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(54) Title: SHEEP PRIMERS

(57) Abstract: The present invention provides primer sets for the isolation of sheep antibody variable region sequences. Further provided are antibodies comprising these regions which are useful in the development of therapeutics and, in particular, in the development of humanised antibodies.



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SHEEP PRIMERS

The present invention relates primer sets and their use in the isolation of sheep antibody variable regions which are useful as reagents for the development of therapeutic, prognostic and diagnostic agents. In particular, these antibodies may be humanised.

As ethical considerations generally preclude the immunisation of humans for the production of antibodies, alternative species are used. In particular, sheep can be used as a source of antibodies for humanisation.

Antibodies have long been viewed as potential agents for therapeutic interventions and targeted drug delivery, largely with a view to exploiting the combination of high specificity and affinity of the antibody-antigen interaction. Hence, the number of antibody variable region heavy and light gene sequences, their organisation into sequence specific families and their relative utilisation is of interest with respect to antibody engineering. For example, in man there are a large number of V_H , V_L and D region genes and several J region (J_H and J_L) genes which make up the variable region (nb. light chains have no D regions). Combination of such sequences provide large primary repertoires of variable heavy and light chain sequences with assembly of light and heavy chains in the B cell providing further combinatorial diversity. In contrast, in the sheep, it has been proposed that a process of antigen-independent hypermutation of antibody variable regions is the principle means of primary antibody diversity from a small initial pool of sequences (Reynaud *et al.*, 1991, Cell 64:995-1005; Reynaud *et al.*, 1995, Cell 80:115-125) although more recently it has been suggested that combinatorial rearrangement makes a much larger contribution, and somatic hypermutation makes a much smaller contribution to the generation of diversity within the sheep antibody repertoire (Jenne *et al.*, 2003, J. Immunol. 170:3739-3750).

Hybridoma technology for the production of monoclonal antibodies is well-known and historically used mice as the immunised species. Production of monoclonal antibodies requires immortalization of splenocytes by somatic fusion to a myeloma cell line partner to produce a hybridoma. One disadvantage of hybridomas is that, although they can be immortal, their maintenance may depend on a feeder cell layer and they may be genetically unstable. A second disadvantage is that only a fraction of B cells from a spleen population can be recovered as stable lines after the

fusion and screening process. Since the inception of hybridoma technology (Kohler, G. & Milstein C., 1975, Nature 256:495-497) efforts to improve the efficiency and stability of monoclonal antibody-producing cell lines have not brought about substantial progress.

5 Other methods include the isolation of antibodies from bacterially expressed libraries, *e.g.* phage display libraries but these are limited in that they are restricted by practical limits to the size of libraries meaning that all possible antibody sequences may not be represented within a library.

One advantageous method is the selected lymphocyte antibody method
10 (Babcook *et al.*, 1996, Proc. Natl. Acad. Sci, 93, 7843-7848; WO 92/02551; de Wildt *et al.*, 1997, J. Immunol. Methods, 207:61-67 and in Lagerkvist, *et al.*, 1995, BioTechniques 18(5):862-869) which enables cells producing high affinity antibodies generated during *in vivo* immune responses to be isolated from any species. A major difficulty after that isolation then lies in the ability to obtain the antibody sequence, in
15 particular the variable region sequences.

Known methods for obtaining DNA sequences coding for antibody variable regions exist, *e.g.* cloning rearranged genomic DNA, or mRNA via cDNA. Methods to obtain full length cDNAs or to extend short cDNAs are also known in the art, for example RACE (Rapid amplification of cDNA ends; *e.g.* Frohman *et al.*, 1988, Proc.
20 Natl. Acad. Sci USA 85:8998-9002) and for example, using Marathon™ technology (Clontech Laboratories, Inc.). However, the mechanism by which antibody diversity is generated causes particular issues when considering the cloning methodology. In humans, mice and rats, due to the large number of possible variable region sequences, a considerable problem arises in that in order to ensure that all possible expressed
25 variable region sequences will be represented, the utilisation of large numbers of primers, *i.e.* a high complexity primer set, is required. For example, the usual method for obtaining antibody sequence is to use primers designed to anneal to the mature nucleic sequence encoding the antibody, for example within framework 1; often large numbers of possible variable region sequences exist necessitating a large primer set to
30 ensure coverage. Thus, primer design is influenced by the mechanism by which antibody diversity is generated. In particular, there is a need for sets of primers that will efficiently recover a large majority of possible expressed variable regions, ensuring cloning of said regions from a single, or small numbers of, B cells or other

antibody producing cells without alteration to variable region sequences. Sets of suitable primers that permit coverage of most or all of the possible expressed variable region sequences in the sheep have not been previously described. Indeed, a limited number of sheep antibody sequences are publicly available (see, for example, 5 Charlton *et al.*, 2000, J. Immunol. 164:6221-6229, who use a bacteriophage library approach).

Thus, the invention described herein includes novel primer sets which provide coverage of a large majority of possible expressed variable regions in the sheep, permitting the isolation of a nucleic acid comprising a sheep antibody variable region 10 sequence expressed by a single or low numbers of antibody producing cells, for example an antibody of interest from B cells, B cell lines, or other antibody producing cells, *e.g.* a hybridoma cell. It will be apparent to one skilled in the art that use of the primer sets provided includes the use of a forward primer set in conjunction with a reverse primer set.

15 Accordingly, provided is an oligonucleotide primer set comprising or consisting of:

- (a) SEQ ID NO:1 and/or SEQ ID NO:2 and/or SEQ ID NO:3.

Suitably, use of any one of the primer sets defined in (a), above, in a PCR (polymerase chain reaction) enables the isolation of a nucleic acid comprising the 20 variable region sequence of a sheep antibody heavy chain. In one embodiment, the primer set comprises SEQ ID NO:1 (Set A). Most preferably, the primer set comprises or consists of SEQ ID NOS:1, 2 and 3 (Set B), and use of said primer set in a PCR enables the isolation of a nucleic acid comprising the variable heavy chain region sequence a sheep antibody heavy chain; and hence the corresponding 25 polypeptide sequence of those regions. Such nucleic acid sequences are of use in cloning the full length sequence of a sheep antibody heavy chain. They are also of use in designing a humanised antibody or fragment thereof.

Table 1

30

SEQ ID NO:	Primer Name	Primer Sequence
1	VH	atgaaccactgtggaccctctc
2	JH1	tgaggagacggtgaccaggagtc
3	JH2	tgaagaacgctgatcaggagcc

SEQ ID NO:	Primer Name	Primer Sequence
4	Vk 1/2.1	atgaggttccctgctcagctcctg
5	Vk 4	atgagggcccccactcagctcctc
6	Vk 3	atgggtgtcctgactcaactctcc
7	Jk1	tttgatttcacgttggtccc
8	Jk2	ttgatctctagcttggtcc
9	VL1	atggcctgggtcccctctgctcctc
10	VL2	atggcctgggctctgctcctcatc
11	VL6	atggcctggaccctctcctgctg
12	JL	accagagcgggtcagcctggtcc
13	VHinner	cacacaaagcttgccgccaccatgaaccactgtggaccctcctc
14	JH1inner	cacacatgggccccttcgtagaagcgctcgagacgggtgaccaggagtc
15	JH2inner	cacacatgggccccttcgtagaagcgctcgagacgctgatcaggagcc
16	Vk1inner	cacacaaagcttcgaagccgccaccatgaggttccctgctcagctcctg
17	Vk2inner	cacacaaagcttcgaagccgccaccatgagggcccccactcagctcctc
18	Vk3inner	cacacaaagcttcgaagccgccaccatgggtgtcctgactcaactctcc
19	JKinner	cacacacgtacgtttgatttcacgttggtccc
20	VL1inner	cacacaaagcttcgaagccgccaccatggcctgggtcccctctgctcctc
21	VL2inner	cacacaaagcttcgaagccgccaccatggcctgggctctgctcctcatc
22	VL3inner	cacacaaagcttcgaagccgccaccatggcctggaccctctcctgctg
23	JLinner	cacacacgtacgaccagagcgggtcagcctggtcc

Primers of SEQ ID NOS:1, 4-6, 9-11, 13, 16-18 and 20-22 are forward primers; SEQ ID NOS:2, 3, 7, 8, 12, 14, 15, 19 and 22 are reverse primers.

A primer set as used herein includes a primer set comprising at least one primer. In particular, the primer sets described herein are useful for the isolation of sheep antibody variable regions.

It will be apparent to one skilled in the art that, in particular, the amount of template will influence the selection of primer sets. Where the template DNA is obtained from a single or low numbers of antibody producing cells, for example but not limited to, using reverse transcription of mRNA into cDNA, one may screen for the presence of template before committing the use of expensive reagents. Alternatively, no screen is performed.

A DNA template may be prepared using standard means known in the art, for example by cloning rearranged genomic DNA, or mRNA via cDNA (reviewed by *e.g.* Mountain and Adair, 1992, Biotech. Gen. Eng. Rev. 10:1-142; Ehlich and Koppers

1995, Curr. Opin. Immunol. 7:281-284). In one embodiment, RNA obtained from a single antibody producing cell by lysis is used to prepare a DNA template. It may be preferable to culture the antibody producing cells to increase the cell number. In a particular embodiment, a single antibody producing cell, e.g. a B cell or a hybridoma cell, is cultured to increase the cell number before preparation of cDNA from mRNA for use as a template for cloning in order to determine the sequence of, in particular, the variable heavy and light chain regions of a sheep antibody of interest. In another embodiment, multiple antibody producing cells are used as a source for deriving template DNA, which cells may or may not be cultured to increase cell number. In one embodiment, the multiple antibody producing cells are clonal. Alternatively, said cells are not clonal.

The invention also provides an oligonucleotide primer set comprising or consisting of:

- (b) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6;
- (c) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, and SEQ ID NO:7, and/or SEQ ID NO:8; or
- (d) SEQ ID NO:7 and/or SEQ ID NO:8.

Suitably, use of the primer sets defined in (b) to (d), above, in a PCR enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain. In one embodiment, the primer set comprises SEQ ID NOS:4 to 6 (Set C). Most preferably, the oligonucleotide primer set comprises or consists of SEQ ID NOS:4 to 8 (Set D) and use of said primer set in a PCR enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain; and hence the corresponding polypeptide sequence. Such nucleic acid sequences are of use in cloning the full length sequence of a sheep antibody kappa light chain. They are also of use in designing humanised antibodies or fragments thereof.

The invention further provides primer sets designed for the isolation of nucleic acids comprising the variable regions of sheep antibody lambda chain sequences. Accordingly, provided is an oligonucleotide primer set comprising or consisting of: .

- (e) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11;
- (f) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11, and SEQ ID NO:12; or
- 5 (g) SEQ ID NO:12.

Suitably, use of any one of the primer sets defined in (e) to (g), above, in a PCR enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain. In one embodiment, the oligonucleotide primer set comprises SEQ ID NO:9 to 11 (Set E). Most preferably, the primer set comprises
10 or consists of SEQ ID NOS:9 to 12 (Set F) and enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain; and hence the corresponding polypeptide sequence. Such nucleic acid sequences are of use in cloning the full length sequence of a sheep antibody lambda light chain sequence. They are also of use in designing humanised antibodies or fragments
15 thereof.

It can be desirable to amplify a PCR product to increase specific yield and to add restriction sites to facilitate cloning, *e.g.* into an expression vector. Thus, the invention further provides oligonucleotide primer sets for performing nested PCR.
20 These primer sets can alternatively be used to perform a first round PCR.

Accordingly, provided is an oligonucleotide primer set comprising or consisting of:

- (h) SEQ ID NO:13 and/or SEQ ID NO:14 and/or SEQ ID NO:15.

Suitably, use of any of the primer sets defined in (h), above, in a PCR enables
25 the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain. These primer sets have the following restriction sites incorporated: 5'-Hind III (), 5'-Apa I () and 3'-Xho I (). It will be understood by one skilled in the art that any restriction site desired may be incorporated into the primers, for example by replacing the restriction
30 site encoded with coding for the desired restriction site. In another embodiment the upstream restriction site and bases for annealing to the vector may be omitted. This applies to any of the primers with restriction sites within this application, ie those for heavy and light chains. In one embodiment, the primer set comprises SEQ ID NO:13

(Set G). Most preferably, the primer set comprises or consists of SEQ ID NO:13, SEQ ID NO:14 and SEQ ID NO:15 (Set H) and use of said primer set in a PCR enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain; and hence the corresponding polypeptide sequence.

- 5 Such nucleic acid sequences are of use in cloning full length sheep antibody heavy chain sequences. They are also of use in designing humanised antibodies or fragments thereof.

The invention also provides an oligonucleotide primer set comprising or consisting of:

- 10 (i) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18;
- (j) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18, and SEQ ID NO:19; or
- (k) SEQ ID NO:19.

- 15 Suitably, use of the primer sets defined in (i) to (k), above, in a PCR enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain. These primer sets have the following restriction sites incorporated: 5'-Sfu I (*italic*), 5'-Hind III (underlined) and 3'-Bsi WI (underlined and italic). It will be understood by one skilled in the art that any restriction site desired
- 20 may be incorporated into the primers, for example by replacing the restriction site encoded with coding for the desired restriction site. In one embodiment, the oligonucleotide primer set comprises SEQ ID NO:16, SEQ ID NO:17 and SEQ ID NO:18 (Set I). Most preferably, the primer set comprises or consists of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18 and SEQ ID NO:19 (Set J) and enables the
- 25 isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain; and hence the corresponding polypeptide sequence. Such nucleic acid sequences are of use in cloning full length sheep antibody kappa light chain sequences. They are also of use in designing humanised antibodies or fragments thereof.

- 30 The invention further provides an oligonucleotide primer set comprising or consisting of:

- (l) at least one primer selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22;

- (m) at least one primer selected from the group consisting of SEQ ID NO: 20, SEQ ID NO:21 and SEQ ID NO:22, and SEQ ID NO:23; or
- (n) SEQ ID NO:23.

Suitably, use of any one of the primers defined in (l) to (n), above, in a PCR
5 enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain. These primer sets have the following restriction sites incorporated: 5'-Sfu I (*italic*), 5'-Hind III (underlined) and 3'-Bsi WI (*italic* and underlined). It will be understood by one skilled in the art that any restriction site desired may be incorporated into the primers, for example by replacing the restriction
10 site encoded with coding for the desired restriction site. In one embodiment, the oligonucleotide primer set comprises SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22 (Set K). Most preferably, the primer set comprises or consists of SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23 (Set L) enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep
15 antibody lambda light chain; and hence the corresponding polypeptide sequence of that region. Such antibody regions isolated are useful for the cloning of the full sequence of a sheep antibody lambda light chain. They are also of use in designing humanised antibodies or fragments thereof.

It will be apparent to one skilled in the art that, in particular, the amount of
20 template, and hence the number of PCRs possible, will influence the selection of primer sets. Preferably, one primer set is used to isolate a nucleic acid comprising the variable heavy chain region sequence, and one primer set is used to isolate a nucleic acid comprising the variable light chain region sequence of a sheep antibody. Alternatively, two or more primer sets may be used. Suitably: (1) any one of the sets
25 defined in (a) and (h), above, may be used in conjunction with any one of the sets defined in (b)-(d), or (i)-(k), above, enabling the isolation of a nucleic acid comprising the variable region sequences of a sheep antibody heavy chain and kappa light chain; and (2) any one of the sets defined in (a) and (h), above, may be used in conjunction with any one of the sets defined in (e)-(g) or (l)-(n), above, enabling the isolation of a
30 nucleic acid comprising the variable region sequences of a sheep antibody heavy chain and lambda light chain. In particular, when cloning from a single or small numbers of antibody producing cells, it is desirable to perform the minimum number of PCRs; hence, it is desirable to use the optimal primer set for maximum coverage of

possible antibody sequences. Thus, preferably, primer set B or primer set H, above, is used in conjunction with primer set D or J (or primer set F or L), above, and even more preferably, primer set B is used in conjunction with primer set D (or primer set F) and primer set H is used in conjunction with primer set J (or primer set L) enables the isolation of nucleic acids comprising the variable region sequence of the heavy and light chain region sequences of a sheep antibody. Such nucleic acid sequences are of use in cloning full length sheep antibody heavy and light chain sequences.

The invention described herein also provides methods for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain comprising use of an oligonucleotide primer set in a PCR, wherein the primer set comprises or consists of:

(I) SEQ ID NO:1 and/or SEQ ID NO:2 and/or SEQ ID NO:3.

In one embodiment, any one of the primer sets defined in (I), above, is used in a PCR in conjunction with template derived from at least one antibody producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a most preferred embodiment, the method comprises use of the oligonucleotide primer set consisting of Set B, defined above, in a PCR, preferably a first PCR, and enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain. Alternatively, any of said primer sets defined in (I), above, may be used in a second PCR, using template derived from a first PCR. In a particular embodiment, any one of the primer sets defined in (I), above, can be used in a first and a second PCR.

The invention further provides a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain comprising use of an oligonucleotide primer set in a PCR, wherein the primer set comprises or consists of:

(II) SEQ ID NO:13 and/or SEQ ID NO:14 and/or SEQ ID NO:15.

In one embodiment, any one of the primer sets defined in (II), above, is used in a PCR. In a most preferred embodiment, the method comprises use of the primer set of Set H, defined above, in a PCR, most preferably a second PCR, using as template nucleic acid derived from a first PCR, said method enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain. Alternatively, any of the primer sets defined in (II), above, is used in a first PCR in

conjunction with template derived from at least one antibody producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a particular embodiment, the primers set defined in (II), above, can be used in a first and a second PCR.

5 In a more preferred embodiment, the method comprises two PCRs; a first PCR and a second PCR using as template, DNA from the first PCR. Accordingly, provided is a method for the isolation of the variable region of a sheep antibody heavy chain comprising use of at least one primer set in a first PCR and at least one primer set in a second PCR, said first primer set comprising or consisting of any one of the
10 primer sets defined in (I), above, and said second primer set comprising or consisting of any one of the primer sets defined in (II), above. In a most preferred embodiment, the method comprises use of primer Set B, defined above, in a first PCR and primer Set H, defined above, in a second PCR using as template, DNA from the first PCR. Said method enables the isolation of a nucleic acid comprising the variable region
15 sequence of a sheep antibody heavy chain. Preferably, said PCRs are performed individually. Alternatively, said PCRs are performed jointly. Such antibody regions are useful for the cloning of the full sequence of a sheep antibody heavy chain.

In further embodiments, more than two PCRs may be used, for example, but not limited to three, four, five or more PCRs. Thus, one skilled in the art will
20 appreciate that if, for example, fewer numbers of cycles are used then one or more additional PCRs may be performed to produce the amplification required.

The invention described herein also provides methods for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain, said method comprising use of an oligonucleotide primer set in a PCR wherein
25 the primer set comprises or consists of:

- (III) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6;
- (IV) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, and SEQ ID NO:7, and/or SEQ ID
30 NO:8; or
- (V) SEQ ID NO: 7 and/or SEQ ID NO:8;

In one embodiment, any one of the primer set defined in, (III)-(V), above, is used in a PCR in conjunction with template derived from at least one antibody

producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a most preferred embodiment, the method comprises use of the oligonucleotide primer Set D, defined above, in a PCR, preferably a first PCR enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain. Alternatively, any one of the primer sets defined in (III)-(V), above, may be used in a second PCR in conjunction with template derived from a first PCR. In a particular embodiment, any one of the primer sets defined in (III)-(V), above, can be used in a first and a second PCR.

Also, provided is a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain, said method comprising use of an oligonucleotide primer set in a PCR, wherein the primer set comprises or consists of:

- (VI) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18;
- (VII) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18, and SEQ ID NO:19; or
- (VIII) SEQ ID NO:19.

In one embodiment, any one of the primer sets defined in (VI)-(VIII), above, is used in a PCR. In a most preferred embodiment, the method comprises use of the oligonucleotide primer Set I, defined above, in a PCR, preferably a second PCR and enables the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain. Alternatively, any of the primer sets defined in (VI)-(VIII), above, is used in a first PCR in conjunction with template derived from at least one antibody producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a particular embodiment, any one of said primer sets can be used in a first and a second PCR.

In a more preferred embodiment, the method comprises two PCRs, a first PCR and a second PCR using as template, DNA from the first PCR. Accordingly, provided is a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain comprising use of at least one primer set in a first PCR and at least one primer set in a second PCR, said first primer set comprising or consisting of any one of the primer sets defined in (III)-(V), above, and said second primer set comprising or consisting of any one of the primer sets defined

in (VI)-(VIII), above. In a most preferred embodiment, the method comprises use of primer Set D, defined above, in a first PCR and primer Set J, defined above, in a second PCR, using as template, DNA from the first PCR enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain. Preferably, the PCRs are performed individually. Alternatively, said PCRs are performed jointly. Such antibody regions isolated are useful for the cloning of the full sequence of a sheep antibody kappa light chain.

In further embodiments, more than two PCRs may be used, for example, but not limited to three, four, five or more PCRS. Thus, one skilled in the art will appreciate that if, for example, fewer numbers of cycles are used then one or more additional PCRs may be performed to produce the amplification required.

The invention described herein further provides a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain comprising use of an oligonucleotide primer set in a PCR, wherein the primer set comprises or consists of:

- (IX) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11;
- (X) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11, and SEQ ID NO:12; or
- (XI) SEQ ID NO:12.

In one embodiment, any one of the primer sets defined in (IX)-(XI), above, is used in a first PCR in conjunction with template derived from at least one antibody producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a most preferred embodiment, the method comprises use of the oligonucleotide primer Set F, defined above, in a PCR, preferably a first PCR enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain. Alternatively, any one of the primer sets defined in (IX)-(XI), above, may be used in a second PCR in conjunction with template derived from a first PCR. In a particular embodiment, any one of said primer sets can be used in a first and a second PCR.

Further provided is a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain, said method

comprising use of an oligonucleotide primer set in a PCR, wherein the primer set comprises:

- (XII) at least one primer selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22;
- 5 (XIII) at least one primer selected from the group consisting of SEQ ID NO: 20, SEQ ID NO:21 and SEQ ID NO:22, and SEQ ID NO:23; or
- (XIV) SEQ ID NO:23.

In one embodiment, any one of the primer sets defined in (XII)-(XIV), above, is used in a PCR. In a most preferred embodiment, the method comprises use of the
10 oligonucleotide primer set L, defined above, in a PCR, most preferably a second PCR, using as template nucleic acid derived from a first PCR, said method enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain. Alternatively, any one of the primer sets defined in (XII)-(XIV), above, is used in a first PCR in conjunction with template derived from
15 at least one antibody producing cell of interest, for example by reverse transcription of mRNA into cDNA. In a particular embodiment, any one of said primer sets can be used in a first and a second PCR.

In a more preferred embodiment, the method comprises two PCRs, a first PCR and a second PCR using as template, DNA from the first PCR. Accordingly,
20 provided is a method for the isolation of a nucleic acid comprising the variable region sequence of a sheep lambda light chain comprising use of at least one primer set in a first PCR and at least one primer set in a second PCR, said first primer set comprising or consisting of the primer set defined in (IX)-(XI), above, and said second primer set comprising or consisting of any one of the primer sets defined in (XII)-(XIV), above.
25 In a most preferred embodiment, the method comprises use of primer Set F, defined above, in a first PCR and primer Set L, defined above, in a second PCR, using as template, DNA from the first PCR enabling the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain. Preferably, said PCRs are performed individually. Alternatively, said PCRs are performed jointly.
30 Such antibody regions isolated are useful for the cloning of the full sequence of a sheep antibody lambda light chain.

In further embodiments, more than two PCRs may be used, for example, but not limited to three, four, five or more PCRs. Thus, one skilled in the art will

appreciate that if, for example, fewer numbers of cycles are used then one or more additional PCRs may be performed to produce the amplification required.

Thus, using the methods of the invention, the sequence of at least the variable
5 regions of both the heavy and light chains of a sheep antibody can be determined by use of the primer sets and methods described above.

All methods described herein for the isolation of at least the variable region of a sheep antibody sequence comprise the performance of at least one PCR and provision of:

- 10 1. at least one primer set as provided herein;
2. optionally one or more additional primer sets;
3. template DNA from an antibody producing cell of interest; and
4. PCR performance reagents.

15 It will be understood by one skilled in the art that the additional primer set as required can be a forward or a reverse primer set depending upon whether the primer set provided herein is a forward or a reverse primer set. Such additional primer sets may be designed to anneal to any desired region of an antibody, particularly a sheep antibody. It will also be understood that suitable use of primer sets requires that
20 forward and reverse primers are used.

Reverse transcription and PCR performance reagents include reagents as known in the art, such as without limitation, DNA polymerase, dNTPs, buffers such as but not limited to Tris buffers, cations such as Mg^{2+} , detergents, for example but not limited, to Nonidet NP-40, reducing agents such as dithiothreitol or
25 mercaptoethanol and RNAasin.

In particular, when cloning an antibody from a single or small numbers of antibody producing cells, it is desirable to perform the minimum numbers of PCRs necessary. Therefore, most preferably, any one of the primer Sets B, D, F, H, J and L provided herein are utilised in a single PCR. One skilled in the art will understand
30 that when utilising Sets A, C, E, G, I or K, preferably in a single PCR, suitable use means that one or more additional primers will be required. Alternatively, one or more PCRs may be performed utilising a portion of the primers present within a primer set, for example but without limitation, one or two or more primers from any

one of Sets A to L, plus additional primers where required, may be utilised in a single PCR.

It will be apparent to one skilled in the art that one or more of the primer sets A, C, E, G, I or K (which are designed to complement the variable regions of an antibody, in particular a sheep antibody), or one or more primers comprising any of the sets, may be used in combination with primers designed to anneal to downstream regions of a nucleic acid encoding an antibody of interest. In one embodiment, one or more of the primer sets A, C, E, G, I or K, or one or more primers comprising any of the sets, may be used for RACE, where the source cDNA can be homopolynucleotide tailed at the 3'-end using terminal transferase enzyme, providing a location for strand synthesis using a complementary primer.

It will be also be apparent to one skilled in the art that modifications to the primers described herein can be made as desired. For example, the sequence of one or more primers may be shortened or lengthened. In particular, the restriction sites may be altered or removed altogether. Suitable annealing temperatures for using such primers may be selected as known in the art, for example but without limitation, lowering the annealing temperature may allow less stringent priming and result in a PCR product where previously, at higher temperatures, no product was isolated.

Any antigen of interest can be used for immunisation of an animal, particularly a sheep. Such antigens include any substance that can be recognised by an antibody, including proteins, glycoproteins and carbohydrates. Antigens also include biologically active proteins, such as hormones, cytokines, and their cell surface receptors, bacterial or parasitic cell membranes or purified components thereof, and viral antigens. Antigens may also include cells. Such cells may express endogenous proteins as antigens, for example, presented on the cell surface. Such cells may also transiently or stably express antigens. Antigens of particular interest are those involved in diseases and which show aberrant expression or aberrant activity associated with a disease. Such diseases include cancers, inflammatory disorders and immune disorders.

Antibody sequences provided using the methods of the invention described herein include sequences of functionally active fragments, derivatives or analogues and may be, but are not limited to, polyclonal, monoclonal, bi-, tri- or tetra-valent antibodies, humanised or chimeric antibodies, single chain antibodies, Fab fragments,

Fab' and Fab'₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Humanised antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a
5 framework region from a human immunoglobulin molecule (see *e.g.*, US 5,585,089). Antibodies include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.* molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (*e.g.* IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin
10 molecule.

An antibody, optionally conjugated to a therapeutic moiety, can be used therapeutically alone or in combination with a cytotoxic factor(s) and/or cytokine(s). In particular, antibodies provided using the primer sets and methods of the invention can be conjugated to a therapeutic agent, such as a cytotoxic agent, a radionuclide or
15 drug moiety to modify a given biological response. The therapeutic agent is not to be construed as limited to classical chemical therapeutic agents. For example, the therapeutic agent may be a drug moiety which may be a protein or polypeptide possessing a desired biological activity. Such moieties may include, for example and without limitation, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria
20 toxin, maytansinoid (DM1), a protein such as tumour necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin; angiogenin, gelonin, dolstatins, minor groove-binders, bis-iodo-phenol mustard, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-
25 1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Therapeutic agents also include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples include taxol, cytochalasin B,
30 gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vinca alkaloids, *e.g.* vincristine, vinblastine, 4-desacetylvinblastine-3-carbohydrazide, vindesine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids,

procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, but are not limited to, anti-folates (*e.g.* aminopterin and methotrexate), antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine, 5-fluoro-2'-deoxyuridine),
 5 alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin, adriamycin, idarubicin, morpholinodoxorubicin, epirubicin, doxorubicin hydrazides),
 10 antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins, CC-1065, enediyenes, neocarzinostatin), and anti-mitotic agents (*e.g.* vincristine and vinblastine). See Garnett, 2001, *Advanced drug Delivery Reviews* 53:171-216 for further details.

Other therapeutic moieties may include radionuclides such as ^{131}I , ^{111}In and
 15 ^{90}Y , Lu^{177} , Bismuth ^{213} , Bismuth ^{212} , Californium ^{252} , Iridium ^{192} and Tunsten ^{188} /Rhenium ^{188} , $^{211}\text{astatine}$; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Techniques for conjugating such therapeutic agents to antibodies are well known in the art (see, *e.g.* Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting
 20 Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.*, eds., 1985 pp. 243-56, ed. Alan R. Liss, Inc; Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery*, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53, Marcel Dekker, Inc.; Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*; Pinchera *et al.*, 1985, eds., pp. 475-506;
 25 "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabelled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), 1985, pp. 303-16, Academic Press; Thorpe *et al.*, 1982 "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates",
 30 *Immunol. Rev.*, 62:119-58 and Dubowchik *et al.*, 1999, *Pharmacology and Therapeutics*, 83, 67-123).

The antibody sequences provided using the primer sets and methods of the invention can be modified, for example but without limitation, by the covalent

attachment of any type of molecule. Preferably, said attachment does not impair immunospecific binding. In one aspect, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate (see US 4,676,980).

In other embodiments, the antibody sequences provided using the primer sets
5 and methods of the invention are provided as fusion proteins of the antibodies (or functionally active fragments thereof), for example but without limitation, where the antibody or fragment thereof is fused via a covalent bond (*e.g.* a peptide bond), at optionally the N-terminus or the C-terminus, to an amino acid sequence of another protein (or portion thereof; preferably at least a 10, 20 or 50 amino acid portion of the
10 protein). Preferably the antibody, or fragment thereof, is linked to the other protein at the N-terminus of the constant domain of the antibody. In another aspect, an antibody fusion protein may facilitate depletion or purification of a polypeptide as described herein, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

15 Where the fusion protein is an antibody fragment linked to an effector or reporter molecule, this may be prepared by standard chemical or recombinant DNA procedures. A preferred effector group is a polymer molecule, which may be attached to the modified Fab fragment to increase its half-life *in vivo*.

The polymer molecule may, in general, be a synthetic or a naturally occurring
20 polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, *e.g.* a homo- or hetero- polysaccharide.

Particular optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.
25 Particular examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Particular naturally occurring polymers include lactose, amylose, dextran,
30 glycogen or derivatives thereof.

“Derivatives” as used above is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be

appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, preferably from 5000 to 40000Da and more preferably from 25000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product. Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 25000Da to 40000Da.

Particularly preferred polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 25000Da to about 40000Da.

Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond.

Where desired, the antibody fragment may have one or more effector or reporter molecules attached to it. The effector or reporter molecules may be attached to the antibody fragment through any available amino acid side-chain or terminal amino acid functional group located in the fragment, for example any free amino, imino, hydroxyl or carboxyl group.

An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially, for example from Nektar Therapeutics, Inc (Huntsville, AL), or may be prepared from commercially available starting materials using conventional chemical procedures.

Standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector or reporter molecule either before or after reaction with the activated polymer as appropriate may be used. Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 90/09195, WO 89/01476, WO 99/15549 and
5 WO03/031581. Alternatively, where the effector or reporter molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP 0392745.

Most preferably antibodies are attached to poly(ethyleneglycol) (PEG) moieties. Preferably, a modified Fab fragment is PEGylated, *i.e.* has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.* according to the method disclosed in EP 0948544 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris
15 and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one embodiment, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified
20 hinge region. A lysine residue may be covalently linked to the maleimide group. To each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000 Da. The total molecular weight of the entire effector molecule may therefore be approximately 40,000 Da.

25 Methods known in the art for isolating cells producing antibody of interest include hybridoma technology (Kohler, G. & Milstein C., 1975, Nature 256:495-497), the selected lymphocyte antibody method (Babcook *et al.*, 1996, Proc. Natl. Acad. Sci, 93, 7843-7848; WO 92/02551; de Wildt *et al.*, 1997, J. Immunol. Methods, 207:61-67 and in Lagerkvist, *et al.*, 1995, BioTechniques 18(5):862-869) and
30 isolation of antibodies from bacterially expressed libraries, *e.g.* phage display libraries. These methods rely on the identification of a pool of cells producing an antibody of interest followed by the isolation of individual antibody producing cells which are then clonally expanded and, if desired, the subsequent identification of the

sequence of their V_H and V_L chain genes. Alternatively, where more than one cell is present in a sample positive for the antibody of interest, these cells may be cultured together and used as a source of, *e.g.*, template cDNA produced from mRNA by reverse transcription, for the isolation of the nucleic acid encoding an antibody.

5 Thus, using the oligonucleotide primer sets described above enables the cloning and sequence derivation of, in particular, sheep antibodies, from antibody producing cells, especially B cells, B cell lines and hybridoma cells. Accordingly, in a further aspect, the invention includes an antibody or fragment thereof isolated according to any one of the methods described, above. Repertoires of such
10 antibodies, or fragments thereof, are also useful in bacteriophage libraries. Such antibodies are particularly useful as therapeutic agents. In one embodiment, antibodies isolated using the methods of the invention are humanised (see, for example, Adair *et al.*, 1992, Immunol Rev. 130:5-40 and WO91/09967). The antibodies of the invention are also of use as diagnostic and prognostic reagents.
15 Thus, further provided is the use of an antibody for the manufacture of a medicament for the treatment and/or prophylaxis of a disease involved in aberrant expression or activity of an antigen recognised by said antibody. Also provided is a method for the treatment and/or prophylaxis of a disease associated with aberrant expression or aberrant activity of an antigen recognised by an antibody isolated according to the
20 methods of the invention comprising administering a therapeutically effective amount of a composition comprising said antibody.

Antibodies isolated according to the methods of the invention are also useful in diagnosis. Thus, provided is a method of screening for and/or diagnosis or prognosis of a disease in a subject, and/or monitoring the effectiveness of therapy for
25 said disease, which comprises the step of detecting and/or quantifying in a biological sample obtained from said subject, the expression of an antigen recognised by an antibody isolated according to the methods of the invention. In particular, the step of detecting comprises contacting the sample with the antibody and detecting whether binding has occurred between the antibody and the antigen in the sample.

30 Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis*. All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by

reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The invention will now be described with reference to the following examples, which are merely illustrative and should not in any way be construed as limiting the scope of the present invention.

EXAMPLES

1. Preparation of cDNA

(i) A sheep was immunised with an antigen, X. B cells were collected and screened for production of antibody recognising antigen X. A single B cell was selected.

(ii) cDNA was prepared by reverse transcription using a Superscript™ III Reverse Transcriptase kit (Invitrogen cat.# 18080-044) and RNasin, an RNAase Inhibitor (Promega cat. # N2511). The following master mix (20µl) was added directly to the selected single sheep B cell:

RNase-free dH ₂ O	7.5µl
5X Buffer	4µl
Oligo dTVx primers (2.5µM)	4µl
RNasin	0.5µl
DTT	1µl
dNTP (10 mM)	1µl
Superscript III RT	1µl
10 % NP-40	1µl

Oligo dTVx primers were as follows: tttttttttttttttttttttva (SEQ ID NO:24), ttttttttttttttttttttvg (SEQ ID NO:25), ttttttttttttttttttttvc (SEQ ID NO:26), and tttttttttttttttttttvt (SEQ ID NO:27), where v is a, g, or t. RT-PCR was performed at 50°C for 60min followed by 70°C for 15min.

2. Primary PCR

Primary PCR fragments were prepared by adding 2µl of the cDNA and the appropriate primary primers in conjunction with a TaqPlus Precision PCR system (Stratagene Cat. No. # 600211) mix in a total volume of 50µl as described below:

dH ₂ O	37.5µl
10X Precision PCR buffer	5µl
TaqPlus Precision	0.5µl
dNTPs (10 mM)	1µl
1° forward outer primers [V _H (Set A) or V _L (Set E) at 0.25 µM or 0.25 pmol/µl]	2µl

1° reverse outer primers [V_H (SEQ ID NOS:2 & 3) or V_L (SEQ ID NO:12) at 0.25 μ M or 0.25 pmol/ μ l) 2 μ l

The following thermal cycle program used:

- | | | | |
|----|------|-------------------------------------|-------|
| 5 | i. | 94°C | 3min |
| | ii. | 94°C | 30sec |
| | iii. | 50°C | 30sec |
| | iv. | 72°C | 1min |
| | v. | go to step 2 for 40 cycles in total | |
| 10 | vi. | 72°C | 5min |
| | vii. | 4°C | hold |

3. Secondary PCR

Secondary PCR fragments were prepared by adding 2 μ l of the primary PCR
 15 product and the appropriate primary primers in conjunction with a TaqPlus Precision
 PCR system (Stratagene cat No. # 600211) mix in a total volume of 50 μ l as described
 below:

- | | | |
|----|---|--------------|
| | dH ₂ O | 33.5 μ l |
| | 10X Precision PCR buffer | 5 μ l |
| 20 | TaqPlus Precision | 0.5 μ l |
| | dNTPs (10 mM) | 1 μ l |
| | 2° forward inner primers, V_H (Set G) or V_L (Set K) at 0.25 μ M | 4 μ l |
| | 2° reverse inner primers, V_H (SEQ ID NOS:14 & 15) or V_L (SEQ ID NO:19) at
0.25 μ M | 4 μ l |

25 The following thermal cycle program used:

- | | | | |
|----|------|-------------------------------------|-------|
| | i. | 94°C | 3min |
| | ii. | 94°C | 30sec |
| | iii. | 54°C | 30sec |
| | iv. | 72°C | 1min |
| 30 | v. | go to step 2 for 40 cycles in total | |
| | vi. | 72°C | 5min |
| | vii. | 4°C | hold |

The presence of DNA encoding the variable region (V_H and V_{Lkappa}) of the
 35 antibody recognising antigen X was confirmed by running a 5 μ l sample of the product
 on a 2%w/v agarose gel.

The DNA product present in each case (V_H and V_L) was approximately 450bp
 and was cloned into an expression vector using the Hind III/Xho I/Bsi WI/Sfu I/Apa I
 restriction sites.

CLAIMS:

1. An oligonucleotide primer set comprising or consisting of SEQ ID NO:1 and/or SEQ ID NO:2 and/or SEQ ID NO:3.
- 5 2. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:1 to 3.
3. An oligonucleotide primer set comprising or consisting of:
 - 10 (a) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6;
 - (b) at least one primer selected from the group consisting of SEQ ID NO:4, SEQ ID NO:5 and SEQ ID NO:6, and SEQ ID NO:7, and/or SEQ ID NO:8; or
 - (c) SEQ ID NO:7 and/or SEQ ID NO:8.
- 15 4. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:4 to 8.
5. An oligonucleotide primer set comprising or consisting of:
 - 20 (a) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11;
 - (b) at least one primer selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10 and SEQ ID NO:11, and SEQ ID NO:12; or
 - (c) SEQ ID NO:12.
- 25 6. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:9 to 12.
7. An oligonucleotide primer set comprising or consisting of SEQ ID NO:13 and/or SEQ ID NO:14 and/or SEQ ID NO:15.
- 30 8. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:13 to 15.
- 35 9. An oligonucleotide primer set comprising or consisting of:

- (a) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18;
 - (b) at least one primer selected from the group consisting of SEQ ID NOS:16, 17 and 18, and SEQ ID NO:19; or
 - 5 (c) SEQ ID NO:19.
10. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:16 to 19.
- 10 11. An oligonucleotide primer set comprising or consisting of:
- (a) at least one primer selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22;
 - (b) at least one primer selected from the group consisting of SEQ ID NO:20, SEQ ID NO:21 and SEQ ID NO:22, and SEQ ID NO:23; or
 - 15 (c) SEQ ID NO:23.
12. An oligonucleotide primer set comprising or consisting of SEQ ID NOS:20 to 23.
- 20 13. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain comprising use of a primer set in one or more polymerase chain reactions, said primer set according to any one of claims 1, 2, 7 or 8.
- 25 14. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain comprising use of a primer set in one or more polymerase chain reactions, said primer set according to any one of claims 3, 4, 9 or 10.
- 30 15. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain comprising use of a primer set in one or more polymerase chain reactions, said primer set according to any one of claims 5, 6, 11 or 12.

16. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody heavy chain comprising use of a primer set in one or more polymerase chain reactions, said primer set according to claim 2 or claim 8.
- 5 17. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody kappa light chain comprising use of a primer set in one or more polymerase chain reactions, said primer according to claim 4 or claim 10.
18. A method for the isolation of a nucleic acid comprising the variable region
10 sequence of a sheep antibody lambda light chain comprising use of a primer set in one or more polymerase chain reactions, said primer set according to claim 6 or claim 12.
19. A method for the isolation of a nucleic acid comprising the variable region
15 sequence of a sheep antibody heavy chain comprising use of the primer set according to claim 2 in a first polymerase chain reaction, and use of the primer set according to claim 8 in a second polymerase chain reaction using template DNA from the first polymerase chain reaction.
20. A method for the isolation of a nucleic acid comprising the variable region
20 sequence of a sheep antibody kappa light chain comprising use of the primer set according to claim 4 in a first polymerase chain reaction, and use of the primer set according to claim 10 in a second polymerase chain reaction using template DNA from the first polymerase chain reaction.
- 25 21. A method for the isolation of a nucleic acid comprising the variable region sequence of a sheep antibody lambda light chain comprising use of the primer set according to claim 6 in a first polymerase chain reaction, and use of the primer set according to claim 12 in a second polymerase chain reaction using template DNA from the first polymerase chain reaction.
- 30 22. A sheep antibody isolated using a method according to any one of claims 13 to 21.

23. The use of an antibody according to claim 22, for the manufacture of a medicament for the treatment and/or prophylaxis of a disease involving aberrant expression or aberrant activity of an antigen recognised by said antibody.
- 5 24. A method of screening for and/or diagnosis or prognosis of a disease in a subject, and/or monitoring the effectiveness of therapy for said disease, which comprises the step of detecting and/or quantifying in a biological sample obtained from said subject, the expression of an antigen recognised by an antibody according to claim 22.

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CROMIE, Karen

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