr	Arg	Lys	Lys	Ser	Gly	Ser	Thr	Asn	Glu	Glu	Ser
		35					40					45			

Ile	Ser	Lys	Gly	Gly	Arg	Tyr	Val	Glu	Thr	Val	Asn	Ser	Gly	Ser	Lys
	50					55					60				

Ser	Phe	Ser	Leu	Arg	Ile	Asn	Asp	Leu	Thr	Val	Glu	Asp	Gly	Gly	Thr
65					70					75					80

Tyr	Arg	Cys	Gly	Leu	Ala	Tyr	Thr	Gly	Arg	Cys	Gly	Phe	Cys	Ala	Leu
				85					90					95	

Asp	Arg	Leu	Arg	Lys	Tyr	Ala	Asp	Cys	Gly	Asp	Gly	Thr	Ala	Val	Thr
			100					105					110		

Val Asn

<210> 40

<211> 120

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 40

Ala	Arg	Val	Asp	Gln	Thr	Pro	Arg	Ser	Val	Thr	Lys	Glu	Thr	Gly	Glu
1				5					10					15	

Ser	Leu	Thr	Ile	Asn	Cys	Val	Leu	Arg	Asp	Ala	Ser	Tyr	Ala	Leu	Gly
			20					25					30		

Ser	Thr	Cys	Trp	Tyr	Arg	Lys	Lys	Ser	Gly	Ser	Thr	Asn	Glu	Glu	Ser
		35					40					45			

Ile	Ser	Lys	Gly	Gly	Arg	Tyr	Val	Glu	Thr	Val	Asn	Ser	Gly	Ser	Lys
	50					55					60				

Ser	Phe	Ser	Leu	Arg	Ile	Asn	Asp	Leu	Thr	Val	Glu	Asp	Gly	Gly	Thr
65					70					75					80

Tyr Arg Cys Gly Val Cys His Arg Ile Ala Gly Val Glu Ile Ala Val
85 90 95

Thr Gln Val Cys Ala Leu Asn Arg Met Tyr Asn Tyr Ala Ala Cys Gly
100 105 110

Asp Gly Thr Ala Val Thr Val Asn
115 120

<210> 41

<211> 114

<212> PRT

<213> Ginglymostoma cirratum

<400> 41

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Ile Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Gln Leu Glu Trp Ser Pro Ala Val Thr Thr Ser Pro
85 90 95

Ala Val Leu Ser Arg His Ala Ala Cys Gly Asp Gly Thr Ala Val Thr
100 105 110

Val Asn

<210> 42

<211> 114

<212> PRT

<213> Ginglymostoma cirratum

<400> 42

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45
 Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60
 Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
 65 70 75 80
 Tyr Arg Cys Gly Val Ser Val Tyr Ser Trp Cys Pro Thr Val Thr Gly
 85 90 95
 Met Val Cys Ser Pro Tyr Ala Ala Cys Gly Gly Gly Thr Val Val Thr
 100 105 110

Val Asn

<210> 43

<211> 114

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 43

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

Val Asn

<210> 44

<211> 113

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 44

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

Asn

<210> 45

<211> 114

<212> PRT

<213> Ginglymostoma cirratum

<400> 45

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

Val Asn

<210> 46

<211> 110

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 46

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

<210> 47

<211> 112

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 47

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

Glu Leu Ile Asp Asp Val Tyr Gly Asp Gly Thr Ala Val Thr Val Asn
 100 105 110

<210> 48

<211> 108

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 48

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

<210> 49

<211> 104

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 49

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

Tyr Arg Cys Lys Val Ser Arg Cys Ser Thr Asn Leu Ile Gly Tyr Gly
 85 90 95

Gly Gly Thr Val Val Thr Val Asn
 100

<210> 50

<211> 108

<212> PRT

<213> Ginglymostoma cirratum

<400> 50

Ala Arg Val Asp Gln Thr Pro Gln Thr Ile Thr Lys Glu Thr Gly Glu
 1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ser Asn Cys Ala Leu Ser
 20 25 30

Ser Thr Tyr Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser
 35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
 50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Ser Gly Thr
 65 70 75 80

Tyr Ala Cys Lys Ala Glu Gly Met Asp Arg Glu Ile Arg Leu Asn Cys
 85 90 95

Val Ile Tyr Gly Gly Gly Thr Val Val Thr Val Asn
 100 105

<210> 51

<211> 113

<212> PRT

<213> Ginglymostoma cirratum

<400> 51

Ala Arg Val Asp Gln Thr Pro Gln Thr Ile Thr Lys Glu Thr Gly Asp
 1 5 10 15

Thr Leu Thr Ile Asn Cys Val Leu Arg Asp Ser Asn Cys Ala Leu Ser
 20 25 30

Asp Met Tyr Trp Ser Arg Lys Lys Ser Gly Ser Thr His Glu Glu Asn
 35 40 45

Ile Ala Lys Glu Gly Arg Tyr Val Glu Thr Phe Asn Arg Ala Ser Lys
 50 55 60

Ser Ser Ser Leu Arg Ile Asn Asp Leu Thr Val Ala Asp Ser Gly Thr
65 70 75 80

Tyr Arg Cys Arg Leu Asp Leu Val Cys Asp Glu Thr Ala Tyr Gln Asp
85 90 95

Glu Leu Glu Phe Asp Asp Ile Tyr Gly Asp Gly Thr Ala Val Thr Val
100 105 110

Asn

<210> 52

<211> 113

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 52

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
1 5 10 15

Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly
20 25 30

Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Glu Gly Asn Glu Glu Ser
35 40 45

Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys
50 55 60

Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr
65 70 75 80

Tyr Arg Cys Gly Leu Gly Val Ala Gly Gly Tyr Cys Asp Tyr Ala Leu
85 90 95

Cys Ser Ser Arg Tyr Ala Glu Cys Gly Asp Gly Thr Ala Val Thr Val
100 105 110

Asn

<210> 53

<211> 339

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 53

gctcgagtgg accaaacacc gagatcagta acaaaggaga cgggcgaatc actgaccatc

60

aactgtgtcc tacgagatgc gagctatgca ttgggcagca cgtgctggta tcgaaaaaaa 120
 tcgggcgaag gaaacgagga gagcatatcg aaaggtggac gatatgttga aacagttaac 180
 agcggatcaa agtccttttc ttgagaatt aatgatctaa cagttgaaga cggtaggcacg 240
 tatcgttgcg gtctcggggg agctggaggg tactgtgact acgctctgtg ctcttcccgc 300
 tatgctgaat gcggagatgg cactgccgtg actgtgaat 339

<210> 54

<211> 339

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 54

cgagctcacc tggtttgtgg ctctagtcac tgtttcctct gcccgcttag tgactggtag 60
 ttgacacagg atgctctacg ctcgatacgt aaccgcgtcg gcacgaccat agcttttttt 120
 acgccgcttc ctttgctcct ctcgtatagc tttccacctg ctatacaact ttgtcaattg 180
 tcgcctagtt tcaggaaaag aaactcttaa ttactagatt gtcaacttct gccaccgtgc 240
 atagcaacgc cagagcccca tcgacctccc atgacactga tgcgagacac gagaagggcg 300
 atacgactta cgcctctacc gtgacggcac tgacactta 339

<210> 55

<211> 113

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 55

Ala Arg Val Asp Gln Thr Pro Arg Ser Val Thr Lys Glu Thr Gly Glu
 1 5 10 15
 Ser Leu Thr Ile Asn Cys Val Leu Arg Asp Ala Ser Tyr Ala Leu Gly

	20		25		30
Ser Thr Cys Trp Tyr Arg Lys Lys Ser Gly Ser Thr Asn Glu Glu Ser	35	40	45		
Ile Ser Lys Gly Gly Arg Tyr Val Glu Thr Val Asn Ser Gly Ser Lys	50	55	60		
Ser Phe Ser Leu Arg Ile Asn Asp Leu Thr Val Glu Asp Gly Gly Thr	65	70	75	80	
Tyr Arg Cys Gly Leu Gly Val Ala Gly Gly Tyr Cys Asp Tyr Ala Leu	85	90	95		
Cys Ser Ser Arg Tyr Ala Glu Cys Gly Asp Gly Thr Ala Val Thr Val	100	105	110		

Asn

<210> 56

<211> 339

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 56

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gctcgagtgg accaaacacc gagatcagta acaaaggaga cgggcgaatc actgaccatc      60
aactgtgtcc tacgagatgc gagctatgca ttgggcagca cgtgctggta tcgaaaaaaaa      120
tcgggctcaa caaacgagga gagcatatcg aaaggtggac gatatgttga aacagttaac      180
agcggatcaa agtccttttc tttgagaatt aatgatctaa cagttgaaga cgggtggcacg      240
tatcgttgcg gtctcgggggt agctggaggg tactgtgact acgctctgtg ctcttcccgc      300
tatgctgaat gcggagatgg cactgccgtg actgtgaat                                339

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<210> 57

<211> 339

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 57

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cgagctcacc tggtttgtgg ctctagtcac tgtttctctc gcccgcttag tgactggtag      60

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ttgacacagg atgctctacg ctcgatacgt aaccgctcgt gcacgaccat agcttttttt 120
agcccgagtt gtttgctcct ctcgtatagc tttccacctg ctatacaact ttgtcaattg 180
tcgcctagtt tcaggaaaag aaactcttaa ttactagatt gtcaacttct gccaccgtgc 240
atagcaacgc cagagcccca tcgacctccc atgacactga tgcgagacac gagaagggcg 300
atacgactta cgcctctacc gtgacggcac tgacactta 339

<210> 58

<211> 114

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 58

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

<210> 59

<211> 341

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 59

gctcgagtgg accaaacacg agatcagtaa caaaggagac gggcgaatca ctgaccatca 60

actgtgtcct acgagatgcg aactatgcat tgggcagcac gtgttggtat cgaaaaaat 120

cgggctcaac aaactgggac agcatatcga aagggtggacg atatgttgaa acagttaaca 180

gcggatcaaa gtccttttct ttgagaatta atgatctaac agttgaagac ggtggcacgt 240

atcgttgcgg tcgagagggc cggtatcata tggatagctg tgactacagt cggtgtcgct 300

actatgctgc atgcggagat ggcactgccg tgactgtgaa t 341

<210> 60

<211> 342

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 60

cgagctcacc tggtttgtgg ctctagtcac tgtttcctct gcccgcttag tgactggtag 60

ttgacacagg atgctctacg cttgatacgt aaccgcgtcg gcacaacacat agcttttttt 120

agcccgagtt gtttgaccct gtcgtatagc tttccacctg ctatacaact ttgtcaattg 180

tcgcctagtt tcaggaaaag aaactcttaa ttactagatt gtcaacttct gccaccgtgc 240

atagcaacgc cagctctccc ggccatagta tacctatcga cactgatgtc agccacagcg 300

atgatacgac gtacgcctct accgtgacgg cactgacact ta 342

<210> 61

<211> 114

<212> PRT

<213> *Ginglymostoma cirratum*

<400> 61

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

<210> 62

<211> 342

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 62

gctcgagtgg accaaacacc gagatcagta acaaaggttg cgggcgaatc actgaccatc 60

aactgtgtcc tacgagatgc gaactaccca ttgggcagta cgtgctggta tcgaaaaaaaa 120

tcgggctcaa caaacgagga gagcatatcg aaaggtggac gatatgttga aacagttaac 180

agcggatcaa agtccttttc tttgagaatt aatgatctaa cagttgaaga cgggtggcacg 240

tatcgttgcg gaagagaggg ccggtatcat atggatagct gtgactacag tcggtgtcgc 300

tactatgggtg catgcggaga tggcactgcc gtgactgtga at 342

<210> 63

<211> 342

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 63

cgagctcacc tggtttgtgg ctctagtcac tgtttccaac gcccgcttag tgactggtag 60

ttgacacagg atgctctacg cttgatgggt aaccogtcat gcacgaccat agcttttttt 120

agcccgagtt gtttgctcct ctcgatatagc tttccacctg ctatacaact ttgtcaattg 180

tcgcctagtt tcaggaaaag aaactcttaa ttactagatt gtcaacttct gccaccgtgc 240

atagcaacgc cttctctccc ggccatagta tacctatcga cactgatgtc agccacagcg 300

atgataccac gtacgcctct accgtgacgg cactgacact ta 342

<210> 64

<211> 46

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 64

ataatcaagc ttgcggccgc attcacagtc acgacagtgc cacctc 46

<210> 65

<211> 46

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 65

ataatcaagc ttgcggccgc attcacagtc acggcagtgc catctc 46

<210> 66

<211> 39

<212> DNA

<213> *Ginglymostoma cirratum*

<400> 66

ataataagga attccatggc tcgagtggac caaacaccg

39

<210> 67

<211> 16

<212> DNA

<213> M13 reverse primer

<400> 67

ttcacacagg aaacag

16

<210> 68

<211> 21

<212> DNA

<213> HuCk forward primer

<400> 68

gaagatgaag acagatgggtg c

21

<210> 69

<211> 17

<212> PRT

<213> LMB3 primer

<400> 69

Cys Ala Gly Gly Ala Ala Ala Cys Ala Gly Cys Thr Ala Thr Gly Ala
1 5 10 15

Cys

<210> 70

<211> 17

<212> PRT

<213> pHEN primer

<400> 70

Cys Thr Ala Thr Gly Cys Gly Gly Cys Cys Cys Cys Ala Thr Thr Cys
1 5 10 15

Ala

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Claims

1. A process for the production of an antigen specific antigen binding domain using a transformed host containing an
5 expressible DNA sequence encoding the antigen specific antigen binding domain, wherein the antigen specific antigen binding domain is derived from a variable region of the immunoglobulin isotype NAR found in a species of *Elasmobranchii* subclass and wherein the specificity of the
10 antigen specific antigen binding domain is determined by an antigen which is introduced into a member of the *Elasmobranchii* subclass.
2. The process according to claim 1, wherein the transformed
15 host is a prokaryote or a lower eukaryote.
3. The process according to claim 2, wherein the prokaryote host is *Escherichia coli*.
- 20 4. The process according to any one of claims 1 to 3, wherein the expressible DNA sequence is in the form of a phagemid vector.
5. The process according to any one of claims 1 to 4,
25 wherein the species of *Elasmobranchii* subclass is a shark or a dog fish.
6. The process according to claim 5, wherein the shark is a nurse shark.
- 30 7. The process according to any one of claims 1 to 6, wherein the antigen specific antigen binding domain is targeted to a specific antigen(s).

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8. The process according to any one of claims 1 to 7, wherein the antigen specific antigen binding domain is raised to a single antigen.

- 5 9. A process for the production of an antigen specific antigen binding domain comprising the steps of:
- a) immunising a member of the *Elasmobranchii* subclass with an antigen;
 - b) isolating lymphocytes from the member;
 - 10 c) isolating RNA from the lymphocytes;
 - d) amplifying DNA sequences encoding the antigen specific antigen binding domain by PCR;
 - e) cloning the amplified DNA into a display vector;
 - f) transforming a host to produce a library;
 - 15 g) selecting the desired clones from the library;
 - h) isolating and purifying the antigen specific antigen binding domain from these clones;
 - i) cloning the DNA sequences encoding the antigen specific antigen binding domain into an expression vector;
 - 20 j) transforming a host to allow expression of the expression vector.

10. The process according to claim 9, wherein before step d) the cDNA of the antigen specific antigen binding domain is
25 generated.

11. The process according to either claim 9 or 10, wherein restriction enzymes are used to digest the amplified DNA sequences encoding the antigen specific antigen binding
30 domain.

12. The process according to claim 11, wherein the restriction enzymes are *NcoI* and *NotI*.

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13. The process according to any one of claims 9 to 12, wherein the display vector is any phagemid vector.

14. The process according to claim 13, wherein the display
5 vector is pHEN2.

15. The process according to any one of claims 9 to 14, wherein the expression vector is a soluble expression vector.

10 16. The process according to claim 15, wherein the soluble expression vector is pIMS100.

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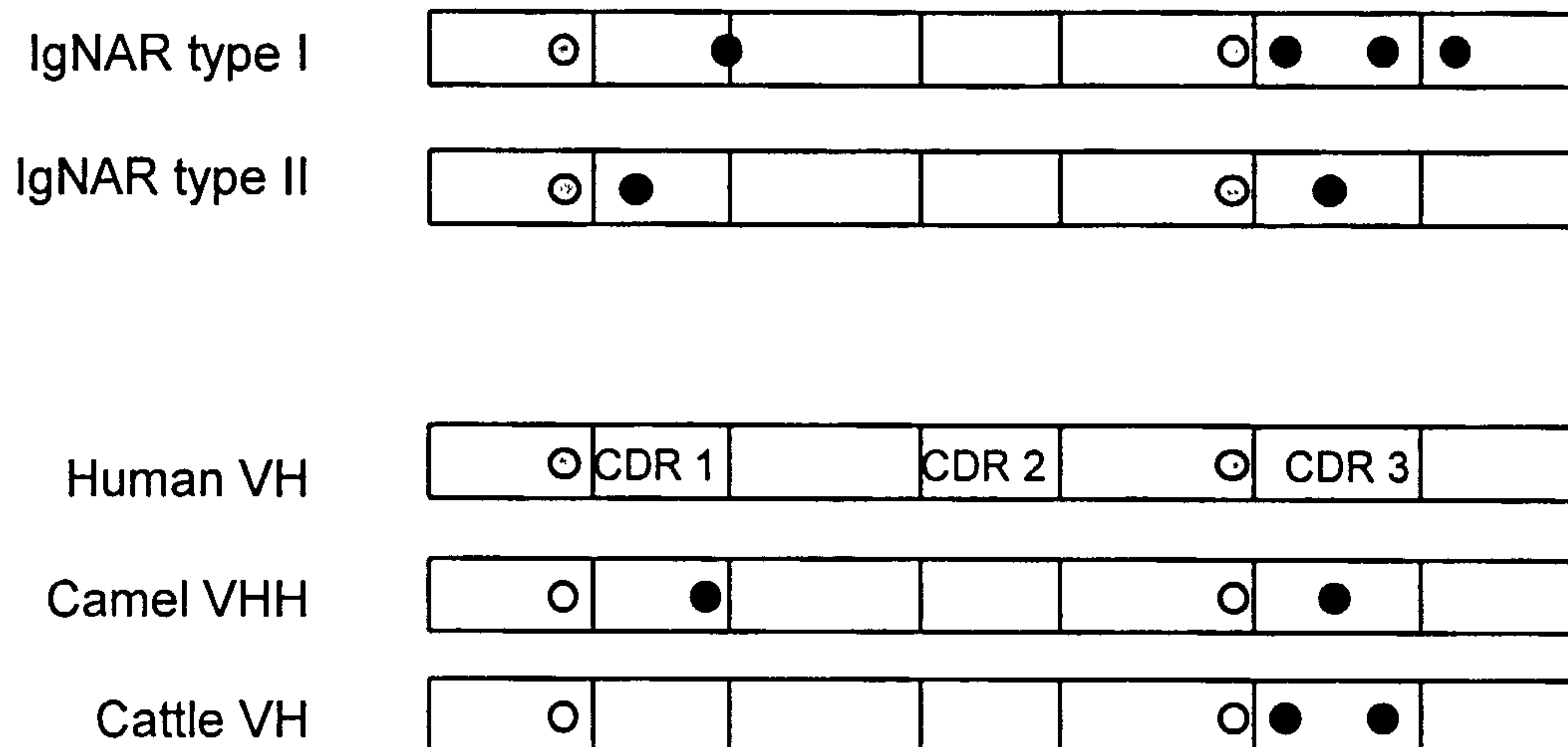


Figure 1

1 10 20 30 40 50 66 70
IgnAR I ARVDQTPRSVTKETGESLTINCVLRDASYGLSTCWYRKKGSTNEESI**SKGG**RYVETVNSGSKS
IgnAR II -----QEI-----S-----D-CA-P--Y-N-----T-----

13 -----A-----
34 -----A-----
25 -----N-A-----
50 -----A-----
19 -----A-----
17 -----A-----

80 abc 90 95 104 112 2 / 21
IgnAR I FSLRINDLTVEDGGTYRCGVSPWGGRSCDYPSCAQRPYAA CGDGTAVTVN
IgnAR II -----S-----K-YRKNWAYDCGLEELDWIYV Y-G--V----

13 -----STWCRCTCCDYETGLCS*AYAA-----
34 -----GSWEPVTG*CAVNYAA-----
25 -----CTVMSLI FHLDRILSNLL*SNFD*DLIDCDNYAA-----
50 -----EPLVWSEL*HACSSPYAA-----
19 -----LNPTL*LLLCSCGSSIYAA-----
17 -----I-----LQLVWIPPL*LRIGGALPYGA-----E-----

Figure 2A

	1	10	20	30	40	50	60	70
IGNAR I	ARVDQTPRSVTKETGESLTINCVLRDASYGLSTCWYRKKGSTNEESI	SKGG	RYVETVNSGSKS					
IGNAR II	-----QEI-----S-----D-CA-P--Y-N-----T-----							
47	-----	-----SNCVFSR-Y-----N-----	-----WSIC*NN*PH*					
05	-----Q-----	-----TET-S-----						
04	-----	-----A-----						
21	-----	-----A-----						
39	-----	-----A-----						
01	-----	-----A-----						
08	-----	-----A-----						
07	-----	-----A-----						
15	-----	-----A-----						
42	-----	-----A-----						
43	-----	-----A-----						
22	-----	-----A-----						
27	-----KITIT-----	-----S-T-CAWD--Y-----LD-----	-----T-----					
28	-----QTIT-----	-----SNCA-S--Y-----						
37	-----QTI-----	-----Y--Q-SIC--S--Y-----R--P--L-----						
14	-----	-----A-----	-----IGLNKRGEHIERWTIC*NS*QRIKV					

Figure 2B

	80	a b c	90	95	104	112
IgNAR I	FSLRINDLT	VEDGGTYRCGV	SPWGWGRSCDY	PSCAQRPYAA	CGDGTAV	TVN
IgNAR II	-----S-----	K-YRKNWAYDCGLEELD	WIYV		Y-G--V----	
47	QRIKVLFFGNG	*SMS	*RKCHVSM	*RGRYTPED	*NNL	-----
05	-----	-----	PGIAGGSGCALL	TLCCMRRWHCR		---
04	-----	-----	WEL	*LRGALYMLHAD	MALP*L*	
21	-----	-----	WIAGVDYDYS	LAVLLSSTSMAM	LHAEMALP*L*	
39	-----	-----	EAHPLRSSVT	TMLHAEMALP*L*		
01	-----	-----	VFLADSWCGSV	VTSCALPPML	LHAEMALP*L*	
08	-----	-----	IW	*RCSLCIGCML	HAEMALP*L*	
07	-----	-----	L---	I	*MVCCDSFGSV	LYRRELHAEMALP*L*
15	-----	-----	CRTWGSRCDL	AHVLLGCMRR	WHCRDCE	
42	-----	-----	AGI	*LVEGSRGCMRR	WHCRDCE	
43	-----	-----	*RRI	*LVWML	*LTVCCMRR	WHCRDCE
22	-----	-----	GVWICDETL	SCALDRAACGD	GTAL*	
27	-----	-----	S-----	RAYPGL	*LYCGYHGAL*	*IWRWHCRDCE
28	-----	-----	S-----	KV	*GYIGGLGVMY	TEVALS*L*
37	-----	-----	L-SAGGTP	LC	KLVPNQ	LAPDLTFRTTLMYTEMALP*L*
14	VLFFEN*	*SNS	*RRWHVSL	RCLDRLGAV	TTYRCALPRG	MLHAEMALP*L*

Figure 2B cont'd

1	ARVDQTPRSVTKETGESLTINCVLRDASYGLSTC	WYRKKS	GSTNEESI	SKGGRY	ETVNSG	SKS
40	IgnAR I	IgnAR II	---	---	---	---
46	---	---	---	---	---	---
06	---	---	---	---	---	---
02	---	---	---	---	---	---
09	---	---	---	---	---	---
23	---	---	---	---	---	---
12	---	---	---	---	---	---
30	---	---	---	---	---	---
20	---	---	---	---	---	---
38	---	---	---	---	---	---
18	---	---	---	---	---	---
26	---	---	---	---	---	---
49	---	---	---	---	---	---
24	---	---	---	---	---	---
31	---	---	---	---	---	---
29	---	---	---	---	---	---
36	---	---	---	---	---	---
45	---	---	---	---	---	---
03	---	---	---	---	---	---
32	---	---	---	---	---	---
33	---	---	---	---	---	---
48	---	---	---	---	---	---
16	---	---	---	---	---	---
11	---	---	---	---	---	---
41	---	---	---	---	---	---
44	---	---	---	---	---	---
10	---	---	---	---	---	---

Figure 2C

	80	abc	90	95	104	111
IGNAR I	FSLRINDLTVEDGGTYRCGVSPWGRSCDYPSCAQRPYAA				CGDGTAVTVN	
IGNAR II	-----S-----K-YRKNWAYDCGLEELDWIYV				Y-G--V----	
40	-----WGQLHVRCALGDAA				-----	
46	-----PDSWWRFAVVCALPDAA				-----	
06	-----CPHFSWCRLHEQCALA				G-----	
02	-----H-----CDSSIAVVAGCGYCLCTLVHSV				-----	
09	-----ARAGGPFLCSCVYAA				-----	
23	-----PVGRSCDYPQLCSWGLNYAA				-----	
12	-----STAGVDCDYTCALWDYAA				-----	
30	-----SHAVAGGVCDYSSGLCSWSYAA				-----	
20	-----SWAYSCDYILCSDEYAA				-----	
38	-----SLGARYSCDYNPCSSGYAA				--G--V----	
18	-----RIFLYSCDYACALDGYAA				-----	
26	-----T-----ARPVGSCDYDLCSEFRPYAA				-----	
49	-----ELVWGYHSCDYDMCSFRYAA				-----	
24	-----SLVWIGYIAVTTLDVLLRAA				-----	
31	-----LAYTGRCGFCALDRLRKYAD				-----	
29	-----CHRIAGVEIAVTQVCALNRMVNYAA				-----	
36	-----I-----QLEWSPAVTTS PAVLSRHAA				-----	
45	-----SVYSWCPTVTGMVCSFYAA				--G--V----	
03	-----GGAYSCVTTYRGCALYAA				-----	
32	-----A-SSIAIRCDHAELCSRYGA				-----	
33	-----AAATIQYSCDRLC SWDFAV				-----	
48	-----G-K---SW-----KAYTEPKTRRLIKCCRE				Y-----	
16	-----S-----K-PSRYSYDCVRFELIDDV				Y-----	
11	-----S-----RAELYCGAELDSFDE				Y-----	
41	-----S-----K-SRCSTNLIG				Y-G--V----	
44	-----S-----A-KAEGMDREIRLNCVI				Y-G--V----	
10	S-----A-S-----RLDLVCDETAYQDELEFDDI				Y-----	

Figure 2C cont'd

```

1      10      20      30      40      50      66
IgNAR I  ARVDQTPRSVTKETGESTLTINCVLRDASYGLGSTCWYRKKSGSTNEESISKGGRYVE
IgNAR II -----QEIT-----S-----D-CA-P-Y-N-----T-----

70      80      abc      90      95      100      b c d e f g h i j k l m      104      113
TVNSGSKSFLRINDLTVEDGGTYRCGVSPWGRSCDYPSCAQRPYAACGDGTAVTNP
-----S-----K-YRKNWAYD-GLEELDWIY  VY-G--V-----

```

Figure 3

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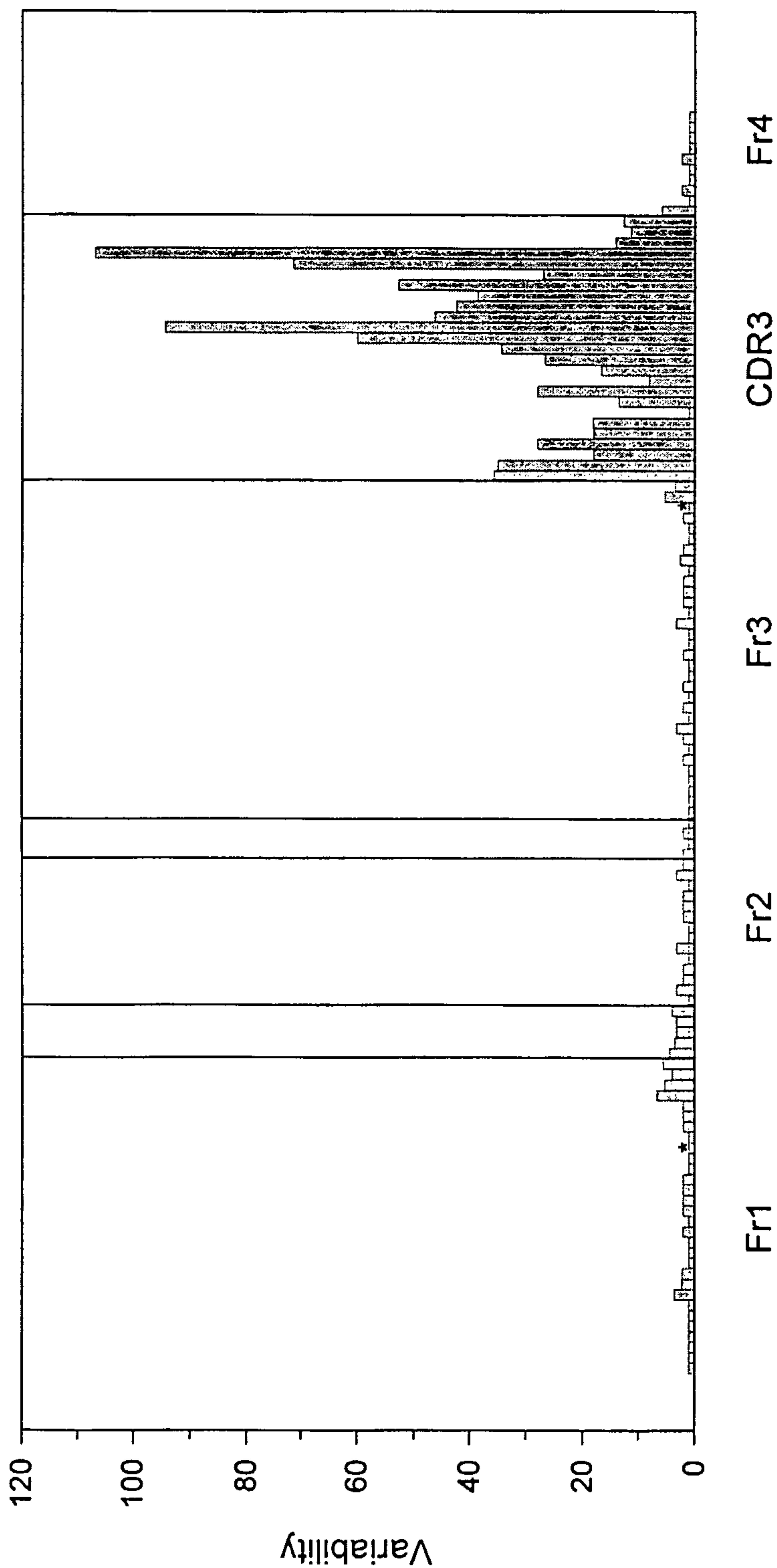
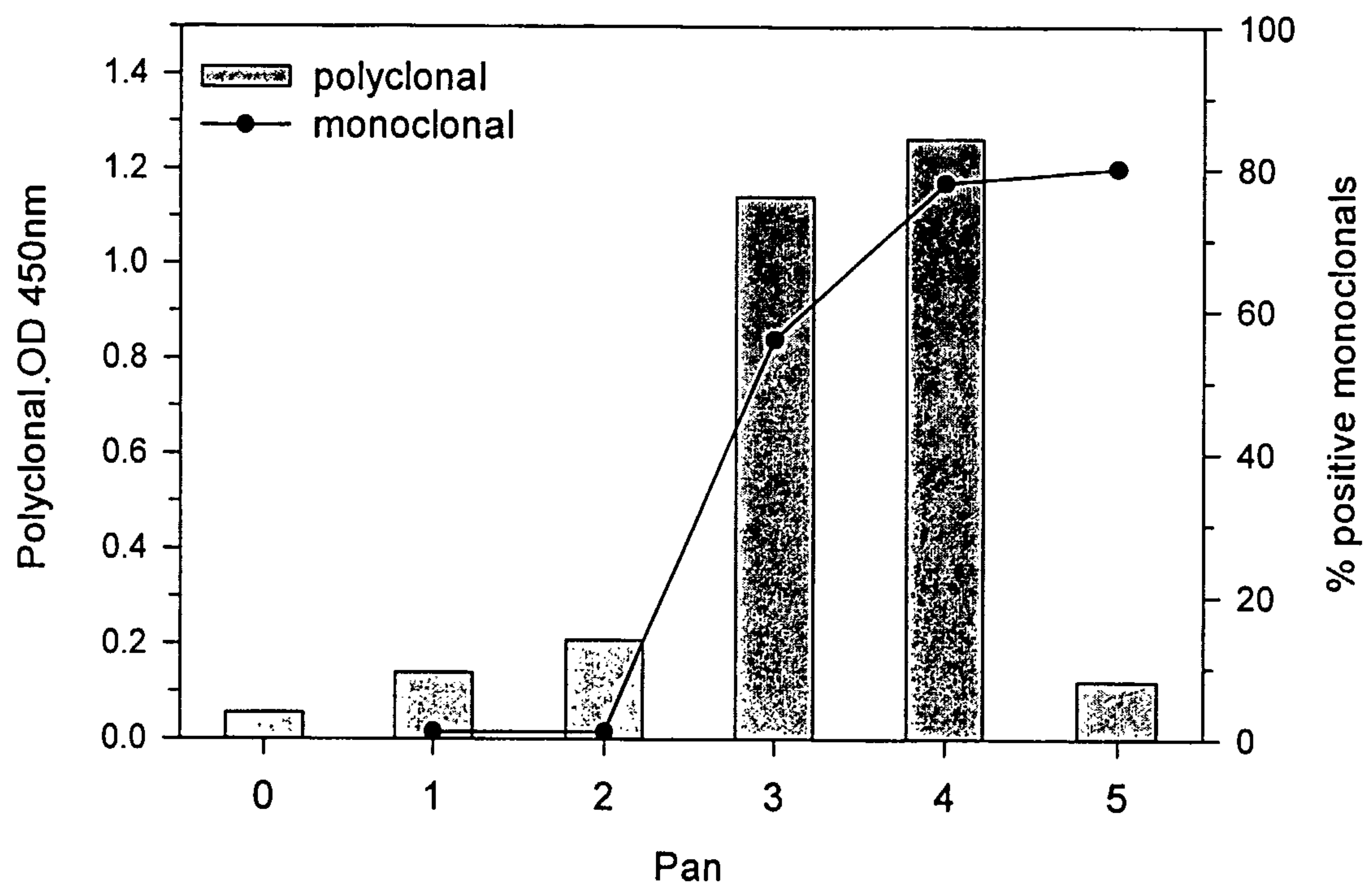


Figure 4

Figure 5A

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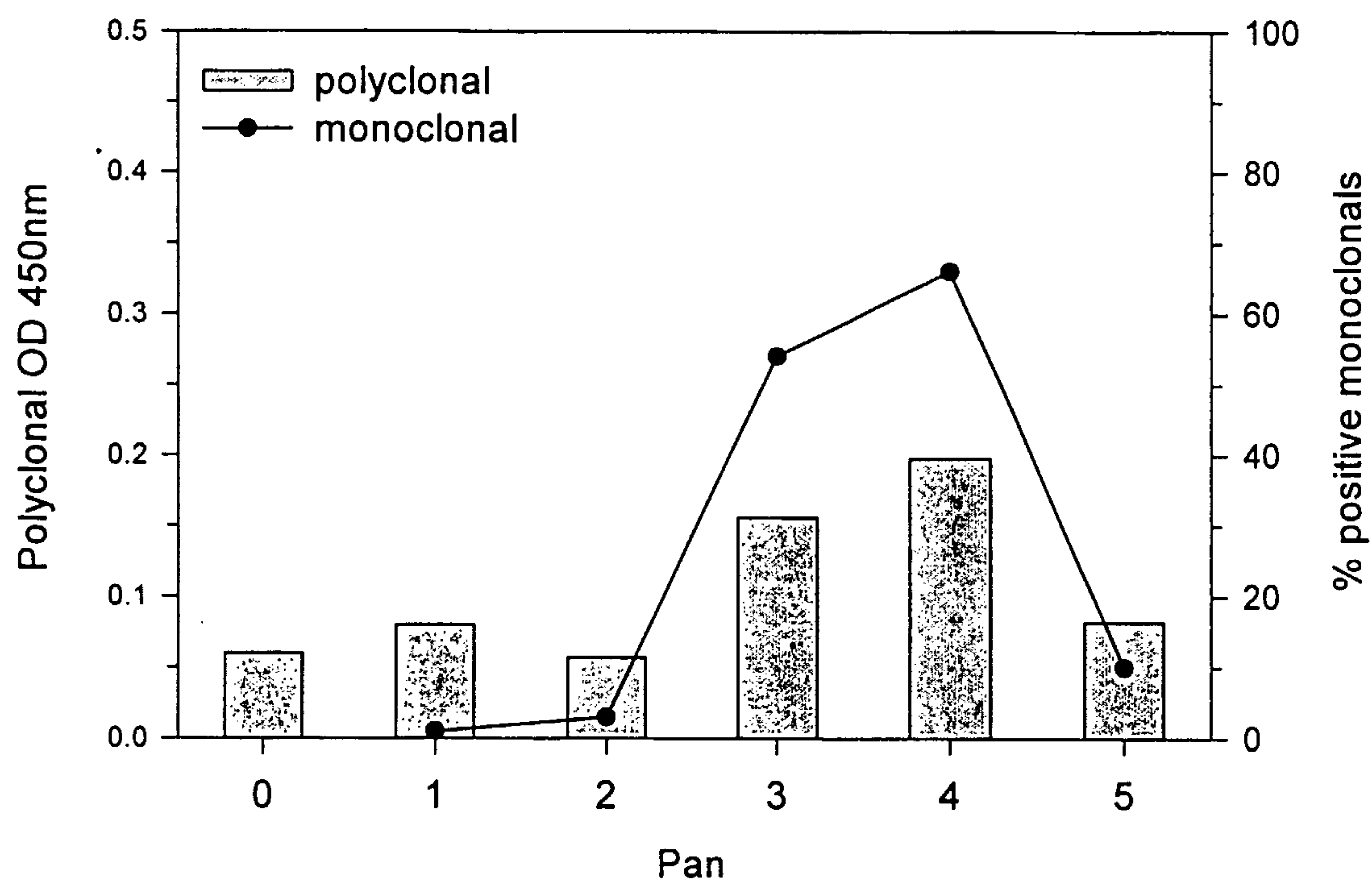


Figure 5B

ALA	ARG	VAL	ASP	GLN	THR	PRO	ARG	SER	VAL	THR	LYS	GLU	THR	GLY	GLU	SER	LEU	THR	ILE	ASN	CYS	VAL
GCT	CGA	GTG	GAC	CAA	ACA	CCG	AGA	TCA	GTA	ACA	AAG	GAG	ACG	GGC	GAA	TCA	CTG	ACC	ATC	AAC	TGT	GTC
CGA	GCT	CAC	CTG	GTT	TGT	GGC	TCT	AGT	CAT	TGT	TTC	CTC	TGC	CCG	CTT	AGT	GAC	TGG	TAG	TTG	ACA	CAG
LEU	ARG	ASP	ALA	SER	TYR	ALA	LEU	GLY	SER	THR	CYS	TRP	TYR	ARG	LYS	LYS	SER	GLY	GLU	GLY	ASN	GLU
CTA	CGA	GAT	GCG	AGC	TAT	GCA	TTG	GGC	AGC	ACG	TGC	TGG	TAT	CGA	AAA	AAA	TCG	GGC	GAA	GGA	AAC	GAG
GAT	GCT	CTA	CGC	TCG	ATA	CGT	AAC	CCG	TCG	TGC	ACG	ACC	ATA	GCT	TTT	TTT	AGC	CCG	CTT	CCT	TTG	CTC
GLU	SER	ILE	SER	LYS	GLY	GLY	ARG	TYR	VAL	GLU	THR	VAL	ASN	SER	GLY	SER	LYS	SER	PHE	SER	LEU	ARG
GAG	AGC	ATA	TCG	AAA	GGT	GGA	CGA	TAT	GTT	GAA	ACA	GTT	AAC	AGC	GGA	TCA	AAG	TCC	TTT	TCT	TTG	AGA
CTC	TCG	TAT	AGC	TTT	CCA	CCT	GCT	ATA	CAA	CTT	TGT	CAA	TTG	TCG	CCT	AGT	TTC	AGG	AAA	AGA	AAC	TCT
ILE	ASN	ASP	LEU	THR	VAL	GLU	ASP	GLY	GLY	THR	TYR	ARG	CYS	GLY	LEU	GLY	VAL	ALA	GLY	GLY	TYR	CYS
ATT	AAT	GAT	CTA	CTA	ACA	GTT	GAA	GAC	GAC	ACG	TAT	CGT	TGC	GGT	CTC	GGG	GTA	GCT	GGA	GGG	TAC	TGT
TAA	TTA	CTA	GAT	TGT	CAA	CTT	CTG	CCA	CCG	TGC	ATA	GCA	ACG	CCA	GAG	CCC	CAT	CGA	CCT	CCC	ATG	ACA
ASP	TYR	ALA	LEU	CYS	SER	SER	ARG	TYR	ALA	GLU	CYS	GLY	ASP	GLY	THR	ALA	VAL	THR	VAL	ASN		
GAC	TAC	GCT	CTG	TGC	TCT	TCC	CGC	TAT	GCT	GAA	TGC	GGA	GAT	GGC	ACT	GCC	GTG	ACT	GTG	AAT		
CTG	ATG	CGA	GAC	ACG	AGA	AGG	GCG	ATA	CGA	CTT	ACG	CCT	CTA	CCG	TGA	CGG	CAC	TGA	CAC	TTA		

Figure 6

ALA	ARG	VAL	ASP	GLN	THR	PRO	ARG	SER	VAL	THR	LYS	GLU	THR	GLY	GLU	SER	LEU	THR	ILE	ASN	CYS	VAL
GCT	CGA	GTG	GAC	CAA	ACA	CCG	AGA	TCA	GTA	ACA	AAG	GAG	ACG	GGC	GAA	TCA	CTG	ACC	ATC	AAC	TGT	GTC
CGA	GCT	CAC	CTG	GTT	TGT	GGC	TCT	AGT	CAT	TGT	TTC	CTC	TGC	CCG	CTT	AGT	GAC	TGG	TAG	TTG	ACA	CAG
LEU	ARG	ASP	ALA	SER	TYR	ALA	LEU	GLY	SER	THR	CYS	TRP	TYR	ARG	LYS	LYS	SER	GLY	SER	THR	ASN	GLU
CTA	CGA	GAT	GCG	AGC	TAT	GCA	TTG	GGC	AGC	ACG	TGC	TGG	TAT	CGA	AAA	AAA	TCG	GGC	TCA	ACA	AAC	GAG
GAT	GCT	CTA	CGC	TCG	ATA	CGT	AAC	CCG	TCG	TGC	ACG	ACC	ATA	GCT	TTT	TTT	AGC	CCG	AGT	TGT	TTG	CTC
GLU	SER	ILE	SER	LYS	GLY	GLY	ARG	TYR	VAL	GLU	THR	VAL	ASN	SER	GLY	SER	LYS	SER	PHE	SER	LEU	ARG
GAG	AGC	ATA	TCG	AAA	GGT	GGA	CGA	TAT	GTT	GAA	ACA	GTT	AAC	AGC	GGA	TCA	AAG	TCC	TTT	TCT	TTG	AGA
CTC	TCG	TAT	AGC	TTT	CCA	CCT	GCT	ATA	CAA	CTT	TGT	CAA	TTG	TCG	CCT	AGT	TTC	AGG	AAA	AGA	AAC	TCT
ILE	ASN	ASP	LEU	THR	VAL	GLU	ASP	GLY	GLY	THR	TYR	ARG	CYS	GLY	LEU	GLY	VAL	ALA	GLY	GLY	TYR	CYS
ATT	AAT	GAT	CTA	ACA	GTT	GAA	GAC	GAT	GGT	ACG	TAT	CGT	TGC	GGT	CTC	GGG	GTA	GCT	GGA	GGG	TAC	TGT
TAA	TTA	CTA	GAT	TGT	CAA	CTT	CTG	CCA	CCG	TGC	ATA	GCA	ACG	CCA	GAG	CCC	CAT	CGA	CCT	CCC	ATG	ACA
ASP	TYR	ALA	LEU	CYS	SER	SER	ARG	TYR	ALA	GLU	CYS	GLY	ASP	GLY	THR	ALA	VAL	THR	VAL	ASN		
GAC	TAC	GCT	CTG	TGC	TCT	TCC	CGC	TAT	GCT	GAA	TGC	GGA	GAT	GGC	ACT	GCC	GTG	ACT	GTG	AAT		
CTG	ATG	CGA	GAC	ACG	AGA	AGG	GCG	ATA	CGA	CTT	ACG	CCT	CTA	CCG	TGA	CGG	CAC	TGA	CAC	TTA		

Figure 7

IGNAR I
HEL 5A7
HEL 5E11

IGNAR I
HEL 5A7
HEL 5F11

Figure 8

ALA ARG VAL ASP GLN THR PRO ARG SER VAL THR LYS GLU THR GLY GLU SER LEU THR ILE ASN CYS VAL
GCT CGA GTG GAC CAA ACA CCG AGA TCA GTA ACA AAG GAG GGC GGC GAA TCA CTG ACC ATC AAC TGT GTC
CGA GCT CAC CTG GTT TGT GGC TCT AGT CAT TGT TTC CTC TGC CCG CTT AGT GAC TGG TAG TTG ACA CAG

LEU ARG ASP ALA ASN TYR ALA LEU GLY SER THR CYS TRP TYR ARG LYS LYS SER GLY SER THR ASN TRP
CTA CGA GAT GCG AAC TAT GCA TTG GCG AGC ACG TGT TGG TAT CGA AAA AAA TCG GGC TCA ACA AAC TGG
GAT GCT CTA CGC TTG ATA CGT AAC CCG TCG TGC ACA ACC ATA GCT TTT TTT AGC CCG AGT TGT TTG ACC

ASP SER ILE SER LYS GLY GLY ARG TYR VAL GLU THR VAL ASN SER GLY SER PHE SER LEU ARG
GAC AGC ATA TCG AAA GGT GGA CGA TAT GTT GAA ACA GTT AAC AGC GGA TCA AAG TCC TTT TCT TTG AGA
CTG TCG TAT AGC TTT CCA CCT GCT ATA CAA CTT TGT CAA TTG TCG CCT AGT TTC AGG AAA AGA AAC TCT

ILE ASN ASP LEU THR VAL GLU ASP GLY GLU THR TYR ARG CYS GLY ARG GLY GLU GLY ARG TYR HIS MET ASP
ATT AAT GAT CTA ACA GTT GAA GAC GGT GGC ACCT GGC ACCT GGC TAT TAT CAT ATG GAT
TAA TTA CTA GAT TGT CAA CTT CTG CCA CTT CCA CCA CCA GCT CTC CTC CCG GCC ATA GTA TAC CTA

SER CYS ASP TYR SER ARG CYS ARG TYR TYR ALA ALA CYS GLY ASP GLY THR VAL ASN
AGC TGT GAC TAC AGT CGG TGT CGC TAC TAT GCT GCA TGC GGA GAT GGC ACT GCC GTG ACT GTG AAT
TCG ACA CTG ATG TCA GCC ACA GCG ATG ATA CGA CGT ACG CCT CTA CCG TGA CGG CAC TGA CAC TTA

Figure 9

ALA	ARG	VAL	ASP	GLN	THR	PRO	ARG	SER	VAL	THR	LYS	VAL	ALA	GLY	GLU	SER	LEU	THR	ILE	ASN	CYS	VAL
GCT	CGA	GTG	GAC	CAA	ACA	CCG	AGA	TCA	GTA	ACA	AAG	GTT	GCG	GGC	GAA	TCA	CTG	ACC	ATC	AAC	TGT	GTC
CGA	GCT	CAC	CTG	GTT	TGT	GGC	TCT	AGT	CAT	TGT	TTC	CAA	CGC	CCG	CTT	AGT	GAC	TGG	TAG	TTG	ACA	CAG
LEU	ARG	ASP	ALA	ASN	TYR	PRO	LEU	GLY	SER	THR	CYS	TRP	TYR	ARG	LYS	LYS	SER	GLY	SER	THR	ASN	GLU
CTA	CGA	GAT	GCG	AAC	TAC	CCA	TTG	GGC	AGT	ACG	TGC	TGG	TAT	CGA	AAA	AAA	TCG	GGC	TCA	ACA	AAC	GAG
GAT	GCT	CTA	CGC	TTG	ATG	GGT	AAC	CCG	TCA	TGC	ACG	ACC	ATA	GCT	TTT	TTT	AGC	CCG	AGT	TGT	TTG	CTC
GLU	SER	ILE	SER	LYS	GLY	GLY	ARG	TYR	VAL	GLU	THR	VAL	ASN	SER	GLY	SER	LYS	SER	PHE	SER	LEU	ARG
GAG	AGC	ATA	TCG	AAA	GGT	GGA	CGA	TAT	GTT	GAA	ACA	GTT	AAC	AGC	GGA	TCA	AAG	TCC	TTT	TCT	TTG	AGA
CTC	TCG	TAT	AGC	TTT	CCA	CCT	GCT	ATA	CAA	CTT	TGT	CAA	TTG	TCG	CCT	AGT	TTC	AGG	AAA	AGA	AAC	TCT
ILE	ASN	ASP	LEU	THR	VAL	GLU	ASP	GLY	GLY	THR	TYR	ARG	CYS	GLY	ARG	GLU	GLY	ARG	TYR	HIS	MET	ASP
ATT	AAT	GAT	CTA	ACA	GTT	GAA	GAC	GGT	GGC	ACG	TAT	CGT	TGC	GGA	AGA	GAG	GGC	CGG	TAT	CAT	ATG	GAT
TAA	TTA	CTA	GAT	TGT	CAA	CTT	CTG	CCA	CCG	TGC	ATA	GCA	ACG	CCT	TCT	CTC	CCG	GCC	ATA	GTA	TAC	CTA
SER	CYS	ASP	TYR	SER	ARG	CYS	ARG	TYR	TYR	GLY	ALA	CYS	GLY	ASP	GLY	THR	ALA	VAL	THR	VAL	ASN	
AGC	TGT	GAC	TAC	AGT	CGG	TGT	CGC	TAC	TAT	GGT	GCA	TGC	GGA	GAT	GGC	ACT	GCC	GTG	ACT	GTG	AAT	
TCG	ACA	CTG	ATG	TCA	GCC	ACA	GCG	ATG	ATA	CCA	CGT	ACG	CCT	CTA	CCG	TGA	CGG	CAC	TGA	CAC	TTA	

Figure 10

IgNAR I
Ova 4H11
Ova 3E4

1
ARVDQTPRSVTKETGESLTINCVLRDASYGLSTCWRKKSGSTNEESI**SKGGRYVETVN**
ARVDQTPRSVTKETGESLTINCVLRDANYA**LGSTC**WRKKSGSTN**WDSISKGGRYVETVN**
ARVDQTPRSVTKVAGE**SLTINCVLRDANYPLGSTC**WRKKSGSTNE**ESISKGGRYVETVN**
***** : ***** : *****

10203040506670

IgNAR I
Ova 4H11
Ova 3E4

8090104112
SGSKSFSLRINDLTVEDGGTYRCGVSPWGWRSCDYPSCAQR**PYAA**CGDGTAVTN
SGSKSFSLRINDLTVEDGGTYRCGR**EGRYHMDSCDYSRC** RY**YAAC**GDGTAVTN
SGSKSFSLRINDLTVEDGGTYRCGR**EGRYHMDSCDYSRC** RY**YGAC**GDGTAVTN
***** : ***** : *****

Figure 11

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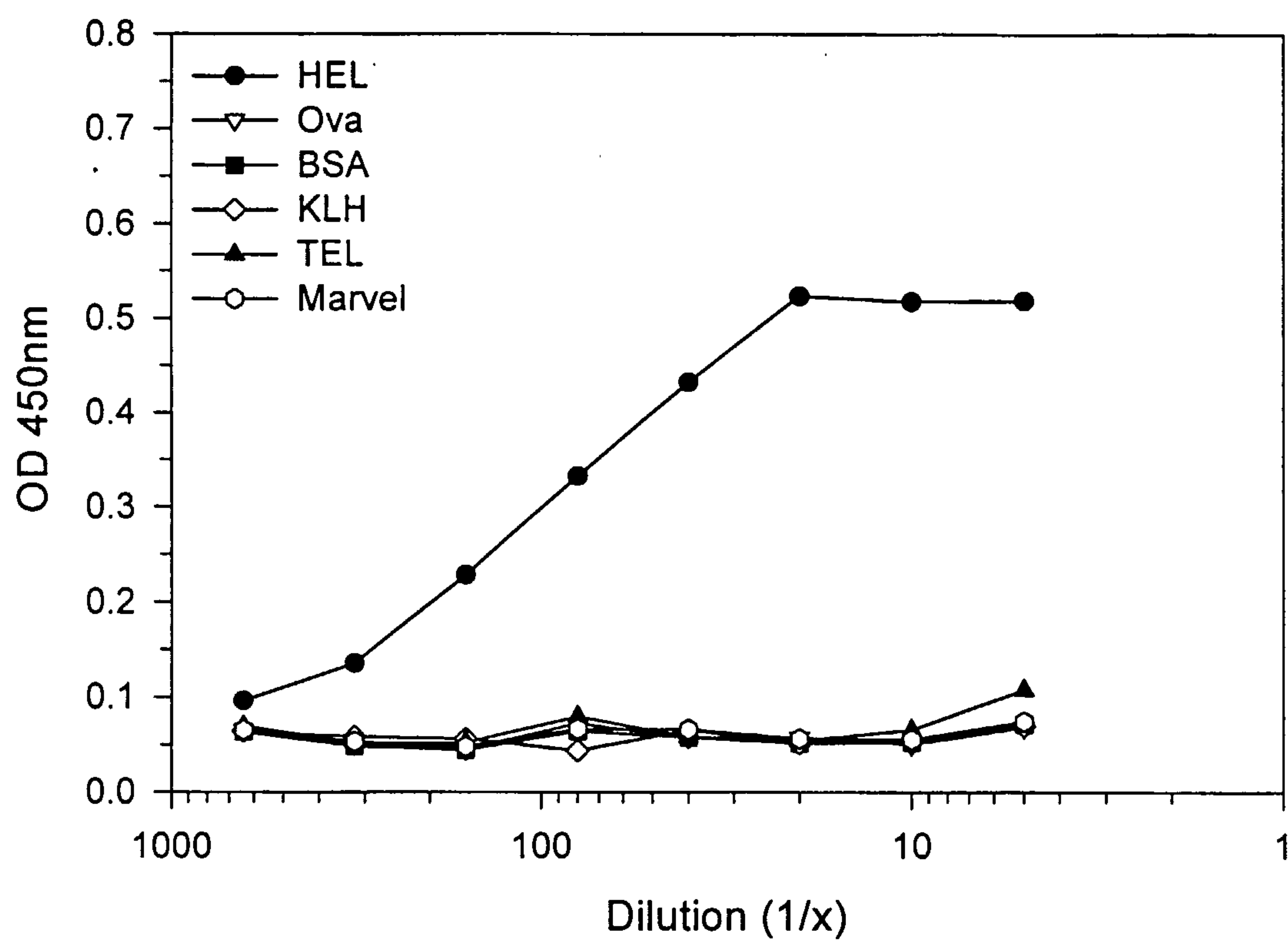


Figure 12

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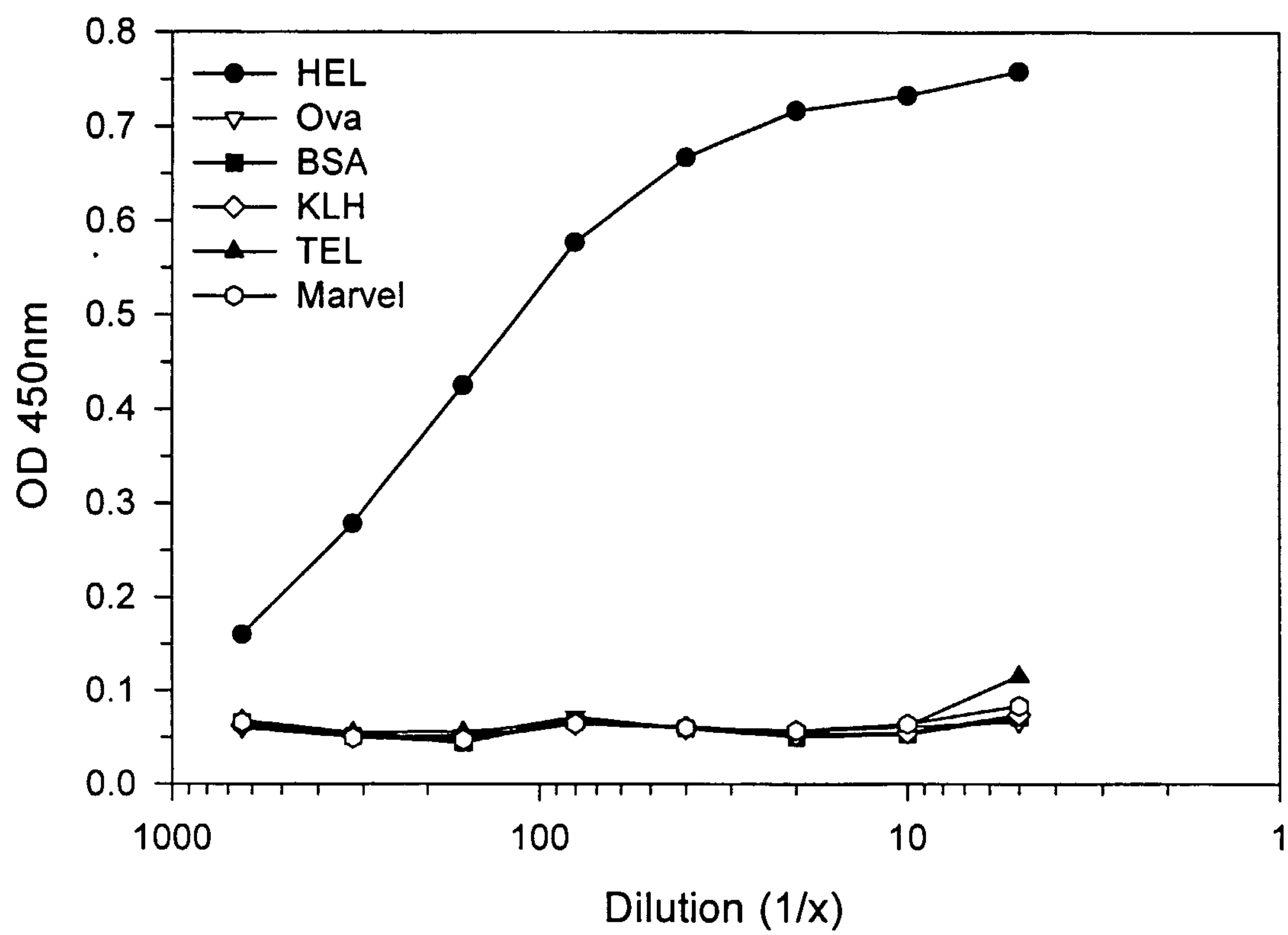


Figure 13

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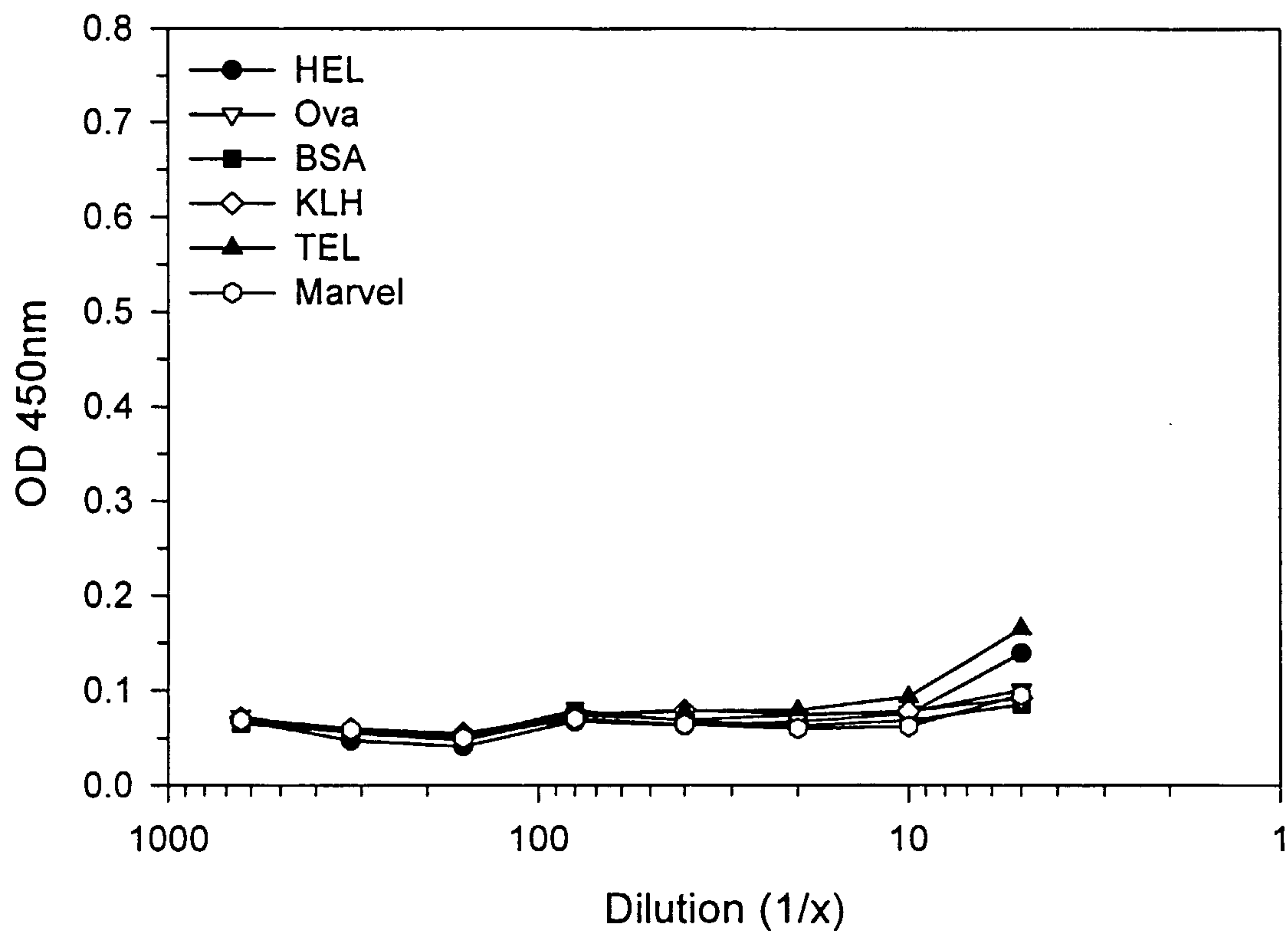


Figure 14

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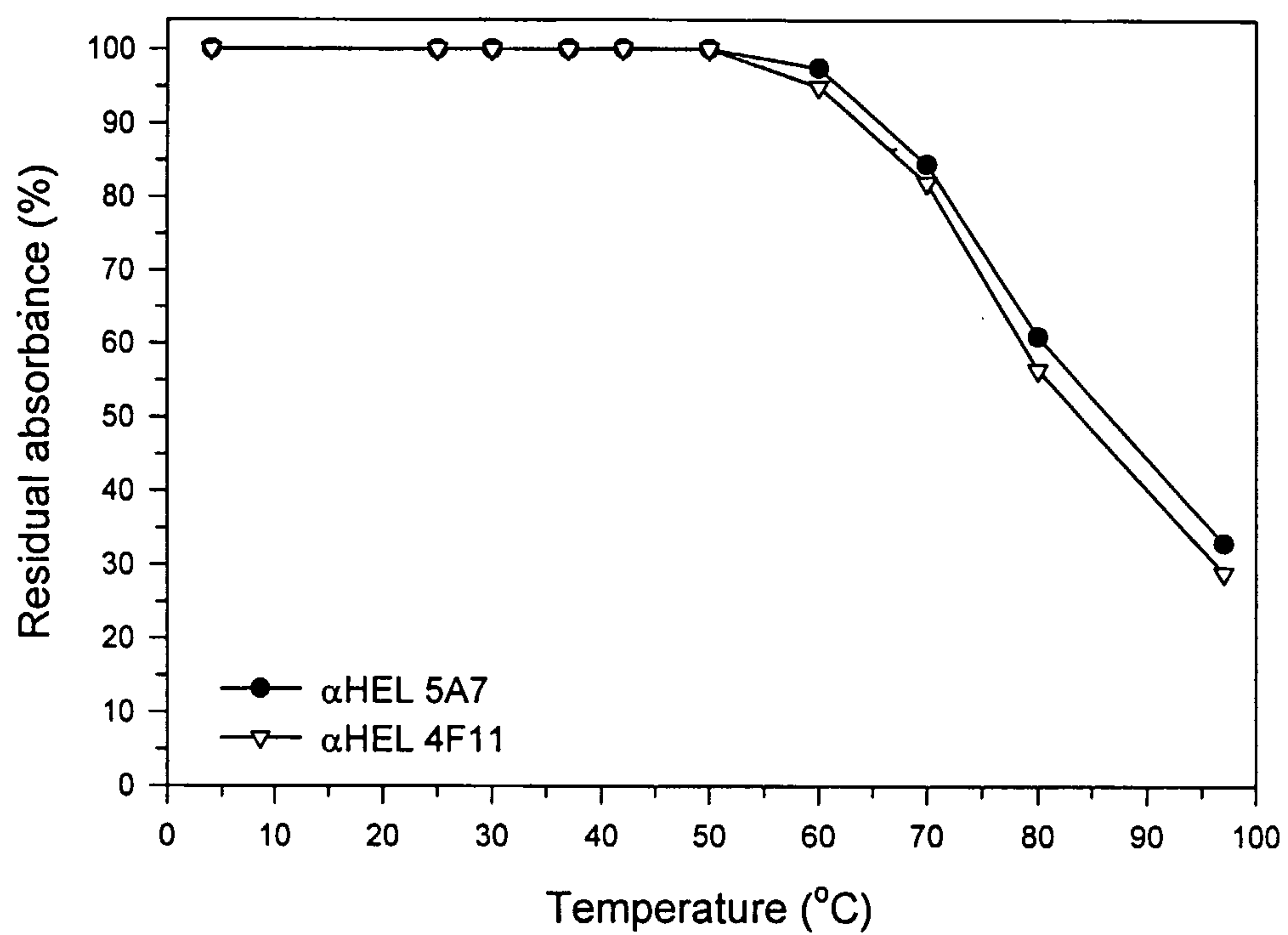


Figure 15

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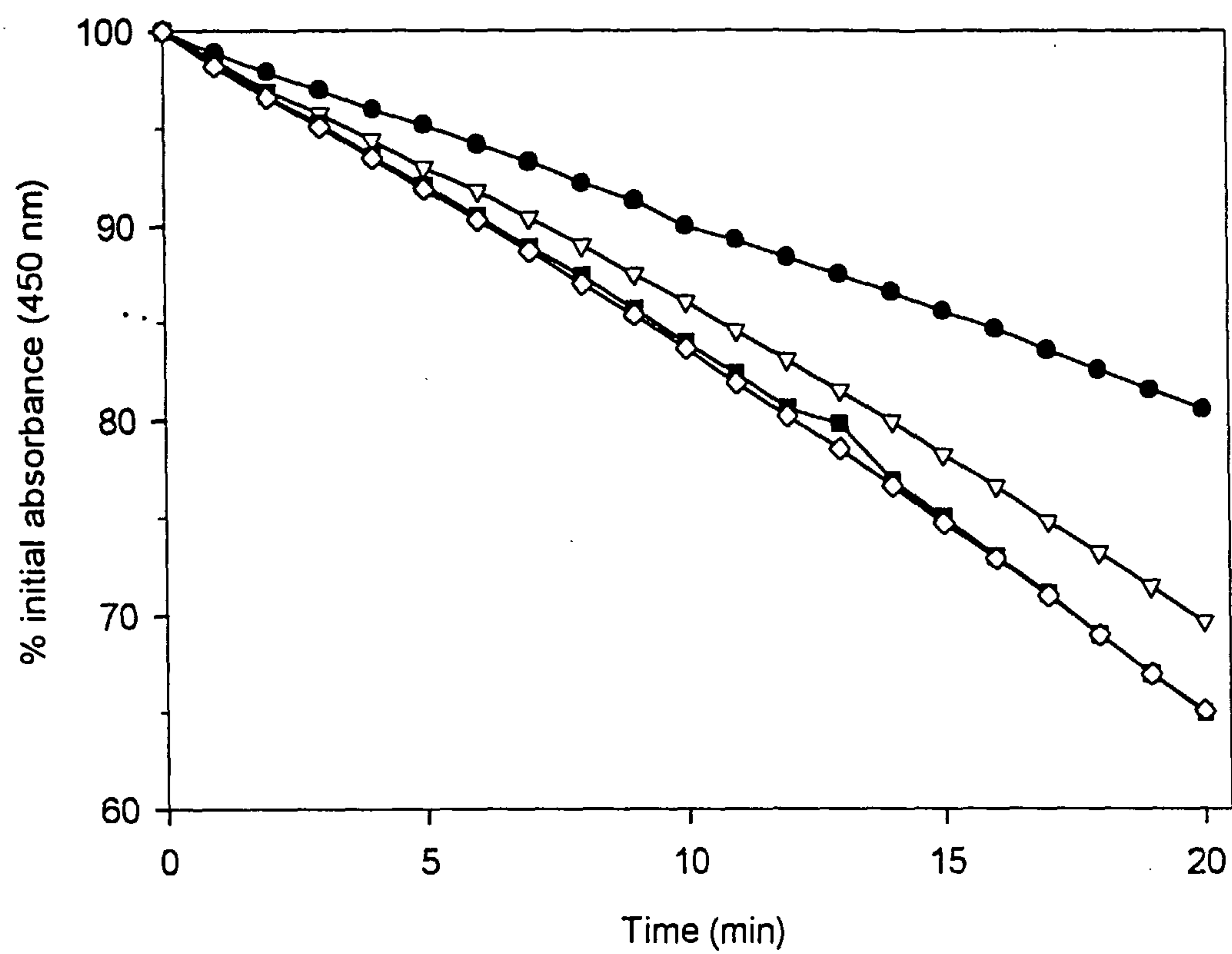


Figure 16

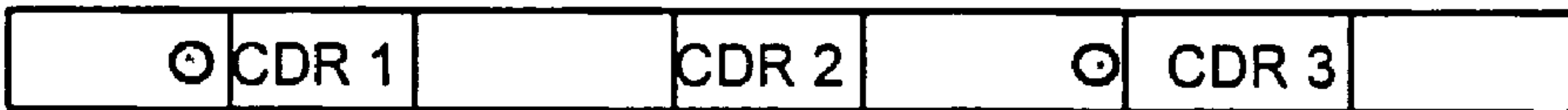
IgNAR type I



IgNAR type II



Human VH



Camel VHH



Cattle VH

