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(54) Title: ANTISENSE OLIGONUCLEOTIDE COMPOSITIONS

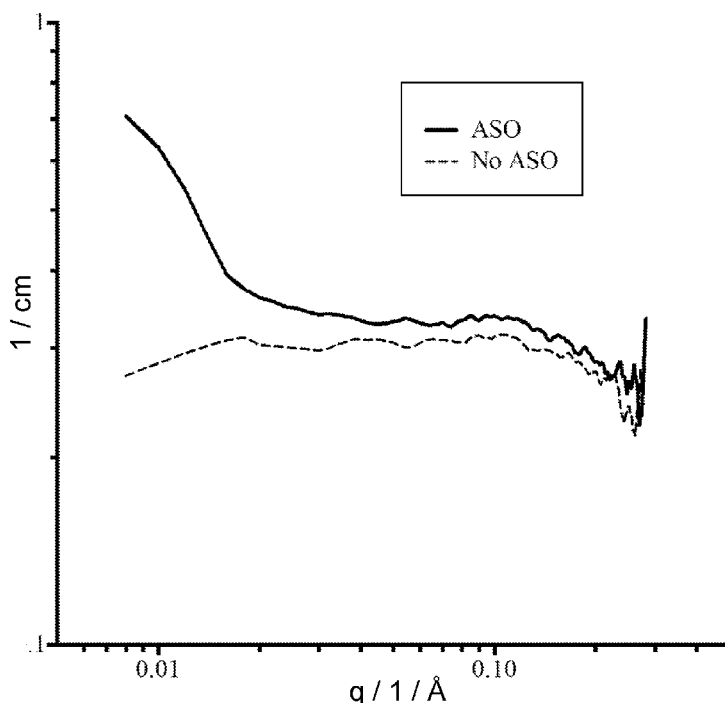


Figure 3

(57) Abstract: The present invention relates to antisense oligonucleotide (ASO) compositions and particularly to compositions and methods for the cytosolic delivery of antisense oligonucleotides (ASOs). Hybrid ASOs, part single-stranded and part double-stranded, are provided, hybridising to form a double-stranded region that can non-covalently bond to nucleic-acid-binding protein regions. In this way, ASO:protein complexes may be produced that facilitate delivery of antisense DNA into target cells. Such complexes may be used to down-regulate gene expression in cells.



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Antisense Oligonucleotide Compositions

The present invention relates to antisense oligonucleotide (ASO) compositions and particularly to compositions and methods for the cytosolic delivery of antisense oligonucleotides (ASOs). Hybrid ASOs, part single-stranded and part double-stranded, are provided, hybridising to form a double-stranded region that can non-covalently bond to nucleic-acid-binding protein regions. In this way, ASO::protein complexes may be produced that facilitate delivery of antisense DNA into target cells. Such complexes may be used to down-regulate gene expression in cells.

Antisense oligonucleotides have been approved by the FDA for use as antivirals, for the treatment of cytomegalovirus-mediated retinitis and chronic ulcerative colitis (Roehr, 1998; Yacychyn *et al.*, 1998). ASOs are comprised of segments of single-stranded DNA, or analogues thereof, that are designed to hybridise to a messenger RNA (mRNA) transcript, derived from a specific gene. The mRNA::ASO hybrids so formed may be degraded by RNase H. In an organism infected with a virus, the genetic material of the virus enters specific cells in order to replicate, a process requiring the translation of virus-specific mRNA. By treating an infected cell with an antisense oligonucleotide specific for virus mRNA, it is possible to prevent the expression of a target viral gene and to block the viral life cycle.

An important factor in the effectiveness of antisense oligonucleotide treatment is the bioavailability of the oligonucleotides (Biroccio *et al.*, 2003). Bioavailability can be limited by the inability of antisense oligonucleotides to penetrate the plasma membrane or endomembrane system of cells. Since the targets of antisense oligonucleotides (*e.g.*, mRNA) are located within the cytosol of a cell, the oligonucleotides need to be able to traverse cell membranes before they can access the cytosol.

One way to address this problem of cytosolic access of antisense oligonucleotides is to administer the treatment locally rather than systemically, and in particular, to administer the antisense oligonucleotide into a discrete pharmacokinetic compartment, raising the local concentration of the antisense oligonucleotide. For example, an antisense oligonucleotide treatment of cytomegalovirus-mediated retinitis involves administering

fomivirsen (Vitravene<sup>®</sup>) into a discrete pharmacokinetic compartment by intravitreal injection. However, cytosolic access *i.e.*, access of the antisense oligonucleotides to their target mRNA, remains a substantial limitation, even after intravitreal administration (Lysik and Wu-Pong, 2003).

- 5 Other approaches to antisense oligonucleotide intracellular delivery may involve: mechanical and electrical cell damage to the cell membrane, polymeric and lipid carriers (known to non-specifically destabilise membranes), or viral vectors. However, these and similar approaches have not proved to be safe or reliable in the clinic and have not resulted (to date) in any delivery technologies licensed for antisense products in the market place.

10

The present invention aims to provide compositions and methods for use in a system for the down-regulation of one or more genes within a cell by means of a novel arrangement of an active antisense sequence (e.g., of single-stranded DNA) flanking a partially overlapping second oligonucleotide strand that forms a binding site that can bind to a  
15 proteinaceous, nucleic-acid-binding domain. We term these partly single- and partly double-stranded nucleotides, "ASO hybrids". Unexpectedly, we have discovered and have shown empirically (unpublished data):

20

- 1) That ASO hybrids can show an equivalent antisense activity profile to the control single-stranded antisense oligonucleotide in a cell-free assay, for example at an ASO concentration of 30 pMol.

25

- 2) That ASO hybrids bind to a nucleic-acid-binding domain (such as *S. cerevisiae* GAL4) to form a complex without requiring a polycationic affinity handle (such as poly(L-lysine), used previously (Gaur *et al.*, 2002; WO97/23236)) or other covalent chemical conjugation methods.

30

- 3) That if, in an ASO hybrid complex (for example, GAL4::ASO) the nucleic-acid-binding domain is fused to attenuated lethal factor domain 1 (from *Bacillus anthracis*, *i.e.*, LFn), this supramolecular assembly can pass through a pore derived from a bacterial virulence factor (*Bacillus anthracis* PA63). This is unexpected as it is reported that passage through the PA pore requires the structural disassembly of cargo (molten globular transition) (Zorretta *et al.*, 2010). For a supramolecular complex to transit into the cytosol via the PA pore, molten globular transition of the

cargo, *i.e.*, a transition from an ordered structure to a random coil, must not be taking place. Again this transition has been shown without the use of a polycationic affinity handle (such as poly(L-lysine) (Gaur *et al.*, 2002: WO97/23236)).

5 It is a particular aim of the present invention to provide a conjugation strategy to enable compositions of antisense oligonucleotides to cross a cell membrane. In this way, antisense oligonucleotides can be delivered to the cytosol of a cell providing direct access to RNA or DNA targets within the cytosol (such as viral mRNA transcripts) via the described antisense-to-protein conjugation strategy.

10

In one aspect, the present invention provides an antisense oligonucleotide composition comprising a pair of antisense oligonucleotides, wherein the two antisense oligonucleotides hybridise to give at least one single-stranded antisense sequence and at least one double-stranded protein-binding sequence. Preferably the composition further comprises a shuttle  
15 protein (*e.g.*, recombinant LFn-GAL4), wherein the shuttle protein comprises a nucleic-acid-binding domain (for example GAL4) that recognises the double-stranded protein-binding sequence and wherein the shuttle protein is non-covalently bonded to the pair of antisense oligonucleotides.

20 In another aspect, the present invention provides a system for delivering an antisense oligonucleotide across a membrane of a cell, the system comprising (i) a pair of antisense oligonucleotides, the pair comprising a double-stranded protein-binding sequence (*e.g.*, GAL4) and at least one single-stranded antisense sequence (as shown for example in Figure 1), and (ii) a shuttle protein having a nucleic-acid-binding domain that is capable of  
25 recognising a specific sequence (*e.g.*, CGG-N<sub>11</sub>-CCG) incorporated within the double-stranded protein-binding nucleic acid sequence to non-covalently bind the shuttle protein to the pair of antisense oligonucleotides.

In a further aspect, the present invention provides a method of non-covalently conjugating  
30 an antisense oligonucleotide to a shuttle protein (*e.g.*, LFn-GAL4), the method comprising: (i) providing two antisense oligonucleotides, (ii) hybridising the two antisense oligonucleotides to form an antisense hybrid, wherein the antisense hybrid comprises at least one single-stranded antisense sequence and at least one double-stranded protein-

binding sequence, (iii) providing a shuttle protein containing a nucleic-acid-binding domain that recognises a double-stranded protein-binding target sequence, and (iv) non-covalently conjugating the shuttle protein to the antisense hybrid by means of the nucleic-acid-binding domain of the protein and the protein-binding sequence of the antisense  
5 hybrid.

The method of conjugation and membrane translocation does not require the use of (polycationic or ionic) DNA condensing agents (such as poly(ethylene imine) or poly(L-Lysine)) as these materials are often toxic and this toxicity is thought to be due, at least in  
10 part, to their propensity to destabilise cell membranes (Richardson *et al.*, 1999) (and see Table 1 below). In the context of the present invention, a shuttle protein is defined as a protein that is capable of conveying genetic material through a pore in a cell membrane. The pore may comprise a pore-forming protein, such as *Bacillus anthracis* PA83 or PA63.

15 The oligonucleotides of the invention comprise nucleic acid sequences, which may be of DNA or DNA analogues, for example sulphonated analogues in which certain oxygen atoms are replaced by sulphur. Other DNA analogues that may be found suitable include those disclosed by Leumann (2002). The sequences may directly adjoin each other or be connected by an intervening DNA sequence, preferably at least partly double-stranded.

20

In a preferred embodiment, the shuttle protein is an attenuated toxin protein. In particular, the shuttle protein may be *B. anthracis* lethal factor domain I (LFn).

25 Preferably, in the attenuated toxin, at least one toxin domain, *e.g.*, one or more of toxic domains II-IV of the *B. anthracis* lethal factor protein toxin, is replaced by a nucleic-acid-binding domain. For example, the nucleic-acid-binding domain may be *Saccharomyces cerevisiae* GAL4 (fused with LFn).

30 Advantageously, the antisense oligonucleotide composition further comprises a pore-forming protein. Preferably, the pore-forming protein is a non-toxic protein. One preferred pore-forming protein is *B. anthracis* virulence factor Protective Antigen (PA). In particular, the pore-forming protein may be *B. anthracis* PA83 or PA63.

In one embodiment, (in the instance of targeting syntaxin5 (Syn5) for down-regulation) one of the oligonucleotides includes the antisense sequence

5' AATTTGTTTGTTGAGGCTA 3' (SEQ ID No: 18). Each member of the antisense oligonucleotide pair comprises one of the two complementary strands that hybridise to form the protein-binding sequence (see Figure 1). The antisense sequence may be downstream (3') or upstream (5') of the protein-binding sequence (or both, if there is more than one antisense sequence in the hybrid that results) (see Figure 1a).

In one embodiment, the protein-binding sequence is CGG-N<sub>11</sub>-CCG where "N" is any nucleotide comprising a purine or pyrimidine base. Suitably, the protein-binding sequence is 5' **CGGCTGCTCTGATGCCG** 3' (SEQ ID No: 19).

Advantageously, the antisense oligonucleotide composition comprises oligonucleotide pairs that hybridise to form a protein-binding sequence that includes the sequence: 5' **CGGCATCAGAGCAGCCG** 3' (SEQ ID No: 20). Examples of such sequences are SEQ ID Nos 9, 11 and 13 shown below (Example 4).

In one embodiment, the oligonucleotide further comprises a flanking sequence between the protein-binding sequence and the antisense sequence (see Figure 1a).

20

Advantageously, the oligonucleotides used in the present invention may be sulphonated, thereby increasing their stability. In sulphonated oligonucleotides, one or more non-bridging oxygen atoms in the oligonucleotide chain may be replaced by sulphur atoms. Sulphonation may be effected by various known methods, for example by use of Beaucage Reagent (3H-1,2-benzodithiole-3-one-1,1,-dioxide) (Cieślak *et al.*, 2005). Other ways of increasing stability of oligonucleotides may be employed in the invention: for example, those disclosed in Leumann (2002).

The present invention also provides an *in vitro* method of down-regulating the expression of a target gene in the cytosol of a cell by delivering an antisense oligonucleotide across a membrane of the cell using an antisense oligonucleotide composition as described above.

30

An alternative aspect of the present invention is an antisense oligonucleotide composition as described above for use in a method of down-regulating the expression of a gene from Human Papilloma Virus (HPV) in a human cervical epithelial cell (see Figure 5 and Example 7).

- 5 In a further aspect, the present invention provides an antisense oligonucleotide composition as described above as a potential treatment or prophylaxis for cancer, in particular cancers caused by human papilloma virus (HPV), including cervical cancer and oral cancers.

10 Many plant and bacterial toxins (virulence factors) have evolved to deliver a toxin, in the form of a macromolecular catalytically-active protein domain, to the cytosol of a cell. Examples include cholera toxin (Sandvig *et al.*, 2005), ricin toxin (Sandvig *et al.*, 2010) and anthrax toxin (Gaur *et al.*, 2002). These agents are known to operate *in vivo*, causing human morbidity and mortality.

15 The present inventors have prepared a known attenuated version of *Bacillus anthracis* lethal factor (LFn), where the wild-type domains II-IV (responsible for catalytic toxic activity) have been removed using recombinant PCR. These domains (II-IV) have subsequently been replaced with a nucleic-acid-binding domain such as *Saccharomyces cerevisiae* GAL4. All of this is known in the art (Gaur *et al.*, 2002). The inventors have  
20 shown that GAL4 can bind, indirectly, to active antisense oligonucleotides under suitable conditions *i.e.*, when two suitable oligonucleotides are hybridised together to form a hybrid containing a single-stranded antisense sequence and a double-stranded (DS) protein-binding sequence. The inventors have further shown that the complete complex (LFn-GAL4::ASO) can be used to translocate antisense oligonucleotides into the cytosol of a  
25 cell via interaction with another protein, PA83. Significantly, the processes and materials employed by the inventors to generate the complete antisense oligonucleotide - protein complex mean that condensing agents or polycation affinity handles hitherto regarded as necessary for DNA complex formation in the art (such as poly(L-lysine) (Gaur *et al.*, 2002; WO97/23236), which is toxic and known to destabilise membranes (Richardson *et al.*, 1999)) are not required.  
30

A preferred embodiment of a system in accordance with the present invention provides a single-stranded antisense oligonucleotide sequence 3' and/or 5' to a double-stranded (DS)

protein-binding sequence. Proteins such as LFn may be used in conjunction with pore-forming proteins, for example PA83. PA83 has the capacity to enhance the membrane translocation of LFn. The interaction between LFn and PA83 is described elsewhere (Krantz *et al.*, 2005) though it has been documented to require the molten-globular transition of cargo during pore translocation. We have now shown that it is possible to use a supramolecular assembly as cargo and that an antisense sequence associated with that supramolecular assembly can translocate into the cytosol. This could not have been predicted.

The inventors have further developed the system to use hybridised oligonucleotide containing a double-stranded LFn-GAL4 binding sequence and a 3' and/or 5' overhanging single-stranded antisense sequence, which they have shown to be no less effective than the antisense (single-stranded nucleotide) sequence without any flanking sequence at antisense oligonucleotide (ASO) concentrations above 30pMol. The complex developed by the inventors can be used as an antisense oligonucleotide conjugation system to facilitate antisense oligonucleotide cytosolic delivery without a covalent attachment between the oligonucleotides and the LFn-GAL4 protein. The present invention provides a useful tool for *in vitro* research as well as a basis for an antisense oligonucleotide pharmaceutical composition and a critical part of the delivery methodology of any suitable antisense therapy. In particular, the present invention may form the basis for an antisense antiviral composition for the treatment of HPV-infected cervical epithelial cells.

The dimerisation of the LFn-GAL4 protein in response to the presence of a double-stranded protein-recognition nucleic acid sequence adjacent to the antisense sequence has been shown using small-angle neutron scattering (SANS) (see Example 5). Example 5 shows that the protein LFn-GAL4 forms high molecular weight complexes (protein radius of gyration ( $R_g$ ) expanding from approximately 5nm to 25nm) upon the addition of the hybrid oligonucleotide compositions of the invention.

In order to test a three-component system in accordance with the present invention, the inventors initially produced a prototype. The system's ability to down-regulate genes was tested in human epithelial cells (Hela). The LFn-GAL4::oligonucleotide composite, when mixed with PA83 facilitates the down-regulation of the specific, selected gene (see

Example 6), corresponding to the antisense sequence. With reference to Example 6, this illustrates the down-regulation of the protein Syntaxin5 by Western blotting and immunodetection (Suga *et al.*, 2005). Gene specificity (as well as controlling for cell number) is further emphasised by normalising the levels of target gene expression to the expression levels of a “housekeeper” gene such as Derlin1 and through the inclusion of “non-sense” controls (see Figure 4). ‘Non-sense’ controls are controls that include single-stranded DNA that is not complementary to target RNA in the gene being expressed, and accordingly are not expected to inhibit its expression.

- 10 In addition antisense activity is also shown against the expression of the HPV gene E7 (see Example 7). HPV E7 is critical to the propagation of the HPV viral life cycle and is a target for therapeutic intervention (Jonson *et al.*, 2008).

The arrangement of two oligonucleotide strands to provide a double-stranded protein-binding region or sequence, whilst leaving free single-stranded regions of sequence that have antisense activity provides a convenient conjugation method that can be used to bind the antisense domains to agents already described in the art (such as LFn-GAL4 and PA83). This facilitates the cytosolic delivery of the antisense agents that can mediate the down-regulation of genes coding for potential drug targets, for example HPV E7.

20

Specific embodiments to the invention are now described in more detail with reference to the accompanying drawings, in which:

Figure 1 comprises Figures 1a to 1e showing five different compositions according to the invention, illustrating different topologies for creating a double-stranded protein-binding site from a pair of single-stranded antisense oligonucleotides;

25

Figure 2 compares inhibitory activity of a composition according to the invention with other antisense compositions in a cell-free assay.

30

Figure 3, by using small-angle neutron scattering (SANS), shows that in the presence of antisense compositions of the invention, LFn-GAL4 dimerise to form compositions of higher molecular weight.

Figures 4 and 5 illustrate how the described system can down-regulate expression of two different genes: down-regulating the expression of Syntaxin5 is shown in Figure 4 and of HPV (serotype 18) Early (E)7 is shown in Figure 5;

Figure 6 is the LFn-GAL4 nucleotide sequence (SEQ ID No: 1);

5 Figure 7 is the LFn (LF domain I) protein sequence (SEQ ID No: 2);

Figure 8 is the GAL4 (amino acids 1-147) protein sequence (SEQ ID No: 3);

Figure 9 is the V5-LFn-GAL4-6His DNA sequence (SEQ ID No: 4);

Figure 10 is the V5-LFn-GAL4-6His protein sequence (SEQ ID No: 5);

Figure 11 is the MRGS-6His-PA83 DNA sequence (SEQ ID No: 6); and

10 Figure 12 is the MRGS-6His-PA83 protein sequence (SEQ ID No: 7).

### Example 1: Formation of shuttle protein

LFn-GAL4 was enriched from cultures of *E. coli* with a yield of approximately 5 mg/l. The DNA sequence coding for the protein LFn-GAL4 is SEQ ID No: 4 (Figure 9) . This  
15 sequence is within the plasmid pET151/D (Invitrogen), an *E.coli* expression cassette.

The DNA sequence for LFn (LF domain 1) and GAL4 (amino-acids 1-147) (known in the art - Gaur *et al.*, 2002) was sub-cloned into the pET151/D bacterial expression cassette. The addition of a V5 epitope tag at the N-terminus and 6x histidine affinity tag at the C-  
20 terminus allowed for the rapid immunodetection and affinity purification, respectively, of the fusion protein from bacterial lysate.

Recombinant protein production: Chemically competent bacteria (*E.coli* BL21\*DE3 (Invitrogen)) were transformed with purified plasmid (described above) and cultured  
25 overnight (10 mL) in 2x yeast extract tryptone (2xYT) bacterial culture, containing ampicillin (200 µg/ml) media prior to sub-culturing in large volumes (1L) of 2xYT also containing a similar concentration of ampicillin. Bacterial cultures were then incubated in an orbital shaker set at 180 rpm (at 37°C) for 3 hours. Subsequently isopropyl β-D-1-thiogalactopyrnoside (IPTG) was added to a final concentration of 500 µM and incubated  
30 for a further 3 hours. Bacterial pellets were prepared by centrifugation (6 000xg for 10 min at 4°C) and subject to lysis using a French Press set to 1500 PSI. Bacterial lysates were subjected to further centrifugation (20 000xg for 20 min). The resultant supernatant was passed over a 6xHis (Co<sup>2+</sup> Tallon® resin (Clontech)) affinity chromatography column.

LFn-GAL4 was eluted using 150 mM imidazole in fractions of 1 mL. Fractions were analysed for protein purity and concentration, pooled and dialysed in phosphate buffered saline. The final protein preparation was evaluated by SDS-PAGE and subjected to Coomassie staining (to determine protein purity) and Western blot analysis (using an antibody specific to V5 and 6xHis).

### Example 2: Production of pore-forming protein

PA83 was enriched from cultures of *E. coli* BL21\*DE3 with a yield of approximately 5 mg/l. A plasmid containing the known DNA sequence encoding PA83 was obtained as a gift from Professor Les Baillie (University of Cardiff). It contained the PA83-encoding sequence sub-cloned into the bacterial expression vector pQE30 providing an N-terminal 6x histidine affinity tag (Baillie *et al.*, 2010). Chemically competent bacteria were transformed with purified plasmid and cultured overnight as before. During the growth phase, the 1/ bacterial cultures were incubated in an orbital shaker set at 180 rpm (at 37°C) for 3 hours. Subsequently IPTG was added to a final concentration of 500 µM and incubated for a further 2 hours. Bacterial pellets were prepared by centrifugation (6 000xg for 10 min) and subjected to lysis using a French Press as before. Bacterial lysates were subjected to further centrifugation (20 000xg for 20 min). The resultant supernatant was passed over a 6x histidine affinity chromatography column. PA was eluted using 150 mM imidazole in fractions of 1 mL. Protein fractions were analysed for purity and concentration, pooled, and dialysed in phosphate-buffered saline (PBS). The final protein preparation was evaluated by SDS-PAGE and subjected to Coomassie staining (to determine purity) and western blot analysis (using an antibody specific to PA).

The amino-acid sequence for PA83 is shown in SEQ ID No: 7 (Figure 12 -MRGS-6His-PA83 protein).

### Example 3: Formation of antisense oligonucleotide with double-stranded protein-binding region

Two complementary oligonucleotides, each encoding one strand of a GAL4 recognition sequence, were annealed, to form a double-stranded (GAL4) protein-binding sequence with flanking single-stranded antisense sequences. The ASO compositions described (SEQ ID Nos: 8 & 9: see Example 4) were used in this instance. The resulting composition is

shown in Figure 1a. Antisense oligonucleotide hybridization was performed by repeated (x10) thermal cycling, melting (1 min. at 94°C) and re-annealing (1 min. at 55°C) the two partially overlapping oligonucleotides, leaving single-strand antisense oligonucleotide sequence free to interact with mRNA in a sequence-specific manner.

5

Figures 1a - 1e show several possible topologies of the protein-binding (DS) sequence of DNA (or DNA analogues) in relation to the antisense nucleic acid sequence(s) using the protein::oligonucleotide conjugation strategy disclosed herein. Figure 1a shows two sequences SEQ ID Nos: 8 and 9 oriented in opposite senses and bonded together by  
 10 complementary sections – bases 23 to 48 in SEQ ID No: 8 bonding with bases 48 to 23 of SEQ ID No. 9 (SEQ ID No. 9 is shown 3' to 5' in Figure 1a). The ASO hybrids of our invention may or may not include an additional DNA sequence flanking the antisense sequence(s). As shown in Figures 1a - 1e, the double-stranded (protein-binding) region of the ASO hybrid can be formed in a number of different ways. For example, the double-  
 15 stranded region can be positioned between two antisense sequences (which may be identical or different), as in Figure 1a. Alternatively, the double-stranded region can be positioned at one side of a single antisense oligonucleotide, as in Figures 1b-1e.

#### 20 **Example 4: Production of DNA sequences useful for inhibiting expression of specific proteins**

*Antisense oligonucleotide sequence specific for Syntaxin5 with flanking and GAL4 binding sequence:*

Forward oligonucleotide (SEQ ID No: 8)

5' AATTTGTTTGTTGAGGCTAATGCATGCCGGCTGCTCTGATGCCGGCAT 3'

25 Reverse oligonucleotide (SEQ ID No: 9)

5' AATTTGTTTGTTGAGGCTAATGCATGCCGGCATCAGAGCAGCCGGCAT 3'

*Antisense oligonucleotide sequence specific for HPV (serotype 18) Early (E) 7 mRNA transcripts, with flanking and GAL4 binding sequence:*

30 Forward oligonucleotide (SEQ ID No: 10)

5' GGTCGTCTGCTGAGCTTTCTATGCATGCCGGCTGCTCTGATGCCGGCAT 3'

Reverse oligonucleotide (SEQ ID No: 11)

5' GGTCGTCTGCTGAGCTTTCTATGCATGCCGGCATCAGAGCAGCCGGCAT 3'

*Antisense oligonucleotide sequence specific for TurboGFP mRNA transcripts, with flanking and GAL4 binding sequence:*

Forward oligonucleotide (SEQ ID No: 12)

5' GGTGCTCTTCATCTTGTTGGTATGCATGCCGGCTGCTCTGATGCCGGCAT 3'

Reverse oligonucleotide (SEQ ID No: 13)

5' GGTGCTCTTCATCTTGTTGGTATGCCGGCATCAGAGCAGCCGGCATGCAT 3'

10

The above oligonucleotides (SEQ ID Nos: 8-13) were synthesised with phosphorothioate modification to enhance the lifetime of the antisense oligonucleotide.

Forward and reverse primer pairs (SEQ ID Nos 8 with 9; 10 with 11; 12 with 13) were hybridized using a PCR cyclor using the following conditions: Heated to 94°C for 1 min., and cooled to 55°C for 1 min., x10. This annealing process produced hybrid single- and double-stranded DNA sequences according to the invention.

#### **Example 5: Formation of an antisense oligonucleotide-shuttle (LFn-GAL4) complex**

Annealed ASO hybrids were produced (as in Example 4). These ASO hybrids were co-incubated with LFn-GAL4 in phosphate-buffered saline (PBS) for 30 min at room temperature at the appropriate stoichiometry (*i.e.*, at approximately a 3:1 protein to oligonucleotide molar ratio).

One such product so obtained was investigated by small-angle neutron scattering (SANS). The profile resulting is compared in Figure 3 with that obtained in the absence of any antisense oligonucleotide. Figure 3 shows LFn-GAL4 dimerisation as measured by SANS in response to the addition of the antisense oligonucleotide composition shown in Figure 1a - small angle neutron scattering by LFn-GAL4 and LFn-GAL4::DS-antisense oligonucleotide in PBS (at approximately 0.5 mg/ml of LFn-GAL4 in each instance). Units of the x-axis of Figure 3 are q/l/angstrom and of the y-axis are 1/cm. The upper thick black line shows results in the presence of the ASO, in comparison with the lower thin line where the ASO is absent. The differential in scattering profile between 0.01 and 0.04 (q/1/Å) is attributable to the LFn-GAL4 dimerising, caused by association with the double-stranded

protein-binding sequence forming part of the oligonucleotide (Figure 1a). In this instance 2mm cuvettes (Hellman Analytics, Essex, UK) were loaded with LFn-GAL4 and LFn-GAL4::oligonucleotide stoichiometry of 3:1 respectively (molar ratio).

The intensity of such scattered radiation is a reflection of the scatterer size and shape, as well as composition (*i.e.*, LFn-GAL4). Both the individual proteins and their blends were examined. For the individual components, generally no scattering was observed, indicating their relatively small size. In the mixture of LFn-GAL4 and the hybridised oligonucleotide, scattering was observed, confirming that an interaction was occurring to form larger structures (*i.e.*, LFn-GAL4::ASO complexes).

#### **Example 6: Down-regulation of target gene expression (Syntaxin5)**

One of the complexes produced in Example 5 was employed to target the expression of the gene Syntaxin5 in Hela cells. The pore-forming protein PA83 was used to help mediate antisense oligonucleotide membrane translocation via an interaction with LFn-GAL4. The ASO portion of the complex consisted of two annealed (as in Example 4) ASO sequences (SEQ ID Nos: 8 & 9). Hela cells produce Syntaxin5 when cultured under normal conditions. Hela cells were treated in various ways at a range of ASO complex concentrations: 0 (control), 1, 10, 50, 100 and 200 pMol per litre.

In Figure 4: the black filled bar represents Hela cells treated with PA83, LFn-GAL4 and a “non-Syntaxin5” (GFP specific) ASO (formed from hybridised SEQ ID No:12 and 13). The bar with no fill represents Hela cells treated with PBS only. These two treatments show sequence specificity for Syntaxin5 down-regulation (since the anti-GFP ASO sequence here is inactive against Syntaxin5) and serve as negative controls. Bars filled with horizontal hatching represent Hela cells electroporated with hybrid ASO specific for Syntaxin5 (formed from SEQ ID Nos: 8 and 9). Bars filled with vertical hatching represent Hela cells treated with PA83, LFn-GAL4 and hybrid ASO specific for Syntaxin5 (formed from SEQ ID Nos: 8 and 9). The bars filled with diagonal hatching represent Hela cells treated with hybrid ASO specific for Syntaxin5 (formed from SEQ ID Nos: 8 and 9) mixed with Oligofectamine (Invitrogen), following the manufacturer's recommendations.

Syntaxin5 expression levels in the cells were measured after 24 hours using immunoblotting. Results are shown as % expression of control (Hela cells treated with

PBS only) (y axis). The data show that compositions according to the invention down-regulate Syntaxin5 expression in a gene-specific manner. This is shown at a population level by Western blotting and immunodetection. The graph of Figure 4 displays Syntaxin5 expression levels and shows the effect of loading antisense oligonucleotide hybrids (3.2µg/mL) (SEQ ID Nos: 8 and 9) onto LFn-GAL4 (50µg/mL) co-incubated with PA (50µg/mL) on Hela cells relative to the expression of a housekeeper gene, Derlin1 (to control for any variation in cell number). The data shown is derived from 3 separate experiments and demonstrates that the addition of the compositions described can mediate the knockdown of a specific gene antisense oligonucleotide encoding an antisense sequence (*i.e.*, Syntaxin5). The data represent three repeats of each experiment and the error bars represent the standard error of the mean.

#### **Example 7: Down-regulation of target gene expression (HPV18 E7)**

An experiment to examine the efficacy of anti-HPV E7 antisense oligonucleotides against the expression of Human Papilloma Virus (serotype 18) Early 7 in Hela cells was undertaken as described in Example 6 above, but using different oligonucleotide sequences. In this instance the Syntaxin5-specific ASO hybrids (formed from SEQ ID No: 8 and SEQ ID No: 9) of Example 6 were replaced with HPV-E7-specific ASO hybrid also containing the hybrid protein-binding region and a small flanking region (formed from SEQ ID No:10 and SEQ ID No:11). In this example 200 pM of oligonucleotide, associated with LFn-GAL4 and PA83, was used (this is the black bar on the graph in Figure 5). The result is shown in Figure 5: % gene expression is shown on the y axis. The control treatment, PBS, is taken as 100%: this is the white bar on the graph in Figure 5. The data represent three repeats of each experiment and the error bars represent the standard error of the mean. There was a statistical difference between the two treatments ( $p=0.0104$  as measured by a one-tailed, unpaired t-test).

#### **Example 8: *In vitro* toxicity of compositions of the invention compared with poly(ethyleneimine)**

The ASO hybrid-shuttle complexes produced as in Example 5 were tested for toxicity in relation to poly(ethyleneimine) (PEI) (Table 1). These data show a differential in toxicity relative to cells exposed to varying concentrations of either PEI, a cationic polymer well characterised in the literature as being able to mediate transfection, and LFn-GAL4 protein

after hybridisation with ASO hybrids in the presence of 50µg/mL PA over 72h. Cell viability was normalised to untreated cells and measured by the addition of MTT (Richardson *et al.*, 1999).

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**Table 1**

|                     | IC <sub>50</sub> in HeLa (micrograms/ml) | IC <sub>50</sub> in Vero (micrograms/ml) |
|---------------------|------------------------------------------|------------------------------------------|
| 25KDa Branched PEI  | 2.9 +/- 0.6                              | 7.3 +/- 0.1                              |
| 0.8KDa Branched PEI | 2.4 +/- 0.2                              | 7.4 +/- 0.3                              |
| 20KDa Linear PEI    | 3.0 +/- 0.1                              | 6.9 +/- 0.5                              |
| PA83                | >100                                     | >100                                     |
| ASO                 | >100                                     | >100                                     |
| GAL4-LFn            | >100                                     | >100                                     |
| ASO ::GAL4-LFn+PA83 | >100                                     | >100                                     |

**Example 9: Antisense inhibition of an *in vitro* translation assay using different antisense oligonucleotide compositions**

Figure 2 shows the effect of compositions of the invention as compared with component ASOs in a cell-free assay (where membrane translocation is not rate-limiting).

Control reactions were performed using a “One step human high yield mini- *in vitro* translation kit” (Thermo Scientific), in conjunction with a control plasmid encoding the protein turboGFP (green fluorescent protein – Evrogen). The reactions were performed in 4.5 µl volumes (incubated for 3h at 30°C) and the expression of turboGFP was monitored by Western immunoblotting using an anti-6His primary antibody (1:1000 dilution) and an anti-mouse HRP-conjugated secondary antibody (1:1000). Detection was performed using a Picostable ECL kit (GE Healthcare). Three anti-turbo GFP antisense oligonucleotide (ASO) compositions were added to the reaction at differing concentrations at the beginning of the 3h incubation period previously described.

Results are shown in Figure 2, (n=3 ± SEM) relative to a control containing no antisense oligonucleotides. In Figure 2 the y-axis represents the expression of turboGPF (%) relative to an untreated control, while the x-axis represents concentration of antisense

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oligonucleotide added to the reaction (in pMol). The data points represented with a square were derived from a single-stranded ASO oligonucleotide, SEQ ID No: 12, consisting of the turboGPF antisense sequence and (one strand of the) protein-binding sequence. The data points represented by triangles represent data generated from only the antisense  
5 sequence incorporated into SEQ ID No: 12 (*i.e.*, the 21-mer GGTGCTCTTCATCTTGTTGGA, SEQ ID No: 21). The data points represented by circles were generated from the ASO hybrid of the invention formed by annealing together SEQ ID Nos: 12 and 13. The results shown in Figure 2 illustrate that (*in vitro*) the presence of the protein-binding sequence in the ASO hybrid does not inhibit the activity of the  
10 antisense sequence above an ASO concentration of 30 pMol.

Our invention may be used to produce antisense compositions having substantially no significantly reduced antisense activity (as measured in cell-free assay), relative to an antisense control having no protein-binding sequence or flanking sequence, at or above a  
15 30pMol concentration of antisense oligonucleotide (ASO). Example 9 above (Figure 2) illustrates this.

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**CLAIMS:**

1. A pharmaceutically acceptable antisense oligonucleotide composition useful for delivering antisense oligonucleotide into the cytosol of a cell, comprising a complex formed from:

(i) a pair of partially complementary, single-stranded oligonucleotide hybridised to give at least one single-stranded antisense sequence and at least one double-stranded protein-binding sequence; and

(ii) a shuttle protein capable of conveying genetic material through a pore in cell membrane, the shuttle protein comprising a nucleic-acid-binding domain that recognises the double-stranded protein-binding sequence and is non-covalently bound thereto;

wherein the shuttle protein is an attenuated toxin protein, in which at least one toxin domain has been replaced by a said nucleic acid binding domain;

and wherein the composition further comprises:

(iii) a pore-forming protein which is capable of interaction with the said complex to facilitate translocation of the complex into the cytosol of the cell.

2. A composition as claimed in claim 1, comprising an oligonucleotide that is modified by sulfonation to increase stability.

3. An antisense oligonucleotide composition as claimed in any one of the previous claims, wherein the protein-binding sequence is CGG-N11-CCG, where "N" is any purine or pyrimidine base.

4. An antisense oligonucleotide composition as claimed in any one of the preceding claims, wherein the protein-binding sequence is:

5'-CGGCTGCTCTGATGCCG-3' (SEQ ID NO: 19) or 5'-CGGCATCAGAGCAGCCG-3' (SEQ ID NO: 20).

5. An antisense oligonucleotide composition as claimed in any one of the preceding claims, wherein the shuttle protein contains *B. anthracis* lethal factor (LF) domain I (LFn).

6. An antisense oligonucleotide composition as claimed in claim 5, wherein at least one of domains (II-IV) of LF is replaced by the nucleic-acid-binding domain.

7. An antisense oligonucleotide composition as claimed in any one of claims 1 to 6, wherein the nucleic-acid-binding domain of the shuttle protein is *Saccharomyces cerevisiae* GAL4.
8. An antisense oligonucleotide composition as claimed in claim 1, in which the pore-forming protein is *B. anthracis* virulence factor Protective Antigen PA83.
9. An antisense oligonucleotide composition as claimed in any one of claims 1 to 8, in which the antisense sequence is single-stranded DNA designed to hybridise to messenger RNA derived from a target gene.
10. An antisense oligonucleotide composition as claimed in claim 9, in which the target gene is expressed by a virus, preferably the target gene is expressed by Human Papilloma Virus (HPV).
11. A method of transporting an antisense oligonucleotide across a membrane of a cell, the method comprising: providing an antisense oligonucleotide composition as claimed in any one of claims 1 to 10, and applying the antisense oligonucleotide composition to the cell.
12. A method of treating a subject infected with Human Papilloma Virus or having cervical cancer, comprising applying to the subject a therapeutically effective amount of a composition as claimed in any one of claims 1 to 10, whereby the composition is pharmaceutically acceptable.
13. A method of non-covalently conjugating an antisense oligonucleotide to a shuttle protein, the method comprising:
  - (i) providing two partially complementary single-stranded oligonucleotides,
  - (ii) hybridising the two oligonucleotides to form an oligonucleotide hybrid, wherein the hybrid comprises at least one single-stranded antisense sequence and at least one double-stranded protein-binding sequence,
  - (iii) providing a shuttle protein capable of conveying genetic material through a pore in a cell membrane and having a nucleic-acid-binding domain that recognises the double-stranded protein-binding sequence, wherein the shuttle protein is an attenuated toxin protein in which at least one toxin domain has been replaced by a said nucleic acid binding domain, and

(iv) non-covalently conjugating the shuttle protein to the antisense hybrid by means of the nucleic-acid-binding domain of the protein and the protein-binding sequence of the oligonucleotide hybrid.

14. The method of claim 13, wherein the antisense oligonucleotide is modified by sulfonation to increase stability.

15. The method of claim 13, wherein the protein-binding sequence is CGG-N11-CCG, where "N" is any purine or pyrimidine base; or the protein-binding sequence is:

5'-CGGCTGCTCTGATGCCG-3' (SEQ ID NO: 19) or 5'-  
CGGCATCAGAGCAGCCG-3' (SEQ ID NO: 20).

16. The method as claimed in claim 13, wherein the attenuated toxin protein is *Bacillus anthracis* lethal factor domain I (LFn); and preferably wherein at least one of domains (II-IV) of LF is replaced by the nucleic-acid-binding domain.

17. The method as claimed in claim 13, wherein the nucleic-acid-binding domain of the shuttle protein is *Saccharomyces cerevisiae* GAL4.

18. The method as claimed in claim 13, wherein the antisense sequence is single-stranded DNA designed to hybridize to messenger RNA derived from a target gene.

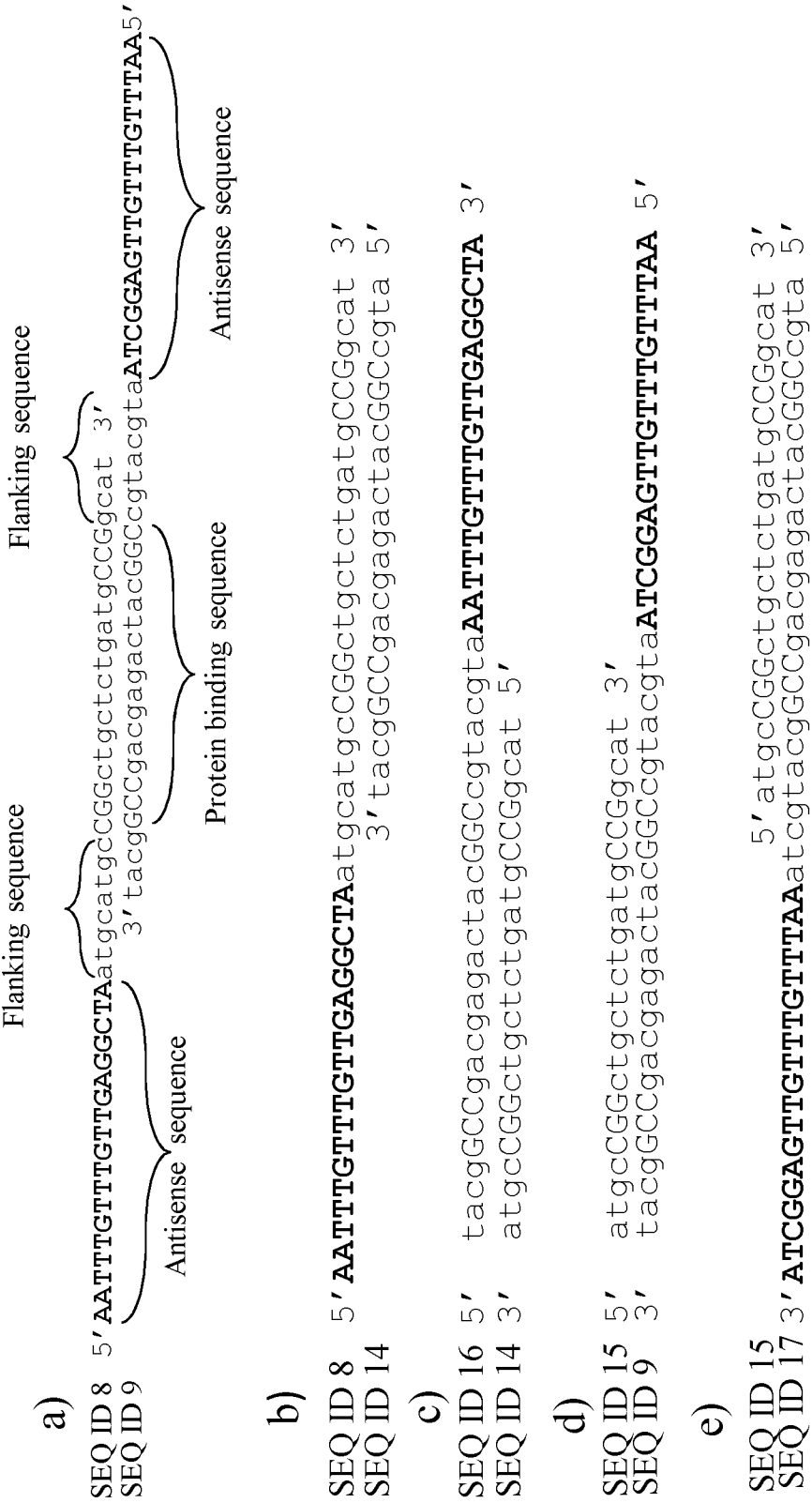
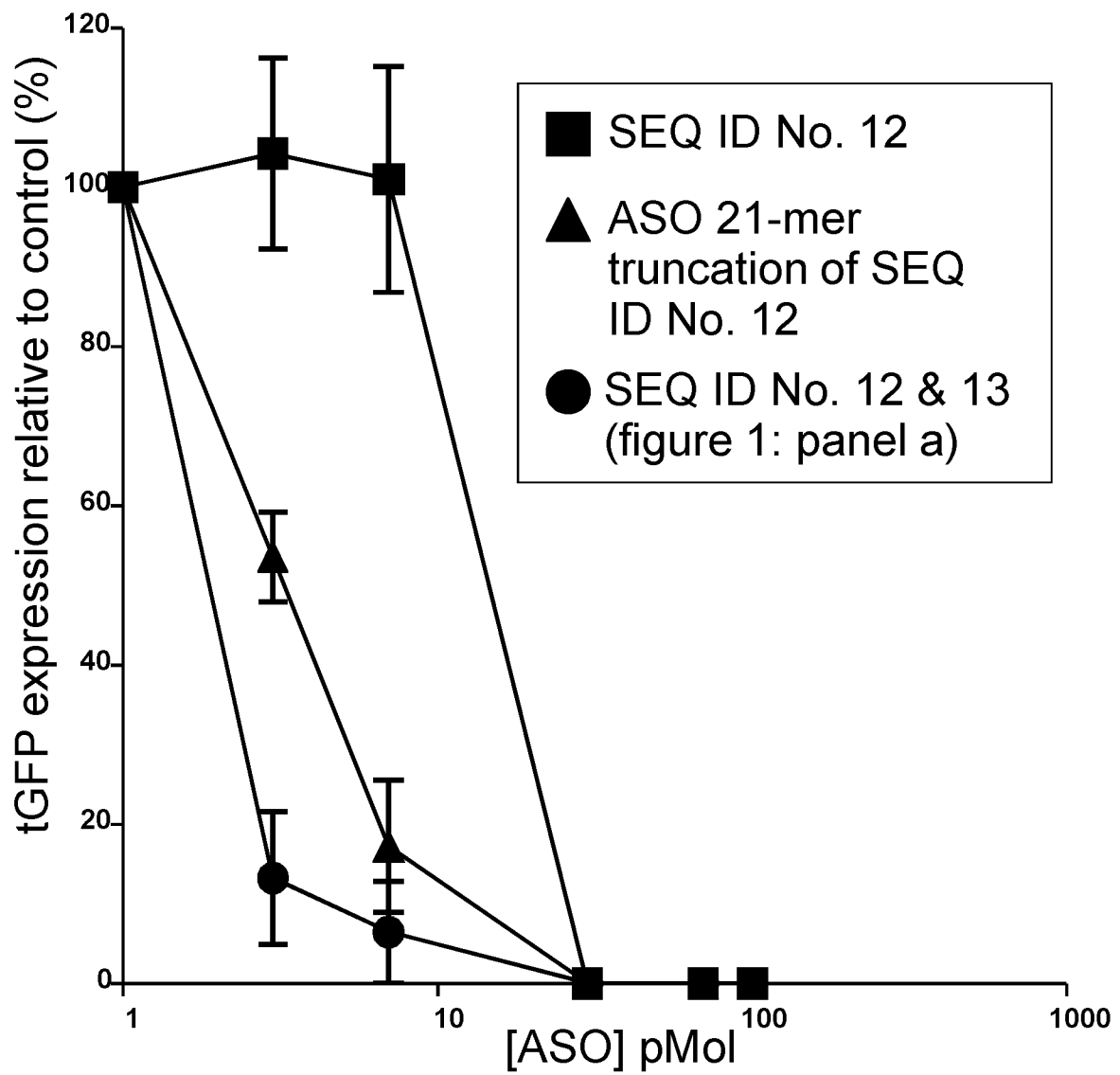
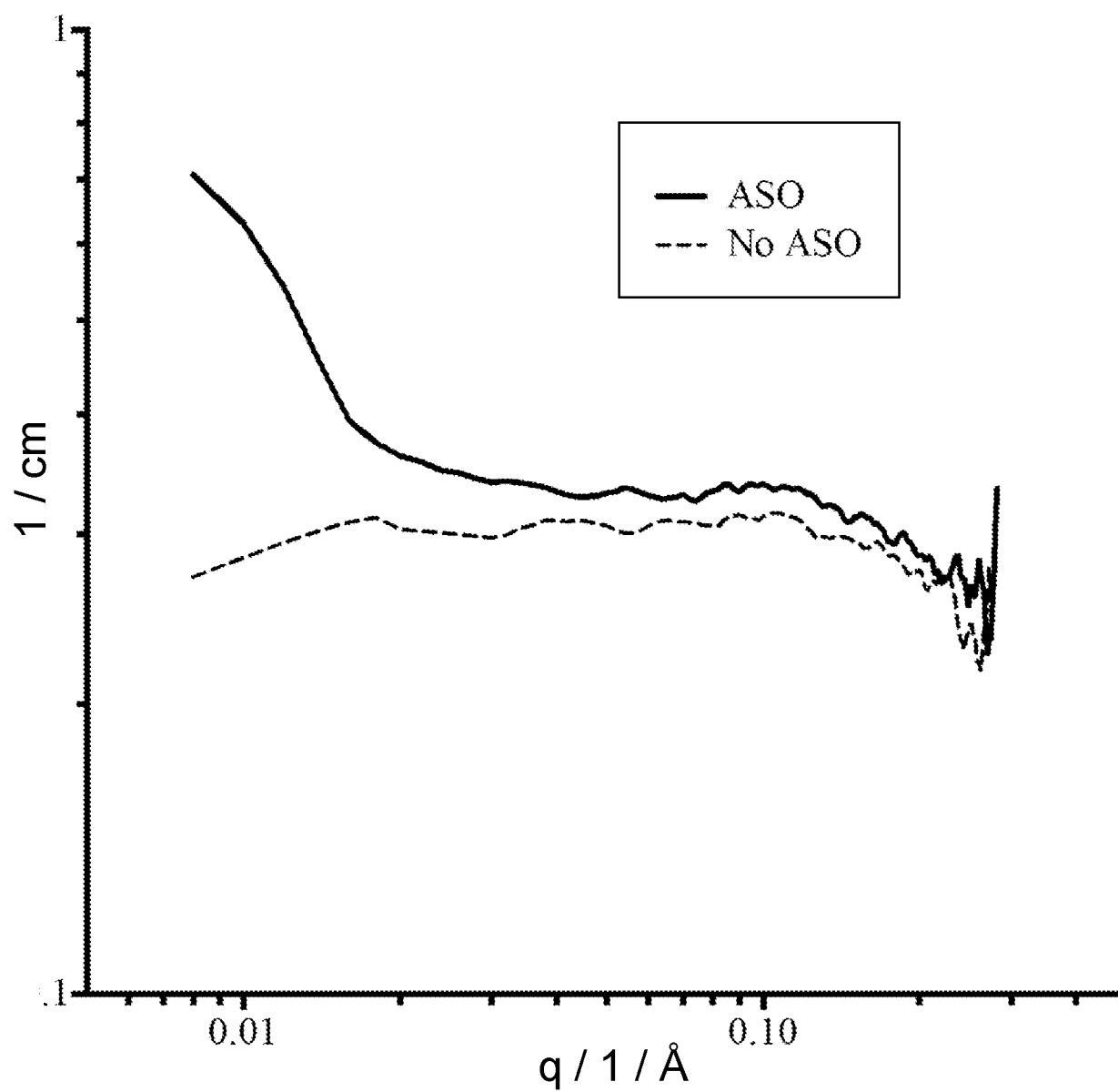


Figure 1

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**Figure 2**

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**Figure 3**

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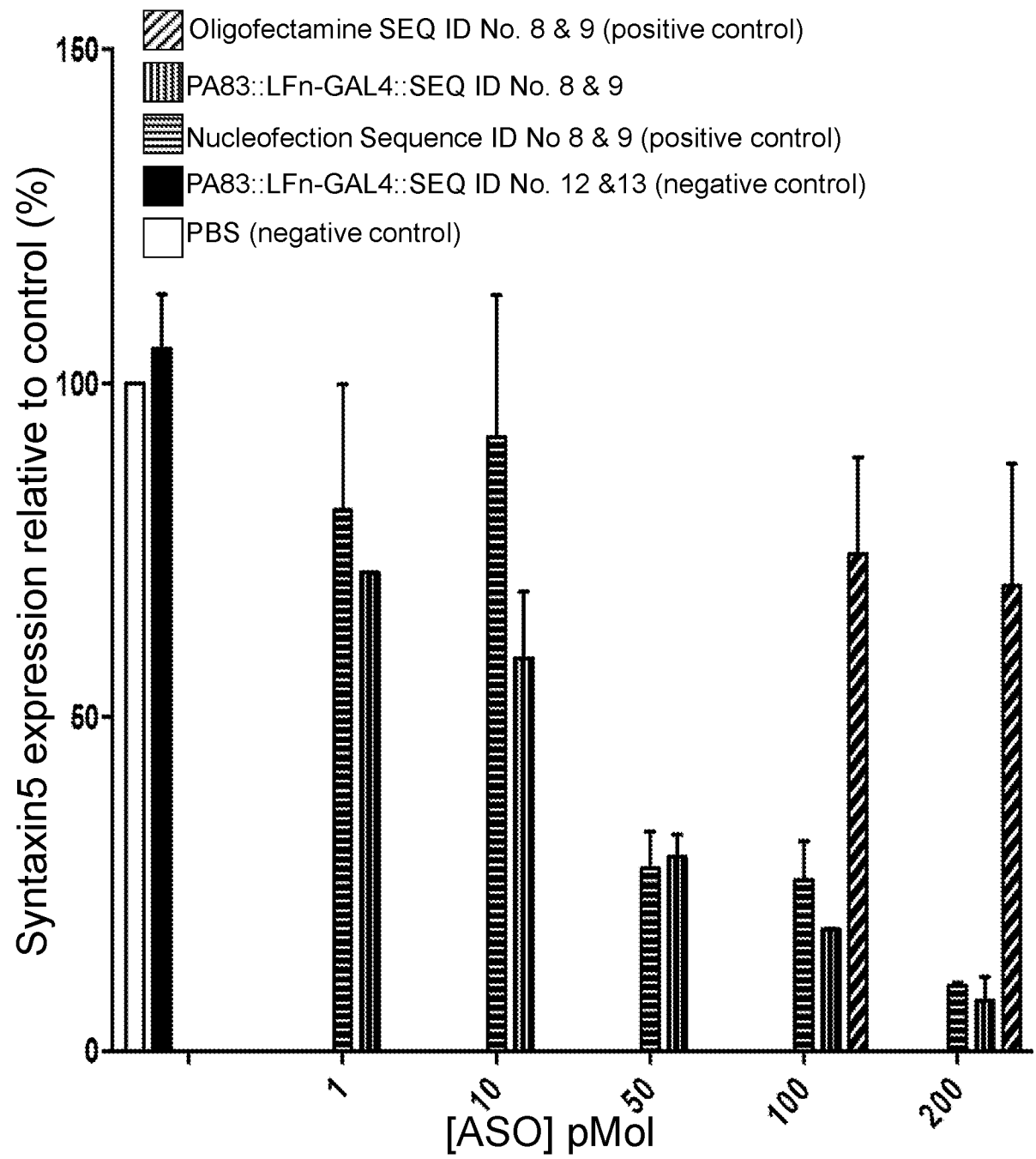
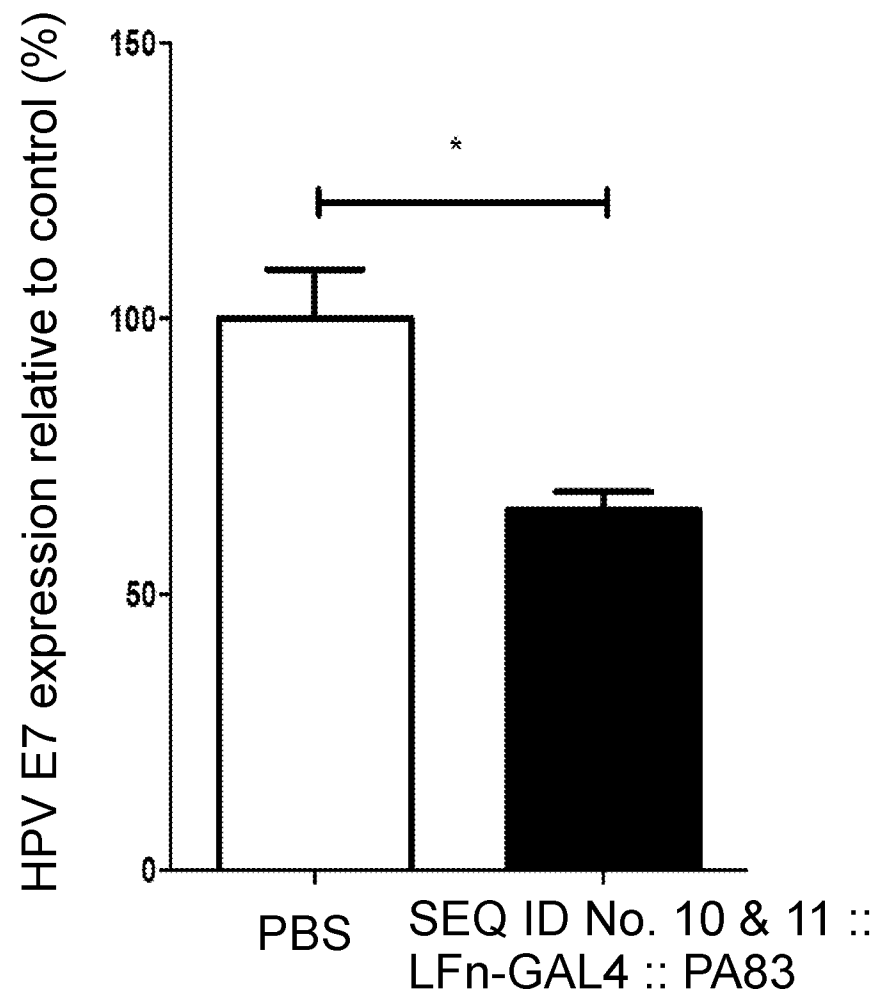


Figure 4

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**Figure 5**

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**SEQ ID No: 1**

ATGGAACGCAACAAAACCTCAGGAGGAACACCTGAAAGAGATCATGAAACACATTGTTAAAT  
CGAGGTGAAAGGCGAAGAAGCGGTTAAAAAGGAGGCTGCCGAAAAGCTGCTGGAGAAGGT  
ACCGTCTGATGTGCTGGAAATGTATAAAGCGATTGGTGGCAAAATCTACATCGTGGATGGTGAT  
ATTACCAAACACATCTCCCTGGAAGCACTGAGCGAAGACAAGAAGAAGATCAAAGATATCTAC  
GGCAAGGACGCGCTGCTGCACGAGCATTACGTTTACGCAAAAGAAGGTTACGAACCGGTGCT  
GGTTATCCAGTCCAGCGAGGATTACGTCGAAAATACGGAAAAAGCTCTGAACGTATATTACGA  
AATTGGTAAAATCCTGTCTCGTGACATTCTGAGCAAAATTAACCAACCTTATCAGAAGTTCCTG  
GACGTTCTGAACACCATCAAAAACGCTTCTGACTCCGACGGCCAGGACCTGCTGTTCACTAAT  
CAGCTGAAAGAACATCCGACCGATTTCTCTGTAGAATTCCTGGAACAGAACTCTAACGAGGTC  
CAAGAAGTTTTTGCCAAAGCATTGCGTACTACATCGAGCCGCAGCATCGCGACGTGCTGCAG  
CTGTACGCTCCAGAAGCCTTCAACTATATGGACAAATTCAATGAACAAGAAATCAACCTGTCTA  
TGAAACTGCTGTCCTCCATCGAACAGGCTTGGGATATCTGTCGTCTGAAGAACTGAAATGCTC  
TAAAGAAAAACCGAAATGCGCGAAATGCCTGAAAAACAACCTGGGAATGTCGCTATCCCCCTAA  
AACCAAACGTTCTCCACTGACCCGTGCGCACCTGACCGAAGTAGAATCCCGTCTGGAACGTCT  
GGAGCAGCTGTTTCTGCTGATTTTCCCGCGTGAAGACCTGGACATGATCCTGAAAATGGATAG  
CCTGCAGGATATCAAAGCACTGCTGACCGGTCTGTTTGTGCAGGACAACGTCAACAAAGACG  
CTGTTACTGATCGCCTGGCGAGCGTTGAACTGATATGCCGCTGACCCTGCGTCAGCACCGTAT  
CTCCGCAACGAGCTCCAGCGAAGAATCTAGCAACAAAGGTCAGCGCCAGCTGACCGTTAGC

**Figure 6****SEQ ID No: 2**

MERNKTQEEHLKEIMKHIVKIEVKGEEAVKKEAAEKLEKVPDVLEMYKAIGGKIYIVDGDITKHI  
SLEALSEDKKKIKDIYKGDALLHEHYVYAKEGYEPVLVIQSSDYVENTEKALNVYYEIGKILSRDILSK  
INQPYQKFLDVLNTIKNASDSGQDLLFTNQLKEHPTDFSVEFLEQNSNEVQEVFAKAFAYYIEPQ  
HRDVLQLYAPEAFNYMDKFNEQEINLS

**Figure 7**

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**SEQ ID No: 3**

MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVESRLERLEQLF  
LLIFPREDLDMILKMDSLQDIKALLTGFLVQDNVNKDAVTDRLASVETDMPLTLRQHRISATSSSEE  
SSNKGQRQLTVS

**Figure 8****SEQ ID No: 4**

ATGTAAACCGATTCCGAACCCGCTGCTGGGCCTGGACTCTACTATGGAACGCAACAAAACCTCA  
GGAGGAACACCTGAAAAGAGATCATGAAACACATTGTTAAAATCGAGGTGAAAGGCGAAGAA  
GCGGTTAAAAAGGAGGCTGCCGAAAAGCTGCTGGAGAAGGTACCGTCTGATGTGCTGGAAA  
TGTATAAAGCGATTGGTGGCAAAATCTACATCGTGGATGGTGATATTACCAAACACATCTCCCT  
GGAAGCACTGAGCGAAGACAAGAAGAAGATCAAAGATATCTACGGCAAGGACGCGCTGCTG  
CACGAGCATTACGTTTACGCAAAAGAAGGTTACGAACCGGTGCTGGTTATCCAGTCCAGCGA  
GGATTACGTCGAAAATACGGAAAAAGCTCTGAACGTATATTACGAAATTGGTAAAATCCTGTCT  
CGTGACATTCTGAGCAAAATTAACCAACCTTATCAGAAGTTCCTGGACGTTCTGAACACCATCA  
AAAACGCTTCTGACTCCGACGGCCAGGACCTGCTGTTCACTAATCAGCTGAAAGAACATCCGA  
CCGATTTCTCTGTAGAATTCTGGAACAGAACTCTAACGAGGTCCAAGAAGTTTTTGCCAAAG  
CATTCGCGTACTACATCGAGCCGCAGCATCGCGACGTGCTGCAGCTGTACGCTCCAGAAGCCT  
TCAACTATATGGACAAATTCAATGAACAAGAAATCAACCTGTCTATGAAACTGCTGTCCTCCATC  
GAACAGGCTTGCGATATCTGTCGTCTGAAGAAACTGAAATGCTCTAAAGAAAAACCGAAATGC  
GCGAAATGCCTGAAAAACAACCTGGGAATGTCGCTATTCCCCTAAAACCAAACGTTCTCCACTG  
ACCCGTGCGCACCTGACCGAAGTAGAATCCCGTCTGGAACGTCTGGAGCAGCTGTTTCTGCTG  
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CTGCTGACCGGTCTGTTTGTGCAGGACAACGTCAACAAAGACGCTGTTACTGATCGCTGGCG  
AGCGTTGAAACTGATATGCCGCTGACCCTGCGTCAGCACCGTATCTCCGCAACGAGCTCCAGC  
GAAGAATCTAGCAACAAAGGTCAGCGCCAGCTGACCGTTAGCCACCACCATCACCACCACTAA  
GG

**Figure 9**

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**SEQ ID No: 5**

MGKPIPNPLLGLDSTMERNKTQEEHLKEIMKHIVKIEVKGEEAVKKEAAEKLEKVPSPDVLEMYKA  
IGGKIYVDGDITKHISLEALSEDKKKIKDIYGKDALLHEHYVYAKEGYEPVLVIQSSDYVENTEKAL  
NVYYEIGKILSRDILSKINQPYQKFLDVLNTIKNASDSDGQDLLFTNQLKEHPTDFSVEFLEQNSNE  
VQEVFAKAFAYYIEPQHRDVLQLYAPEAFNYMDKFNEQEINLSMKLLSSIEQACDICRLKKLKCSKE  
KPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVESRLERLEQLFLLIFPREDLDMILKMDSLQDIKAL  
LTGLFVQDNVNKDAVTDRLASVETDMPLTLRQHRISATSSSEESSNKGQRQLTVSHHHHHH

**Figure 10**

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**SEQ ID No: 6**

ATGAGAGGATCGCATCACCATCACCATCACGGATCCGAAGTTAAACAGGAGAACCGGTTATTAAAT  
GAATCAGAATCAAGTTCAGGGGTTACTAGGATACTATTTTAGTGATTGGAATTTCAAGCACCCAT  
GGTGGTTACCTCTTCTACTACAGGGGATTATCTATTCCTAGTTCTGAGTTAGAAAATATTCCATCGG  
AAAACCAATATTTTCAATCTGCTATTTGGTCAGGATTATCAAAGTTAAGAAGAGTGATGAATATACA  
TTTGCTACTTCCGCTGATAATCATGTAACAATGTGGGTAGATGACCAAGAAGTGATTAATAAAGCTTC  
TAATTCTAACAAAATCAGATTAGAAAAAGGAAGATTATATCAAATAAAAATTCAATATCAACGAGAA  
AATCCTACTGAAAAAGGATTGGATTTCAGTTGTACTGGACCGATTCTCAAATAAAAAAGAAGTG  
ATTTCTAGTGATAACTTACAATTGCCAGAATTAACAAAAATCTTCGAACTCAAGAAAAAAGCGAA  
GTACAAGTGCTGGACCTACGGTTCAGACCGTGACAATGATGGAATCCCTGATTCATTAGAGGTAG  
AAGGATATACGGTTGATGTCAAAAATAAAGAAGCTTTTCTTCACCATGGATTCTAATATTCATGAA  
AAGAAAGGATTAACCAATATAAATCATCTCCTGAAAAATGGAGCACGGCTTCTGATCCGTACAGTG  
ATTCGAAAAGGTTACAGGACGGATTGATAAGAATGTATCACCAGAGGCAAGACACCCCTTGTGG  
CAGCTTATCCGATTGTACATGTAGATATGGAGAATATTATTCTCTCAAAAATGAGGATCAATCCACA  
CAGAATACTGATAGTCAACGAGAACAATAAGTAAAAATACTTCTACAAGTAGGACACATACTAGTG  
AAGTACATGGAAATGCAGAAGTGCATGCGTCGTTCTTTGATATTGGTGGGAGTGATCTGCAGGAT  
TTAGTAATTCGAATTCAGTACGGTCGCAATTGATCATTCACTATCTCTAGCAGGGGAAAGAAGCTTG  
GGCTGAAACAATGGGTTTAAATACCGCTGATACAGCAAGATTAAATGCCAATATTAGATATGTAAATA  
CTGGGACGGCTCCAATCTACAACGTGTTACCAACGACTTCGTTAGTGTTAGGAAAAAATCAAACAC  
TCGCGACAATTAAAGCTAAGGAAAACCAATTAAGTCAAATACTTGCACCTAATAATTATTATCCTTCT  
AAAACTTGGCGCCAATCGCATTAAATGCACAAGACGATTTCAAGTTCTACTCCAATTACAATGAATT  
ACAATCAATTTCTTGAGTTAGAAAAACGAAACAATTAAGATTAGATACGGATCAAGTATATGGGAA  
TATAGCAACATACAATTTTGAAGTGAAGAGTGAGGGTGGATACAGGCTCGAACTGGAGTGAAG  
TGTTACCGCAAATTCAGAAACAACGACGATCATTTTTAATGGAAAAGATTTAAATCTGGTAGA  
AAGGCGGATAGCGGCGGTTAATCCTAGTGATCCATTAGAAACGACTAAACCGGATATGACATTA  
AGAAGCCCTTAAATAGCATTGGATTAAACGAACCGAATGGAACTTACAATATCAAGGGAAAGA  
CATAACCGAATTTGATTTAATTCGATCAACAAACATCTCAAATATCAAGAATCAGTTAGCGGAAT  
TAAACGCACTAACATATATACTGTATTAGATAAAATCAAATTAATGCAAAAATGAATATTTAATAA  
GAGATAAACGTTTTTATTATGATAGAAATAACATAGCAGTTGGGGCGGATGAGTCAGTAGTTAAGG  
AGGCTCATAGAGAAGTAATTAATTCGTCACAGAGGGATTATTGTTAAATATTGATAAGGATATAAG  
AAAAATATTATCAGGTTATATTGTAGAAATTGAAGATACTGAAGGGCTTAAAGAAGTTATAAATGAC  
AGATATGATATGTTGAATATTTCTAGTTTACGGCAAGATGGAAAAACATTTATAGATTTTAAAAAATAT  
AATGATAAATTACCGTTATATATAAGTAATCCCAATTATAAGGTAAATGTATATGCTGTACTAAAGAAA  
ACACTATTATTAATCCTAGTGAGAATGGGGATACTAGTACCAACGGGATCAAGAAAATTTTAATCTTT  
TCTAAAAAAGGCTATGAGATAGGATGA

**Figure 11**

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**SEQ ID No: 7**

MRGSHHHHHHGSEVKQENRLLNESESSSQGLLGYFSDLNFAQPMVVTSSTTGDLSPSSELENI  
PSENQYFQSAIWSGFIKVKKSDEYTFATSADNHVTMWVDDQEVINKASNSNKIRLEKGRLYQIKI  
QYQRENPTKEGLDFKLYWTD SQNKKEVISSDNLQLPELKQKSSNSRKKRSTSAGPTVPDRDNDGI  
PDSLEVEGYTVDVKNKRTFLSPWISNIHEKKGLTKYKSSPEKWSTASDPYSDFEKTGRIDKNVSP  
EARHPLVAAYPIVHVDMENIILSKNEDQSTQNTDSQTRTISKNTSTSRTHTSEVHGNAEVHASFF  
DIGGSVSAGFSNSNSSTVAIDHSLSLAGERTWAETMGLNTADTARLNANIRYVNTGTAPIYNVLP  
TTSVLGKNQTLATIKAKENQLSQILAPNNYYPSKNLAPIALNAQDDFSSTPITMNYNQFLELEKTK  
QLRLDTDQVYGNIATYNFENGRRVVDTGSNWSEVLPQIQETTARIIFNGKDLNLVERRIAAVNPS  
DPLETTKPDMTLKEALKIAFGFNPNLQYQKGDITEFDNFDDQTSQNIKNQLAELNATNIYT  
VLDKIKLNAKMNILIRDKRFHYDRNNIAVGADSVVKEAHREVINSSTEGLLLNIDKDIRKILSGYIV  
EIEDTEGLKEVINDRYDMLNISSLRQDGKTFIDFKKYNDKLPYISNPYKVN VYAVTKENTIINPSE  
NGDTSTNGIKKILIFS KKGYEIG

**Figure 12**

pctgb2014051918-seql  
SEQUENCE LISTING

<110> Greenwich University

<120> Antisense oligonucleotide compositions

<130> 120120W0

<150> UK1131057.2

<151> 2013-06-21

<160> 21

<170> PatentIn version 3.5

<210> 1

<211> 1131

<212> DNA

<213> Artificial sequence

<220>

<223> Derived from more than one source

<400> 1

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| atggaacgca acaaaactca ggaggaacac ctgaaagaga tcatgaaaca cattgttaaa  | 60   |
| atcgaggtga aaggcgaaga agcgggttaa aaggaggctg ccgaaaagct gctggagaag  | 120  |
| gtaccgtctg atgtgctgga aatgtataaa gcgattggtg gcaaaatcta catcgtggat  | 180  |
| ggtgatatta ccaaacacat ctccctggaa gactgagcg aagacaagaa gaagatcaaa   | 240  |
| gatatctacg gcaaggacgc gctgctgcac gagcattacg tttagcaaa agaaggttac   | 300  |
| gaaccggtgc tgggttatcca gtccagcgag gattacgtcg aaaatacggg aaaagctctg | 360  |
| aacgtatatt acgaaattgg taaaatcctg tctcgtgaca ttctgagcaa aattaaccaa  | 420  |
| ccttatcaga agttcctgga cgttctgaac accatcaaaa acgcttctga ctccgacggc  | 480  |
| caggacctgc tgttcactaa tcagctgaaa gaacatccga ccgatttctc tgtagaattc  | 540  |
| ctggaacaga actctaacga ggtccaagaa gtttttgcca aagcattcgc gtactacatc  | 600  |
| gagccgcagc atcgcgacgt gctgcagctg tacgctccag aagccttcaa ctatatggac  | 660  |
| aaattcaatg aacaagaaat caacctgtct atgaaactgc tgtcctccat cgaacaggct  | 720  |
| tgcgatatct gtcgtctgaa gaaactgaaa tgctctaaag aaaaaccgaa atgcgcgaaa  | 780  |
| tgctgaaaa acaactggga atgtcgctat tcccctaaaa ccaaacgttc tccactgacc   | 840  |
| cgtgcgcacc tgaccgaagt agaatcccgt ctggaacgtc tggagcagct gtttctgctg  | 900  |
| attttccgc gtgaagacct ggacatgatc ctgaaaatgg atagcctgca ggatatcaaa   | 960  |
| gactgctga ccggtctgtt tgtgcaggac aacgtcaaca aagacgctgt tactgatcgc   | 1020 |
| ctggcgagcg ttgaaactga tatgccgctg accctgcgtc agcaccgtat ctccgcaacg  | 1080 |
| agctccagcg aagaatctag caacaaaggt cagcgccagc tgaccgttag c           | 1131 |

<210> 2

<211> 230

<212> PRT

<213> Bacillus anthracis

## pctgb2014051918-seql

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Met Glu Arg Asn Lys Thr Gln Glu Glu His Leu Lys Glu Ile Met Lys  
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His Ile Val Lys Ile Glu Val Lys Gly Glu Glu Ala Val Lys Lys Glu  
 20 25 30

Ala Ala Glu Lys Leu Leu Glu Lys Val Pro Ser Asp Val Leu Glu Met  
 35 40 45

Tyr Lys Ala Ile Gly Gly Lys Ile Tyr Ile Val Asp Gly Asp Ile Thr  
 50 55 60

Lys His Ile Ser Leu Glu Ala Leu Ser Glu Asp Lys Lys Lys Ile Lys  
 65 70 75 80

Asp Ile Tyr Gly Lys Asp Ala Leu Leu His Glu His Tyr Val Tyr Ala  
 85 90 95

Lys Glu Gly Tyr Glu Pro Val Leu Val Ile Gln Ser Ser Glu Asp Tyr  
 100 105 110

Val Glu Asn Thr Glu Lys Ala Leu Asn Val Tyr Tyr Glu Ile Gly Lys  
 115 120 125

Ile Leu Ser Arg Asp Ile Leu Ser Lys Ile Asn Gln Pro Tyr Gln Lys  
 130 135 140

Phe Leu Asp Val Leu Asn Thr Ile Lys Asn Ala Ser Asp Ser Asp Gly  
 145 150 155 160

Gln Asp Leu Leu Phe Thr Asn Gln Leu Lys Glu His Pro Thr Asp Phe  
 165 170 175

Ser Val Glu Phe Leu Glu Gln Asn Ser Asn Glu Val Gln Glu Val Phe  
 180 185 190

Ala Lys Ala Phe Ala Tyr Tyr Ile Glu Pro Gln His Arg Asp Val Leu  
 195 200 205

Gln Leu Tyr Ala Pro Glu Ala Phe Asn Tyr Met Asp Lys Phe Asn Glu  
 210 215 220

Gln Glu Ile Asn Leu Ser  
 225 230

&lt;210&gt; 3

&lt;211&gt; 147

&lt;212&gt; PRT

&lt;213&gt; Saccharomyces cerevisiae

&lt;400&gt; 3

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Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu  
1 5 10 15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu  
20 25 30

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

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu  
50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile  
65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu  
85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala  
100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser  
115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu  
130 135 140

Thr Val Ser  
145

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<211> 1197  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Derived from more than one source

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gaagaagcgg ttaaaaagga ggctgccgaa aagctgctgg agaaggtacc gtctgatgtg 180  
ctggaaatgt ataaagcgat tgggtggcaaa atctacatcg tggatggtga tattaccaa 240  
cacatctccc tggaagcact gagcgaagac aagaagaaga tcaaagatat ctacggcaag 300  
gacgcgctgc tgcacgagca ttacgtttac gcaaaagaag gttacgaacc ggtgctggtt 360  
atccagtcca gcgaggatta cgtcgaaaat acggaaaaag ctctgaacgt atattacgaa 420  
attggtaaaa tcctgtctcg tgacattctg agcaaaatta accaacctta tcagaagttc 480  
ctggacgttc tgaacaccat caaaaacgct tctgactccg acggccagga cctgctgttc 540

pctgb2014051918-seql

|                                                                   |      |
|-------------------------------------------------------------------|------|
| actaatcagc tgaagaaca tccgaccgat ttctctgtag aattcctgga acagaactct  | 600  |
| aacgaggtcc aagaagtttt tgccaaagca ttcgcgtact acatcgagcc gcagcatcgc | 660  |
| gacgtgctgc agctgtacgc tccagaagcc ttcaactata tggacaaatt caatgaacaa | 720  |
| gaaatcaacc tgtctatgaa actgctgtcc tccatcgaac aggcttgcca tatctgtcgt | 780  |
| ctgaagaaac tgaaatgctc taaagaaaaa ccgaaatgcg cgaaatgcct gaaaaacaac | 840  |
| tgggaatgtc gctattcccc taaaacaaa cgttctccac tgaccctgctc gcacctgacc | 900  |
| gaagtagaat cccgtctgga acgtctggag cagctgtttc tgctgatttt cccgcgtgaa | 960  |
| gacctggaca tgatcctgaa aatggatagc ctgcaggata tcaaagcact gctgaccggt | 1020 |
| ctgtttgtgc aggacaacgt caacaaagac gctgttactg atcgcttggc gagcgttgaa | 1080 |
| actgatatgc cgctgaccct gcgtcagcac cgtatctccg caacgagctc cagcgaagaa | 1140 |
| tctagcaaca aaggtcagcg ccagctgacc gttagccacc accatcacca ccactaa    | 1197 |

<210> 5  
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 <212> PRT  
 <213> Artificial sequence

<220>  
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<400> 5

|                                                                 |  |
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| Met Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser Thr Met |  |
| 1 5 10 15                                                       |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Glu Arg Asn Lys Thr Gln Glu Glu His Leu Lys Glu Ile Met Lys His |  |
| 20 25 30                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Ile Val Lys Ile Glu Val Lys Gly Glu Glu Ala Val Lys Lys Glu Ala |  |
| 35 40 45                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Ala Glu Lys Leu Leu Glu Lys Val Pro Ser Asp Val Leu Glu Met Tyr |  |
| 50 55 60                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Lys Ala Ile Gly Gly Lys Ile Tyr Ile Val Asp Gly Asp Ile Thr Lys |  |
| 65 70 75 80                                                     |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| His Ile Ser Leu Glu Ala Leu Ser Glu Asp Lys Lys Lys Ile Lys Asp |  |
| 85 90 95                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Ile Tyr Gly Lys Asp Ala Leu Leu His Glu His Tyr Val Tyr Ala Lys |  |
| 100 105 110                                                     |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Glu Gly Tyr Glu Pro Val Leu Val Ile Gln Ser Ser Glu Asp Tyr Val |  |
| 115 120 125                                                     |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Glu Asn Thr Glu Lys Ala Leu Asn Val Tyr Tyr Glu Ile Gly Lys Ile |  |
| 130 135 140                                                     |  |

pctgb2014051918-seql

Leu Ser Arg Asp Ile Leu Ser Lys Ile Asn Gln Pro Tyr Gln Lys Phe  
145 150 155 160

Leu Asp Val Leu Asn Thr Ile Lys Asn Ala Ser Asp Ser Asp Gly Gln  
165 170 175

Asp Leu Leu Phe Thr Asn Gln Leu Lys Glu His Pro Thr Asp Phe Ser  
180 185 190

Val Glu Phe Leu Glu Gln Asn Ser Asn Glu Val Gln Glu Val Phe Ala  
195 200 205

Lys Ala Phe Ala Tyr Tyr Ile Glu Pro Gln His Arg Asp Val Leu Gln  
210 215 220

Leu Tyr Ala Pro Glu Ala Phe Asn Tyr Met Asp Lys Phe Asn Glu Gln  
225 230 235 240

Glu Ile Asn Leu Ser Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys  
245 250 255

Asp Ile Cys Arg Leu Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys  
260 265 270

Cys Ala Lys Cys Leu Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys  
275 280 285

Thr Lys Arg Ser Pro Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser  
290 295 300

Arg Leu Glu Arg Leu Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu  
305 310 315 320

Asp Leu Asp Met Ile Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala  
325 330 335

Leu Leu Thr Gly Leu Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val  
340 345 350

Thr Asp Arg Leu Ala Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg  
355 360 365

Gln His Arg Ile Ser Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys  
370 375 380

Gly Gln Arg Gln Leu Thr Val Ser His His His His His His  
385 390 395

<210> 6  
<211> 2244  
<212> DNA

&lt;213&gt; Bacillus anthracis

&lt;400&gt; 6

|                                                                    |      |
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| ttaaataaat cagaatcaag ttcccagggg ttactaggat actatatttag tgatttgaat | 120  |
| tttcaagcac ccatgggtgt tacctcttct actacagggg atttatctat tcctagttct  | 180  |
| gagttagaaa atattccatc ggaaaaccaa ttttttcaat ctgctatttg gtcaggattt  | 240  |
| atcaaagtta agaagagtga tgaatataca tttgctactt ccgctgataa tcatgtaaca  | 300  |
| atgtgggtag atgaccaaga agtgattaat aaagcttcta attctaaca aatcagatta   | 360  |
| gaaaaaggaa gattatatca aataaaaatt caatatcaac gagaaaatcc tactgaaaaa  | 420  |
| ggattggatt tcaagttgta ctggaccgat tctcaaaata aaaaagaagt gatttctagt  | 480  |
| gataacttac aattgccaga attaaaacaa aaatcttcga actcaagaaa aaagcgaagt  | 540  |
| acaagtgtgt gacctacgtg tccagaccgt gacaatgatg gaatccctga ttcattagag  | 600  |
| gtagaaggat atacggttga tgtcaaaaat aaaagaactt ttctttcacc atggatttct  | 660  |
| aatattcatg aaaagaaagg attaaccaaa tataaatcat ctctgaaaa atggagcacg   | 720  |
| gcttctgatc cgtacagtga tttcgaaaag gttacaggac ggattgataa gaatgtatca  | 780  |
| ccagaggcaa gacacccctt tgtggcagct tatccgattg tacatgtaga tatggagaat  | 840  |
| attattctct caaaaaatga ggatcaatcc acacagaata ctgatagtca aacgagaaca  | 900  |
| ataagtaaaa atacttctac aagtaggaca catactagtg aagtacatgg aaatgcagaa  | 960  |
| gtgcatgcgt cgttctttga tattggtggg agtgtatctg caggatttag taattcgaat  | 1020 |
| tcaagtacgg tcgcaattga tcattcacta tctctagcag gggaaagaac ttgggctgaa  | 1080 |
| acaatgggtt taaataccgc tgatacagca agattaaatg ccaatattag atatgtaaat  | 1140 |
| actgggacgg ctccaatcta caacgtgtta ccaacgactt cgttagtgtt agggaaaaat  | 1200 |
| caaacactcg cgacaattaa agctaaggaa aaccaattaa gtcaaatact tgcacctaata | 1260 |
| aattattatc cttctaaaaa cttggcgcca atcgcattaa atgcacaaga cgatttcagt  | 1320 |
| tctactccaa ttacaatgaa ttacaatcaa tttcttgagt tagaaaaaac gaaacaatta  | 1380 |
| agattagata cggatcaagt atatgggaat atagcaacat acaattttga aaatggaaga  | 1440 |
| gtgaggggtg atacaggctc gaactggagt gaagtgttac cgcaaattca agaaacaact  | 1500 |
| gcacgtatca tttttaatgg aaaagattta aatctggtag aaaggcggat agcggcgggt  | 1560 |
| aatcctagtg atccattaga aacgactaaa ccggatatga cattaaaaga agcccttaaa  | 1620 |
| atagcatttg gatttaacga accgaatgga aacttacaat atcaaggga agacataacc   | 1680 |
| gaatttgatt ttaatttcga tcaacaacaa tctcaaaata tcaagaatca gttagcggaa  | 1740 |
| ttaaacgcaa ctaacatata tactgtatta gataaaatca aattaaatgc aaaaatgaat  | 1800 |
| attttaataa gagataaacg ttttcattat gatagaaata acatagcagt tggggcggat  | 1860 |
| gagtcagtag ttaaggaggc tcatagagaa gtaattaatt cgtcaacaga gggattattg  | 1920 |
| ttaaataattg ataaggatat aagaaaaata ttatcaggtt atattgtaga aattgaagat | 1980 |

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actgaagggc ttaaagaagt tataaatgac agatatgata tgttgaatat ttctagttta 2040  
 cggcaagatg gaaaaacatt tatagatttt aaaaaatata atgataaatt accgttatat 2100  
 ataagtaatc ccaattataa ggtaaattgta tatgctgtta ctaaagaaaa cactattatt 2160  
 aatcctagtg agaattggga tactagtacc aacgggatca agaaaatttt aatcttttct 2220  
 aaaaaaggct atgagatagg atga 2244

<210> 7  
 <211> 747  
 <212> PRT  
 <213> Bacillus anthracis

<400> 7

Met Arg Gly Ser His His His His His His Gly Ser Glu Val Lys Gln  
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Glu Asn Arg Leu Leu Asn Glu Ser Glu Ser Ser Ser Gln Gly Leu Leu  
 20 25 30

Gly Tyr Tyr Phe Ser Asp Leu Asn Phe Gln Ala Pro Met Val Val Thr  
 35 40 45

Ser Ser Thr Thr Gly Asp Leu Ser Ile Pro Ser Ser Glu Leu Glu Asn  
 50 55 60

Ile Pro Ser Glu Asn Gln Tyr Phe Gln Ser Ala Ile Trp Ser Gly Phe  
 65 70 75 80

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

Asn His Val Thr Met Trp Val Asp Asp Gln Glu Val Ile Asn Lys Ala  
 100 105 110

Ser Asn Ser Asn Lys Ile Arg Leu Glu Lys Gly Arg Leu Tyr Gln Ile  
 115 120 125

Lys Ile Gln Tyr Gln Arg Glu Asn Pro Thr Glu Lys Gly Leu Asp Phe  
 130 135 140

Lys Leu Tyr Trp Thr Asp Ser Gln Asn Lys Lys Glu Val Ile Ser Ser  
 145 150 155 160

Asp Asn Leu Gln Leu Pro Glu Leu Lys Gln Lys Ser Ser Asn Ser Arg  
 165 170 175

Lys Lys Arg Ser Thr Ser Ala Gly Pro Thr Val Pro Asp Arg Asp Asn  
 180 185 190

Asp Gly Ile Pro Asp Ser Leu Glu Val Glu Gly Tyr Thr Val Asp Val

195

200

205

Lys Asn Lys Arg Thr Phe Leu Ser Pro Trp Ile Ser Asn Ile His Glu  
 210 215 220

Lys Lys Gly Leu Thr Lys Tyr Lys Ser Ser Pro Glu Lys Trp Ser Thr  
 225 230 235 240

Ala Ser Asp Pro Tyr Ser Asp Phe Glu Lys Val Thr Gly Arg Ile Asp  
 245 250 255

Lys Asn Val Ser Pro Glu Ala Arg His Pro Leu Val Ala Ala Tyr Pro  
 260 265 270

Ile Val His Val Asp Met Glu Asn Ile Ile Leu Ser Lys Asn Glu Asp  
 275 280 285

Gln Ser Thr Gln Asn Thr Asp Ser Gln Thr Arg Thr Ile Ser Lys Asn  
 290 295 300

Thr Ser Thr Ser Arg Thr His Thr Ser Glu Val His Gly Asn Ala Glu  
 305 310 315 320

Val His Ala Ser Phe Phe Asp Ile Gly Gly Ser Val Ser Ala Gly Phe  
 325 330 335

Ser Asn Ser Asn Ser Ser Thr Val Ala Ile Asp His Ser Leu Ser Leu  
 340 345 350

Ala Gly Glu Arg Thr Trp Ala Glu Thr Met Gly Leu Asn Thr Ala Asp  
 355 360 365

Thr Ala Arg Leu Asn Ala Asn Ile Arg Tyr Val Asn Thr Gly Thr Ala  
 370 375 380

Pro Ile Tyr Asn Val Leu Pro Thr Thr Ser Leu Val Leu Gly Lys Asn  
 385 390 395 400

Gln Thr Leu Ala Thr Ile Lys Ala Lys Glu Asn Gln Leu Ser Gln Ile  
 405 410 415

Leu Ala Pro Asn Asn Tyr Tyr Pro Ser Lys Asn Leu Ala Pro Ile Ala  
 420 425 430

Leu Asn Ala Gln Asp Asp Phe Ser Ser Thr Pro Ile Thr Met Asn Tyr  
 435 440 445

Asn Gln Phe Leu Glu Leu Glu Lys Thr Lys Gln Leu Arg Leu Asp Thr  
 450 455 460

Asp Gln Val Tyr Gly Asn Ile Ala Thr Tyr Asn Phe Glu Asn Gly Arg

## pctgb2014051918-seql

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 Val Arg Val Asp Thr Gly Ser Asn Trp Ser Glu Val Leu Pro Gln Ile  
                                  485                      490                      495  
 Gln Glu Thr Thr Ala Arg Ile Ile Phe Asn Gly Lys Asp Leu Asn Leu  
                                  500                      505                      510  
 Val Glu Arg Arg Ile Ala Ala Val Asn Pro Ser Asp Pro Leu Glu Thr  
                                  515                      520                      525  
 Thr Lys Pro Asp Met Thr Leu Lys Glu Ala Leu Lys Ile Ala Phe Gly  
                                  530                      535                      540  
 Phe Asn Glu Pro Asn Gly Asn Leu Gln Tyr Gln Gly Lys Asp Ile Thr  
                                  545                      550                      555                      560  
 Glu Phe Asp Phe Asn Phe Asp Gln Gln Thr Ser Gln Asn Ile Lys Asn  
                                  565                      570                      575  
 Gln Leu Ala Glu Leu Asn Ala Thr Asn Ile Tyr Thr Val Leu Asp Lys  
                                  580                      585                      590  
 Ile Lys Leu Asn Ala Lys Met Asn Ile Leu Ile Arg Asp Lys Arg Phe  
                                  595                      600                      605  
 His Tyr Asp Arg Asn Asn Ile Ala Val Gly Ala Asp Glu Ser Val Val  
                                  610                      615                      620  
 Lys Glu Ala His Arg Glu Val Ile Asn Ser Ser Thr Glu Gly Leu Leu  
                                  625                      630                      635                      640  
 Leu Asn Ile Asp Lys Asp Ile Arg Lys Ile Leu Ser Gly Tyr Ile Val  
                                  645                      650                      655  
 Glu Ile Glu Asp Thr Glu Gly Leu Lys Glu Val Ile Asn Asp Arg Tyr  
                                  660                      665                      670  
 Asp Met Leu Asn Ile Ser Ser Leu Arg Gln Asp Gly Lys Thr Phe Ile  
                                  675                      680                      685  
 Asp Phe Lys Lys Tyr Asn Asp Lys Leu Pro Leu Tyr Ile Ser Asn Pro  
                                  690                      695                      700  
 Asn Tyr Lys Val Asn Val Tyr Ala Val Thr Lys Glu Asn Thr Ile Ile  
                                  705                      710                      715                      720  
 Asn Pro Ser Glu Asn Gly Asp Thr Ser Thr Asn Gly Ile Lys Lys Ile  
                                  725                      730                      735  
 Leu Ile Phe Ser Lys Lys Gly Tyr Glu Ile Gly

740

<210> 8  
<211> 48  
<212> DNA  
<213> Artificial sequence

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<223> Synthetic

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48

<210> 9  
<211> 48  
<212> DNA  
<213> Artificial sequence

<220>  
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<400> 9  
aatttgtttg ttgaggctaa tgcattgccgg catcagagca gccggcat

48

<210> 10  
<211> 49  
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<220>  
<223> Derived from more than one source

<400> 10  
ggctgctgc tgagctttct atgcattgccg gctgctctga tgccggcat

49

<210> 11  
<211> 49  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Derived from more than one source

<400> 11  
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49

<210> 12  
<211> 50  
<212> DNA  
<213> Artificial sequence

<220>  
<223> Derived from more than one source

<400> 12  
ggctgcttc atcttggttg tatgcattgcc ggctgctctg atccggcat

50

<210> 13  
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<212> DNA  
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pctgb2014051918-seq1

<220>  
 <223> Derived from more than one source

<400> 13  
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<210> 14  
 <211> 25  
 <212> DNA  
 <213> Artificial sequence

<220>  
 <223> Derived from more than one source

<400> 14  
 atgccggcat cagagcagcc ggcac 25

<210> 15  
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<220>  
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<400> 15  
 atgccggctg ctctgatgcc ggcac 25

<210> 16  
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 <212> DNA  
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<400> 16  
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<210> 17  
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 <212> DNA  
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<400> 17  
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<210> 18  
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<400> 18  
 aatttgtttg ttgaggcta 19

<210> 19

pctgb2014051918-seql

<211> 17  
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<400> 19  
 cggctgctct gatgccg 17

<210> 20  
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 <212> DNA  
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<400> 20  
 cggcatcaga gcagccg 17

<210> 21  
 <211> 21  
 <212> DNA  
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 <223> Derived from more than one source

<400> 21  
 ggtgctcttc atcttggttg a 21