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(54) **NESTED OLIGONUCLEOTIDES CONTAINING A HAIRPIN FOR NUCLEIC ACID AMPLIFICATION**

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C12Q 1/68	(2006.01)
C07H 21/04	(2006.01)

(52) **U.S. Cl.** **435/91.2**; 435/91.1; 435/6; 536/23.1; 536/24.3; 536/24.32; 536/24.33

(58) **Field of Classification Search** 435/91.2, 435/91.21, 6; 536/23.1, 24.33, 24.3, 24.32
See application file for complete search history.

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(57) **ABSTRACT**

Templates that are engineered to contain a predetermined sequence and a hairpin structure are provided by a nested oligonucleotide extension reaction. The engineered template allows Single Primer Amplification (SPA) to amplify a target sequence within the engineered template. In particularly useful embodiments, the target sequences from the engineered templates are cloned into expression vehicles to provide a library of polypeptides or proteins, such as, for example, an antibody library.

38 Claims, 8 Drawing Sheets

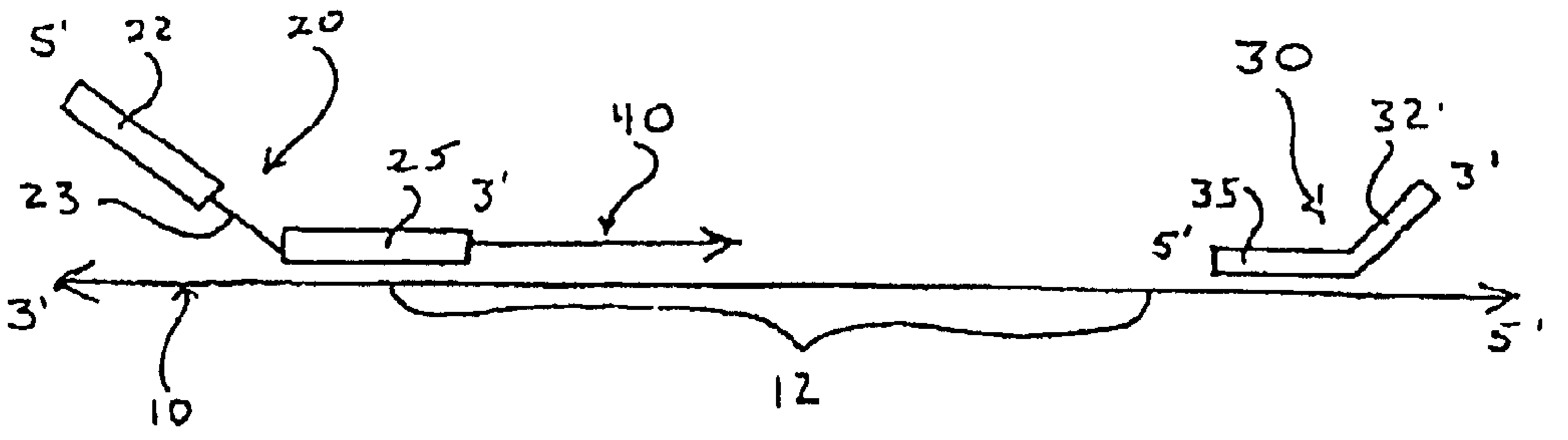

Fig. 1

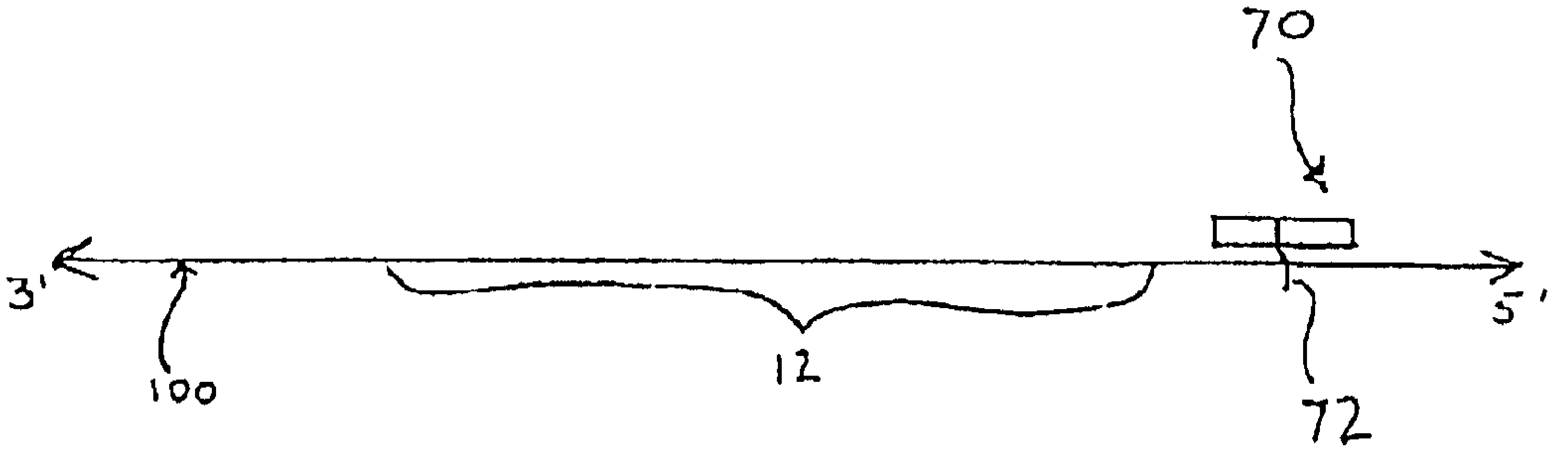

Fig. 2A

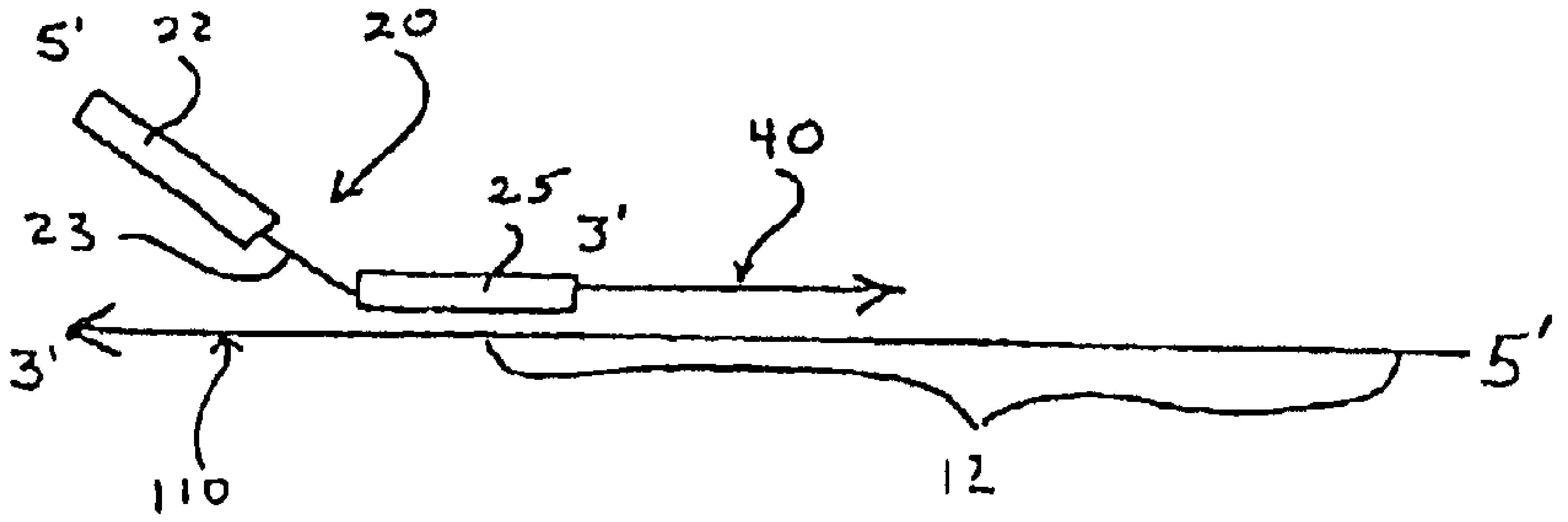

Fig. 2B

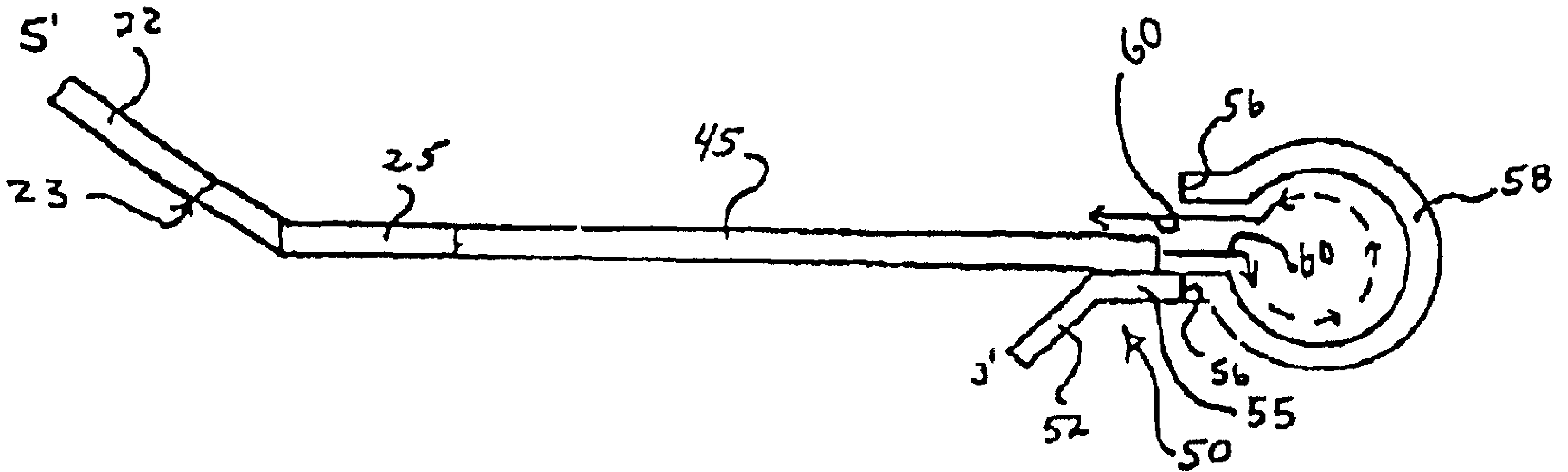

Fig. 3

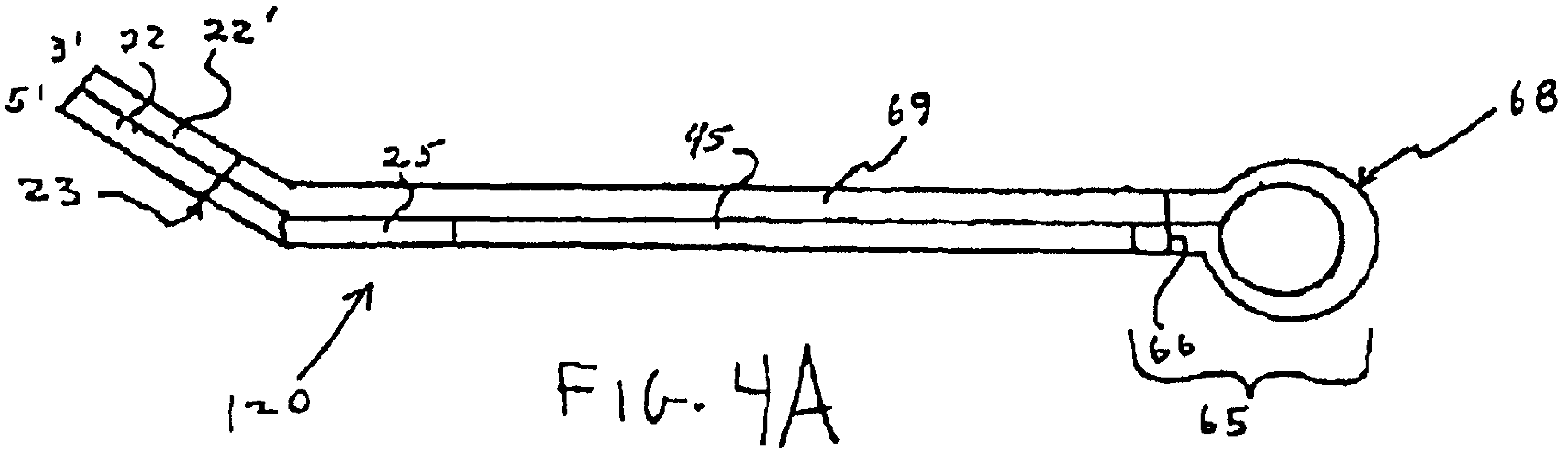

FIG. 4A

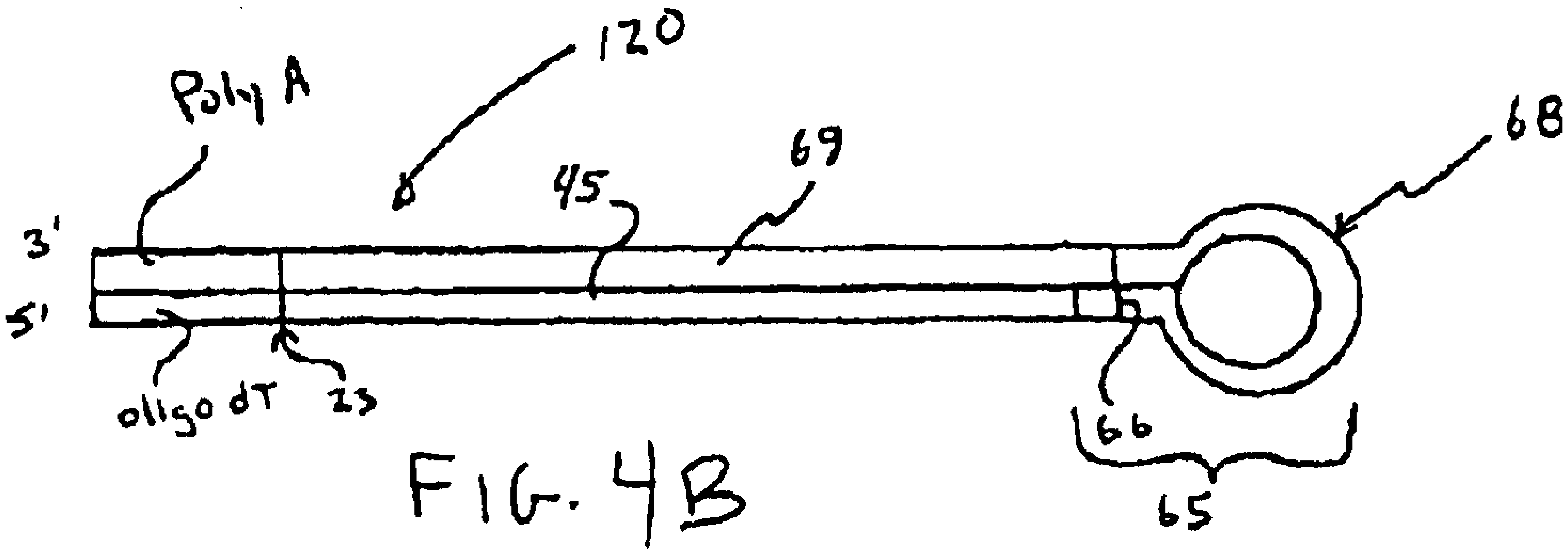

FIG. 4B

clones	SEQ ID NO:	FR1	CDR1	FR2
A7, B1, F8, G2	SEQ ID NO: 209	ESDGAVVQPGGSLRLSCAASGF	IFDDFAMH	WLRQVPGKGLQWVGL
C2, E6	SEQ ID NO: 210	QPGGSLRLSCAASGF	TLSSSAMS	WVRQAPGKGLEFVAV
A4	SEQ ID NO: 211	QPGGSLRLSCAASGF	TLSSSAMS	WVRQAPGKGLEFVAV
F6	SEQ ID NO: 212	AWYSRGSPCLSCAASGF	TLSSSAMS	WVRQAPGKGLEFVAV
E9, G7	SEQ ID NO: 213	ESDPGLVKPSETPSLTCTVSGG	SISSTMYFWG	WIRQPPGKGLEWIAS
F3, G4	SEQ ID NO: 214	PGLVKPSETLSLTCTVSGG	SISNIMYFWG	WIRQPPGKGLEWIAS
A12, B5, B8, B9	SEQ ID NO: 215	ESDPGLVQPSQTLSLTCTVSGG	SLRSDDYYWS	WIRQSPGKGLEWIAY
E8	SEQ ID NO: 216	PVQPLEF	TFTDHWMH	WVRQAPGKGLVWLAR
F7	SEQ ID NO: 217	ESEGGLVQPGGSLRLSCAASGF	TFSSYAMT	WVRQAPGKGLEWVST
E11	SEQ ID NO: 218	LAGVEVVQPGGSLRLSCAASGF	TFDDYAMH	WLRQIPGKGLQWVSL

Figure 5A

clones	SEQ ID NO:	CDR2	FR3
A7, B1, F8, G2	SEQ ID NO: 209 (cont'd)	MSWDGVSAYYADSVEG	RFTISRDNKKNALYLQMNSLGVEDTALYYCAK
C2, E6	SEQ ID NO: 209 (cont'd)	SSGNGFSTYYGDSVKG	RFTISRDNSKNMVYLQMDSLRAEDTAKYHCAK
A4	SEQ ID NO: 209 (cont'd)	SSGNGFSTYYGDSVKG	RFTISRDNSKNMVYLQMDSLRAEDTAKYHCAK
F6	SEQ ID NO: 209 (cont'd)	SSGNGFSTYYGDSVKG	RFTISRDNSKNMVYLQMDSLRAEDTAKYHCAK
E9, G7	SEQ ID NO: 209 (cont'd)	IYYSGTT-YYNPSLRS	RVTMSVDTSKNQLSLKLNSVTAADTAVYYCAR
F3, G4	SEQ ID NO: 209 (cont'd)	IYYSGTT-YYNPSLRS	RVTMSVDTSKNQLSLKLNSVTAADTAVYYCAR
A12, B5, B8, B9	SEQ ID NO: 209 (cont'd)	ISYTGGT-YYNPSLKS	RVTISVDTSRNQFSLRLRSVTAADSAVYFCAS
E8	SEQ ID NO: 209 (cont'd)	INRDGSDTTYADSVTG	RFTISRDNGKNTVSLQMDSLSVDDTAVYYCAR
F7	SEQ ID NO: 209 (cont'd)	MTGSGGVTYYADVLKG	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK
E11	SEQ ID NO: 209 (cont'd)	LSWDGVSAYYADSVEG	RFTISRDNKKNSLYLQMNSLRAEDVALYYCAK

Figure 5B

clones	SEQ ID NO:	CDR3	FR4
A7, B1, F8, G2	SEQ ID NO: 209 (cont'd)	DMGGGLRFPHF	WGQGTPVTVSA
C2, E6	SEQ ID NO: 210 (cont'd)	VRYGPRSHFFFDP	WGQGTLVTVSS
A4	SEQ ID NO: 211 (cont'd)	VRYGPRSHFFFDP	WGPGNPGHRLL
F6	SEQ ID NO: 212 (cont'd)	VRYGPRSHFFFDP	WGQGTLVTVSS
E9, G7	SEQ ID NO: 213 (cont'd)	PTIYYFDGRTSYYPGEAAFDI	WGQGTTV
F3, G4	SEQ ID NO: 214 (cont'd)	PTIYYFDGRTSYYPGEAAFDI	WGQGTTVTV
A12, B5, B8, B9	SEQ ID NO: 215 (cont'd)	TTAVTTTFDY	WGRGTLVTVS
E8	SEQ ID NO: 216 (cont'd)	GGHHTVLSPLSNWFDP	WGQGTLVTVS
F7	SEQ ID NO: 217 (cont'd)	GYGLFDY	WGQGTLVTVS
E11	SEQ ID NO: 218 (cont'd)	DMGGAQRLPDH	WGQGTLVTVSS

Figure 5C

clones	SEQ ID NO:	FR1	CDR1	FR2
4D, 10C, 4G	SEQ ID NO: 219	GGGLVQPGASVKVSCKASGY	TFSDYFMH	CVRQAPGQGLEWMGL
8A	SEQ ID NO: 220	RCPAKLLDT	PFSVYFMH	WVRQAPGQGLEWMGL
3G	SEQ ID NO: 221	RCPAKLLDT	PSGDYFMH	WVRQAPGQGLEWMGL
1A	SEQ ID NO: 222	SGGLVQRGAKVLRLSCVASGF	TFSSSAMS	WVRQAPGKGLEWVSV
7H	SEQ ID NO: 223	LGS	PYSSSAMS	WVRQAPGKGLE?VSF
6F	SEQ ID NO: 224	VESGGVVQPGAKVLRLSCAASGF	SFEDYAMH	WVRQPPGKGLEWVAL
4F	SEQ ID NO: 225	AASGF	IFDDFAMH	WFQAVPGKGLQWVGL
5A	SEQ ID NO: 226	FWLGGPWRLSCAVSGY	TLSSSAMI	WVRQPPGKGLEFVSV
1D	SEQ ID NO: 227	GGGLVQPGASLRLSCVASGF	TLSSSAMS	CVRQAPGKGLEWVSV
7E	SEQ ID NO: 228	WGRRGPAWGVPVGSPVQPLGY	TFDDYAMH	WLRQIPGKGLQWVSL
9E	SEQ ID NO: 229	WTGGGVVQPGGSLRVSVAASGY	TFDDYAMH	WLRQIPGKGLQWVSL
12B	SEQ ID NO: 230	AESGGGVVQPGGSLRLSCAASGF	TFSRYTLS	WVRQAPGKGLEWVSY

Figure 6A

clones	SEQ ID NO:	CDR2	FR3
4D, 10C, 4G	SEQ ID NO: 219 (cont'd)	VNPTNGYTAYAPKFQG	RVTMTRQRFTSTVYMELSSLRSEDTAVYFCAR
8A	SEQ ID NO: 220 (cont'd)	VNPTNGYTAYAPKFQG	RVTMTRQRFTSTVYMELSSLRSEDTAVYFCAR
3G	SEQ ID NO: 221 (cont'd)	VNPTNGYTAYAPKFQG	RVTMTRQRFTSTVYMELSSLRSEDTAVYFCAR
1A	SEQ ID NO: 222 (cont'd)	ISGNGFSTYYADSVK	RFTISRDNSKNTLYLQMNSLRAEDTAEYYCTK
7H	SEQ ID NO: 223 (cont'd)	IS?NGLSAYYADSVKG	RFTISRDNS?NTVYLQMNSLRSEDTAEYYCVK
6F	SEQ ID NO: 224 (cont'd)	ISWDVISAYYADSVKG	RFTISRDNSKNSLYLQMDSLRPEDSGLYYCGR
4F	SEQ ID NO: 225 (cont'd)	MSWDGVSAYYADSVEG	RFTISRDNKKNALYLQMNSLGVEDTALYFCAK
5A	SEQ ID NO: 226 (cont'd)	ISGNGLSAYYADSVKG	RFTISRDNSKNTVYLQMNSLRAEDTAEYYCVK
1D	SEQ ID NO: 227 (cont'd)	SSGNGFSAYYADSVKG	RFTISRDNSKNTLYLQMNSLVAEDTAEYYCTK
7E	SEQ ID NO: 228 (cont'd)	LSWDGVSAYYADSVEG	RFTISRDNKKNSLYLQMNSLVAEDTALYFCAK
9E	SEQ ID NO: 229 (cont'd)	LSWDGVSAYYADSVEG	RFTISRDN?KNSLYLQMNSLIAEDTALYFCAK
12B	SEQ ID NO: 230 (cont'd)	ISTDGSTIYYTDSVKG	RFTISRDNAKNSLSLQMISLRDEDTAVYYCAR

Figure 6B

clones	SEQ ID NO:	CDR3	FR4
4D, 10C, 4G	SEQ ID NO: 219 (cont'd)	VKSSDSIDAFDI	WGQGTMVTVSS
8A	SEQ ID NO: 220 (cont'd)	VKSSDSIDAFDI	WGQGTMVTVSS
3G	SEQ ID NO: 221 (cont'd)	VKSSDSIDAFDI	WGQGTMVTVSS
1A	SEQ ID NO: 222 (cont'd)	VKYGSGSHFWFDP	WGQGTLVTVSS
7H	SEQ ID NO: 223 (cont'd)	V?YGSRSHF	
6F	SEQ ID NO: 224 (cont'd)	DIGQQRTMDV	WGQGTTVTVSS
4F	SEQ ID NO: 225 (cont'd)	DMGGGLRFPHF	WGQGTPVTVSA
5A	SEQ ID NO: 226 (cont'd)	VKYGSRSHFFFDS	WGQGTLVSVSP
1D	SEQ ID NO: 227 (cont'd)	VNYGSRSHFYFGS	WGHGTLVIVSS
7E	SEQ ID NO: 228 (cont'd)	DMGGAQRLPDH	WGQGTLVTVSS
9E	SEQ ID NO: 229 (cont'd)	DMGGAQRLPDH	WGQGTLVTVSS
12B	SEQ ID NO: 230 (cont'd)	VFFGGNFRAHWYFDL	WGRGTLVAVSS

Figure 6C

NESTED OLIGONUCLEOTIDES CONTAINING A HAIRPIN FOR NUCLEIC ACID AMPLIFICATION

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 60/254,669 filed December 11, 2000 and to U.S. Provisional Application No. 60/323,400 filed Sep. 19, 2001. The disclosures of both these Provisional Applications are incorporated herein in their entirety by this reference.

TECHNICAL FIELD

This disclosure relates to engineered templates useful for amplification of a target nucleic acid sequence. More specifically, templates which are engineered to contain a predetermined sequence and a hairpin structure are provided by a nested oligonucleotide extension reaction. The engineered templates allow Single Primer Amplification (SPA) to amplify a target sequence within the engineered template. In particularly useful embodiments, the target sequences from the engineered templates are cloned into expression vehicles to provide a library of polypeptides or proteins, such as, for example, an antibody library.

BACKGROUND OF RELATED ART

Methods for nucleic acid amplification and detection of amplification products assist in the detection, identification, quantification, isolation and sequence analysis of nucleic acid sequences. Nucleic acid amplification is an important step in the construction of libraries from related genes such as, for example, antibodies. These libraries can be screened for antibodies having specific, desirable activities. Nucleic acid analysis is important for detection and identification of pathogens, detection of gene alteration leading to defined phenotypes, diagnosis of genetic diseases or the susceptibility to a disease, assessment of gene expression in development, disease and in response to defined stimuli, as well as the various genome projects. Other applications of nucleic acid amplification method include the detection of rare cells, detection of pathogens, and the detection of altered gene expression in malignancy, and the like. Nucleic acid amplification is also useful for qualitative analysis (such as, for example, the detection of the presence of defined nucleic acid sequences) and quantification of defined gene sequences (useful, for example, in assessment of the amount of pathogenic sequences as well as the determination of gene multiplication or deletion, and cell transformation from normal to malignant cell type, etc.). The detection of sequence alterations in a nucleic acid sequence is important for the detection of mutant genotypes, as relevant for genetic analysis, the detection of mutations leading to drug resistance, pharmacogenomics, etc.

There are many variations of nucleic acid amplification, for example, exponential amplification, linked linear amplification, ligation-based amplification, and transcription-based amplification. One example of exponential nucleic acid amplification method is polymerase chain reaction (PCR) which has been disclosed in numerous publications. See, for example, Mullis et al. Cold Spring Harbor Symp. Quant. Biol. 51:263–273 (1986); Mullis K. EP 201,184; Mullis et al. U.S. Pat. No. 4,582,788; Erlich et al. EP 50,424, EP 84,796, EP 258,017, EP 237,362; and Saiki R. et al. U.S. Pat. No. 4,683,194. In fact, the polymerase chain reaction (PCR) is the most commonly used target amplification method. PCR is based on multiple cycles of denaturation, hybridization of two different oligonucleotide primers, each to opposite strand of the target strands, and primer extension by a nucleotide polymerase to produce multiple double stranded copies of the target sequence.

Amplification methods that employ a single primer, have also been disclosed. See, for example, U.S. Pat. Nos. 5,508,178; 5,595,891; 5,683,879; 5,130,238; and 5,679,512. The primer can be a DNA/RNA chimeric primer, as disclosed in U.S. Pat. No. 5,744,308.

Some amplification methods use template switching oligonucleotides (TSOs) and blocking oligonucleotides. For example, a template switch amplification in which chimeric DNA primer are utilized is disclosed in U.S. Pat. Nos. 5,679,512; 5,962,272; 6,251,639; and by Patel et al. Proc. Natl. Acad. Sci. U.S.A. 93:2969–2974 (1996).

However the previously described target amplification methods have several drawbacks. For example, the transcription base amplification methods, such as Nucleic Acid Sequence Based Amplification (NASBA) and transcription mediated amplification (TMA), are limited by the need for incorporation of the polymerase promoter sequence into the amplification product by a primer, a process prone to result in non-specific amplification. Another example of a drawback of the current amplification methods is the requirement of two binding events which may have optimal binding at different temperatures. This combination of factors results in increased likelihood of mis-priming and resultant amplification of sequences other than the target sequence. Therefore, there is a need for improved nucleic acid amplification methods that overcome these drawbacks. The invention provided herein fulfills this need and provides additional benefits.

SUMMARY

A method of amplifying nucleic acid has been discovered which includes the steps of a) annealing a primer to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the end of the template, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

In an alternative embodiment, the method of amplifying nucleic acid includes the steps of a) annealing a primer and a boundary oligonucleotide to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

In yet another embodiment, the method of amplifying nucleic acid includes the steps of a) annealing an oligo dT primer and a boundary oligonucleotide to an mRNA template; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide an extended polynucleotide that includes a portion that is complementary to the hairpin structure and a poly A terminal portion; and f) amplifying the extended polynucleotide using a single primer.

In another aspect an engineered nucleic acid strand is disclosed which has a predetermined sequence at a first end thereof, a sequence complementary to the predetermined sequence at the other end thereof, and a hairpin structure therebetween.

In yet another aspect, a method of amplifying a nucleic acid strand has been discovered which includes the steps of providing an engineered nucleic acid strand having a predetermined sequence at a first end thereof, a sequence complementary to the predetermined sequence at the other end thereof and a hairpin structure therebetween, and contacting the engineered nucleic acid strand with a primer containing at least a portion of the predetermined sequence in the presence of a polymerase and nucleotides under conditions suitable for polymerization of the nucleotides.

Once the engineered nucleic acid is amplified a desired number of times, restriction sites can be used to digest the strand so that the target nucleic acid sequence can be ligated into a suitable expression vector. The vector may then be used to transform an appropriate host organism using standard methods to produce the polypeptide or protein encoded by the target sequence. In particularly useful embodiments, the techniques described herein are used to amplify a family of related sequences to build a complex library, such as, for example, an antibody library.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1** is a schematic illustration of a primer and boundary oligo annealed to a template;

FIG. **2**A is a schematic illustration of a restriction oligo annealed to a nucleic acid strand;

FIG. **2**B is a schematic illustration of a primer annealed to a template that has a shortened 5' end;

FIG. **3** is a schematic illustration of a nested oligo having a hairpin structure annealed to a newly synthesized nucleic acid strand;

FIG. **4**A is a schematic illustration of an engineered template in accordance with this disclosure; and

FIG. **4**B is a schematic illustration of an engineered template in accordance with an alternative embodiment.

FIGS. **5**A–**5**C is a chart showing the sequences of clones produced in Example 4.

FIGS. **6**A–**6**C is a chart showing the sequences of clones produced in Example 7.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present disclosure provides a method of amplifying a target nucleic acid sequence. In particularly useful embodiments, the target nucleic acid sequence is a gene encoding a polypeptide or protein. The disclosure also describes how the products of the amplification may be cloned and expressed in suitable expression systems. In particularly useful embodiments, the techniques described herein are used to amplify a family of related sequences to build a complex library, such as, for example, an antibody library.

The target nucleic acid sequence is exponentially amplified through a process that involves only a single primer. The ability to employ a single primer (i.e., without the need for both forward and reverse primers each having different sequences) is achieved by engineering a strand of nucleic acid that contains the target sequence to be amplified. The engineered strand of nucleic acid (sometimes referred to herein as the "engineered template") is prepared from two templates; namely, 1) a starting material that is a natural or synthetic nucleic acid (e.g., RNA, DNA or cDNA) containing the sequence to be amplified and 2) a nested oligonucleotide that provides a hairpin structure. The starting material can be used directly as the original template, or, alternatively, a strand complementary to the starting material can be prepared and used as the original template. The nested oligonucleotide is used as a template to extend the nucleotide sequence of the original template during creation of the engineered strand of nucleic acid. The engineered strand of nucleic acid is created from the original template by a series of manipulations that result in the presence of a predetermined sequence at one end thereof and a hairpin structure. It is these two features that allow amplification using only a single primer.

Any nucleic acid, in purified or nonpurified form, can be utilized as the starting material for the processes described herein provided it contains or is suspected of containing the target nucleic acid sequence to be amplified. Thus, the starting material employed in the process may be, for example, DNA or RNA, including messenger RNA, which DNA or RNA may be single stranded or double stranded. In addition, a DNA-RNA hybrid which contains one strand of each may be utilized. A mixture of any of these nucleic acids may also be employed, or the nucleic acids produced from a previous amplification reaction herein using the same or different primers may be utilized. The target nucleic acid sequence to be amplified may be a fraction of a larger molecule or can be present initially as a discrete molecule. The starting nucleic acid may contain more than one desired target nucleic acid sequence which may be the same or different. Therefore, the present process may be useful not only for producing large amounts of one target nucleic acid sequence, but also for amplifying simultaneously more than one different target nucleic acid sequence located on the same or different nucleic acid molecules.

The nucleic acids may be obtained from any source, for example: genomic or cDNA libraries, plasmids, cloned DNA or RNA, or from natural DNA or RNA from any source, including bacteria, yeast, viruses, and higher organisms such as plants or animals. The nucleic acid can be naturally occurring or synthetic, either totally or in part. Techniques for obtaining and producing the nucleic acids used in the present processes are well known to those skilled in the art. If the nucleic acid contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as the original template, either as a separate step or simultaneously with the synthesis of the primer extension products. Additionally, if the starting material is first strand DNA, second strand DNA may advantageously be created by processes within the purview of those skilled in the art and used as the original template from which the engineered template is created.

First strand cDNA and mRNA are particularly useful as the original template for the present methods. Suitable methods for generating DNA templates are known to and readily selected by those skilled in the art. In one embodiment, 1^{st} strand cDNA is synthesized in a reaction where reverse transcriptase catalyzes the synthesis of DNA complementary to any RNA starting material in the presence of an oligodeoxynucleotide primer and the four deoxynucleoside triphosphates, dATP, dGTP, dCTP, and TTP. The reaction is initiated by the non-covalent bonding of the oligodeoxynucleotide primer to the 3' end of mRNA followed by stepwise addition of the appropriate deoxynucleotides as determined by base pairing relationships with the mRNA nucleotide sequence, to the 3' end of the growing chain. As those skilled in the art will appreciate, all mRNA in a sample can be used to generate first strand cDNA through the annealing of oligo dT to the poly A tail of the mRNA.

Once the original template is obtained, a primer **20** and a boundary oligonucleotide **30** arc annealed to the original template **10**. (See FIG. **1**.) A strand of nucleic acid complementary to the portion of the original template beginning at the 3' end of the primer up to about the 5' end of the boundary oligonucleotide is polymerized.

The primer **20** that is annealed to the original template includes a portion **25** that anneals to the original template and optionally a portion **22** of predetermined sequence that preferably does not anneal to the template, and optionally a restriction site **23** between portions **22** and **25**. Thus, for example, where the original template is mRNA, a portion having a predetermined sequence that does not anneal to the template is not needed, but rather the primer can be any gene-specific internal sequence of the mRNA or oligo dT which will anneal to the unique poly A tail of the mRNA.

The primer anneals to the original template adjacent to the target sequence **12** to be amplified. It is contemplated that the primer can anneal to the original template upstream of the target sequence (or downstream in the case, e.g., of an mRNA original template) to be amplified, or that the primer may overlap the beginning of the target sequence **12** to be amplified as shown in FIG. **1**. The predetermined sequence of portion **22** of the primer is selected so as to provide a sequence to which the single primer used during the amplification process can hybridize as described in detail below. Preferably, the predetermined sequence is not native in the original template and does not anneal to the original template, as shown in FIG. **1**. Optionally, the predetermined sequence may include a restriction site useful for insertion of a portion of the engineered template into an expression vector as described more fully hereinbelow.

The boundary oligonucleotide **30** that is annealed to the original template serves to terminate polymerization of the nucleic acid. Any oligonucleotide capable of terminating nucleic acid polymerization may be utilized as the boundary oligonucleotide **30**. In a preferred embodiment the boundary oligonucleotide includes a first portion **35** that anneals to the original template **10** and a second portion **32** that is not susceptible to an extension reaction. Techniques to prevent the boundary oligo from acting as a site for extension are within the purview of one skilled in the art. By way of example, portion **32** of the boundary oligo **30** may be designed so that it does not anneal to the original template **10** as shown in FIG. **1**. In such embodiments, the boundary oligonucleotide **30** prevents further polymerization but does not serve as a primer for nucleic acid synthesis because the 3' end thereof does not hybridize with the original template **10**. Alternatively, the 3' end of the boundary oligo **30** might be designed to include locked nucleic acid to achieve the same effect. Locked nucleic acid is disclosed for example in WO 99/14226, the contents of which are incorporated herein by reference. Those skilled in the art will envision other ways of ensuring that no extension of the 3' end of the boundary oligo occurs.

Primers and oligonucleotides described herein may be synthesized using established methods for oligonucleotide synthesis which are well known in the art. Oligonucleotides, including primers of the present invention include linear oligomers of natural or modified monomers or linkages, such as deoxyribonucleotides, ribonucleotides, and the like, which are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to monomer interactions such as Watson-Crick base pairing. Usually monomers are linked by phosphodiester bonds or their analogs to form oligonucleotides ranging in size from a few monomeric units e.g., 3–4, to several tens of monomeric units. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers known in the art may be useful for the methods of the present disclosure. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the template depends on the stringency of the hybridization conditions. Primers may be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

Polymerization of nucleic acid can be achieved using methods known to those skilled in the art. Polymerization is generally achieved enzymatically, using a DNA polymerase or reverse transcriptase which sequentially adds free nucleotides according to the instructions of the template. The selection of a suitable enzyme to achieve polymerization for a given template and primer is within the purview of those skilled in the art. In certain embodiments, the criteria for selection of polymerases includes lack exonuclease activity or DNA polymerases which do not possess a strong exonuclease activity. DNA polymerases with low exonuclease activity for use in the present process may be isolated from natural sources or produced through recombinant DNA techniques. Illustrative examples of polymerases that may be used, are, without limitation, T7 Sequenase v. 2.0, the Klenow Fragment of DNA polymerase I lacking exonuclease activity, the Klenow Fragment of Taq Polymerase, exo.- Pfu DNA polymerase, Vent. (exo.-) DNA polymerase, and Deep Vent. (exo-) DNA polymerase.

In a particularly useful embodiment, the use of a boundary oligonucleotide is avoided by removing unneeded portions of the starting material by digestion. In this embodiment, which is shown schematically in FIG. **2**A, a restriction oligonucleotide **70** is annealed to the starting material **100** at a preselected location. The restriction oligonucleotide provides a double stranded portion on the starting material containing a restriction site **72**. Suitable restriction sites, include, but are not limited to Xho I, Spe I, Nhe1, Hind III, Nco I, Xma I, Bgl II, Bst I, and Pvu I. Upon exposure to a suitable restriction enzyme, the starting material is digested and thereby shortened to remove unnecessary sequence while preserving the desired target sequence **12** (or portion thereof) to be amplified on what will be used as the original template **110**. Once the original template **10** is obtained, a primer **20** is annealed to the original template **110** (see FIG. **2**B) adjacent to or overlapping with the target sequence **12** as described above in connection with previous embodiments. A strand of nucleic acid **40** complementary to the portion of the original template between the 3' end of the primer **20** and the 5' end of the original template **110** is polymerized. As those skilled in the art will appreciate, in this embodiment where a restriction oligonucleotide is employed to generate the original template, there is no need to use a boundary oligonucleotide, because primer extension can be allowed to proceed all the way to the 5' end of the shortened original template **110**.

Once polymerization is complete (i.e., growing strand **40** reaches the boundary oligonucleotide **30** or the 5' end of the shortened original template **110**), the newly synthesized complementary strand is separated from the original template by any suitable denaturing method including physical, chemical or enzymatic means. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or the enzyme RecA, which has helicase activity and in the presence of riboATP is known to denature DNA. The reaction conditions suitable for separating the strands of nucleic acids with helicases are described by Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIII "DNA: Replication and Recombination" (New York: Cold Spring Harbor Laboratory, 1978), B. Kuhn et al., "DNA Helicases", pp. 63–67, and techniques for using RecA are reviewed in C. Radding, Ann. Rev. Genetics, 16:405–37 (1982).

The newly synthesized complementary strand thus includes sequences provided by the primer **20** (e.g., the predetermined sequence **22**, the optional restriction site **23** and the annealing portion **25** of the primer) as well as the newly synthesized portion **45** that is complementary to the portion of the original template **10** between the location at which the primer **20** was annealed to the original template **10** and either the portion of the original template **10** to which the boundary oligonucleotide **30** was annealed or through to the shortened 5' end of the original template. See FIG. **3**.

Optionally, multiple rounds of polymerization using the original template and a primer are performed to produce multiple copies of the newly synthesized complementary strand for use in subsequent steps. It is contemplated that 2 to 10 rounds or more (preferably, 15–25 rounds) of linear amplification can be performed when a DNA template is used. Making multiple copies of the newly synthesized complementary strand at this point in the process (instead of waiting until the entire engineered template is produced before amplifying) helps ensure that accurate copies of the target sequence are incorporated into the engineered templates ultimately produced. It is believed that multiple rounds of polymerization based on the original template provides a greater likelihood that a better representation of all members of the library will be achieved, therefore providing greater diversity compared to a single round of polymerization.

The next step in preparing the engineered template involves annealing a nested oligonucleotide **50** to the 3' end of the newly synthesized complementary strand, for example as shown in FIG. **3**. As seen in FIG. **3**, the nested oligonucleotide **50** provides a template for further polymerization necessary to complete the engineered template. Nested oligonucleotide **50** includes a portion **52** that does not hybridize and/or includes modified bases to the newly synthesized complementary strand, thereby preventing the nested oligonucleotide from serving as a primer. Nested oligonucleotide **50** also includes a portion **55** that hybridizes to the 3' end of the newly synthesized complementary strand. Nested oligonucleotide **50** may optionally also define a restriction site **56**. The final portion **58** of nested oligonucleotide **50** contains a hairpin structure. From the point at which portion **55** extends beyond the 3' end of the beginning the newly synthesized complementary strand, the nested oligonucleotide serves as a template for further polymerization to form the engineered template. It should be understood that the nested oligo may contain part of the target sequence (if part thereof was truncated in forming the original template) or may include genes that encode a polypeptide or protein (or portion thereof) such as, for example, one or more CDR's or Framework regions or constant regions of an antibody. It is also contemplated that a collection of nested oligonucleotides having different sequences can be employed, thereby providing a variety of templates which results in a library of diverse products. Thus, polymerization will extend the newly synthesized complementary strand by adding additional nucleic acid **60** that is complementary to the nested oligonucleotide as shown in FIG. **3**. Techniques for achieving polymerization are within the purview of one skilled in the art. As previously noted, in selecting a suitable polymerase, an enzyme lacking exonuclease activity may be employed to prevent the 3' end of the nested oligo from acting as a primer. Because of hairpin structure **50** of the nested oligonucleotide, eventually the newly synthesized complementary strand will turn back onto portion **45** of the same strand which will then serve as the template for further polymerization. Polymerization will continue until the end of the primer is reached, at which point the newly synthesized strand will terminate with a portion whose sequence is complementary to the primer.

Once polymerization is complete, the engineered template **120** is separated from the nested oligonucleotide **50** by techniques well known to those skilled in the art such as, for example, heat denaturation. The resulting engineered template **120** contains a portion derived from the original primer **20**, portion **45** that is complementary to a portion of the original template, and portion **65** that is complementary to a portion of the nested oligonucleotide and includes a hairpin structure **68**, and a portion **69** that is complementary to portion **45**. (See FIGS. **4**A and B.) The 3' end of engineered template **120** includes a portion containing a sequence that is complementary to primer **20**. Thus, for example, as shown in FIG. **4**A, the 3' end of engineered template **120** includes portion **22'** containing a sequence that is complementary to the predetermined sequence of portion **22** of primer **20**. This allows for amplification of the desired sequence contained within engineered template **120** using a single primer having the same sequence as the predetermined sequence of primer portion **22** (or a primer that is complementary thereto) using techniques known to those of ordinary skill in the art.

As another example (shown in FIG. **4**B), where mRNA is used as the template and oligo dT is used as the primer, the 3' end of engineered template **120** includes poly A portion that is complementary to the oligo dT primer. In this case, any sequence along portion **45** can be selected for use as the primer to be annealed to portion **69** once the engineered template is denatured for single primer amplification. Optionally, the primer may include a non-annealing portion, such as, for example, a portion defining a restriction site.

During single primer amplification, the presence of a polymerase having exonuclease activity is preferred because such enzymes are known to provide a "proofreading" function and have relatively higher processivity compared to polymerases lacking exonuclease activity.

Due to hairpin structure **68** there is internal self annealing between the 5' end predetermined sequence and the 3' end sequence which is complementary to the predetermined sequence on the engineered template. Upon denaturation and addition of a primer having the predetermined sequence, the primer will hybridize to the template and amplification can proceed.

After amplification is performed, the products may be detected using any of the techniques known to those skilled in the art. Examples of methods used to detect nucleic acids include, without limitation, hybridization with allele specific oligonucleotides, restriction endonuclease cleavage, single-stranded conformational polymorphism (SSCP), analysis-.gel electrophoresis, ethidium bromide staining, fluorescence resonance energy transfer, hairpin FRET essay, and TaqMan assay.

Once the engineered nucleic acid is amplified a desired number of times, restriction sites **23** and **66** or any internal restriction site can be used to digest the strand so that the target nucleic acid sequence can be ligated into a suitable expression vector. The vector may then be used to transform an appropriate host organism using standard methods to produce the polypeptide or protein encoded by the target sequence.

In particularly useful embodiments, the methods described herein are used to amplify target sequences encoding antibodies or portions thereof, such as, for example the variable regions (either light or heavy chain) using cDNA of an antibody. In this manner, a library of antibodies can be amplified and screened. Thus, for example, starting with a sample of antibody mRNA that is naturally diverse, first strand cDNA can be produced and digested to provide an original template. A primer can be designed to anneal upstream to a selected complementary determining region (CDR) so that the newly synthesized nucleic acid strand includes the CDR. By way of example, if the target sequence is heavy chain CDR3, the primer may be designed to anneal to the heavy chain framework one (FR1) region. Those skilled in the art will readily envision how to design appropriate primers to anneal to other upstream sites or to reproduce other selected targets within the antibody cDNA based on this disclosure.

The following Examples are provided to illustrate, but not limit, the present invention(s):

Example 1

Amplification of a Repertoire of Ig Kappa Light Chain Variable Genes

First Strand cDNA Synthesis

First strand cDNA to be used as the original template was generated from 2 µg of human peripheral blood lymphocyte

(PBL) mRNA with an oligo-dT primer using the SuperScript II First Strand Synthesis Kit (Invitrogen) according to the manufacturer's instructions. The 1st strand cDNA product was purified over a QIAquick spin column (QIAGEN PCR Purification Kit) and eluted in 400 µL of nuclease-free water.

Second Strand Linear Amplification (SSLA) in the Presence of Blocking Oligonucleotide

The second strand cDNA reaction contained 5 µL of 1st strand cDNA original template, 0.5 µM primer JMX26VK1a, 0.5 µM blocking oligo CKLNA1, 0.2 mM dNTPs, 5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems), 1×GeneAmp Gold Buffer(15 mM Tris-HCl, pH 8.0, 50 mM KCl), and 1.5 mM $MgCl_2$. The final volume of the reaction was 98 µL. The sequence of primer JMX26VK1a, which hybridizes to the framework 1 region of VK1a genes, was 5' GTC ACT CAC GAA CTC ACG ACT CAC GGA GAG CTC RAC ATC CAG ATG ACC CAG 3' (SEQ ID NO: 1) where R is an equal mixture of A and G. The sequence of the blocking oligo CKLNA1, which hybridizes to the 5' end of the VK constant region, was 5' GAA CTG TGG CTG CAC CAT CTG 3' (SEQ ID NO: 2), where the underlined bases are locked nucleic acid (LNA) nucleotide analogues. After an initial heat denaturation step of 94° C. for 3 minutes, linear amplification of 2nd strand cDNA was carried out for 20 cycles of 94° C. for 15 seconds, 56° C. for 15 seconds, and 68° C. for 1 minute.

Nested Oligo Extension Reaction

After the last cycle of linear amplification, 2 µL of a nested/hairpin oligo designated "JK14TSHP" was added to give a final concentration of 20 µM. The sequence of JK14TSHP was 5' CCT TAG AGT CAC GCT AGC GAT TGA TTG ATT GAT TGATTG TTT GTG ACT CTA AGG TTG GCG CGC CTT CGT TTG ATY TCC ACC TTG GTC C(ps)T(ps)G(ps)P 3' (SEQ ID NO: 3) where Y is an equal mixture of C and T and (ps) are phosphorothioate backbone linkages and P is a 3' propyl group. For nested oligo extension reaction, two cycles of 94° C. for 1 minute, 56° C. for 15 seconds, and 72° C. for 1 minute were performed, followed by a 10 minute incubation at 72° C. to allow complete extension of the hairpin. The reaction products were purified over a QIAquick spin column (QIAgen PCR Purification Kit) and eluted in 50 µL of nuclease-free water.

Analysis of Engineered Template

The efficiency of the nested oligo extension reaction was determined by amplifying the products with either a primer set specific for the engineered product or a primer set that detects all VK1a/JK14 second strand cDNA products (including the engineered product). For specific detection of engineered product, a 10 µL aliquot was amplified for 20 or 25 cycles with primers designated "JMX26" and "TSDP". Primer JMX26 hybridizes to the 5' end of JMX26VK1a, the framework 1 primer used in the second strand cDNA reaction. Primer TSDP hybridizes to the hairpin-loop sequence added to the 3' ends of the second strand cDNAs in the nested oligo extension reaction. The sequence of primer JMX26 was 5' GTC ACT CAC GAA CTC ACG ACT CAC GG 3' (SEQ ID NO: 4). The sequence of primer TSDP was 5' CAC GCT AGC GAT TGA TTG ATT G 3' (SEQ ID NO: 5). For detection of all VK1a/JK14 second strand cDNA products a 10 µL aliquot was amplified for 20 or 25 cycles with primers JMX26 and JK14. The sequence of primer JK 14, which hybridizes to the framework 4 region of JK1 and JK4 genes, was 5' GAG GAG GAG GAG GAG GAG GGC GCG CCT GAT YTC CAC CTT GGT CCC 3' (SEQ ID NO: 6). Both reactions contained 1×GeneAmp Gold Buffer, 1.5 mM $MgCl_2$, 7.5% glycerol, 0.2 mM dNTPs, and 0.5 µM of each primer in a final volume of 50 µL.

The results with primers JMX26 and TSDP demonstrated the successful production of nested oligo and extended VK stem-loop DNA when using SSLA DNA that was blocked specifically with a boundary oligo. Suitable controls showed that when using the nested oligo in the presence of SSLA DNA that was not blocked, only a minimal amount of amplified product was produced. Additional controls without the nested oligo were negative. However, VK1a/JK14 second strand cDNA products were detected equally among all tested samples.

Single Primer Amplification of the Stem-Loop cDNA Template

Conditions that were previously shown to amplify a 352 bp stem-15 bp loop DNA product were as follows: 10 pg of the stem-loop DNA, 2 μM primer, 50 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 15 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 1.7 M betaine, 0.2 mM dNTPs, and 2.5 units of Z-Taq DNA Polymerase (Takara Shuzo) in a final volume of 50 μL. The thermal cycling conditions were an initial denaturation step of 96° C. for 2.5 minutes, 35 cycles of 96° C. for 30 seconds, 64° C. for 30 seconds, 74° C. for 1.5 minutes, and a final extension step of 74° C. for 10 minutes. Oligonucleotides containing the modified bases 5-methyl-2'-deoxycytidine and/or 2-amino-2'-deoxyadenosine have been shown to prime much more efficiently than unmodified oligonucleotides at primer binding sites located within hairpin structures (Lebedev et al. 1996. Genetic Analysis: Biomolecular Engineering 13, 15–21). These modifications work by increasing the melting temperature of the primer, allowing the annealing step of the amplification to be performed at a higher temperature. JMX26 primers containing ten 5-methyl-2'-deoxycytidines or seven 2-amino-2'-deoxyadenosines have been synthesized.

Cloning VK products

Amplified fragments are cloned by Sac I/Asc I into an appropriate expression vector that contains, in frame, the remaining portion of the kappa constant region. Suitable vectors include pRL5 and pRL4 vectors (described in U.S. Provisional Application 60/254,411, the disclosure of which is incorporated herein by reference), fdtetDOG, PHEN1, and pCANTAB5E. Individual kappa clones can be sequenced.

Expanding the Repertoire of VKappa Amplified Products

Further coverage of the VK repertoire is achieved by using the above protocols with a panel of primers for the generation of the second strand DNA. The primers contain JMX26 sequence, a Sac I restriction site, and a region that anneals to 1st strand cDNA in the framework 1 region of human antibody kappa light chain genes. The antibody annealing sequences were derived from the VBase database primers (www.mrc-cpe.cam.ac.uk/imt-doc/publicINTRO.html) which were designed based on the known sequences of human antibodies and are reported to cover the entire human antibody repertoire of kappa light chain genes. Below is a list of suitable primers:

JMX26Vk1a (SEQ ID NO:7)
GTCACTCACGAACTCACGACTCACGGAGAGCTCRACATCCAGATGACCCAG
JMX26Vk1b (SEQ ID NO: 8)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGMCATCCAGTTGACCCAG
JMX26Vk1C (SEQ ID NO: 9)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGCCATCCRGATGACCCAG
JMX26Vk1d (SEQ ID NO: 10)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGTCATCTGGATGACCCAG
JMX26Vk2a (SEQ ID NO: 11)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATATTGTGATGACCCAG
JMX26Vk2b (SEQ ID NO: 12)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATRTTGTGATGACTCAG
JMX26Vk3a (SEQ ID NO: 13)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTGTTGACRCAG
JMX26Vk3b (SEQ ID NO: 14)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATAGTGATGACGCAG
JMX26Vk3c (SEQ ID NO: 15)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTAATGACACAG
JMX26Vk4a (SEQ ID NO: 16)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGACATCGTGATGACCCAG
JMX26Vk5a (SEQ ID NO: 17)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAACGACACTCACGCAG
JMX26Vk6a (SEQ ID NO:18)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTGCTGACTCAG
JMX26 Vk6b (SEQ ID NO: 19)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATGTTGTGATGACACAG

In the foregoing sequences, R is an equal mixture of A and G, M is an equal mixture of A and C, Y is an equal mixture of C and T, W is an equal mixture of A and T, and S is an equal mixture of C and G.

Example 2

Amplification of a Repertoire of IgM or IgG Heavy Chain or Lambda Light Chain Variable Genes

Similar protocols are applied to the amplification of both heavy chain and lambda light chain genes. JMX26, or another primer without antibody specific sequences, is used for each of those applications. If JMX26 is used, the second strand DNA is generated with the primers listed below which contain JMX26 sequence, a restriction site (Sac I for lambda, Xho I for heavy chains), and a region that anneals to 1st strand cDNA in the framework 1 region of human antibody lambda light chain or heavy chain genes. The antibody annealing sequences were derived from the VBase database primers (www.mrc-cpe.cam.ac.uk/imt- doc/public/INTRO.html) which were designed based on the known sequences of human antibodies and are reported to cover the entire human antibody repertoire of lambda light chain and heavy chain genes.

Lambda Light Chain Framework 1 Specific Primers:
JMX26VL1a (SEQ ID NO:20)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTGCTGACTCAG
JMX26VL1b (SEQ ID NO: 21)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTGYTGACGCAG
JMX264VL1C (SEQ ID NO: 22)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTCGTGACGCAG
JMX26VL2 (SEQ ID NO: 23)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGCCCTGACTCAG
JMX26VL3a (SEQ ID NO:24)
GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGWGCTGACTCAG
JMX26VL3b (SEQ ID NO: 25)

GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGAGCTGACACAG

JMX26VL3c (SEQ ID NO: 26)
GTCACTCACGAACTCACGACTCACGGAGAGCTCTCTTCTGAGCTGACTCAG

JMX26VL3d (SEQ ID NO: 27)
GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGAGCTGATGCAG

JMX26VL4 (SEQ ID NO: 28)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGCYTGTGCTGACTCAA

JMX26VL5 (SEQ ID NO: 29)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGSCTGTGCTGACTCAG

JMX26VL6 (SEQ ID NO:30)
GTCACTCACGAACTCACGACTCACGGAGAGCTCAATTTTATGCTGACTCAG

JMX26VL7 (SEQ ID NO: 31)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGRCTGTGGTGACTCAG

JMX26VL8 (SEQ ID NO:32)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGACTGTGGTGACCCAG

JMX26VL4/9 (SEQ ID NO: 33)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCWGCCTGTGCTGACTCAG

JMX26VL10 (SEQ ID NO: 34)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGGCAGGGCTGACTCAG

In the foregoing sequences (and throughout this disclosure), R is an equal mixture of A and G, M is an equal mixture of A and C, Y is an equal mixture of C and T, W is an equal mixture of A and T, and S is an equal mixture of C and G.

Heavy Chain Framework 1 Specific Primers:

JMX24VH1a (SEQ ID NO: 35)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTKCAGCTGGTGCAG

JMX24VH1b (SEQ ID NO: 36)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTCCAGCTTGTGCAG

JMX26VH1c (SEQ ID NO: 37)
GTCACTCACGAACTCACGACTCACGGActcgagSAGGTCCAGCTGGTACAG

JMX26VH1d (SEQ ID NO: 38)
GTCACTCACGAACTCACGACTCACGGActcgagCARATGCAGCTGGTGCAG

JMX26VH2a (SEQ ID NO: 39)
GTCACTCACGAACTCACGACTCACGGActcgagCAGATCACCTTGAAGGAG

JMX26VH2b (SEQ ID NO: 40)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTCACCTTGARGGAG

JMX26VH3a (SEQ ID NO: 41)
GTCACTCACGAACTCACGACTCACGGActcgagGARGTGCAGCTGGTGGAG

JMX26VH3b (SEQ ID NO: 42)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTGCAGCTGGTGGAG

JMX26VH3c (SEQ ID NO: 43)
GTCACTCACGAACTCACGACTCACGGActcgagGAGGTGCAGCTGTTGGAG

JMX26VH4a (SEQ ID NO: 44)
GTCACTCACGAACTCACGACTCACGGActcgagCAGSTGCAGCTGCAGGAG

JMX26VH4b (SEQ ID NO: 45)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTGCAGCTACAGCAG

JMX26VH5a (SEQ ID NO: 46)
GTCACTCACGAACTCACGACTCACGGActcgagGARGTGCAGCTGGTGCAG

JMX26VH6a (SEQ ID NO: 47)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTACAGCTGCAGCAG

JMX26VH7a (SEQ ID NO: 48)
GTCACTCACGAACTCACGACTCACGGActcgagCAGGTSCAGCTGGTGCAA

In the foregoing sequences (and throughout this disclosure), R is an equal mixture of A and G, K is an equal mixture of G and T, and S is an equal mixture of C and G.

Blocking oligos for the constant domain of IgM, IgG, and lambda chains are designed. Essentially, a region downstream of that required for cloning the genes is identified, and within that region, a sequence useful for annealing a blocking oligo is determined. For example with IgG heavy chains, a native Apa I restriction site present in the CH1 domain can be used for cloning. Generally, the boundary oligo is located downstream of that native restriction site. Compatible nested oligos are then designed which contained all the elements described previously.

Once amplified, the lambda light chain genes are cloned as is described above for the kappa light chain genes. Likewise, amplified IgG heavy chain fragments are cloned by Xho I/Apa I into an appropriate expression vector that contains, in frame, the remaining portion of the CHI constant region. Suitable vectors include pRL5, pRL4, fdtetDOG, PHEN1, and pCANTAB5E. Amplified IgM heavy chain fragments are cloned by Xho I/EcoR I into an appropriate expression vector that contains, in frame, the remaining portion of the CH1 constant region. Like the Apa I present natively in IgG genes, the EcoR I site is native to the IgM CH1 domain. Libraries co-expressing both light chains and heavy chains can be screened or selected for Fabs with the desired binding activity.

Example 3

Amplification of a Repertoire of Human IgM Heavy Chain Genes

First Strand cDNA Synthesis

Human peripheral blood lymphocyte (PBL) mRNA was used as the original template to generate the first strand cDNA with ThermoScript RT-PCR System (Invitrogen Life Technologies). In addition to oligo dT primer, a phosphoramidate oligonucleotide (synthesized by Annovis Inc. Aston, PA) was also included in the reverse transcription reaction. The phosphoramidate oligonucleotide serves as a boundary for reverse transcriptase. The first strand cDNA synthesis was terminated at the location where the phosphoramidate oligonucleotide anneals with the mRNA. The phosphoramidate oligonucleotide, PN-1, was designed to anneal with the framework 1 region of immunoglobulin (Ig) heavy chain VH3 genes and PN-VH5 was designed to anneal with the framework 1 region of all the Ig heavy chain genes. A control for first strand cDNA synthesis was also set up by not including the phosphoramidate blocking oligonucleotide. The first strand cDNA product was purified by QIAquick PCR Purification Kit (QIAGEN).

Phosphoramidate Framework 1 Blocking Oligonucleotides for Ig Heavy Chain Genes have the Following Sequences:

PN-1 5' GCCTCCCCCAGACTC 3'	(SEQ ID NO:49)
PN-VH5 5' GCTCCAGACTGCACCAGCTGCAC(C/T)TCGG 3'	(SEQ ID NO:50)

Examination of the Blocking Efficiency

The blocking efficiency in first strand cDNA synthesis was examined by PCR reactions using blocking check primers and primer CM1, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. PCR was performed on a PTC-200 thermal cycler (MJ Research) by heating to 94° C. for 30 seconds and followed by cycles of 94° C. for 15 second, 60° C. for 15 second, and 72° C. for one minute. The blocking check primers were designed to anneal with the leader sequences of Ig heavy chain genes. The sequence of CM1, which hybridizes with the CH1 region of IgM, was 5' GCTCACACTAGTAGGCAGCTCAGCAATCAC 3' (SEQ ID NO: 51). Blocking was analyzed by gel electrophoresis of the PCR products. With appropriate number of cycles, less PCR product was observed from the reverse transcription reactions containing the blocking oligonucleotides than the one does not contain the blocking oligonucleotides, an indication that termination of first strand cDNA synthesis was provided by the hybridization of the blocking oligonucleotides.

The sequences of the blocking check Primers for Ig heavy chain genes have the following sequences:

H1/7blck 5' C TGG ACC TGG AGG ATC C 3'	(SEQ ID NO:52)
H1blck2 5' C TGG ACC TGG AGG GTC T 3'	(SEQ ID NO:53)
H1blck3 5' C TGG ATT TGG AGG ATC C 3'	(SEQ ID NO:54)
H2blck 5, GACACACTTTGCTCCACG 3'	(SEQ ID NO:55)
H2blck2 5' GAC ACA CTT TGC TAC ACA 3'	(SEQ ID NO:56)
H3blck 5' TGGGGCTGAGCTGGGTTT 3'	(SEQ ID NO:57)
H3blck2 5' TG GGA CTG AGC TGG ATT T 3'	(SEQ ID NO:58)
H3blck3 5' TT GGG CTG AGC TGG ATT T 3'	(SEQ ID NO:59)
H3blck4 5' TG GGG CTC CGC TGG GTT T 3'	(SEQ ID NO:60)
H3blck5 5' TT GGG CTG AGC TGG CTT T 3'	(SEQ ID NO:61)
H3blck6 5' TT GGA CTG AGC TGG GTT T 3'	(SEQ ID NO:62)
H3blck7 5' TT TGG CTG AGC TGG GTT T 3'	(SEQ ID NO:63)
H4blck 5' AAACACCTGTGGTTCTTC 3'	(SEQ ID NO:64)
H4blck2 5' AAG CAC CTG TGG TTT TTC 3'	(SEQ ID NO:65)
H5blck 5' GGGTCAACCGCCATCCT 3'	(SEQ ID NO:66)
H6blck 5' TCTGTCTCCTTCCTCATC 3'	(SEQ ID NO:67)

Second Strand cDNA Synthesis and Nesting Oligonucleotide Extension Reaction:

The purified first strand cDNA synthesis product was used in a nested oligo extension reaction with a hairpin-containing nesting oligonucleotide, dNTPs, Advantage-2 DNA polymerase mix (Clontech), and the reaction buffer. The extension reaction was performed with a GeneAmp PCR System 9700 thermocyler (PE Applied Biosystems). It was heated to 94° C. for 30 seconds and followed by ten cycles of 94° C. for 15 seconds, appropriate annealing temperature for each nesting oligonucleotide for 15 seconds, ramping the temperature to 90° C. at 10% of the normal ramping rate, and 90° C. for 30 seconds. The resulted heavy chain gene should contain a hairpin structure.

Nesting Oligonucleotides for Ig VH1 Heavy Chain genes had the following sequences:

hpVH1-1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAGGTGCAGCTGGTGCAG TCTGGGGCT GAGGTGAAGAAGCCTG AAG 3' (SEQ ID NO: 68)

hpVH1-2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGaTGCAGCTGGTGCAG TCTGGGGCTGAGGTGAAGAAGaCTAAT 3' (SEQ ID NO: 69)

hpVH 1-3
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATG CAG CTG GTG CAG TCT GGGCCT GAG GTG AAG AAG CCT ATT 3' (SEQ ID NO: 70)

hpVH1-4
5' CTCGAGGGCCCGCGAAAGCGGOCCCTCGAGGAGGTGCAGCTGGTGCAG TCTGGGGCTGAGGTGAAGAAGCCTGAAG 3' (SEQ ID NO: 71)

Nesting Oligonucleotides for Ig VH2 Heavy Chain Genes:

hpVH2-1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACATAA 3' (SEQ ID NO: 72)

hpVH2-2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTC ACC TTG AAG GAG TCT GGT CCT GYG CTG GTG AAA CCC AC TAA 3' Y:C/T (SEQ ID NO: 73)

Nesting Oligonucleotides for Ig VH3 Heavy Chain Genes:

hpVH3A1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GT(C/A) CAG CCT GGGAAA 3' C/A: M(SEQ ID NO: 74)

hpVH3A2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGTCTGGG GGAGGC (T/C)TGGT(A/C)AAGCCTGGGAAA 3' (SEQ ID NO: 75)

hpVH3A3
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CTGGGGGAGGTGTGGTACGGCCTGGGAAA 3' (SEQ ID NO: 76)

hpVH3A4
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGA CTGGAGGAGGCTTGATCCAGCCTGGGAAG 3' (SEQ ID NO: 77)

hpVH3A5
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CTGGGGGAGTCGTGGTACAGCCTGGGAAA 3' (SEQ ID NO: 78)

hpVH3A6
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CT CGGGGAGTCTTGGTACAGCCTGGGAAA 3' (SEQ ID NO: 79)

hpVH3A7
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTA CAG CCT GGCAAA 3' (SEQ ID NO: 80)

hpVH3A8
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTC CAG CCT GGAAAA 3' (SEQ ID NO: 81)

hpVH3A9
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTA GTT CAG CCT GGGAAA 3' (SEQ ID NO: 82)

hpVH3A10
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTA CAG CCA GGGAAA 3' (SEQ ID NO: 83)

ots-hp-VH3b
5 CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGGTGCAGCTGGTGGAGT CTGGGGGAGGCGTGGTCCAGCCTGGGTTT 3' (SEQ ID NO: 84)
hp-VH3B2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGGTGCAGCTGGTGGAGT CTGGGGGAGGCTTGGTCAAGCCTGGAAAG 3' (SEQ ID NO: 85)
hpVH3C
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTGTTG GA G TCT GGG GGA GGC TTG GTA CAG CCT GGGAAA 3' (SEQ ID NO: 86)

Nesting Oligonucleotides for Ig VH4 Heavy Chain Genes:
hpVH4-1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG STG CAG CTG CAG GA G TCG GGC CCA GGA CTG GTG AAG CCT T AAA 3' S: C/G (SEQ ID NO: 87)
hpVH4-2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG CTG CAG CTG CAG GAG TCG GGC TCA GGA CTG GTG AAG CCT T AAA 3' (SEQ ID NO: 88)
hpVH4-3
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG AG GTG CAG CTG CAGCAG TGG GGC GCA GGA CTG TTG AAG CCT T AAT 3' (SEQ ID NO: 89)

Nesting Oligonucleotides for Ig VH5 Heavy Chain Genes:
othpVH52
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGCAGT CT GGAGCAGAGGTGAAAAAGCCCGGGGAAAA 3' (SEQ ID NO: 90)

Nesting Oligonucleotides for Ig VH6 Heavy Chain Genes:
hpVH6
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC AAA 3' (SEQ ID NO: 91)

Nesting Oligonucleotides for Ig VH7 Heavy Chain Genes:
hpVH7
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT ATA 3' (SEQ ID NO: 92)

Additional Ig Heavy Chain Nesting Oligonucleotides:
hpVH 3 kb1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCGACTGGTGGAG TCTGGGGGAGACTTGGTAGAACCGGGGAAG 3' (SEQ ID NO: 93)
hpVH 3 kb2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGATGCAACTGGTGGAG TCTGGGGGAGCCTTCGTCCAGCCGGGGAAG 3' (SEQ ID NO: 94)

Single Primer Amplification of IgM Hairpin-Containing Fd Fragments

Products from the nesting oligo extension reaction (i.e. the engineered template) were amplified using Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, dNTPs, and a single primer named CM3 primer. The sequence for the CM3 primer, which anneals with the CHI region of IgM, was:
5' AGAATTTGACTAGTTGGCAAGAGGCACGTTCTTTTCTTTGTTGCCGT 3' (SEQ ID NO: 231).

The amplification reaction was performed with a GeneAmp PCR System 9700 thermocyler (PE Applied Biosystems). It was initially heated to 94° C. for 30 seconds and followed by thirty to forty cycles of 94° C. for 15 seconds, appropriate annealing temperature for 15 seconds, ramping the temperature to 90° C. at 10% of the normal ramping speed, and at 90° C. for 30 seconds. The amplified product was examined by electrophoresis to be of the expected size, ~0.7 kb. The amplified fragments were cloned into an expression vector and their sequences were confirmed to be human IgM.

Example 4

Amplification of a Repertoire of Human IgG Heavy Chain Genes from a Donor Immunized with Hepatitis B Surface Antigen

First Strand cDNA Synthesis

The same protocol as example 3 is employed using mRNA of PBL from a human donor immunized with hepatitis B surface antigen and the phosphoramidate boundary oligonucleotides designed to anneal with the leader sequence of the Ig heavy chain genes. The phosphoramidate leader boundary oligonucleotides for Ig heavy chain genes have the following sequences:

PNVR3ld 5'CACCTCACACTGGACACCTTT 3' (SEQ ID NO:95)

PNVH4ld 5'CTGGGACAGGACCCATCTGGG 3' (SEQ ID NO:96)

PNVH1ld 5'TGGGAGTGGGCACCTGTGG 3' (SEQ ID NO:97)

PNVH2ld 5'CTGGGACAAGACCCATGAAG 3' (SEQ ID NO:98)

PNVH5ld 5'TCGGAACAGACTCCTTGGAGA 3' (SEQ ID NO:99)

PNVH6ld 5'CTGTGACAGGACACCCCATGG 3'(SEQ ID NO:100)

Examination of the Blocking Efficiency

The blocking efficiency in first strand cDNA synthesis is examined by PCR reactions using dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, the first strand cDNA synthesis product, the blocking check primers in Example 3, and the pooled primer mixture of CG1Z, CG2speI, CG3speI, and CG4SpeI. The sequence of primer CG1Z, which hybridized with the CH1 region of IgG1, is 5' GCATGTACTAGTTTTGTCACAAGATTTGGG 3'. (SEQ ID NO: 101) The sequence of primer CG2speI, which hybridized with the CH1 region of IgG2, is 5' AAGGAAACTAGTTTTGCGCTCAACTGTCTTGTCCACCT 3'. (SEQ ID NO: 102) The sequence of primer CG3speI, which hybridized with the CHI region of IgG3, is 5' AAGGAAACTAGTGTCACCAAGTGGGGTTTTGAGCTC 3'. (SEQ ID NO: 103) The sequence of primer CG4speI, which hybridized with the CHI region of IgG4, is 5' AAGGAAACTAGTACCATAGGACTCAACTCTCTTG 3'. (SEQ ID NO: 104) PCR is performed on a PTC-200 thermal cycler (MJ Research) by heating to 94° C. for 30 seconds before the following cycle is run, 94° C. for 15 second, 60° C. for 15 second, and 72° C. for one minute. The PCR products were analyzed by gel electrophoresis. With appropriate number of cycles less PCR products were observed from reverse transcription reactions containing the blocking oligonucleotide than the one does not contain blocking oligonucleotide, an indication that termination of first strand cDNA synthesis was provided by hybridization of the leader boundary oligonucleotides.

Second Strand cDNA Synthesis and Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 is employed with nesting oligonucleotides having the following sequences are used.

Nesting Oligonucleotides for Ig Heavy Chain VH3 Genes:
HpH3 L1
5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGSAGGTGCAGCTGGTGGAG TCYGAAA 3' where S is an equal mixture of C and G, and Y is an equal mixture of T and C (SEQ ID NO: 105)

HpH3L2
5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAG CTG TTG GAG TCT GAAT 3' (SEQ ID NO: 106)

HpH3L3
5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG ACT GATA 3' (SEQ ID NO: 107)

HpH3L4
5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG TCT CAAA 3' (SEQ ID NO: 108)

Nesting Oligonucleotides for Ig Heavy Chain VH4 Genes:

HpH4L1
5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG STG CAG CTG CAG GAG TCG GAAA 3' where S is an equal mixture of C and G (SEQ ID NO: 109)

HpH4L2
5 CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG CTG CAG CTG CAG GAG TCC AAA 3' (SEQ ID NO: 110)

HpH4L3
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTA CAG CAG TGG GAAA 3' (SEQ ID NO: 111)

Nesting Oligonucleotides for Ig Heavy Chain VH1 Genes:

HpH1L1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTB CAG CTK GTG CAG AAA 3' where B is an equal mixture of C, G and T and K is an equal mixture of G and T (SEQ ID NO: 112)

HpH1L2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG SAG GTC CAG CTG GTA CAG AAA 3' where S is an equal mixture of C and G (SEQ ID NO: 113)

HpH1L3
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATG CAG CTG GTG CAG AAA 3' (SEQ ID NO: 114)

HpH1L4
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAA ATG CAG CTG GTG CAG AAA 3' (SEQ ID NO: 115)

Nesting Oligonucleotides for Ig Heavy Chain VH2 Genes:

HpH2L1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATC ACC TTG AAG GAG TCT AAA 3' (SEQ ID NO: 116)

HpH2L2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTC ACC TTG AAG GAG TCT AAA 3' (SEQ ID NO: 117)

Nesting Oligonucleotides for Ig Heavy Chain VH5 Genes:

HpH5L1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG CAG AAA 3' (SEQ ID NO: 118)

HpH5L2
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAA GTG CAG CTG GTG CAG AAA 3' (SEQ ID NO: 119)

Nesting Oligonucleotides for Ig Heavy Chain VH6 Genes:

HpH6L1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTA CAG CTG CAG CAG TC AAA 3' (SEQ ID NO: 120)

Nesting Oligonucleotides for Ig Heavy Chain VH7 Genes:

HpH7L1
5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTG GTG CAA TAAA 3' (SEQ ID NO: 121)

Single Primer Amplification of Human IG Heavy Chain Fd Hairpin Containing Fragments

The sample protocol as Example 3 was employed using CGIZ, CG2speI, CG3speI, or CG4SpeI as the primer.

Cloning of Amplified IgG Heavy Chain Fd Fragments into a Phage Display Vector

The amplified IgG heavy chain fd hairpin fragments are analyzed by gel electrophoresis. The ~0.7 kb fragment is separated from the primers by cutting out the gel slice and the DNA was collected by electroelution. The eluted DNA was precipitated by ethanol and resuspended in water. It is digested with restriction enzymes XhoI and SpeI and purified by the QIAquick PCR Purification Kit (QIAGEN). The purified XhoI-SpeI fragment is ligated into a suitable plasmid into which the light chain kappa genes amplified from the same donor had previously been cloned. The ligated reaction was transformed into E. coli XL-1 Blue strain {F'proA$^+$B$^+$lac1$_q$ Δ (lacZ) M15 Tn10/recA1 endA1 gyrA96thi-1 hsdR17 supE44 relA1 lac} by electroporation.

Selection of Human IgG Antibodies that Bind with the Hepatitis B Surface Antigen

The XL-1 Blue cells electroporated with the ligation reaction of the phagemid vector and the heavy chain Fd fragments were grown in SOC medium at 37° C. with shaking for one hour. SOC medium is 20 mM glucose in SB medium which contains 1% MOPS hemisodium salt, 3% Bacto Tryptone, and 2% Bacto Yeast Extract. Cells transformed with the plasmid were selected by adding carbenicillin to the culture and they were grown for two hours before infected with a helper phage, VCSM13. After two hours XL-1 Blue cells infected with the helper phage were selected by adding Kanamycin to the culture and the infected cells were amplified overnight by growing at 37° C. with shaking. The next morning the amplified phages were harvested by precipitating with polyethylene glycol (PEG) from the culture supernatant. The PEG precipitated phages were collected by centrifugation. They were resuspended in 1% bovine serum albumin (BSA) in TBS buffer and used in panning for selecting human IgG antibodies that bind with the hepatitis B surface antigen. The resuspended phages were bound with the hepatitis B surface antigen immobilized on the ELISA plate (Costar). The unbound phages were washed off with a washing buffer (0.5% Tween 20 in PBS) and the bound phages were eluted off the plate with a phage elution buffer (0.1M HCl/glycine, pH 2.2, 1 mg/ml BSA) and neutralized with a neutralization buffer (2M Tris Base). The eluted phages were infected with E. coli ER strain {F' proA$_+$B$^+$ lac1^q Δ (lacZ) M15/fhuA2 (ton A)Δ(lac-proAB) supE thi-1 (hsdMS-mcrB) 5}, followed by infection with VCSM13 helper phage. The panning procedure for selecting antibodies bound to hepatitis B surface antigen were repeated three more times.

ELISA Screening of Antibody Clones that Bind with the Hepatitis B Surface Antigen

Phages eluted at the fourth round of panning were infected with E. coli Top 10F' strain {F' lac$_1$^{q}, Tn10 (Tet^R mcrA Δ(mrr-hsdRMS-mcrBC) Φ8(lacZ Δm15 Δlacx74 deoR recA1 araD13 Δ(ara-leu)7697 galU galK [sL(Str^R) end A1 nupG) and plated on LB-agar plates containing carbenicilin and tetracycline. Individual clones were picked from the plates and grown overnight in SB medium containing carbenicilin and tetracycline. The IgG Fab fragment will be secreted into the culture supernatant. The next morning cells were removed from these cultures by centrifugation and the culture supernatant was screened in ELISA assay for binding to hepatitis B surface antigen immobilized on the ELISA plates. To reduce false positives the ELISA plates were preblocked with BSA before binding with the Fab fragments in culture supernatant. The non-binding Fab fragments were washed off by a washing solution (0.05% Tween 20 in PBS). Following the wash, plates were incubated with anti-human

IgG (Fab')$_2$ conjugated with alkaline phosphatase (Pierce) which reacts with p-Nitrophenyl phosphate (Sigma), a chromogenic substrate that shows absorbance at OD405. Positive binding clones were identified by a plate reader (Bio RAD Model 1575) with light absorbance at OD405. Among the ninety-four clones screened there were twenty-eight positive clones.

Characterization of the Hepatitis B Surface Antigen Binding Clones

The IgG heavy chain genes of positive clones from ELISA screening were characterized by DNA sequencing. Plasmid DNA was extracted from the positive clones and sequenced using primers leadVHpAX, NdP, or SeqGZ (Retrogen, San Diego, Calif.). The sequencing primers have the following sequences:

VBVH3A 5' GAGCCGCACGAGCCCCTCGAGGARGTGCAGCTGGTGGAG 3' (SEQ ID NO: 122)

VBVH 3B 5' GAGCCGCACGAGCCCCTCGAGGAGGTGCAGCTGGTGGAG 3' (SEQ ID NO: 123)

VBVH 3C$_5$' GAGCCGCACGAGCCCCTCGAGGAGGTGCAGCTGTTGGAG 3' (SEQ ID NO: 124)

VBVH4A 5' GAGCCGCACGAGCCCCTCGAGCAG(CG) TGCAGCTGCAGGAG 3' (SEQ ID NO: 125)

VBVH4B 5' GAGCCGCACGAGCCCCTCGAGCAGGTGCAGCTACAGCAG 3' (SEQ ID NO: 126)

LeadVHPAX 5' GCGGCGCAGCCGGCGATGGCG 3' (SEQ ID NO: 127)

NdP 5' AGCGTAGTCCGGAACGTCGTACGG (SEQ ID NO: 128)

SeqGZ 5' GAAGTAGTCCTTGACCAG 3' (SEQ ID NO: 129)

The sequences of the variable region of these IgG heavy chain genes from nineteen positive clones are shown in FIG. **5**. The great diversity of these IgG heavy chain genes shows this method can efficiently amplify the repertoire of human IgG heavy chain genes from immunized donors.

Example 5

Amplification of a Repertoire of Human Light Chain Kappa Genes

First Strand cDNA Synthesis

The same protocol as example 3 is employed using the phosphoramidate boundary oligonucleotides designed to hybridize with the leader sequence of the kappa light chain genes. The phosphoramidate leader boundary oligonucleotides for kappa light chain genes have the following sequences:

PNK1ld: 5' T GTC ACA TCT GGC ACC TGG 3' (SEQ ID NO: 130)

PNK2ld: 5' TC CCC ACT GGA TCC AGG GAC 3' (SEQ ID NO: 131)

PNK3ld: 5° C. TCC GOT GGT ATC TOG GAG 3' (SEQ ID NO: 132)

PNK4ld: 5' TC CCC GTA GGC ACC AGA GA 3' (SEQ ID NO: 133)

PNK5ld: 5' TC TGC CCT GGT AT C AGA GAT 3' (SEQ ID NO: 134)

PNK6ld: 5' ACC CCT GGA GGC TGG AAC 3' (SEQ ID NO: 135)

Examination of the Blocking Efficiency

The blocking efficiency in first Strand cDNA Synthesis was examined by PCR reactions using blocking check primers and primer CK1DX2, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. PCR was performed on a PTC-200 thermal cycler (MJ Research) by heating to 94° C. for 30 seconds and followed by cycles of 94° C. for 15 second, 60° C. for 15 second, and 72° C. for one minute. The blocking check primers were designed to anneal with the leader sequences of kappa light chain genes. The sequence of CK1DX2, which hybridizes with the constant region of Kappa light chain, was 5' AGACAGTGAGCGCCGTCTAGAATTAACACTCTCCCCTGTTGAAGCTCTTTGTGAC GGGCGAACTCAG 3'. (SEQ ID NO: 136) Blocking was analyzed by gel electrophoresis of the PCR products. With appropriate number of cycles less PCR products was observed from reverse transcription reactions containing the blocking oligonucleotide than one that does not contain blocking oligonucleotide, an indication that termination of first strand cDNA synthesis was provided by hybridization of the leader boundary oligonucleotides.

Blocking Check Primers for Kappa Light Chain Genes have the Following Sequences:

K1blck: 5'CTCCGAGGTGCCAGATGT 3' (SEQ ID NO: 137)

K1/2blck2: 5' GCT CAG CTC CTG GGG CT 3' (SEQ ID NO: 138)

K2blck: 5' GTCCCTGGATCCAGTGAG 3' (SEQ ID NO: 139)

K3blck: 5' CTCCCAGATACCACCGGA 3' (SEQ ID NO: 140)

K3blck2: 5' GCG CAG CTT CTC TTC CT 3' (SEQ ID NO: 141)

K3blck3: 5' CAC AGC TTC TTC TTC CTC 3' (SEQ ID NO: 142)

K4blck: 5' ATCTCTGGTGCCTACGGG 3' (SEQ ID NO: 143)

K5blck: 5' ATCTCTGATACCAGGGCA 3' (SEQ ID NO: 144)

K6blck: 5' GTTCCAGCCTCCAGGGGT 3' (SEQ ID NO: 145)

Second Strand cDNA Synthesis and Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 is employed using nesting oligonucleotides having the following sequences:

Nesting Oligonucleotides for Light Chain Kappa Vk1:

HpK1L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GMC ATC CAG ATG ACC CAG TCT CCTAA 3' wherein M is an equal mixture of A and C (SEQ ID NO: 146)

HpK1L2

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC AAC ATC CAG ATG ACC CAG TCT CC TAA 3' (SEQ ID NO: 147)

HpK1L3

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GMC ATC CAG TTG ACC CAG TCT CC TAA 3' wherein M is an equal mixture of A and C (SEQ ID NO: 148)

HpK1L4

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GCC ATC CGG ATG ACC CAG TCT CCTAT 3' (SEQ ID NO: 149)

HpK1L5

5' GAGCTCGGCCCGCGAAAGCOGGCCGAGCTC GTC ATC TGG ATG ACC CAG TCT CCTAT 3' (SEQ ID NO: 150)

Nesting Oligonucleotides for Light Chain Kappa Vk2:

HpK2L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT ATT GTG ATG ACC CAG ACT CTTA 3' (SEQ ID NO: 151)

HpK2L2

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT GTT GTG ATG ACT CAG TCT CCTAA 3' (SEQ ID NO: 152)

HpK2L3

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT ATT GTG ATG ACT CAG TCT CCTAA 3' (SEQ ID NO: 153)

Nesting Oligonucleotides for Light Chain Kappa Vk3:

HpK3L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTG TTG ACG CAG TCT CCTAA 3' (SEQ ID NO: 154)

HpK3L2

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATA GTG ATG ACG CAG TCT CCTAA3' (SEQ ID NO: 155)

HpK3L3

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTA ATG ACA CAG TCT CCTAA3' (SEQ ID NO: 156)

Nesting Oligonucleotides for Light Chain Kappa Vk4:

HpK4L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAC ATC GTG ATG ACC CAG TCT CCTAT3' (SEQ ID NO: 157)

Nesting Oligonucleotides for Light Chain Kappa Vk5:

HpK5L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ACG ACA CTC ACG CAG TCT CCTAA3' (SEQ ID NO: 158)

Nesting Oligonucleotides for Light Chain Kappa Vk6:

HpK6L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTG CTG ACT CAG TCT CCTAT3' (SEQ ID NO: 159)

Single Primer Amplification of Kappa Hairpin Fragments

The same protocol as Example 3 is employed using CK1DX2 as the primer.

Example 6

Amplification of a Repertoire of Human Light Chain Lambda Genes

First Strand cDNA Synthesis

The same protocol as example 3 is employed using the following phosphoramidate boundary oligonucleotides designed to hybridize with the leader sequence of the lambda light chain genes. The phosphoramidate boundary oligonucleotides for lambda light chain genes have the sequences:

PNL1Id: 5' CTG GGC CCA GGA CCC TGT GC 3' (SEQ ID NO: 160)

PNL2Id: 5' CTG GGC CCA GGA CCC TGT 3'. (SEQ ID NO: 161)

PNL3Id: 5' GA GGC CAC AGA GCC TGT GCA GAG AGT GAG 3' (SEQ ID NO: 162)

PNL4Id1: 5' CAG AGC ACA GAG ACC TGT GGA3'(SEQ ID NO: 163)

PNL4Id2: 5' CTG GGA GAG AGA CCC TGT CCA3' (SEQ ID NO: 164)

PNL5Id1: 5' CTG GGA GAG GGA ACC TGT GCA3' (SEQ ID NO: 165)

PNL6Id1: 5' ATT GGC CCA AGA ACC TGT GCA3' (SEQ ID NO: 166)

PNL7Id1: 5' CTG AGA ATT GGA CCC TGG GCA3' (SEQ ID NO: 167)

PNL8Id1: 5' CTG AGA ATC CAC TCC TGA TCC3' (SEQ ID NO: 168)

PNL9Id1: 5' CTG GGA GAG GGA CCC TGT GAG3' (SEQ ID NO: 169)

PNL10Id1: 5' CTG GAC CAC TGA CAC TGC AGA3' (SEQ ID NO: 170)

Examination of the Blocking Efficiency

The same protocol as example 3 is employed using the following blocking check primers and primer CL2DX2, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. The blocking check primers have the following sequences:

L1blck: 5' CAC TGY GCA GGG TCC TGG 3' (SEQ ID NO: 171)

L2blck: 5' CAG GGC ACA GGG TCC TGG 3' (SEQ ID NO: 172)

L3blck1: 5' TAC TGC ACA GGA TCC GTG 3' (SEQ ID NO: 173)

L3blck2: 5' CAC TTT ACA GGT TCT GTG 3' (SEQ ID NO: 174)

L3blck3: 5' TTC TGC ACA GTC TCT GAG 3' (SEQ ID NO: 175)

L3blck4: 5' CTC TGC ACA GGC TCT GAG 3' (SEQ ID NO: 176)

L3blck5: 5' CTT TGC TCA GGT TCT GTG 3' (SEQ ID NO: 177)

L3blck6: 5' CAC TGC ACA GGC TCT GTG 3' (SEQ ID NO: 178)

L3blck7: 5' CTC TAC ACA GGC TCT ATT 3' (SEQ ID NO: 179)

L3blck7: 5' CTC TGC ACA GTC TCT GTG 3' (SEQ ID NO: 180)

L4blck1: 5' TTC TCC ACA GGT CTC TGT 3' (SEQ ID NO: 181)

L4blck2: 5' CAC TGG ACA GGG TCT CTC 3' (SEQ ID NO: 182)

L5blck1: 5' CAC TGC ACA GGT TCC CTC 3' (SEQ ID NO: 183)

L6blck: 5' CAC TGC ACA GGT TCT TGG 3' (SEQ ID NO: 184)

L7blck: 5' TGC TGC CCA GGG TCC AAT 3' (SEQ ID NO: 185)

L8blck: 5' TAT GGA TCA GGA GTG GAT 3' (SEQ ID NO: 186)

L9blck: 5' CTC CTC ACA GGG TCC CTC 3' (SEQ ID NO: 187)

L10blck: 5' CAC TCT GCA GTG TCA GTG 3' (SEQ ID NO: 188)

The Sequence of CL2DX2, which hybridizes with the CL region of Lambda genes, has this sequence: 5' AGACAGTGACGCCGTCTAGAATTATGAACATTCTGTAGG 3' (SEQ ID NO: 189).

Second Strand cDNA Synthesis and Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 is employed using the nesting oligonucleotides having the following sequences:

Nesting Oligonucleotides for Lambda Light Chain VL1:

HpL1$_L$ 1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GTG CTG ACT CAG CCA CCAAA 3' (SEQ ID NO: 190)

HpL1L2

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GTG YTG ACG CAG CCG CCAAA 3' (SEQ ID NO: 191)

Nesting Oligonucleotides for Lambda Light Chain VL2:

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GCC CTG ACT CAG CCT SAAA3' (SEQ ID NO: 192)

Nesting Oligonucleotides for Lambda Light Chain VL3:

HpL3L1

5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACT CAG CCA CYAAA3' (SEQ ID NO: 193)

HpL3L2
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACA CAG CYA CCAAT 3' (SEQ ID NO: 194)
HpL3L3
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC T CT TCT GAG CTG ACT CAG GAC CCAAA 3' (SEQ ID NO: 195)
HpL3L4
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GTG CTG ACT CAG CCA CCAAA 3' (SEQ ID NO: 196)
HpL3L5
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ATG CAG CCA CCAAA 3' (SEQ ID NO: 197)
HpL3L6
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACA CAG CCA TCAAA3' (SEQ ID NO: 198)

Nesting Oligonucleotides for Lambda Light Chain VL4:
HpL4L1
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CTG CCT GTG CTG ACT CAG CCC CCAAA3' (SEQ ID NO: 199)
HpL4L2
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAA TCA TCAAA3' (SEQ ID NO: 200)
HpL4L3
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CTT GTG CTG ACT CAA TCG CCAAA3' (SEQ ID NO: 201)

Nesting Oligonucleotides for Lambda Light Chain VL5:
HpL5L1 5 e. 5b
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAG CCA YCAAA3' (SEQ ID NO: 202)
HpL5L2 5c
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG GCT GTG CTG ACT CAG CCG GCAAA3' (SEQ ID NO: 203)

Nesting Oligonucleotides for Lambda Light Chain VL6:
HpL6L1 6a
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC AAT TTT ATG CTG ACT CAG CCC CAAAA3' (SEQ ID NO: 204)

Nesting Oligonucleotides for Lambda Light Chain VL7 and VL8:
HpL7/8L1
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG ACT GTG GTG ACY CAG GAG CCAAA3' (SEQ ID NO: 205)
HpL7L2
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC G CAG GCT GTG GTG ACT CAG GAG CCAAA3' (SEQ ID NO: 206)

Nesting Oligonucleotides for Lambda Light Chain VL9:
HpL9L
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAG CCA CCAAA3' (SEQ ID NO: 207)

Nesting Oligonucleotides for Lambda Light Chain VL10:
5' GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG GCA GGG CTG ACT CAG CCA CCAAA3' (SEQ ID NO: 208)

Single Primer Amplification of Lambda Hairpin Containing Fragments

The same protocol as Example 3 is employed using CL2DX2 as the primer.

Example 7

Amplification of a Repertoire of Human IgG Heavy Chain Genes from a Donor Immunized with Hepatitis B Surface Antigen

First Strand cDNA Synthesis

The same protocol as example 3 was employed using mRNA of PBL from a human donor immunized with hepatitis B surface antigen as the original template using blocking oligonucleotides that anneal to FR1 of the variable heavy chain.

Examination of the Blocking Efficiency

The same protocol as example 4 was employed.

Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 was employed.

Single Primer Amplification of Human IgG Heavy Chain Fd Hairpin Containing Fragments

The sample protocol as Example 4 was employed.

Cloning of Amplified IgG Heavy Chain Fd Fragments into a Phage Display Vector

The sample protocol as Example 4 was employed.

Selection of Human IgG Antibodies that Bind with the Hepatitis B Surface Antigen

The sample protocol as Example 4 was employed.

ELISA Screening of Antibody Clones that Bind with the Hepatitis B Surface Antigen

The sample protocol as Example 4 was employed. Among the ninety-four clones screened eighty clones are positive.

Characterization of the Hepatitis B Surface Antigen Binding Clones

The sample protocol as Example 4 was employed. Sequences of the variable regions of the heavy chain genes from fourteen positive clones are listed in FIG. **6**. The sequence diversity of these clones and others produced shows this method can efficiently amplify the repertoire of human heavy chain genes from immunized donors.

It will be understood that various modifications may be made to the embodiments described herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of this disclosure.

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<220> FEATURE:
<223> OTHER INFORMATION: blocking oligonucleotide

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<223> OTHER INFORMATION: n is c with a phosphorothionate backbone
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (91)..(91)
<223> OTHER INFORMATION: n is t with a phosphorothionate backbone
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (93)..(93)
<223> OTHER INFORMATION: n is g with a phosphorothionate backbone
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cgccttcgtt tgatytccac cttggtccnt ngn                                  93


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<220> FEATURE:
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 5
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 5

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<210> SEQ ID NO 6
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<212> TYPE: DNA
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<223> OTHER INFORMATION: primer

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<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 7

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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 8

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<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 9

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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 10

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<220> FEATURE:
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 14
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<220> FEATURE:
<223> OTHER INFORMATION: primer

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<212> TYPE: DNA
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<400> SEQUENCE: 16

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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 17

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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 18

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 21
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 21

gtcactcacg aactcacgac tcacggagag ctccagtctg tgytgacgca g              51


<210> SEQ ID NO 22
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 23
<211> LENGTH: 51
<212> TYPE: DNA
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<210> SEQ ID NO 24
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 24

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<210> SEQ ID NO 25
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 25

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<210> SEQ ID NO 26
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 27

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<210> SEQ ID NO 28
<211> LENGTH: 51
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 28

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<210> SEQ ID NO 29
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 29

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<210> SEQ ID NO 30
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 30

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<210> SEQ ID NO 31
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 31

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<210> SEQ ID NO 32
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 32
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<210> SEQ ID NO 33
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 33

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<210> SEQ ID NO 34
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 34

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<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 36
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 36

gtcactcacg aactcacgac tcacggactc gagcaggtcc agcttgtgca g              51


<210> SEQ ID NO 37
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 37

gtcactcacg aactcacgac tcacggactc gagsaggtcc agctggtaca g              51


<210> SEQ ID NO 38
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<223> OTHER INFORMATION: primer

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 39

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<210> SEQ ID NO 40
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 40

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<210> SEQ ID NO 41
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 41

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<210> SEQ ID NO 42
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 42

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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 43

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<210> SEQ ID NO 44
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<210> SEQ ID NO 45
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 45

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<223> OTHER INFORMATION: primer

<400> SEQUENCE: 46

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 47

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<220> FEATURE:
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<400> SEQUENCE: 48

gtcactcacg aactcacgac tcacggactc gagcaggtsc agctggtgca a              51


<210> SEQ ID NO 49
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<212> TYPE: DNA
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<223> OTHER INFORMATION: blocking oligonucleotide

<400> SEQUENCE: 49

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<210> SEQ ID NO 50
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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: n is c or t

<400> SEQUENCE: 50

gctccagact gcaccagctg cacntcgg                                        28


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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

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<210> SEQ ID NO 52
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 52

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<210> SEQ ID NO 53
<211> LENGTH: 17
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 53

ctggacctgg agggtct                                                    17


<210> SEQ ID NO 54
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 54

ctggatttgg aggatcc                                                    17


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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 55

gacacacttt gctccacg                                                   18


<210> SEQ ID NO 56
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 56

gacacacttt gctacaca                                                   18


<210> SEQ ID NO 57
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 57

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 58
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 59

ttgggctgag ctggattt                                                    18


<210> SEQ ID NO 60
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 60

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<210> SEQ ID NO 61
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 61

ttgggctgag ctggcttt                                                    18


<210> SEQ ID NO 62
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 62

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<210> SEQ ID NO 63
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 63

tttggctgag ctgggttt                                                    18


<210> SEQ ID NO 64
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 64

aaacacctgt ggttcttc                                                    18


<210> SEQ ID NO 65
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 65

aagcacctgt ggtttttc                                                    18


<210> SEQ ID NO 66
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 66

gggtcaaccg ccatcct                                                     17


<210> SEQ ID NO 67
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 67

tctgtctcct tcctcatc                                                    18


<210> SEQ ID NO 68
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 68

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tggtgcagtc tggggctgag      60

gtgaagaagc ctgaag                                                      76


<210> SEQ ID NO 69
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 69

ctcgagggcc cgcgaaagcg ggccctcgag cagatgcagc tggtgcagtc tggggctgag      60

gtgaagaaga ctaat                                                       75


<210> SEQ ID NO 70
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 70

ctcgagggcc cgcgaaagcg ggccctcgag cagatgcagc tggtgcagtc tgggcctgag      60

gtgaagaagc ctatt                                                       75


<210> SEQ ID NO 71
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
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<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 71

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtgcagtc tggggctgag 60

gtgaagaagc ctgaag 76

<210> SEQ ID NO 72
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 72

ctcgagggcc cgcgaaagcg ggccctcgag cagatcacct tgaaggagtc tggtcctacg 60

ctggtgaaac ccacataa 78

<210> SEQ ID NO 73
<211> LENGTH: 77
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 73

ctcgagggcc cgcgaaagcg ggccctcgag caggtcacct tgaaggagtc tggtcctgyg 60

ctggtgaaac ccactaa 77

<210> SEQ ID NO 74
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (66)..(66)
<223> OTHER INFORMATION: n is c or a

<400> SEQUENCE: 74

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

ttggtncagc ctgggaaa 78

<210> SEQ ID NO 75
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (61)..(61)
<223> OTHER INFORMATION: n is t or c
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (66)..(66)
<223> OTHER INFORMATION: n is a or c

<400> SEQUENCE: 75

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

ntggtnaagc ctgggaaa 78

<210> SEQ ID NO 76
<211> LENGTH: 78

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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 76

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggt 60

gtggtacggc ctgggaaa 78

<210> SEQ ID NO 77
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 77

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagac tggaggaggc 60

ttgatccagc ctgggaag 78

<210> SEQ ID NO 78
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 78

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggagtc 60

gtggtacagc ctgggaaa 78

<210> SEQ ID NO 79
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 79

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tcggggagtc 60

ttggtacagc ctgggaaa 78

<210> SEQ ID NO 80
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 80

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

ttggtacagc ctggcaaa 78

<210> SEQ ID NO 81
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 81

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

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ttggtccagc ctggaaaa 78

<210> SEQ ID NO 82
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 82

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

ttagttcagc ctgggaaa 78

<210> SEQ ID NO 83
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 83

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tgggggaggc 60

ttggtacagc cagggaaa 78

<210> SEQ ID NO 84
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 84

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tggtggagtc tgggggaggc 60

gtggtccagc ctgggttt 78

<210> SEQ ID NO 85
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 85

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tggtggagtc tgggggaggc 60

ttggtcaagc ctggaaag 78

<210> SEQ ID NO 86
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 86

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tgttggagtc tgggggaggc 60

ttggtacagc ctgggaaa 78

<210> SEQ ID NO 87
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

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<400> SEQUENCE: 87

ctcgagggcc cgcgaaagcg ggccctcgag cagstgcagc tgcaggagtc gggcccagga 60

ctggtgaagc cttaaa 76

<210> SEQ ID NO 88
<211> LENGTH: 76
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 88

ctcgagggcc cgcgaaagcg ggccctcgag cagctgcagc tgcaggagtc gggctcagga 60

ctggtgaagc cttaaa 76

<210> SEQ ID NO 89
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 89

ctcgagggcc cgcgaaagcg ggccctcgag aggtgcagct gcagcagtgg ggcgcaggac 60

tgttgaagcc ttaat 75

<210> SEQ ID NO 90
<211> LENGTH: 80
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 90

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtgcagtc tggagcagag 60

gtgaaaaagc ccggggaaaa 80

<210> SEQ ID NO 91
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 91

ctcgagggcc cgcgaaagcg ggccctcgag caggtacagc tgcagcagtc aggtccagga 60

ctggtgaagc ccaaa 75

<210> SEQ ID NO 92
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 92

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tggtgcaatc tgggtctgag 60

ttgaagaagc ctata 75

<210> SEQ ID NO 93

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<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 93

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcgac tggtggagtc tgggggagac     60

ttggtagaac cggggaag                                                   78


<210> SEQ ID NO 94
<211> LENGTH: 78
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 94

ctcgagggcc cgcgaaagcg ggccctcgag gagatgcaac tggtggagtc tgggggagcc     60

ttcgtccagc cggggaag                                                   78


<210> SEQ ID NO 95
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 95

cacctcacac tggacacctt t                                               21


<210> SEQ ID NO 96
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 96

ctgggacagg acccatctgg g                                               21


<210> SEQ ID NO 97
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 97

tgggagtggg cacctgtgg                                                  19


<210> SEQ ID NO 98
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 98

ctgggacaag acccatgaag                                                 20


<210> SEQ ID NO 99
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 99

tcggaacaga ctccttggag a                                              21


<210> SEQ ID NO 100
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 100

ctgtgacagg acaccccatg g                                              21


<210> SEQ ID NO 101
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 101

gcatgtacta gttttgtcac aagatttggg                                     30


<210> SEQ ID NO 102
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 102

aaggaaacta gttttgcgct caactgtctt gtccacctt                           39


<210> SEQ ID NO 103
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 103

aaggaaacta gtgtcaccaa gtggggtttt gagctc                              36


<210> SEQ ID NO 104
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 104

aaggaaacta gtaccatatt tggactcaac tctcttg                             37


<210> SEQ ID NO 105
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 105

ctcgagggcc cgcgaaagcg ggccctcgag saggtgcagc tggtggagtc ygaaa         55
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<210> SEQ ID NO 106
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 106

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tgttggagtc tgaat 55

<210> SEQ ID NO 107
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 107

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagac tgata 55

<210> SEQ ID NO 108
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 108

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtggagtc tcaaa 55

<210> SEQ ID NO 109
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 109

ctcgagggcc cgcgaaagcg ggccctcgag cagstgcagc tgcaggagtc ggaaa 55

<210> SEQ ID NO 110
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 110

ctcgagggcc cgcgaaagcg ggccctcgag cagctgcagc tgcaggagtc caaa 54

<210> SEQ ID NO 111
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 111

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tacagcagtg ggaaa 55

<210> SEQ ID NO 112
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

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<400> SEQUENCE: 112

ctcgagggcc cgcgaaagcg ggccctcgag caggtbcagc tkgtgcagaa a 51

<210> SEQ ID NO 113
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 113

ctcgagggcc cgcgaaagcg ggccctcgag saggtccagc tggtacagaa a 51

<210> SEQ ID NO 114
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 114

ctcgagggcc cgcgaaagcg ggccctcgag cagatgcagc tggtgcagaa a 51

<210> SEQ ID NO 115
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 115

ctcgagggcc cgcgaaagcg ggccctcgag caaatgcagc tggtgcagaa a 51

<210> SEQ ID NO 116
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 116

ctcgagggcc cgcgaaagcg ggccctcgag cagatcacct tgaaggagtc taaa 54

<210> SEQ ID NO 117
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 117

ctcgagggcc cgcgaaagcg ggccctcgag caggtcacct tgaaggagtc taaa 54

<210> SEQ ID NO 118
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 118

ctcgagggcc cgcgaaagcg ggccctcgag gaggtgcagc tggtgcagaa a 51

<210> SEQ ID NO 119

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<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 119

ctcgagggcc cgcgaaagcg ggccctcgag gaagtgcagc tggtgcagaa a              51


<210> SEQ ID NO 120
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 120

ctcgagggcc cgcgaaagcg ggccctcgag caggtacagc tgcagcagtc aaa            53


<210> SEQ ID NO 121
<211> LENGTH: 52
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 121

ctcgagggcc cgcgaaagcg ggccctcgag caggtgcagc tggtgcaata aa             52


<210> SEQ ID NO 122
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 122

gagccgcacg agcccctcga ggargtgcag ctggtggag                            39


<210> SEQ ID NO 123
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 123

gagccgcacg agcccctcga ggaggtgcag ctggtggag                            39


<210> SEQ ID NO 124
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 124

gagccgcacg agcccctcga ggaggtgcag ctgttggag                            39


<210> SEQ ID NO 125
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer
<220> FEATURE:
<221> NAME/KEY: misc_feature
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<222> LOCATION: (25)..(25)
<223> OTHER INFORMATION: n is c or g

<400> SEQUENCE: 125

gagccgcacg agcccctcga gcagntgcag ctgcaggag                         39


<210> SEQ ID NO 126
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 126

gagccgcacg agcccctcga gcaggtgcag ctacagcag                         39


<210> SEQ ID NO 127
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 127

gcggcgcagc cggcgatggc g                                            21


<210> SEQ ID NO 128
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 128

agcgtagtcc ggaacgtcgt acgg                                         24


<210> SEQ ID NO 129
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 129

gaagtagtcc ttgaccag                                                18


<210> SEQ ID NO 130
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 130

tgtcacatct ggcacctgg                                               19


<210> SEQ ID NO 131
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 131

tccccactgg atccagggac                                              20
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<210> SEQ ID NO 132
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 132

ctccggtggt atctgggag                                                  19


<210> SEQ ID NO 133
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 133

tccccgtagg caccagaga                                                  19


<210> SEQ ID NO 134
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 134

tctgccctgg tatcagagat                                                 20


<210> SEQ ID NO 135
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 135

caccccctgga ggctggaac                                                 19


<210> SEQ ID NO 136
<211> LENGTH: 67
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 136

agacagtgag cgccgtctag aattaacact ctcccctgtt gaagctcttt gtgacgggcg     60

aactcag                                                               67


<210> SEQ ID NO 137
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 137

ctccgaggtg ccagatgt                                                   18


<210> SEQ ID NO 138
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
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<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 138

gctcagctcc tggggct                                                     17


<210> SEQ ID NO 139
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 139

gtccctggat ccagtgag                                                    18


<210> SEQ ID NO 140
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 140

ctcccagata ccaccgga                                                    18


<210> SEQ ID NO 141
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 141

gcgcagcttc tcttcct                                                     17


<210> SEQ ID NO 142
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 142

cacagcttct tcttcctc                                                    18


<210> SEQ ID NO 143
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 143

atctctggtg cctacggg                                                    18


<210> SEQ ID NO 144
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 144

atctctgata ccagggca                                                    18
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<210> SEQ ID NO 145
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 145

gttccagcct ccaggggt 18

<210> SEQ ID NO 146
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 146

gagctcggcc cgcgaaagcg ggccgagctc gmcatccaga tgacccagtc tcctaa 56

<210> SEQ ID NO 147
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 147

gagctcggcc cgcgaaagcg ggccgagctc aacatccaga tgacccagtc tcctaa 56

<210> SEQ ID NO 148
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 148

gagctcggcc cgcgaaagcg ggccgagctc gmcatccagt tgacccagtc tcctaa 56

<210> SEQ ID NO 149
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 149

gagctcggcc cgcgaaagcg ggccgagctc gccatccgga tgacccagtc tcctat 56

<210> SEQ ID NO 150
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 150

gagctcggcc cgcgaaagcg ggccgagctc gtcatctgga tgacccagtc tcctat 56

<210> SEQ ID NO 151
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

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<400> SEQUENCE: 151

gagctcggcc cgcgaaagcg ggccgagctc gatattgtga tgacccagac tctta 55

<210> SEQ ID NO 152
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 152

gagctcggcc cgcgaaagcg ggccgagctc gatgttgtga tgactcagtc tcctaa 56

<210> SEQ ID NO 153
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 153

gagctcggcc cgcgaaagcg ggccgagctc gatattgtga tgactcagtc tcctaa 56

<210> SEQ ID NO 154
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 154

gagctcggcc cgcgaaagcg ggccgagctc gaaattgtgt tgacgcagtc tcctaa 56

<210> SEQ ID NO 155
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 155

gagctcggcc cgcgaaagcg ggccgagctc gaaatagtga tgacgcagtc tcctaa 56

<210> SEQ ID NO 156
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 156

gagctcggcc cgcgaaagcg ggccgagctc gaaattgtaa tgacacagtc tcctaa 56

<210> SEQ ID NO 157
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 157

gagctcggcc cgcgaaagcg ggccgagctc gacatcgtga tgacccagtc tcctat 56

<210> SEQ ID NO 158

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<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 158

gagctcggcc cgcgaaagcg ggccgagctc gaaacgacac tcacgcagtc tcctaa         56


<210> SEQ ID NO 159
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 159

gagctcggcc cgcgaaagcg ggccgagctc gaaattgtgc tgactcagtc tcctat         56


<210> SEQ ID NO 160
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 160

ctgggcccag gaccctgtgc                                                 20


<210> SEQ ID NO 161
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 161

ctgggcccag gaccctgt                                                   18


<210> SEQ ID NO 162
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 162

gaggccacag agcctgtgca gagagtgag                                       29


<210> SEQ ID NO 163
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 163

cagagcacag agacctgtgg a                                               21


<210> SEQ ID NO 164
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 164
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ctgggagaga gaccctgtcc a 21

<210> SEQ ID NO 165
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 165

ctgggagagg gaacctgtgc a 21

<210> SEQ ID NO 166
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 166

attggcccaa gaacctgtgc a 21

<210> SEQ ID NO 167
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 167

ctgagaattg gaccctgggc a 21

<210> SEQ ID NO 168
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 168

ctgagaatcc actcctgatc c 21

<210> SEQ ID NO 169
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 169

ctgggagagg gaccctgtga g 21

<210> SEQ ID NO 170
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: boundary oligonucleotide

<400> SEQUENCE: 170

ctggaccact gacactgcag a 21

<210> SEQ ID NO 171
<211> LENGTH: 18
<212> TYPE: DNA

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<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 171

cactgygcag ggtcctgg                                                    18


<210> SEQ ID NO 172
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 172

cagggcacag ggtcctgg                                                    18


<210> SEQ ID NO 173
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 173

tactgcacag gatccgtg                                                    18


<210> SEQ ID NO 174
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 174

cactttacag gttctgtg                                                    18


<210> SEQ ID NO 175
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 175

ttctgcacag tctctgag                                                    18


<210> SEQ ID NO 176
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 176

ctctgcacag gctctgag                                                    18


<210> SEQ ID NO 177
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 177

ctttgctcag gttctgtg                                                    18
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<210> SEQ ID NO 178
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 178

cactgcacag gctctgtg                                                  18


<210> SEQ ID NO 179
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 179

ctctacacag gctctatt                                                  18


<210> SEQ ID NO 180
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 180

ctctgcacag tctctgtg                                                  18


<210> SEQ ID NO 181
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 181

ttctccacag gtctctgt                                                  18


<210> SEQ ID NO 182
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 182

cactggacag ggtctctc                                                  18


<210> SEQ ID NO 183
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 183

cactgcacag gttccctc                                                  18


<210> SEQ ID NO 184
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
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<223> OTHER INFORMATION: primer

<400> SEQUENCE: 184

cactgcacag gttcttgg                                                18


<210> SEQ ID NO 185
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 185

tgctgcccag ggtccaat                                                18


<210> SEQ ID NO 186
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 186

tatggatcag gagtggat                                                18


<210> SEQ ID NO 187
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 187

ctcctcacag ggtccctc                                                18


<210> SEQ ID NO 188
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 188

cactctgcag tgtcagtg                                                18


<210> SEQ ID NO 189
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 189

agacagtgac gccgtctaga attatgaaca ttctgtagg                         39


<210> SEQ ID NO 190
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 190

gagctcggcc cgcgaaagcg ggccgagctc cagtctgtgc tgactcagcc accaaa      56
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<210> SEQ ID NO 191
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 191

gagctcggcc cgcgaaagcg ggccgagctc cagtctgtgy tgacgcagcc gccaaa 56

<210> SEQ ID NO 192
<211> LENGTH: 55
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 192

gagctcggcc cgcgaaagcg ggccgagctc cagtctgccc tgactcagcc tsaaa 55

<210> SEQ ID NO 193
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 193

gagctcggcc cgcgaaagcg ggccgagctc tcctatgagc tgactcagcc acyaaa 56

<210> SEQ ID NO 194
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 194

gagctcggcc cgcgaaagcg ggccgagctc tcctatgagc tgacacagcy accaat 56

<210> SEQ ID NO 195
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 195

gagctcggcc cgcgaaagcg ggccgagctc tcttctgagc tgactcagga cccaaa 56

<210> SEQ ID NO 196
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 196

gagctcggcc cgcgaaagcg ggccgagctc tcctatgtgc tgactcagcc accaaa 56

<210> SEQ ID NO 197
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 197

gagctcggcc cgcgaaagcg ggccgagctc tcctatgagc tgatgcagcc accaaa 56

<210> SEQ ID NO 198
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 198

gagctcggcc cgcgaaagcg ggccgagctc tcctatgagc tgacacagcc atcaaa 56

<210> SEQ ID NO 199
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 199

gagctcggcc cgcgaaagcg ggccgagctc ctgcctgtgc tgactcagcc cccaaa 56

<210> SEQ ID NO 200
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 200

gagctcggcc cgcgaaagcg ggccgagctc cagcctgtgc tgactcaatc atcaaa 56

<210> SEQ ID NO 201
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 201

gagctcggcc cgcgaaagcg ggccgagctc cagcttgtgc tgactcaatc gccaaa 56

<210> SEQ ID NO 202
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 202

gagctcggcc cgcgaaagcg ggccgagctc cagcctgtgc tgactcagcc aycaaa 56

<210> SEQ ID NO 203
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 203

gagctcggcc cgcgaaagcg ggccgagctc caggctgtgc tgactcagcc ggcaaa 56

<210> SEQ ID NO 204
<211> LENGTH: 56

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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 204

gagctcggcc cgcgaaagcg ggccgagctc aattttatgc tgactcagcc ccaaaa         56


<210> SEQ ID NO 205
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 205

gagctcggcc cgcgaaagcg ggccgagctc cagactgtgg tgacycagga gccaaa         56


<210> SEQ ID NO 206
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 206

gagctcggcc cgcgaaagcg ggccgagctc gcaggctgtg gtgactcagg agccaaa        57


<210> SEQ ID NO 207
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 207

gagctcggcc cgcgaaagcg ggccgagctc cagcctgtgc tgactcagcc accaaa         56


<210> SEQ ID NO 208
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: nesting oligonucleotide

<400> SEQUENCE: 208

gagctcggcc cgcgaaagcg ggccgagctc caggcagggc tgactcagcc accaaa         56


<210> SEQ ID NO 209
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 209

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

Cys Ala Ala Ser Gly Phe Ile Phe Asp Asp Phe Ala Met His Trp Leu
            20                  25                  30

Arg Gln Val Pro Gly Lys Gly Leu Gln Trp Val Gly Leu Met Ser Trp
        35                  40                  45

Asp Gly Val Ser Ala Tyr Tyr Ala Asp Ser Val Glu Gly Arg Phe Thr
    50                  55                  60
```

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                         -continued
Ile Ser Arg Asp Asn Lys Lys Asn Ala Leu Tyr Leu Gln Met Asn Ser
65                  70                  75                  80

Leu Gly Val Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Lys Asp Met Gly
                85                  90                  95

Gly Gly Leu Arg Phe Pro His Phe Trp Gly Gln Gly Thr Pro Val Thr
            100                 105                 110

Val Ser Ala
        115


<210> SEQ ID NO 210
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 210

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
1               5                   10                  15

Leu Ser Ser Ser Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
            20                  25                  30

Leu Glu Phe Val Ala Val Ser Ser Gly Asn Gly Phe Ser Thr Tyr Tyr
        35                  40                  45

Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
    50                  55                  60

Asn Met Val Tyr Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala
65                  70                  75                  80

Lys Tyr His Cys Ala Lys Val Arg Tyr Gly Pro Arg Ser His Phe Phe
                85                  90                  95

Phe Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110


<210> SEQ ID NO 211
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 211

Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr
1               5                   10                  15

Leu Ser Ser Ser Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly
            20                  25                  30

Leu Glu Phe Val Ala Val Ser Ser Gly Asn Gly Phe Ser Thr Tyr Tyr
        35                  40                  45

Gly Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys
    50                  55                  60

Asn Met Val Tyr Leu Gln Met Asp Ser Leu Arg Ala Glu Asp Thr Ala
65                  70                  75                  80

Lys Tyr His Cys Ala Lys Val Arg Tyr Gly Pro Arg Ser His Phe Phe
                85                  90                  95

Phe Asp Pro Trp Gly Pro Gly Asn Pro Gly His Arg Leu Leu
            100                 105                 110


<210> SEQ ID NO 212
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
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<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 212

Ala Trp Tyr Ser Arg Gly Ser Pro Cys Leu Ser Cys Ala Ala Ser Gly
1               5                   10                  15

Phe Thr Leu Ser Ser Ser Ala Met Ser Trp Val Arg Gln Ala Pro Gly
            20                  25                  30

Lys Gly Leu Glu Phe Val Ala Val Ser Ser Gly Asn Gly Phe Ser Thr
        35                  40                  45

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

Ser Lys Asn Met Val Tyr Leu Gln Met Asp Ser Leu Arg Ala Glu Asp
65                  70                  75                  80

Thr Ala Lys Tyr His Cys Ala Lys Val Arg Tyr Gly Pro Arg Ser His
                85                  90                  95

Phe Phe Phe Asp Pro Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110


<210> SEQ ID NO 213
<211> LENGTH: 122
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 213

Glu Ser Asp Pro Gly Leu Val Lys Pro Ser Glu Thr Pro Ser Leu Thr
1               5                   10                  15

Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Thr Met Tyr Phe Trp Gly
            20                  25                  30

Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Ala Ser Ile
        35                  40                  45

Tyr Tyr Ser Gly Thr Thr Tyr Tyr Asn Pro Ser Leu Arg Ser Arg Val
    50                  55                  60

Thr Met Ser Val Asp Thr Ser Lys Asn Gln Leu Ser Leu Lys Leu Asn
65                  70                  75                  80

Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro Thr
                85                  90                  95

Ile Tyr Tyr Phe Asp Gly Arg Thr Ser Tyr Tyr Pro Gly Glu Ala Ala
            100                 105                 110

Phe Asp Ile Trp Gly Gln Gly Thr Thr Val
        115                 120


<210> SEQ ID NO 214
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 214

Pro Gly Leu Val Lys Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Val
1               5                   10                  15

Ser Gly Gly Ser Ile Ser Asn Ile Met Tyr Phe Trp Gly Trp Ile Arg
            20                  25                  30

Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile Ala Ser Ile Tyr Tyr Ser
        35                  40                  45
```

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Gly Thr Thr Tyr Tyr Asn Pro Ser Leu Arg Ser Arg Val Thr Met Ser
    50                  55                  60

Val Asp Thr Ser Lys Asn Gln Leu Ser Leu Lys Leu Asn Ser Val Thr
65                  70                  75                  80

Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro Thr Ile Tyr Tyr
                85                  90                  95

Phe Asp Gly Arg Thr Ser Tyr Tyr Pro Gly Glu Ala Ala Phe Asp Ile
            100                 105                 110

Trp Gly Gln Gly Thr Thr Val Thr Val
        115                 120


<210> SEQ ID NO 215
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 215

Glu Ser Asp Pro Gly Leu Val Gln Pro Ser Gln Thr Leu Ser Leu Thr
1               5                   10                  15

Cys Thr Val Ser Gly Gly Ser Leu Arg Ser Asp Asp Tyr Tyr Trp Ser
            20                  25                  30

Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile Ala Tyr Ile
        35                  40                  45

Ser Tyr Thr Gly Gly Thr Tyr Tyr Asn Pro Ser Leu Lys Ser Arg Val
    50                  55                  60

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

Ser Val Thr Ala Ala Asp Ser Ala Val Tyr Phe Cys Ala Ser Thr Thr
                85                  90                  95

Ala Val Thr Thr Thr Phe Asp Tyr Trp Gly Arg Gly Thr Leu Val Thr
            100                 105                 110

Val Ser


<210> SEQ ID NO 216
<211> LENGTH: 104
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 216

Pro Val Gln Pro Leu Glu Phe Thr Phe Thr Asp His Trp Met His Trp
1               5                   10                  15

Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Leu Ala Arg Ile Asn
            20                  25                  30

Arg Asp Gly Ser Asp Thr Thr Tyr Ala Asp Ser Val Thr Gly Arg Phe
        35                  40                  45

Thr Ile Ser Arg Asp Asn Gly Lys Asn Thr Val Ser Leu Gln Met Asp
    50                  55                  60

Ser Leu Ser Val Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly Gly
65                  70                  75                  80

His His Thr Val Leu Ser Pro Leu Ser Asn Trp Phe Asp Pro Trp Gly
                85                  90                  95

Gln Gly Thr Leu Val Thr Val Ser
            100
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<210> SEQ ID NO 217
<211> LENGTH: 110
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 217

Glu Ser Glu Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser
1               5                   10                  15

Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Thr Trp Val
            20                  25                  30

Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Thr Met Thr Gly
        35                  40                  45

Ser Gly Gly Val Thr Tyr Tyr Ala Asp Val Leu Lys Gly Arg Phe Thr
    50                  55                  60

Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser
65                  70                  75                  80

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

Leu Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            100                 105                 110


<210> SEQ ID NO 218
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 218

Leu Ala Gly Val Glu Val Val Gln Pro Gly Gly Ser Leu Arg Leu Ser
1               5                   10                  15

Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr Ala Met His Trp Leu
            20                  25                  30

Arg Gln Ile Pro Gly Lys Gly Leu Gln Trp Val Ser Leu Leu Ser Trp
        35                  40                  45

Asp Gly Val Ser Ala Tyr Tyr Ala Asp Ser Val Glu Gly Arg Phe Thr
    50                  55                  60

Ile Ser Arg Asp Asn Lys Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser
65                  70                  75                  80

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

Gly Ala Gln Arg Leu Pro Asp His Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser
        115


<210> SEQ ID NO 219
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 219

Gly Gly Gly Leu Val Gln Pro Gly Ala Ser Val Lys Val Ser Cys Lys
1               5                   10                  15

Ala Ser Gly Tyr Thr Phe Ser Asp Tyr Phe Met His Cys Val Arg Gln
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            20                  25                  30

Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Leu Val Asn Pro Thr Asn
        35                  40                  45

Gly Tyr Thr Ala Tyr Ala Pro Lys Phe Gln Gly Arg Val Thr Met Thr
    50                  55                  60

Arg Gln Arg Phe Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg
65                  70                  75                  80

Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Val Lys Ser Ser Asp
                85                  90                  95

Ser Ile Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val
            100                 105                 110

Ser Ser


<210> SEQ ID NO 220
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 220

Arg Cys Pro Ala Lys Leu Leu Asp Thr Pro Phe Ser Val Tyr Phe Met
1               5                   10                  15

His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Leu
            20                  25                  30

Val Asn Pro Thr Asn Gly Tyr Thr Ala Tyr Ala Pro Lys Phe Gln Gly
        35                  40                  45

Arg Val Thr Met Thr Arg Gln Arg Phe Thr Ser Thr Val Tyr Met Glu
    50                  55                  60

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg
65                  70                  75                  80

Val Lys Ser Ser Asp Ser Ile Asp Ala Phe Asp Ile Trp Gly Gln Gly
                85                  90                  95

Thr Met Val Thr Val Ser Ser
            100


<210> SEQ ID NO 221
<211> LENGTH: 103
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 221

Arg Cys Pro Ala Lys Leu Leu Asp Thr Pro Ser Gly Asp Tyr Phe Met
1               5                   10                  15

His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Leu
            20                  25                  30

Val Asn Pro Thr Asn Gly Tyr Thr Ala Tyr Ala Pro Lys Phe Gln Gly
        35                  40                  45

Arg Val Thr Met Thr Arg Gln Arg Phe Thr Ser Thr Val Tyr Met Glu
    50                  55                  60

Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg
65                  70                  75                  80

Val Lys Ser Ser Asp Ser Ile Asp Ala Phe Asp Ile Trp Gly Gln Gly
                85                  90                  95

Thr Met Val Thr Val Ser Ser
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            100


<210> SEQ ID NO 222
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 222

Ser Gly Gly Leu Val Gln Arg Gly Ala Lys Val Leu Arg Leu Ser Cys
1               5                   10                  15

Val Ala Ser Gly Phe Thr Phe Ser Ser Ser Ala Met Ser Trp Val Arg
            20                  25                  30

Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Val Ile Ser Gly Asn
        35                  40                  45

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

Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
65                  70                  75                  80

Ala Glu Asp Thr Ala Glu Tyr Tyr Cys Thr Lys Val Lys Tyr Gly Ser
                85                  90                  95

Gly Ser His Phe Trp Phe Asp Pro Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser
        115


<210> SEQ ID NO 223
<211> LENGTH: 83
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (23)..(23)
<223> OTHER INFORMATION: Xaa is unknown
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (29)..(29)
<223> OTHER INFORMATION: Xaa is unknown
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (52)..(52)
<223> OTHER INFORMATION: Xaa is unknown
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (76)..(76)
<223> OTHER INFORMATION: Xaa is unknown

<400> SEQUENCE: 223

Leu Gly Ser Pro Tyr Ser Ser Ser Ala Met Ser Trp Val Arg Gln Ala
1               5                   10                  15

Pro Gly Lys Gly Leu Glu Xaa Val Ser Phe Ile Ser Xaa Asn Gly Leu
            20                  25                  30

Ser Ala Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg
        35                  40                  45

Asp Asn Ser Xaa Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ser
    50                  55                  60

Glu Asp Thr Ala Glu Tyr Tyr Cys Val Lys Val Xaa Tyr Gly Ser Arg
65                  70                  75                  80

Ser His Phe
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-continued

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<210> SEQ ID NO 224
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 224

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

Ser Cys Ala Ala Ser Gly Phe Ser Phe Glu Asp Tyr Ala Met His Trp
            20                  25                  30

Val Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile Ser
        35                  40                  45

Trp Asp Val Ile Ser Ala Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe
    50                  55                  60

Thr Ile Ser Arg Asp Asn Ser Lys Asn Ser Leu Tyr Leu Gln Met Asp
65                  70                  75                  80

Ser Leu Arg Pro Glu Asp Ser Gly Leu Tyr Tyr Cys Gly Arg Asp Ile
                85                  90                  95

Gly Gln Gln Arg Thr Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr
            100                 105                 110

Val Ser Ser
        115


<210> SEQ ID NO 225
<211> LENGTH: 98
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 225

Ala Ala Ser Gly Phe Ile Phe Asp Asp Phe Ala Met His Trp Phe Gln
1               5                   10                  15

Ala Val Pro Gly Lys Gly Leu Gln Trp Val Gly Leu Met Ser Trp Asp
            20                  25                  30

Gly Val Ser Ala Tyr Tyr Ala Asp Ser Val Glu Gly Arg Phe Thr Ile
        35                  40                  45

Ser Arg Asp Asn Lys Lys Asn Ala Leu Tyr Leu Gln Met Asn Ser Leu
    50                  55                  60

Gly Val Glu Asp Thr Ala Leu Tyr Phe Cys Ala Lys Asp Met Gly Gly
65                  70                  75                  80

Gly Leu Arg Phe Pro His Phe Trp Gly Gln Gly Thr Pro Val Thr Val
                85                  90                  95

Ser Ala


<210> SEQ ID NO 226
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 226

Phe Trp Leu Gly Gly Pro Trp Arg Leu Ser Cys Ala Val Ser Gly Tyr
1               5                   10                  15

Thr Leu Ser Ser Ser Ala Met Ile Trp Val Arg Gln Pro Pro Gly Lys
            20                  25                  30
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Gly Leu Glu Phe Val Ser Val Ile Ser Gly Asn Gly Leu Ser Ala Tyr
        35                  40                  45

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

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
65                  70                  75                  80

Ala Glu Tyr Tyr Cys Val Lys Val Lys Tyr Gly Ser Arg Ser His Phe
                85                  90                  95

Phe Phe Asp Ser Trp Gly Gln Gly Thr Leu Val Ser Val Ser Pro
            100                 105                 110


<210> SEQ ID NO 227
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 227

Gly Gly Gly Leu Val Gln Pro Gly Ala Ser Leu Arg Leu Ser Cys Val
1               5                   10                  15

Ala Ser Gly Phe Thr Leu Ser Ser Ser Ala Met Ser Cys Val Arg Gln
            20                  25                  30

Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Val Ser Ser Gly Asn Gly
        35                  40                  45

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

Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Val
65                  70                  75                  80

Ala Glu Asp Thr Ala Glu Tyr Tyr Cys Thr Lys Val Asn Tyr Gly Ser
                85                  90                  95

Arg Ser His Phe Tyr Phe Gly Ser Trp Gly His Gly Thr Leu Val Ile
            100                 105                 110

Val Ser Ser
        115


<210> SEQ ID NO 228
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 228

Trp Gly Arg Arg Gly Pro Ala Trp Gly Val Pro Val Gly Ser Pro Val
1               5                   10                  15

Gln Pro Leu Gly Tyr Thr Phe Asp Asp Tyr Ala Met His Trp Leu Arg
            20                  25                  30

Gln Ile Pro Gly Lys Gly Leu Gln Trp Val Ser Leu Leu Ser Trp Asp
        35                  40                  45

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

Ser Arg Asp Asn Lys Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu
65                  70                  75                  80

Val Ala Glu Asp Thr Ala Leu Tyr Phe Cys Ala Lys Asp Met Gly Gly
                85                  90                  95

Ala Gln Arg Leu Pro Asp His Trp Gly Gln Gly Thr Leu Val Thr Val
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            100                 105                 110

Ser Ser


<210> SEQ ID NO 229
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (70)..(70)
<223> OTHER INFORMATION: Xaa is unknown

<400> SEQUENCE: 229

Trp Thr Gly Gly Gly Val Val Gln Pro Gly Gly Ser Leu Arg Val Ser
1               5                   10                  15

Val Ala Ala Ser Gly Tyr Thr Phe Asp Asp Tyr Ala Met His Trp Leu
            20                  25                  30

Arg Gln Ile Pro Gly Lys Gly Leu Gln Trp Val Ser Leu Leu Ser Trp
        35                  40                  45

Asp Gly Val Ser Ala Tyr Tyr Ala Asp Ser Val Glu Gly Arg Phe Thr
    50                  55                  60

Ile Ser Arg Asp Asn Xaa Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser
65                  70                  75                  80

Leu Ile Ala Glu Asp Thr Ala Leu Tyr Phe Cys Ala Lys Asp Met Gly
                85                  90                  95

Gly Ala Gln Arg Leu Pro Asp His Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser
        115


<210> SEQ ID NO 230
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: cloned antibody

<400> SEQUENCE: 230

Ala Glu Ser Gly Gly Gly Val Val Gln Pro Gly Gly Ser Leu Arg Leu
1               5                   10                  15

Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr Thr Leu Ser Trp
            20                  25                  30

Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Tyr Ile Ser
        35                  40                  45

Thr Asp Gly Ser Thr Ile Tyr Tyr Thr Asp Ser Val Lys Gly Arg Phe
    50                  55                  60

Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Ser Leu Gln Met Ile
65                  70                  75                  80

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

Phe Gly Gly Asn Phe Arg Ala His Trp Tyr Phe Asp Leu Trp Gly Arg
            100                 105                 110

Gly Thr Leu Val Ala Val Ser Ser
        115                 120


<210> SEQ ID NO 231
<211> LENGTH: 47
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<212> TYPE: DNA
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer

<400> SEQUENCE: 231

agaatttgac tagttggcaa gaggcacgtt cttttctttg ttgccgt                47
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We claim:

1. A method of amplifying nucleic acid comprising the steps of:

a) annealing a primer to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence;

b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template adjacent to the location at which the first portion of the primer anneals to the template, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer,

c) separating the polynucleotide synthesized in step (b) from the template;

d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure;

e) extending the polynucleotide synthesized in step (b) to provide an extended polynucleotide comprising a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and

f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

2. A method as in claim **1** further comprising the step of providing a nucleic acid template by annealing a restriction oligonucleotide to a nucleic acid strand to form a double stranded portion and digesting the nucleic acid strand at the double stranded portion.

3. A method as in claim **1** wherein the template encodes an immunoglobulin molecule or fragment thereof.

4. A method as in claim **1** wherein the template is selected from the group consisting of full length or truncated mRNA, DNA and cDNA.

5. A method as in claim **1** wherein the nucleic acid being amplified includes a target sequence encoding a polypeptide.

6. A method as in claim **5** wherein the target sequence encodes an immunoglobulin molecule or fragment thereof.

7. A method as in claim **5** further comprising the step of digesting the extended polynucleotide to isolate the target sequence.

8. A method as in claim **7** further comprising the step of ligating the isolated target sequence into an expression vector.

9. A method as in claim **8** further comprising the steps of transforming a host cell with the expression vector and expressing the polypeptide encoded by the target sequence.

10. A method of amplifying nucleic acid comprising the steps of:

a) annealing a primer and a boundary oligonucleotide to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence;

b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer;

c) separating the polynucleotide synthesized in step (b) from the template;

d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide and a second portion having a hairpin structure;

e) extending the polynucleotide synthesized in step (b) to provide an extended polynucleotide comprising a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and

f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

11. A method as in claim **10** further comprising the step of providing a nucleic acid template by generating first strand cDNA from mRNA.

12. A method as in claim **10** wherein the template is selected from the group consisting of full length or truncated mRNA, DNA and cDNA.

13. A method as in claim **10** wherein the extended polynucleotide includes a target sequence encoding a polypeptide.

14. A method as in claim **10** wherein the extended polynucleotide encodes an immunoglobulin molecule or fragment thereof.

15. A method as in claim **14** wherein the target sequence encodes an immunoglobulin molecule or fragment thereof.

16. A method as in claim **14** further comprising the step of digesting the extended polynucleotide to isolate the target sequence.

17. A method as in claim **16** further comprising the step of ligating the isolated target sequence into an expression vector.

18. A method as in claim **17** further comprising the steps of transforming a host cell with the expression vector and expressing the polypeptide encoded by the target sequence.

19. A method of amplifying nucleic acid comprising the steps of:

a) annealing an oligo dT primer and a boundary oligonucleotide to an mRNA template;

b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer;

- c) separating the polynucleotide synthesized in step (b) from the template;
- d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure;
- e) extending the polynucleotide synthesized in step (b) to provide an extended polynucleotide comprising a portion that is complementary to the hairpin structure and a poly A terminal portion; and
- f) amplifying the extended polynucleotide using a single primer.

20. A method as in claim **19** further comprising the step of providing a nucleic acid template by generating first strand cDNA from mRNA.

21. A method as in claim **19** wherein the template is selected from the group consisting of full length or truncated mRNA, DNA and cDNA.

22. A method as in claim **19** wherein the extended polynucleotide includes a target sequence encoding a polypeptide.

23. A method as in claim **19** wherein the extended polynucleotide encodes an immunoglobulin molecule or fragment thereof.

24. A method as in claim **22** wherein the target sequence encodes an immunoglobulin molecule or fragment thereof.

25. A method as in claim **22** further comprising the step of digesting the extended polynucleotide to isolate the target sequence.

26. A method as in claim **25** further comprising the step of ligating the isolated target sequence into an expression vector.

27. A method as in claim **26** further comprising the steps of transforming a host cell with the expression vector and expressing the polypeptide encoded by the target sequence.

28. A method of amplifying a nucleic acid strand comprising the steps of:

- a) providing a nucleic acid strand having i) a predetermined sequence engineered onto a first end thereof, ii) a sequence complementary to the predetermined sequence, and iii) a hairpin structure therebetween; and
- b) contacting the engineered nucleic acid strand with a primer containing at least a portion of the predetermined sequence in the presence of a polymerase and nucleotides under conditions suitable for polymerization of the nucleotides, thereby producing a complementary nucleic acid strand.

29. A method as in claim **28** further comprising the steps of:

- digesting the complementary nucleic acid strand to isolate a target nucleic acid sequence contained therein;
- ligating the target nucleic acid sequence into an expression vector;
- transforming a host organism with the expression vector; and

expressing a polypeptide or protein encoded by the target sequence.

30. A method of amplifying a family of related nucleic acid sequences to build a complex library of polypeptides encoded by the sequences, the method comprising:

- a) annealing a primer to a family of related nucleic acid sequence templates, the primer having a first portion which anneals to the templates and a second portion of predetermined sequence;
- b) synthesizing polynucleotides that anneal to and are complementary to the portion of the templates adjacent to the location at which the first portion of the primer anneals to the templates, the polynucleotides having a first end and a second end, wherein the first end incorporates the primer;
- c) separating the polynucleotides synthesized in step (b) from the templates;
- d) annealing a nested oligonucleotide to the second end of the polynucleotides synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotides, and a second portion having a hairpin structure;
- e) extending the polynucleotides synthesized in step (b) to provide an extended polynucleotide comprising a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and
- f) amplifying the extended polynucleotides using a single primer having the predetermined sequence.

31. A method as in claim **1**, wherein steps a), b) and c) are repeated from 15 to 25 times prior to annealing the nested oligonucleotide.

32. A method as in claim **10**, wherein steps a), b) and c) are repeated from 15 to 25 times prior to annealing the nested oligonucleotide.

33. A method as in claim **19**, wherein steps a), b) and c) are repeated from 15 to 25 times prior to annealing the nested oligonucleotide.

34. A method as in claim **30**, wherein steps a), b) and c) are repeated from 15 to 25 times prior to annealing the nested oligonucleotide.

35. A method as in claim **1** wherein the first end of the polynucleotide is the 5' end.

36. A method as in claim **1** wherein the first end of the polynucleotide is the 5' end.

37. A method as in claim **19** wherein the first end of the polynucleotide is the 5' end.

38. A method as in claim **1** wherein the first end of the nucleic acid strand is the 5' end.

* * * * *