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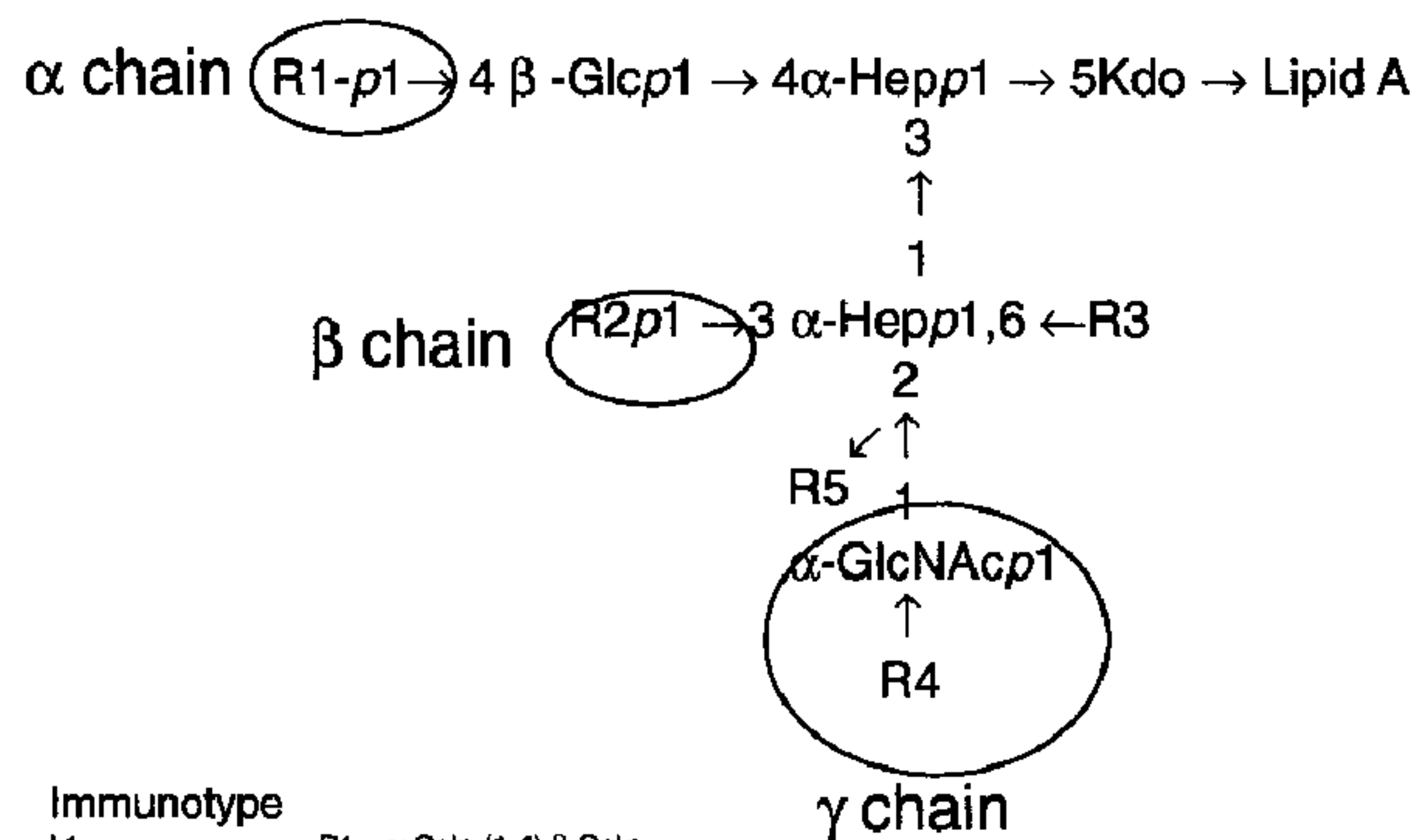
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(54) Titre : O-ACETYLTRANSFERASE PROVENANT DE NEISSERIA MENINGITIDIS, COMPOSITIONS ET METHODES

(54) Title: O-ACETYLTRANSFERASE FROM NEISSERIA MENINGITIDIS, COMPOSITIONS AND METHODS

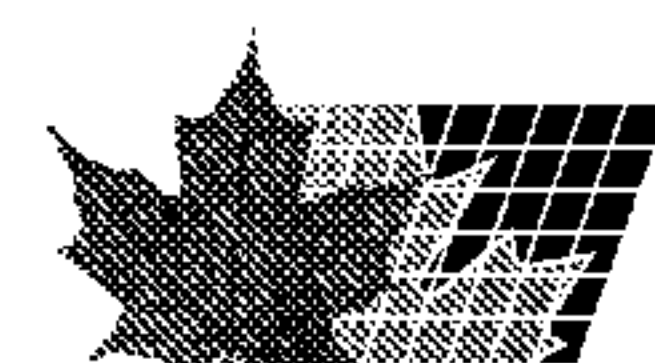


Immunotype

L1	R1 = $\alpha\text{-Gal}p\text{-(1-4)-}\beta\text{-Gal}p$ R2 = 3-PEA R3 = H R4 = Ac
L2	R1 = $\alpha\text{-Neu5Ac-(2}\rightarrow\text{3)-}\beta\text{-Gal}p1\rightarrow\text{4}\beta\text{-Glc}p\text{NAc}1\rightarrow\text{3}\beta\text{-Gal}p$ R2 = $\alpha\text{-Glc}p$ R3 = 6-PEA R4 = Ac (40%) R5 = Gly or O-7 PEA*
L3	R1 = $\alpha\text{-Neu5Ac-(2}\rightarrow\text{3)-}\beta\text{-Gal}p1\rightarrow\text{4}\beta\text{-Glc}p\text{NAc}1\rightarrow\text{3}\beta\text{-Gal}p$ R2 = 3-PEA R3 = H R4 = H R5 = Gly
L4	R1 = $\alpha\text{-Neu5Ac-(2}\rightarrow\text{3)-}\beta\text{-Gal}p1\rightarrow\text{4}\beta\text{-Glc}p\text{NAc}1\rightarrow\text{3}\beta\text{-Gal}p$ R2 = H R3 = 6-PEA R4 = Ac (50%) R5 = Gly
L5	R1 = $\alpha\text{-Neu5Ac-(2}\rightarrow\text{3)-}\beta\text{-Gal}p1\rightarrow\text{4}\beta\text{-Glc}p\text{NAc}1\rightarrow\text{3}\beta\text{-Gal}p1\rightarrow\text{4}\beta\text{-Glc}p1$ R2 = $\alpha\text{-Glc}p$ R3 = H R4 = Ac (60%)
L6	R1 = $\beta\text{-Glc}p\text{NAc}1\rightarrow\text{3}\beta\text{-Gal}p$ R2 = H R3 = H R4 = Ac R5 = 7-PEA
L8	R1 = $\beta\text{-Gal}p$ R2 = 3-PEA R3 = H R4 = H

(57) Abrégé/Abstract:

Provided are recombinant DNA molecules that do not occur in nature encoding a Lot3 O-acetyltransferase, vectors that direct expression of a Lot O-acetyltransferase, recombinant host cells which express a Lot3 O-acetyltransferase, methods for



(57) **Abrégé(suite)/Abstract(continued):**

recombinant production of a Lot3 O-acetyltransferase, methods for acetylating lipooligosaccharides, especially those of a *Neisseria meningitidis* using a recombinant Lot O-acetyltransferase, and immunogenic compositions comprising the acetylated lipooligosaccharide.

ABSTRACT OF THE DISCLOSURE

Provided are recombinant DNA molecules that do not occur in nature encoding a Lot3 O-acetyltransferase, vectors that direct expression of a Lot O-acetyltransferase, recombinant host cells which express a Lot3 O-acetyltransferase, methods for recombinant production of a Lot3 O-acetyltransferase, methods for acetylating lipooligosaccharides, especially those of a *Neisseria meningitidis* using a recombinant Lot O-acetyltransferase, and immunogenic compositions comprising the acetylated lipooligosaccharide.

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
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O-ACETYLTRANSFERASE FROM NEISSERIA MENINGITIDIS,
COMPOSITIONS AND METHODS

STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under United States Public Health Service Grant R01 AI033517 awarded by the National Institutes of Health. The United States Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0002] The field of this invention is molecular biology, in particular as applied to *Neisseria meningitidis*, as well as the preparation of immunogenic compositions comprising lipooligosaccharides and/or protein from *N. meningitidis*.

[0003] *Neisseria meningitidis* is the causative agent of epidemic meningitis and fatal septic shock (1). Being an obligate human pathogen, the human nasopharynx is the only known natural environmental niche from which the organism spreads to other human hosts via inhalation of respiratory droplets. In circumstances which are not entirely understood, nasopharyngeal acquisition may also result in invasive meningococcal disease (1, 2). Comparative studies of invasive and carriage isolates of meningococci implicate lipooligosaccharide (LOS) in the attachment and invasion of the nasopharyngeal epithelium (3, 4) and in the pathogenesis of invasive meningococcal disease (5). Meningococcal isolates express 12 immunologically distinct LOS structures (L1-L12) (6-8) which were subsequently shown to correspond to distinct chemical structures (9-13). Examination of carriage and invasive disease isolates by serology indicates that meningococci commonly co-express patterns of LOS

immunotypes, with L[1,8], L[3,7,9] and L[2,4] being the most prevalent (3, 4).

[0004] Each LOS immunotype structure has a conserved heptose inner core to which α -, β - and γ - chains are added (Figure 1). The length and composition of the α -chain is based upon the phase variable expression of *lgtA*, *lgtD* and *lgtC* (for reviews see 14, 15). The length and composition of the α - and β - chain extensions from HepI as well as the presence or absence of a γ -chain extension and phosphoethanolamine residues (PEA) on HepII appear to determine immunotype. The presence of the γ -chain extension of α 1-3 glucose on HepII, characteristic of L2 and L5 immunotype LOS (Figure 1), is determined by the phase variable expression of *lgtG* (16). In comparison, the presence or absence of the various PEA groups on the inner core is in part determined by whether or not the isolate carries an intact *lpt3* or *lpt6* gene (17, 18). In some meningococcal strains, O-3 linked PEA is attached to HepII by Lpt3 in the absence of LgtG activity, thus producing the L3 immunotype (17). However, an O-3-linked PEA could not be demonstrated in strain NMB which expresses L[2,4] immunotype LOS and contains an intact *lpt3*(18). Ram et al (19) have proposed that PEA variable additions to the inner core play an important role in protecting meningococci from complement mediated lysis, since the O-6 linked PEA groups of L2 and L4 LOS inner core and the exposed O-3 linked PEA groups of L[1,8] LOS are targets for complement component C4b. Mechanisms that mask these PEA inner core-associated epitopes, such as the co-expression of a long α -chain, or prevention of the expression of PEA groups on the cell surface, such as competition by the glucosyltransferase, LgtG (17), increase the resistance to complement mediated lysis (19).

[0005] Recently, it has become clear that at least some strains of meningococci have the capacity to express all immunotype LOS structures and that complex regulatory networks and structural constraints dictate what pattern of immunotypes are expressed during growth (18). Comparison of the meningococcal LOS inner core structures of different

immunotypes reveals that structures without O-3 linked PEA groups are invariably O-acetylated (Fig. 1). We hypothesized that O-acetylation of the terminal LOS inner core N-acetylglucosamine in the presence of a lactoneotetraose α -chain could prevent the enzymatic addition of O-3 linked PEA by Lpt-3 to this substrate. To test this hypothesis, the lipooligosaccharide O-acetyltransferase (*lot3*) was identified in *N. meningitidis* strain Z2491 by amino acid homology with a known O-acetyltransferase, from *Rhizobium leguminosarum* (20).

[0006] Insertional inactivation of *lot3* in *N. meningitidis* strain NMB confirmed that this locus encoded the lipooligosaccharide O-acetyltransferase. In addition, inactivation of both *lot3* and *lgtG* resulted in the appearance of O-3 linked PEA on the LOS inner core of this strain. Therefore, O-acetylation of the terminal N-acetylglucosamine residue of the LOS inner core when expressed in combination with a lactoneotetraose α chain physically prevents the addition of O-3 linked PEA groups to this site by a functional Lpt-3.

BRIEF SUMMARY

[0007] Provided herein are recombinant DNA molecules which do not occur in nature, recombinant host cells and methods of using the foregoing to recombinantly produce a *lot3* O-acetyltransferase derived from *Neisseria meningitidis* or an equivalent O-acetyltransferase from another species of *Neisseria*. Such an acetyltransferase transfers acetyl moieties to lipooligosaccharides, especially those of *N. meningitidis*. The O-acetyltransferase can be purified using specific antibody in an immunoaffinity column, for example, or an affinity tag can be engineered into the recombinant protein by the use of appropriate tag (especially a polyhistidine or His tag) coding sequences fused in frame. Other oligopeptide "tags" which can be fused to a protein of interest by such techniques include, without limitation, strep-tag (Sigma-Genosys, The Woodlands, TX) which directs binding to streptavidin or its derivative streptactin (Sigma-Genosys); a glutathione-S-transferase gene fusion

system which directs binding to glutathione coupled to a solid support (Amersham Pharmacia Biotech, Uppsala, Sweden); a calmodulin-binding peptide fusion system which allows purification using a calmodulin resin (Stratagene, La Jolla, CA); a maltose binding protein fusion system allowing binding to an amylose resin (New England Biolabs, Beverly, MA); and an oligo-histidine fusion peptide system which allows purification using a Ni^{2+} -NTA column (Qiagen, Valencia, CA).

[0008] Further encompassed by the present disclosure is the acetylation (in vitro) of lipooligosaccharides isolated from *N. meningitidis* or other species of Neisseria using acetyltransferase recombinantly produced using the recombinant host cells taught herein.

[0009] Also provided herein are cells of *N. meningitidis* or other Neisseria species into which a functional, expressible *lot* gene is overexpressed, for example by inserting a second active copy of this sequence, or by expressing a *lot3* coding sequence operably linked to a strong promoter in a Lot3 genetic background, either strategy having the result that there is a greater expression of Lot3 protein than in a wild type strain. Such strains produce highly (fully) acetylated lipooligosaccharide. Such LOS is advantageously formulated into immunogenic compositions, for example those destined for human vaccines. The highly acetylated LOS can be combined with other neisserial antigens, including capsular polysaccharides and/or other cellular components. Immunization with a composition of the present invention results in improved protection of a human exposed to a pathogenic Neisseria, especially *N. meningitidis*.

[0010] In addition, there are provided improved immunogenic compositions comprising oligosaccharides and lipooligosaccharides of *N. meningitidis*, or other Neisserial species, where the improvement comprises more complete acetylation of the saccharides than is currently possible in the absence of the enzymatic acetylation by using (*in vivo* or *in vitro*) the acetyltransferase of the present invention, especially those from *N. meningitidis*, with the result that a stronger immune response and greater protection against infection, results. The immunogenic

compositions of the present invention can comprise a pharmaceutically acceptable carrier and optionally can further comprise at least one immunological adjuvant or cytokine. These immunogenic compositions are useful as vaccines and as vaccine components. Also encompassed are non-acetylated oligosaccharides and lipooligosaccharides from *Neisseria*, especially produced by a *lot* mutant of *N. meningitidis* or other species of *Neisseria*.

[0011] It is a further object to provide nonacetylated OS and/or LOS for use in assay kits, for example, diagnostic assay kits. Such kits can be employed with acetylated and nonacetylated LOS for distinguishing *N. meningitidis* producing different immunotypes of LOS, for example. Similar assays and kits can be prepared to application to other species of *Neisseria* which produce such an acetyltransferase, using the OS and/or LOS from the cognate species where it is desired to learn whether acetylated saccharides are produced.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figure 1 shows meningococcal LOS structures of L1- L6 and L8 immunotype strains (9-13). The L7 immunotype structure (not shown) is the non-sialylated version of the L3 immunotype structure (11). The conserved inner core region is shown with variable attachments denoted as R1-R5. The composition of the α chain (R1) is governed by the phase variable expression of the *lgtA-E* transferases (15) and *lst*, which encodes the sialyltransferase that attaches the terminal α -Neu5Ac (sialic acid) group. Note that attachment of glycine to the inner cores of L1 and L5-7 immunotypes have not been investigated using current techniques. This figure is modified from Kogan et al. (11).

[0013] Figure 2 illustrates organization of the *lot3* locus (NMA2202) in *N. meningitidis* strain Z2491. The location of the primers (small arrows) and restriction site (*Sma*I) used during mutagenesis are indicated. NMA2201 and NMA2204 encode hypothetical proteins, whereas NMA2203 encodes

a putative adenylosuccinate lyase (*purB*) and NMA2205 encodes a putative ribonuclease II related protein.

[0014] Figures 3A-3C provide the positive MALDI-TOF MS spectra of the major NMB parent OS structure (Fig. 3A), the major OS from NMBlot3 (Fig. 3B), and the major OS from NMBlot3/lgtG (Fig. 3C). The ions and proposed structures for these ions are as indicated.

[0015] Figure 4 shows the ^1H - ^1H TOCSY NMR of the major OS isolated from NMB wild type LOS. The TOCSY correlations are indicated by the dotted lines. Other correlations not indicated in this figure are given in Table 2.

[0016] Figures 5A-5C provide ^1H - ^{13}C gHSQC NMR spectra of the OS from (Fig. 5A) NMB LOS, (Fig. 5B) NMBlot3, and from (Fig. 5C) NMBlot3/lgtG LOS. The anomeric H/C signals are labeled from A to H for the glycosyl residues shown for these OS structures in Figure 3, and the assignments in Tables 1 and 2. Resonances not indicated in this figure are also given in Tables 1 and 2.

[0017] Figures 6A-6B show the ^{31}P - ^1H HMQC spectra of the major OS from NMBlot3 (Fig. 6A) and the major OS from NMBlot3/lgtG (Fig. 6B). The assignments of the resonances are as indicated.

[0018] Figures 7A-7B show the ^{31}P - ^1H HMQC-TOCSY spectra of the major OS from NMBlot3 (Fig. 7A), and the major OS from NMBlot3/lgtG (Fig. 7B). The assignments of the resonances are as indicated.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The abbreviations used herein are OS, oligosaccharide; LOS, lipooligosaccharide; CPS, capsular polysaccharide; O-Ac CPS, O-acetylated capsular polysaccharide; PCR, polymerase chain reaction; GC-MS, gas-liquid chromatography-mass spectrometry; COSY, ^1H - ^1H correlation spectroscopy; TOCSY, total correlation spectroscopy; mAb,

monoclonal antibody; ELISA: enzyme linked immunosorbant assay; SDS, sodium dodecyl sulfate; Hep, heptose; PEA, phosphatidyl ethanolamine.

[0020] O-acetylation is a common decoration on endotoxins derived from many Gram-negative bacterial species, and has been shown to be instrumental (e.g. *Salmonella typhimurium*) in determining the final tertiary structure of the endotoxin, and the immunogenicity of the molecule. Structural heterogeneity of endotoxins produced by mucosal pathogens such as *N. meningitidis* is determined by decorations on the heptose inner core, including O-acetylation of the terminal N-acetylglucosamine (GlcNAc) attached to HepII. In this report, we show that O-acetylation of the meningococcal LOS inner core has an important role in determining inner core assembly and immunotype expression. The gene encoding the lipooligosaccharide O-acetyltransferase, *lot3*, was identified by homology to NodX from *Rhizobium leguminosarum*. Inactivation of *lot3* in strain NMB resulted in the loss of the O-acetyl group located at the C-3 position of the terminal GlcNAc of the LOS inner core. While inactivation of *lot3* or *lgtG* encoding the HepII glucosyltransferase alone did not result in the appearance of the O-3 linked PEA groups on the LOS inner core, construction of a double mutant in which both *lot3* and *lgtG* were inactivated resulted in the appearance of O-3 linked PEA groups. In conclusion, O-acetylation status of the terminal GlcNAc of the γ -chain of the meningococcal LOS inner core is an important determinant for the appearance or exclusion of the O-3 linked PEA group on the LOS inner core, and contributes to LOS structural diversity. O-acetylation also likely influences resistance to complement mediated lysis and may be important in LOS conjugate vaccine design.

[0021] This specification describes the identification of the lipooligosaccharide C-3 O-acetyl transferase gene (*lot3*) in *N. meningitidis* strain NMB. Meningococcal LOS immunotypes L2 and L4 are O-acetylated at the C-3 position of the non-reducing terminal α -N-acetyl-D-glucosamine (GlcNAc) (11). *Rhizobium leguminosarum* biovar *viciae* expresses lipooligosaccharide nodulation factors containing oligomers of

four to five beta 1-4 linked GlcNAc residues with the reducing-end GlcNAc O-acetylated by NodX at the C-6 position (33). A BlastP search of the complete peptide databases of *N. meningitidis* strain MC58 and strain Z2491 using the amino acid sequence of NodX from *R. leguminosarum* biovar *viviae* strain TOM (34) revealed two potential candidate LOS O-acetyltransferases (Figure 2). NMA0619 encodes a 622 amino acid peptide with 30% identity over the first 191 amino acids of NodX. However, the corresponding open reading frame in *N. meningitidis* strain MC58 (Serogroup B), NMB1836, is intact, and has recently been shown to encode an acyltransferase (PglI) required for the biosynthesis of the basal 2,4-diacetamido-2,4,6-trideoxyhexose residue of the pilin-linked glycan (35). The second candidate, NMA2202, was present as an intact open reading frame in strain Z2491 (Serogroup A) which expresses an L9 LOS (on the internet/worldwide web, address sanger.ac.uk/Projects/N_meningitidis/seroA/strain.shtml) and had 30% amino acid identity over the first 154 amino acids of NodX. In strain MC58, the ORF corresponding to NMA2202, NMB2032, contains a 6 bp poly-G tract which causes a translational frameshift. To further determine whether there was a relationship between an intact NMA2202 open reading frame and O-acetylation of LOS, we examined the locus in strain NMB which expresses an L2 LOS (36). Like strain Z2491, strain NMB contained an intact NMA2202 open reading frame containing a 5 bp poly-G tract.

[0022] NMA2202 encodes the lipooligosaccharide O-acetyltransferase (Lot3). A mutant in which NMA2202 of strain NMB was insertionally inactivated by a tetM antibiotic resistance cassette was constructed and designated NMBlot3 (see below). LOS was extracted and the oligosaccharides (OS) were prepared as described below. Purification of the OSs by gel-filtration chromatography using Bio-Gel P2 resulted in one major and two minor OS peaks from NMBlot3. Glycosyl composition and linkage analysis of the major OS from NMBlot3 showed the glycosyl residues and linkages expected from the published NMB LOS structure (36); namely, terminally linked glucose (Glc), terminally linked galactose (Gal), terminally linked N-acetylglucosamine (GlcNAc), 4-linked Glc, 3-

linked Gal, 4-linked GlcNAc, and 3,4-linked heptosyl (Hep) I. The Hep II residue was not observed since phosphorylated residues are not detected in the method used and Hep II is the location of phosphoethanolamine (PEA) groups. The minor OSs from NMBlot3 were also analyzed by glycosyl composition analysis and mass spectrometry. The results showed that the minor OSs consisted of glycinylated versions of the major OS structure (i.e. the ion masses were 57 mass units greater than those of the major OS), as well as the presence of detectable amounts of sialic acid monosaccharide that was presumably released from the α -chain during mild acid hydrolysis.

[0023] The major OSs from the LOSs of NMB, and NMBlot3 were then analyzed by MALDI-TOF MS and the resulting spectra are shown in Figs. 3A and 3B, respectively. The molecular ions observed for the OS from NMBlot3 (m/z 1804, 1822, 1826, 1844, and 1866; Fig. 3B) are all consistent with sodiated and anhydro-sodiated forms of a single oligosaccharide structure. These masses are 42 mass units less than the corresponding ion masses for the OS from NMB, Fig. 3A. This result is consistent with the NMBlot3 LOS having a structure that is the same as reported for NMB LOS (36) with the notable exception that the NMBlot3 OS lacks an acetyl group. Previous work on the NMB LOS had reported that the OS was acetylated at O-6 of the GlcNAc residue terminally linked to O-2 of Hep II (36). This conclusion was based on comparison of 1-D TOCSY NMR spectra of the NMB OS taken before and after HF treatment which removes PEA as well as the O-acetyl group from the OS. This work on the NMBlot3 LOS required that we re-examine the location of this O-acetyl group in the NMB LOS, as well as confirm its absence in the NMBlot3 LOS. This was accomplished by examining the OS from NMB and from NMBlot3 by gradient correlation spectroscopy (gCOSY), total correlation spectroscopy (TOCSY), and heteronuclear single quantum coherence spectroscopy (gHSQC). From these experiments, it was possible to make assignments for the majority of the protons for both the NMB OS (Table 3) and the NMBlot3 OS (Table 2). Comparison of the assignments for these OSs showed that the α -GlcNAc proton resonances

for the NMB OS were quite different from those for the NMBlot3 OS. In particular, the H3 of this α -GlcNAc residue in NMB OS resonates at δ 5.17, which is considerably downfield from the H3 resonance of this residue in NMBlot3 OS which resonates at δ 3.85. This downfield shift is consistent with O-acetylation at this position in NMB OS and a lack of this acetylation in the NMBlot3 OS. A TOCSY spectrum of the NMB OS showing the coupling of the H1 (δ 5.28), H2 (δ 4.19), H3 (δ 5.17), and H4 (δ 3.68) protons of the α -GlcNAc residue is given in Fig. 4.

[0024] The presence and location, or absence of O-acetyl groups can also be observed in the gHSQC spectra of the NMB and NMBlot3 OS samples; Fig. 5A and 5B, respectively. The NMB OS gHSQC spectrum (Fig. 5A) shows two N-acetyl methyl protons (carbons) at δ 2.04 and 2.06 (δ 22.5), and the O-acetyl methyl protons (carbon) at δ 2.11 (δ 20.6). However, only two N-acetyl methyl protons (carbons) are observed in the NMBlot3 OS spectrum (Fig. 5B) at δ 2.11 (δ 22.5) and δ 2.04 (δ 21.9). The NMBlot3 OS lacks the O-acetyl resonances. The gHSQC spectrum of the NMB OS also contributed to assigning the location of the O-acetyl group to O-3 of the α -linked GlcNAc residue. From the gCOSY spectrum (not shown), the anomeric proton of the α -GlcNAc residue (δ 5.28) was shown to be coupled to H2 at δ 4.19. The gHSQC spectrum (Fig. 5A) shows that this H2 is coupled to the C2 at δ C 51.9 which confirms that this is the α -GlcNAc residue. These data, together with the TOCSY data (see Figure 4) allowed the assignment of the resonances for the GlcNAc residue that are given in Table 2. From these assignments, it was shown that the H3 of this GlcNAc is at δ 5.17 and is coupled to a carbon at δ C 74.0 (see Fig. 5A). This H3/C3 coupling is clearly absent in the gHSQC spectrum of the NMBlot3 OS, Fig. 5B. Thus, these NMR data support the mass spectrometric data which show that the OS from NMBlot3 LOS lacks an O-acetyl group, and that the O-acetyl group on NMB LOS is located at O-3 of the α -GlcNAc residue and not at O-6 as previously reported (36).

[0025] O-acetylation of the terminal GlcNAc residue of meningococcal LOS influences PEA additions to the inner core. The meningococcal LOS

inner core structure is masked by the additions to HepII which are highly variable and heterogeneous (14). One of these additions, the presence of the γ -chain extension of α 1-3 glucose on HepII, characteristic of L2 and L5 immunotype meningococcal LOS, is determined by the phase variable expression of *lgtG* (16). In some meningococcal strains, O-3 linked PEA is attached to HepII by Lpt3 in the absence of LgtG activity, thus producing the L3 immunotype (17). However, in strain NMB which contains an intact Lpt3, inactivation of *lgtG* did not result in the appearance of O-3 linked PEA on the inner core of the LOS (18). Conversely, O-3 linked PEA is an inner core decoration in mutants of strain NMB which lacked the addition of the α -chain (18, 37, 38). These results suggested that the conformation adopted by the inner core with the addition of the α -chain, in combination with the O-acetylation of the terminal GlcNAc residue, precluded the addition of O-3 PEA groups by Lpt3. To test this important hypothesis which has implications for immunotype switching in vivo, a mutant strain in which both *lgtG* and *lot3* were insertionally inactivated, designated NMBlot3/*lgtG*, was analyzed for the appearance of O-3 PEA groups on the inner core in the presence of an intact α -chain. Purification of the OSs by gel-filtration chromatography using Bio-Gel P2 resulted in one major and additional minor OSs from NMBlot3/*lgtG*. The major OS from NMBlot3/*lgtG* had the same glycosyl residue linkages as those for NMBlot3 OS with the exception that terminally linked Glc was absent; a result which is consistent with the mutation in *lgtG*.

[0026] The major OSs were analyzed by MALDI-TOF MS and the resulting spectrum is shown in Fig. 3C. The mass spectrum of the OS from NMBlot3/*lgtG*, shows two clusters of ions. Each cluster represents a single structure. The ions of the first cluster (m/z 1765, 1783, 1787, 1805, 1809, 1827, 1831, and 1849) are all consistent with various sodiated and anhydro sodiated forms of a structure that, compared to the NMBlot3 OS structure, lacks one hexosyl residue and contains an additional (i.e. for a total of two) PEA group. The second cluster ions (m/z 1642, 1660, 1664, 1682, 1686, and 1704) are consistent with sodiated and anhydro sodiated forms of a structure that, compared to the NMBlot3 OS structure, lacks a

hexosyl residue. This missing hexosyl residue is consistent with the methylation data showing that this OS lacks a terminally linked Glc; i.e. the terminal Glc that is linked to O-3 of Hep II. In addition to the glycosyl linkage and MS data showing that the NMBlot3/lgtG OS lacks both the terminal α -Glc residue and the O-acetyl group, the lack of both these moieties was also confirmed by NMR analysis. Comparison with the spectra for NMB and NMBlot3 OSs (Figs. 5A and 5B, respectively), the spectrum for the NMBlot3/lgtG OS (Fig. 5C) lacks the anomeric H/C signal for the α -Glc residue (residue B), and lacks the downfield H3/C3 resonance for the terminal α -GlcNAc as observed for the NMB OS (Fig. 5A). As observed for NMBlot3, the minor OSs from NMBlot3/lgtG consisted of glycinylated versions of the major OS. These results support the conclusions that the NMBlot3/lgtG mutant, which in addition to missing the O-acetyl group, lacks the terminally linked Glc attached to O-3 at Hep II, was able to express a major structure containing two PEA groups attached to Hep II.

[0027] The location of LOS inner core PEA groups is influenced by O-acetylation. In order to determine the location of the PEA groups on NMBlot3 and NMBlot3/lgtG OSs, two NMR experiments were performed. The first was a $^{31}\text{P}/^1\text{H}$ HMQC experiment and the spectra for the NMBlot3 and NMBlot3/lgtG OSs are shown in Figs. 6A-6B. Fig. 6A shows that the phosphorous atom of the PEA group was correlated to the H-6 proton of Hep II and to the $-\text{OCH}_2-$ methylene protons of the PEA group. These results were consistent with NMBlot3 OS containing a single PEA group attached to O-6 of Hep II. The spectrum of NMBlot3/lgtG OS shown in Figure 6B was consistent with an OS that contains two PEA groups. For one PEA group the phosphorous atom was correlated to H-6 of Hep II and to $-\text{OCH}_2-$ of PEA, and for the second PEA group the phosphorous atom was correlated to H-3 of Hep II and to $-\text{OCH}_2-$ of PEA. These results are consistent with the NMBlot3/lgtG OS containing two PEA groups, at O-6 and O-3 of the Hep II residue. The locations of the PEA groups were further confirmed by $^{31}\text{P}/^1\text{H}$ HMQC-TOCSY analysis. The spectrum for the NMBlot3 OS is shown in Fig. 7A and shows that the phosphorus of the

PEA group is correlated to H-6, H-5, H-7a and H-7b of Hep II, and to the –O-CH₂- methylene and –CH₂-NH₂ methylene protons of the PEA group. The spectrum for the NMBlot3/lgtG OS, Fig. 7B, showed that the phosphorus of one PEA group was correlated with Hep II and PEA methylene protons consistent with a location at O-6 of Hep II as just described for the NMBlot3 OS, while the phosphorus of the second PEA group was correlated to H-3, H-2, H-4, and H-5 of Hep II and to the protons of both of the PEA methylene groups. These results confirm that the major OS from NMBlot3/lgtG LOS contains structures with two PEA groups located at O-3 and O-6 of Hep II, as well as with a single PEA group located at O-3 or O-6 of Hep II. Thus, in strain NMB, O-3 linked PEA groups appear on the LOS inner core only in the absence of both O-3 linked glucose at HepII and O-acetylation of the terminal GlcNAc. This is consistent with the hypothesis that in the presence of a full length α -chain and O-acetylation of terminal GlcNAc of the inner core, Lpt3 is unable to gain access to HepII of the inner core.

[0028] Lipooligosaccharide structure and variability in *N. meningitidis* has been shown to be important for the pathogenesis of this organism. An examination of various LOS immunotyped meningococcal strains by Jennings et al (15) demonstrated that one mechanism of LOS phase variation was due to changes in the length of homopolymeric tract regions of *lgtA*, *lgtC* and *lgtG* (Table 1). Interestingly, the most frequently detected reversible LOS immunotype switching event has been that of L[1,8] to L[3,7,9] since many isolates express a mixture of these structures (3, 4). Although immunotype switching involving L2 and L4 immunotypes is less frequent, it does occur with two natural events of L[1,2] (4) and L[2,3] (15) immunotype interconversions being described. Our study indicates that the LOS structural changes resulting in conversion between immunotype pattern expression of L[2,4] and L[3,7,9] LOS is in part dependent upon the phase variable expression of the lipooligosaccharide O-acetyltransferase encoded by *lot3*.

[0029] An L3 immunotype is distinguished from an L2 LOS immunotype by four structural changes to the HepII of the inner core: the loss or exclusion of O-3 linked PEA group from HepII, the addition of O-3 linked glucose by LgtG, the addition of O-6 linked PEA by Lpt6 and as shown in this study, the addition of the O-acetyl group to the terminal N-acetylglucosamine (GlcNAc). Mackinnon et al (17) demonstrated that expression of LgtG was sufficient to exclude the addition of O-3 linked PEA groups from the LOS inner core of L3 immunotype strains and ascribed this dominant effect to increased efficiency for this site by LgtG. However, inactivation of LgtG and Lpt6 in strain NMB, which expresses a mixture of L2 and L4 LOS immunotypes in a 5 to 1 ratio, does not result in the appearance of O-3 linked PEA groups on the inner core even though the strain contains an active Lpt3 (18). Mutations which removed the lactoneotetraose α -chain do result in the appearance of an O-3 linked PEA on LOS inner cores in strain NMB (18, 37, 38). From these data we concluded that the three dimensional structure of the LOS inner core with an intact α chain masks Hep II making the O-3 attachment site unavailable to Lpt-3. The only structural difference between the L3 LOS structure and that of strain NMB without O-3 linked glucose or O-6 linked PEA groups is the presence of an O-acetyl group on the terminal GlcNAc of the γ -chain (36).

[0030] The *N. meningitidis* lipooligosaccharide O-acetyltransferase (*lot3*) was identified by homology to a known LOS O-acetyltransferase from *R. leguminosarum*. Inactivation of *lot3* in strain NMB resulted in the loss of the O-acetyl group located on the terminal GlcNAc of the LOS inner core. We found that O-acetylation of the GlcNAc residue is at O-3 as previously assigned by Kogan et al (11). The proton spectrum of the NMB OS in a previous report (36) clearly shows that it is identical to that obtained for our current analysis of NMB OS, and has the downfield α -GlcNAc H3 resonance at δ 5.17 due to O-acetylation at that position. The O-6 assignment in the earlier report was made by comparing 1-D TOCSY spectra before and after HF treatment of the OS. The fact that this treatment removes both O-acetyl and PEA groups and the rather poor

quality of the 1-D TOCSY spectra resulted in the incorrect assignment of the O-acetyl group. Therefore, the *lot3* locus in strain NMB has been designated *lot3* indicating the linkage of the O-acetyl group at the O-3 position of the terminal GlcNAc residue in the L2 immunotype structure.

[0031] O-3 linked PEA groups did not appear on the LOS inner core of NMBlot3 mutants due to the dominant effect by LgtG as observed by Mackinnon (17). Inactivation of *lgtG* in the absence of Lot3 activity in strain NMB resulted in the appearance of O-3 linked PEA groups on the inner core, supporting the hypothesis that the O-acetyl group was responsible for obstructing access of Lpt-3 to the LOS inner core in the absence of O-3 linked glucose. Based upon previous observations on the effect of O-acetylation on the conformation of *Salmonella typhimurium* O-antigen (39) the most likely mechanism appears to be that the meningococcal LOS undergoes a conformational change with the addition of the O-3 acetylation of GlcNAc, which in the presence of the lactoneotetraose α chain, prevents access to the inner core by Lpt3. A comparison of *lot3* genes found in the meningococcal genome databases with that of strain NMB indicates that a short polymeric G-tract is present in the central region of the open reading frame. The point mutation inactivating *lot3* in strain MC58 which expressed L3 immunotype LOS was found in this region, however, whether the length of the polymeric tract undergoes phase variation at a detectable rate remains unknown.

[0032] The *lot3* locus has been found in all meningococcal strains examined to date, including isolates expressing all twelve immunotype structures. The intact *lot3* gene was found in those strains expressing immunotype LOS structures with an O-acetyl group (Fig. 1, Table 1). Based on these data we believe that immunotypes L9-12 are also O-acetylated, indicating that this substitution is common amongst all immunotypes with the exception of L3 and L7. Based upon the structures for LOS immunotypes L1-8, and the data derived from mutating the LOS biosynthesis pathways in strains NMB and MC58, the following conclusions can be drawn regarding the expression of the O-3 PEA group

on the LOS inner core of meningococcal strains. In meningococcal isolates expressing a LOS structure with a short α -chain (such as L1 and L8), O-3 PEA groups can be added to the inner core by Lpt-3 regardless of the O-acetylation state of the terminal GlcNAc of the γ -chain (18). In comparison, the spectrum of decorations added to the LOS inner core of meningococcal isolates expressing an α -chain consisting of lactoneotetraose is determined by the O-acetylation status of the terminal GlcNAc. When *lot3* is not expressed in the presence of a lactoneotetraose α chain, the phase variable expression of *lgtG* and the presence or absence of functional *lpt3* and *lpt6* determines which decorations are added to the inner core (Table 1). When *lot3* is expressed in the presence of a LOS structure with a full-length α -chain, even if the strain contains an active *lpt-3*, only the O-6 linked PEA groups and O-3 linked glucose residues can be added to the LOS inner core. We cannot completely exclude the possibility that the absence of Lot may affect the stability or function of Lpt-3. However, it appears unlikely that Lot and Lpt-3 form a complex because the predicted structures of these proteins indicate that they reside in separate cell compartments (cytoplasm and periplasm, respectively).

[0033] The biological relevance of these findings is of considerable interest. Ram et al (19) have suggested that there is a strong positive selection pressure for the expression in invasive meningococcal disease of LOS structures with a lactoneotetraose chain and an inner core decorated with an O-3 PEA group, because this structure is unavailable for binding to the complement factor C4b. The O-3 PEA group results in increased resistance to killing by human serum during bloodstream dissemination. The identification of *lot3* and the effect on the exclusion of the addition of O-3 PEA groups to the LOS inner core also suggest that there may be biological selection for preventing or masking the appearance of O-3 linked PEA groups on the surface of meningococci. LOS functions as an adherence molecule and cell invasion ligand (3,4,40,41), and LOS structure influences these events by obstruction of Opa adhesins (42). Alternatively, the effect on the presence of O-3 PEA groups may be an

indirect consequence of immunological selection directed at the O-acetyl group on the LOS inner core. Investigations of the immunological responses generated by meningococcal LOS immunotypes have shown that the O-acetylated L2 and L5 structures induced higher antibody titers than do non-O-acetylated L3,9 structures (43). Polyclonal antiserum raised against L2 and L5 immunotypes was bactericidal, but immunotype specific, whilst the response elicited by L3,9 immunotypes recognized common epitopes preserved between L2 and L3,9 (44) but was not bactericidal.

[0034] Present strategies for the development of LOS glycoconjugates as meningococcal vaccine candidates are providing evidence that such vaccines yield cross protective immunity against meningococci (45). Because the majority of *N. meningitidis* isolated from invasive disease express the L[3,7,9] immunotype LOS (3), (4) and because the expression of the L[3,7,9] immunotype LOS by *N. meningitidis* increases resistance to complement-mediated lysis during disseminated disease (19), most of this work has focused upon utilizing an L3 LOS. However, studies assessing the distribution of LOS immunotypes expressed in meningococcal disease isolates indicate that at least one-quarter of strains causing invasive meningococcal disease express L[2,4], and these strains may utilize other mechanisms for resistance to complement-mediated lysis (46). Based upon our current and previous observations of strain NMB (29), the steps involved for conversion among immunotypes include the phase variable expression of *lgtA*, the carriage of an active *lot3*, the acquisition of islets carrying *lpt-6* and *lgtG*, and the phase variable and regulated expression of *lgtG*. The mechanisms of LOS assembly and the basis of immunotype switching are important in both understanding meningococcal pathogenesis and for LOS vaccine design. O-acetylation of meningococcal LOS represents a potential mechanism for immune escape from a vaccine targeting only L3 immunotype LOS, and therefore multiple endotoxin structures are believed to be required for an effective meningococcal endotoxin-based vaccine.

[0035] Expression refers to the transcription and translation of a structural gene (coding sequence) so that a protein (i.e., expression product) having the biological activity of the O-acetyltransferase as described herein is synthesized. It is understood that post-translational modification(s) in certain types of recombinant host cells may remove portions of the polypeptide which are not essential to enzymatic activity.

[0036] The term expression control sequences refer to DNA sequences that control and regulate the transcription and translation of another DNA sequence (i.e., a coding sequence). A coding sequence is operatively linked to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that coding sequence. Expression control sequences include, but are not limited to, promoters, enhancers, promoter-associated regulatory sequences, transcription termination and polyadenylation sequences, and their positioning and use is well understood by the ordinary skilled artisan.

[0037] The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted in front of the gene. The combination of the expression control sequences and the Lot3 O-acetyltransferase coding sequence form the Lot3 O-acetyltransferase expression cassette.

[0038] As used herein, an exogenous or heterologous nucleotide sequence is one which is not in nature covalently linked to a particular nucleotide sequence, e.g., a Lot3 O-acetyltransferase coding sequence. Examples of exogenous nucleotide sequences include, but are not limited to, plasmid vector sequences, expression control sequences not naturally associated with particular Lot3 O-acetyltransferase coding sequences, and viral or other vector sequences. A non-naturally occurring DNA molecule

is one which does not occur in nature, and it is thus distinguished from a chromosome, or example. As used herein, a non-naturally occurring DNA molecule comprising a sequence encoding an expression product with Lot3 O-acetyltransferase activity is one which comprises said coding sequence and sequences which are not associated therewith in nature.

[0039] Similarly, as used herein an exogenous gene is one which does not naturally occur in a particular recombinant host cell but has been introduced in using genetic engineering techniques well known in the art. An exogenous gene as used herein can comprise a Lot3 O-acetyltransferase coding sequence expressed under the control of an expression control sequence not associated in nature with said coding sequence.

[0040] Another feature is the expression of the sequences encoding Lot3 O-acetyltransferase. As is well-known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate host cell.

[0041] A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences taught herein. Useful expression vectors, for example, may consist of segments of chromosomal, nonchromosomal and synthetic DNA sequences. Suitable vectors include derivatives of SV40 and known bacterial plasmids, e.g., *Escherichia coli* plasmids colE1, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., M13 derivatives, the numerous derivatives of phage λ , e.g., λ gt11, and other phage DNA; yeast plasmids derived from the 2 μ circle; vectors useful in eukaryotic cells, such as insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression control sequences; baculovirus derivatives; and the like. For mammalian cells there are a number of well-known expression vectors available to the art.

[0042] Any of a wide variety of expression control sequences may be used in these vectors to express the DNA sequences taught herein. Such useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus for expression in mammalian cells, the *lac* system, the *trp* system, the *tac* or *trc* system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or phosphatase (e.g., *pho5*), the promoters of the yeast α -mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. The skilled artisan understands which expression control sequences are appropriate to particular vectors and host cells.

[0043] A wide variety of host cells are also useful in expressing the DNA sequences taught herein. These hosts may include well-known prokaryotic and eukaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, such as Chinese Hamster Ovary (CHO), R1.1, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS-7, BSC1, BSC40, and BMT10), insect cells (e.g., Sf9), and human cells and plant cells in culture.

[0044] It is understood that not all combinations of vector, expression control sequence and host cell will function equally well to express the DNA sequences encoding the acetyl-transferase as taught herein. However, one skilled in the art can select the proper vector, expression control sequence, and host cell combination without undue experimentation to accomplish the desired expression.

[0045] In selecting a suitable expression control sequence, a variety of factors are normally considered. These include, for example, the relative strength of the promoter, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, e.g., with regard to potential secondary structure. Suitable hosts are selected by consideration of factors including compatibility with the chosen vector, secretion characteristics, ability to fold proteins correctly, and fermentation

requirements, as well as any toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of purification of the expression products. The practitioner can select the appropriate host cells and expression mechanisms for a particular purpose.

[0046] Several strategies are available for the isolation and purification of recombinant Lot3 O-acetyltransferase after expression in a host system. One method involves expressing the proteins in bacterial cells, lysing the cells, and purifying the protein by conventional means. Alternatively, one can engineer the DNA sequences for secretion from cells. A Lot3 O-acetyltransferase protein can be readily engineered to facilitate purification and/or immobilization to a solid support of choice. For example, a stretch of 6-8 histidine residues can be engineered through polymerase chain reaction or other recombinant DNA technology to allow purification of expressed recombinant protein over a nickel-charged nitrilotriacetic acid (NTA) column using commercially available materials. Other oligopeptide "tags" which can be fused to a protein of interest by such techniques include, without limitation, strep-tag (Sigma-Genosys, The Woodlands, TX) which directs binding to streptavidin or its derivative streptactin (Sigma-Genosys); a glutathione-S-transferase gene fusion system which directs binding to glutathione coupled to a solid support (Amersham Pharmacia Biotech, Uppsala, Sweden); a calmodulin-binding peptide fusion system which allows purification using a calmodulin resin (Stratagene, La Jolla, CA); a maltose binding protein fusion system allowing binding to an amylose resin (New England Biolabs, Beverly, MA); and an oligo-histidine fusion peptide system which allows purification using a Ni^{2+} -NTA column (Qiagen, Valencia, CA).

[0047] Coding sequences which are synonymous to the coding sequence provided herein are within the scope of the present invention, as are sequences encoding O-acetyltransferases carrying out the same acetylations of *Neisseria meningitidis* lipooligosaccharides or oligosaccharides, and where those sequences encode Lot3 O-acetyltransferases with at least 80% amino acid sequence identity with that

of either the Z2491 or the NMB enzyme. All integers between 80 and 100% are included within the scope of the present invention in this context. In calculations of amino acid sequence identity, gaps inserted to optimize alignment are treated as mismatches.

[0048] Lot3 O-acetyltransferase coding sequences from various *N. meningitidis* strains have significant sequence homology to the specifically exemplified O-acetyltransferase coding sequences, and the encoded enzymes have a high degree of amino acid sequence identity as disclosed herein. It is obvious to one normally skilled in the art that equivalent clones and PCR amplification products can be readily isolated using standard procedures and the sequence information provided herein. The ordinary skilled artisan can utilize the exemplified sequences provided herein, or portions thereof, preferably at least 25-30 bases in length, in hybridization probes to identify cDNA (or genomic) clones encoding Lot3 O-acetyltransferase, where there is at least 70% sequence homology to the probe sequence using appropriate art-known hybridization techniques. The skilled artisan understands that the capacity of a cloned cDNA to encode functional Lot3 O-acetyltransferase enzyme can be readily tested as taught herein.

[0049] Hybridization conditions appropriate for detecting various extents of nucleotide sequence homology between probe and target sequences and theoretical and practical consideration are given, for example in B.D. Hames and S.J. Higgins (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, and in Sambrook et al. (1989) *supra*. Under particular hybridization conditions the DNA sequences taught herein hybridize to other DNA sequences having sufficient homology, including homologous sequences from different species. It is understood in the art that the stringency of hybridization conditions is a factor in the degree of homology required for hybridization. The skilled artisan knows how to manipulate the hybridization conditions so that the stringency of hybridization is at the desired level (high, medium). If attempts to identify and isolate the Lot3 O-acetyltransferase coding sequence from another *N. meningitidis* strain fail

using high stringency conditions, the skilled artisan understands how to decrease the stringency of the hybridization conditions so that a sequence with a lower degree of sequence homology hybridizes to the sequence used as a probe. The choice of the length and sequence of the probe is readily understood by the skilled artisan.

[0050] The DNA sequences encoding a Lot3 O-acetyl transferase can be prepared or isolated using recombinant DNA techniques. These include cDNA sequences, sequences isolated using PCR, DNA sequences isolated from their native genome, and synthetic DNA sequences. As used herein, this term is not intended to encompass naturally-occurring chromosomes or genomes. These sequences can be used to direct recombinant synthesis of Lot3 O-acetyltransferase for enzymatic acetylation of isolated OS and/or LOS, especially from *N. meningitidis* strains.

[0051] Isolated oligosaccharides and/or lipooligosaccharides are separated from the cells and culture medium from which it was produced. Further purification is optional and within the realm of the skilled artisan.

[0052] In the present context, an *in vitro* enzymatic reaction, especially acetylation of *N. meningitidis* oligosaccharide and lipooligosaccharide is carried out in the absence of whole, live cells. The enzyme source can be a purified or partly purified enzyme or it can be present in a cell extract, recombinantly produced or otherwise, although greater amounts per cell are produced through recombinant DNA technology. Highly acetylated OS/LOS preparations can be isolated from *N. meningitidis* strains that contain and express an additional *lot3* gene or which have been genetically modified to contain and express a *lot3* coding sequence at levels greater than is wild type *N. meningitidis* cells.

[0053] It is well-known in the biological arts that certain amino acid substitutions can be made within a protein without affecting the functioning of that protein. Preferably such substitutions are of amino acids similar in size and/or charge properties. For example, Dayhoff et al. (1978) in *Atlas*

of *Protein Sequence and Structure*, Volume 5, Supplement 3, Chapter 22, pages 345-352, which is incorporated by reference herein, provides frequency tables for amino acid substitutions which can be employed as a measure of amino acid similarity. Dayhoff et al.'s frequency tables are based on comparisons of amino acid sequences for proteins having the same function from a variety of evolutionarily different sources.

[0054] It will be a matter of routine experimentation for the ordinary skilled artisan to use the DNA sequence information presented herein to optimize Lot3 O-acetyltransferase expression in a particular expression vector and cell line for a desired purpose. A cell line genetically engineered to contain and express a Lot3 O-acetyltransferase coding sequence is useful for the recombinant expression of protein products with the characteristic enzymatic activity of the specifically exemplified enzyme. Any means known to the art can be used to introduce an expressible Lot3 O-acetyltransferase coding sequence into a cell to produce a recombinant host cell, i.e., to genetically engineer such a recombinant host cell. Recombinant host cell lines which express high levels of Lot3 O-acetyltransferase are useful as sources for the purification of this enzyme, especially for in vitro acetylation of isolated OS or LOS polysaccharides, desirably those from *N. meningitidis*.

[0055] The amino acids which occur in the various amino acid sequences referred to in the specification have their usual three- and one-letter abbreviations routinely used in the art: A, Ala, Alanine; C, Cys, Cysteine; D, Asp, Aspartic Acid; E, Glu, Glutamic Acid; F, Phe, Phenylalanine; G, Gly, Glycine; H, His, Histidine; I, Ile, Isoleucine; K, Lys, Lysine; L, Leu, Leucine; M, Met, Methionine; N, Asn, Asparagine; P, Pro, Proline; Q, Gln, Glutamine; R, Arg, Arginine; S, Ser, Serine; T, Thr, Threonine; V, Val, Valine; W, Try, Tryptophan; Y, Tyr, Tyrosine.

[0056] A protein is considered an isolated protein if it is a protein isolated from a host cell in which it is recombinantly produced. It can be purified or it can simply be free of other proteins and biological materials with which it is associated in nature.

[0057] An isolated nucleic acid is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid spanning more than three separate genes. The term therefore covers, for example, a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding or noncoding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Specifically excluded from this definition are nucleic acids present in mixtures of DNA molecules, transformed or transfected cells, and cell clones, e.g., as these occur in a DNA library such as a cDNA or genomic DNA library.

[0058] In the present context, a promoter is a DNA region which includes sequences sufficient to cause transcription of an associated (downstream) sequence. The promoter may be regulated, i.e., not constitutively acting to cause transcription of the associated sequence. If inducible, there are sequences present which mediate regulation of expression so that the associated sequence is transcribed only when an inducer molecule is present in the medium in or on which the organism is cultivated.

[0059] One DNA portion or sequence is downstream of second DNA portion or sequence when it is located 3' of the second sequence. One DNA portion or sequence is upstream of a second DNA portion or sequence when it is located 5' of that sequence.

[0060] One DNA molecule or sequence and another are heterologous to another if the two are not derived from the same ultimate natural source. The sequences may be natural sequences, or at least one sequence can be designed by man, as in the case of a multiple cloning site region. The

two sequences can be derived from two different species or one sequence can be produced by chemical synthesis provided that the nucleotide sequence of the synthesized portion was not derived from the same organism as the other sequence.

[0061] An isolated or substantially pure nucleic acid molecule or polynucleotide is an O-acetyltransferase-encoding polynucleotide which is substantially separated from other polynucleotide sequences which naturally accompany it on the bacterial chromosome. The term embraces a polynucleotide sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, chemically synthesized analogues and analogues biologically synthesized by heterologous systems.

[0062] A polynucleotide is said to encode a polypeptide if, in its native state or when manipulated by methods known to those skilled in the art, it can be transcribed and/or translated to produce the polypeptide or a fragment thereof. The anti-sense strand of such a polynucleotide is also said to encode the sequence.

[0063] A nucleotide sequence is operably linked when it is placed into a functional relationship with another nucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter effects its transcription or expression. Generally, operably linked means that the sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame. However, it is well known that certain genetic elements, such as enhancers, may be operably linked even at a distance, i.e., even if not contiguous.

[0064] The term recombinant polynucleotide refers to a polynucleotide which is made by the combination of two otherwise separated segments of sequence accomplished by the artificial manipulation of isolated segments of polynucleotides by genetic engineering techniques or by chemical synthesis. In so doing one may join together polynucleotide segments of desired functions to generate a desired combination of functions.

[0065] Polynucleotide probes include an isolated polynucleotide attached to a label or reporter molecule and may be used to identify and isolate other O-acetyltransferase coding sequences equivalent in function to the *lot3* specifically exemplified herein, for example, those from other strains of *N. meningitidis*. Probes comprising synthetic oligonucleotides or other polynucleotides may be derived from naturally occurring or recombinant single or double stranded nucleic acids or be chemically synthesized. Polynucleotide probes may be labeled by any of the methods known in the art, e.g., random hexamer labeling, nick translation, or the Klenow fill-in reaction, or with fluors or other detectable moieties.

[0066] Large amounts of the polynucleotides may be produced by replication in a suitable host cell. Natural or synthetic DNA fragments coding for a protein of interest are incorporated into recombinant polynucleotide constructs, typically DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell, especially cultured mammalian cells, wherein protein expression is desired. Usually the construct is suitable for replication in a host cell, such as cultured mammalian cell or a bacterium, but a multicellular eukaryotic host may also be appropriate, with or without integration within the genome of the host cell. Commonly used prokaryotic hosts include strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or a pseudomonad, may also be used. Eukaryotic host cells include mammalian cells, yeast, filamentous fungi, plant, insect, amphibian and avian cell lines. Such factors as ease of manipulation, ability to appropriately glycosylate expressed proteins, degree and control of recombinant protein expression, ease of purification of expressed proteins away from cellular contaminants or other factors influence the choice of the host cell.

[0067] The polynucleotides may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) *Tetra. Letts.* 22: 1859-1862 or the triester method according to Matteuci et al. (1981) *J. Am. Chem. Soc.* 103: 3185, and may

be performed on commercial automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence. DNA constructs prepared for introduction into a prokaryotic or eukaryotic host will typically comprise a replication system (i.e. vector) recognized by the host, including the intended DNA fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide-encoding segment. Expression systems (expression vectors) may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Signal peptides may also be included where appropriate from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes or be secreted from the cell.

[0068] An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) *vide infra*; Ausubel et al. (Eds.) (1995) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley Interscience, New York; and Metzger et al. (1988) *Nature*, 334: 31-36. Many useful vectors for expression in bacteria, yeast, fungal, mammalian, insect, plant or other cells are well known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press,

N.Y. (1983). While such expression vectors may replicate autonomously, they may less preferably replicate by being inserted into the genome of the host cell.

[0069] Expression and cloning vectors will likely contain a selectable marker, that is, a gene encoding a protein necessary for the survival or growth of a host cell transformed with the vector. Although such a marker gene may be carried on another polynucleotide sequence co-introduced into the host cell, it is most often contained on the cloning vector. Only those host cells into which the marker gene has been introduced will survive and/or grow under selective conditions. Typical selection genes encode proteins that confer resistance to antibiotics or other toxic substances, e.g., ampicillin, neomycin, methotrexate, etc.; complement auxotrophic deficiencies; or supply critical nutrients not available from complex media. The choice of the proper selectable marker will depend on the host cell; appropriate markers for different hosts are known in the art.

[0070] Recombinant host cells, in the present context, are those which have been genetically modified to contain an isolated DNA molecule encoding a Lot3 O-acetyltransferase. The DNA can be introduced by any means known to the art which is appropriate for the particular type of cell, including without limitation, transfection, transformation, lipofection or electroporation.

[0071] It is recognized by those skilled in the art that the DNA sequences may vary due to the degeneracy of the genetic code and codon usage. All (synonymous) DNA sequences which code for the O-acetyltransferase protein are included within the scope of this invention, including the DNA sequence as given in Table 5. Also contemplated are coding sequences which encode an O-acetyltransferase as taught herein with at least 80% amino acid sequence identity to that of Table 4.

[0072] Additionally, it is recognized by those skilled in the art that allelic variations may occur in the DNA sequences which do not significantly

change activity of the amino acid sequences of the peptides which the DNA sequences encode. All such equivalent DNA sequences are included within the scope of this invention and the definition of the regulated promoter region. The skilled artisan understands that the sequence of the exemplified O-acetyltransferase protein and the nucleotide sequence encoding it can be used to identify and isolate additional, nonexemplified nucleotide sequences which are functionally equivalent to the sequences given Fig. 8A.

[0073] Hybridization procedures are useful for identifying polynucleotides with sufficient homology to the subject coding sequence to be useful as taught herein. The particular hybridization technique is not essential to the subject invention. As improvements are made in hybridization techniques, they can be readily applied by one of ordinary skill in the art.

[0074] A probe and sample are combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong non-covalent bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical, or completely complementary, if the annealing and washing steps are carried out under conditions of high stringency. The probe's detectable label provides a means for determining whether hybridization has occurred.

[0075] In the use of the oligonucleotides or polynucleotides as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P , ^{35}S , or the like. Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or a chemiluminescent reagent such as luciferin, or fluorescent compounds like fluorescein and its

derivatives. Alternatively, the probes can be made inherently fluorescent as described in International Application No. WO 93/16094.

[0076] Various degrees of stringency of hybridization can be employed. The more stringent the conditions, the greater the complementarity that is required for duplex formation. Stringency can be controlled by temperature, probe concentration, probe length, ionic strength, time, and the like. Preferably, hybridization is conducted under moderate to high stringency conditions by techniques well known in the art, as described, for example in Keller, G.H., M.M. Manak (1987) *DNA Probes*, Stockton Press, New York, NY., pp. 169-170, hereby incorporated by reference.

[0077] As used herein, moderate to high stringency conditions for hybridization are conditions which are particularly advantageous. An example of high stringency conditions are hybridizing at 68° C in 5X SSC/5X Denhardt's solution/0.1% SDS, and washing in 0.2X SSC/0.1% SDS at room temperature. An example of conditions of moderate stringency are hybridizing at 68° C in 5X SSC/5X Denhardt's solution/0.1% SDS and washing at 42° C in 3X SSC. The parameters of temperature and salt concentration can be varied to achieve the desired level of sequence identity between probe and target nucleic acid. See, e.g., Sambrook et al. (1989) *vide infra* or Ausubel et al. (1995) *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, NY, for further guidance on hybridization conditions.

[0078] Specifically, hybridization of immobilized DNA in Southern blots with ³²P-labeled gene specific probes is performed according to standard methods (Maniatis et al.) In general, hybridization and subsequent washes were carried out under moderate to high stringency conditions that allowed for detection of target sequences with homology to the exemplified sequence. For double-stranded DNA gene probes, hybridization can be carried out overnight at 20-25° C below the melting temperature (T_m) of the DNA hybrid in 6X SSPE 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following

formula (Beltzet al. (1983) *Methods of Enzymology*, R.Wu, L, Grossman and K Moldave (eds) Academic Press, New York 100:266-285).

[0079] $T_m = 81.5^{\circ} \text{C} + 16.6 \log[\text{Na}^+] + 0.41(\% \text{G+C}) - 0.61(\% \text{formamide}) - 600/\text{length of duplex in base pairs}$.

[0080] Washes are typically carried out as follows: twice at room temperature for 15 minutes in 1X SSPE, 0.1% SDS (low stringency wash), and once at $T_m - 20^{\circ} \text{C}$ for 15 minutes in 0.2X SSPE, 0.1% SDS (moderate stringency wash).

[0081] For oligonucleotide probes, hybridization is carried out overnight at $10-20^{\circ} \text{C}$ below the melting temperature (T_m) of the hybrid 6X SSPE, 5X Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. T_m for oligonucleotide probes is determined by the following formula:
 $T_m(^{\circ}\text{C}) = 2(\text{number T/A base pairs}) + 4(\text{number G/C base pairs})$ (Suggs et al. (1981) *ICB-UCLA Symp. Dev. Biol. Using Purified Genes*, D.D. Brown (ed.), Academic Press, New York, 23:683-693).

[0082] Washes are typically carried out as follows: twice at room temperature for 15 minutes 1X SSPE, 0.1% SDS (low stringency wash), and once at the hybridization temperature for 15 minutes in 1X SSPE, 0.1% SDS (moderate stringency wash).

[0083] In general, salt and/or temperature can be altered to change stringency. With a labeled DNA fragment >70 or so bases in length, the following conditions can be used: Low, 1 or 2X SSPE, room temperature; Low, 1 or 2X SSPE, 42°C ; Moderate, 0.2X or 1X SSPE, 65°C ; and High, 0.1X SSPE, 65°C .

[0084] Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated. Therefore, the probe sequences useful for binding to a Lot3 O-acetyltransferase coding sequence include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein

said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, and those methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

[0085] Thus, mutational, insertional, and deletional variants of the disclosed nucleotide sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the exemplified primer sequences so long as the variants have substantial sequence identity with the original sequence. As used herein, substantial sequence identity refers to identity which is sufficient to enable the variant polynucleotide to function in the same capacity as the polynucleotide from which the probe was derived. Preferably, this identity is greater than 80%, more preferably, this identity is greater than 85%, even more preferably this identity is greater than 90%, and most preferably, this identity is greater than 95%. All integers between 80 and 100%. The degree of (homology or) identity needed for the variant to function in its intended capacity depends upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are equivalent in function or are designed to improve the function of the sequence or otherwise provide a methodological advantage.

[0086] Polymerase Chain Reaction (PCR) is a repetitive, enzymatic, primed amplification of a nucleic acid sequence. This procedure is well known and commonly used by those skilled in this art (see, e.g., Mullis, U.S. Patent Nos. 4,683,195, 4,683,202, and 4,800,159; Saiki *et al.* (1985) *Science* 230:1350-1354). The thermostable *Taq* polymerase, which is isolated from the thermophilic bacterium *Thermus aquaticus*, allows automation of the amplification process. Other enzymes which can be used are known to those skilled in the art.

[0087] It is well known in the art that the polynucleotide sequences encoding a Lot3 O-acetyltransferase can be truncated and/or mutated

such that certain of the resulting fragments and/or mutants of the original full-length sequence can retain the desired characteristics of the full-length sequence. A wide variety of restriction enzymes which are suitable for generating fragments from larger nucleic acid molecules are well known. In addition, it is well known that *Ba*β1 exonuclease can be conveniently used for time-controlled limited digestion of DNA. See, for example, Maniatis (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, pages 135-139, incorporated herein by reference. See also Wei et al. (1983 *J. Biol. Chem.* 258:13006-13512. By use of *Ba*β1 exonuclease (commonly referred to as “erase-a-base” procedures), the ordinarily skilled artisan can remove nucleotides from either or both ends of the subject nucleic acids to generate a wide spectrum of fragments which are functionally equivalent to the subject nucleotide sequences. One of ordinary skill in the art can, in this manner, generate hundreds of fragments of controlled, varying lengths from locations all along the original O-acetyltransferase coding sequence. The ordinarily skilled artisan can routinely test or screen the generated fragments for their characteristics and determine the utility of the fragments as taught herein. It is also well known that the mutant sequences of the full length sequence, or fragments thereof, can be easily produced with site directed mutagenesis. See, for example, Larionov, O.A. and Nikiforov, V.G. (1982) *Genetika* 18(3):349-59; Shortle, D, DiMaio, D., and Nathans, D. (1981) *Annu. Rev. Genet.* 15:265-94; both incorporated herein by reference. The skilled artisan can routinely produce deletion-, insertion-, or substitution-type mutations and identify those resulting mutants which contain the desired characteristics of the full length wild-type sequence, or fragments thereof, i.e., those which retain O-acetyltransferase activity as determined herein.

[0088] DNA sequences having at least 80, 90, or at least 95% (and all integers and ranges between 80 and 100%) identity to the recited Lot3 coding sequences disclosed herein and functioning to encode a Lot3 O-acetyltransferase protein are within the scope of this invention. Such functional equivalents are included in the definition of an O-

acetyltransferase coding sequence. Additional Lot coding sequences from other immunotypes and from other neisserial species are provided herein below. Following the teachings herein and using knowledge and techniques well known in the art, the skilled worker will be able to make a large number of operative embodiments having equivalent DNA sequences to those listed herein without the expense of undue experimentation.

[0089] As used herein percent sequence identity of two nucleic acids is determined using the algorithm of Altschul et al. (1997) *Nucl. Acids Res.* 25: 3389-3402; see also Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264-2268, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al. (1990) *J. Mol. Biol.* 215:402-410. BLAST nucleotide searches are performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST is used as described in Altschul et al. (1997) *Nucl. Acids. Res.* 25:3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) are used. See the National Center for Biotechnology Information website and programs available on the internet and from other sources.

[0090] In another embodiment, immunogenic compositions for producing polyclonal and/or monoclonal antibodies capable of specifically binding to O-acetyltransferase, OS or LOS from *N. meningitidis* (or fragments thereof) are provided. Similar material can be prepared from other Lot-expressing neisserial strains in for use in immunogenic compositions as well. The term antibody is used to refer both to a homogenous molecular entity and a mixture such as a serum product made up of a plurality of different molecular entities. Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with a particular epitope in a molecule of interest may be made by methods known in the art. See, e.g.,

Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York; and Ausubel et al. (1993) *supra*. Also, recombinant immunoglobulins may be produced by any methods known in the art, including but not limited to, the methods described in U.S. Patent No. 4,816,567, incorporated by reference herein. Monoclonal antibodies with affinities of 10^8 M^{-1} , preferably 10^9 to 10^{10} or more are preferred.

[0091] Antibodies generated against a molecule of interest are useful, for example, as probes for screening DNA expression libraries or for detecting the presence of particular neisserial strains or their isolated LOS or OS polysaccharides in a test sample. Hydrophilic regions of the Lot3 O-acetyltransferase as taught herein can be identified by the skilled artisan, and peptide antigens can be synthesized and conjugated to a suitable carrier protein (e.g., bovine serum albumin or keyhole limpet hemocyanin) for use in vaccines or in raising antibody specific for LOS biosynthetic proteins. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or noncovalently, a substance which provides a detectable signal. Suitable labels include but are not limited to radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. United States Patents describing the use of such labels include but are not limited to Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

[0092] Antibodies specific for the acetylated LOS from *N. meningitidis* are useful in preventing disease resulting from neisseriae, especially *N. meningitidis* infections. Such antibodies can be obtained by the methods described above. Because there is some loss of acetyl residues during isolation of the OS and/or LOS and because there is some loss of immunogenicity of an unacetylated or a poorly acetylated OS or LOS, the quality of a *N. meningitidis* OS and/or LOS-containing immunogenic composition, it is advantageous to treat such a preparation with the Lot3

O-acetyltransferase as taught herein prior to use in immunogenic compositions, including vaccine compositions. Techniques applied to *in vitro* acetylation of capsular polysaccharide can be applied to isolated OS and/or LOS; see, e.g., US Patent Publication 2006/0073168 A1.

[0093] Compositions and immunogenic preparations, including vaccine compositions comprising *in vitro* acetylated oligosaccharides and lipooligosaccharides from *N. meningitidis* or nonacetylated preparations, and a suitable carrier therefor are provided. Immunogenic compositions can be similarly prepared using material from other Neisserial species as well. Immunogenic compositions are those which result in specific antibody production when injected into a human or an animal. Such immunogenic compositions are useful, for example, in immunizing a human, against infection by neisserial pathogenic strains, especially those of *N. meningitidis*. The immunogenic preparations comprise an immunogenic amount of an acetylated LOS (or OS) preparation derived from a *N. meningitidis* strain and a suitable carrier.

[0094] The immunogenic compositions advantageously further comprise lipooligosaccharide(s), proteins and/or neisserial cells of *N. meningitidis* and optionally, one or more other serogroups and/or immunotypes, including, but not limited to, any known to the art. It is understood that where whole cells are formulated into the immunogenic composition, the cells are preferably inactivated, especially if the cells are of a virulent strain. Such immunogenic compositions may comprise one or more LOS preparations, capsular polysaccharides and/or another protein or other immunogenic cellular component. By "immunogenic amount" is meant an amount capable of eliciting the production of antibodies directed against neisserial (acetylated or nonacetylated, as desired) OS or LOS polysaccharides in an animal or human to which the vaccine or immunogenic composition has been administered.

[0095] Immunogenic carriers may be used to enhance the immunogenicity of a component of the immunogenic composition as known to the art. Such carriers include, but are not limited to, proteins and

polysaccharides, liposomes, and bacterial cells and membranes. Protein carriers may be joined to the molecule(s) of interest to form fusion proteins by recombinant or synthetic means or by chemical coupling. Useful carriers and means of coupling such carriers to polypeptide antigens are known in the art. The art knows how to administer immunogenic compositions so as to generate protective immunity on the mucosal surfaces of the upper respiratory system, especially the mucosal epithelium of the nasopharynx, where immunity is specific for *N. meningitidis*, as well as protecting other parts of the body.

[0096] The immunogenic compositions provided herein may be formulated by any means known in the art. Such vaccines are typically prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also, for example, be emulsified, or the protein encapsulated in liposomes.

[0097] The active immunogenic ingredients are often mixed with excipients or carriers which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients include but are not limited to water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. The concentration of the immunogenic polypeptide in injectable formulations is usually in the range of 0.2 to 5 mg/ml.

[0098] In addition, if desired, the vaccines may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide; N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP); N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP); N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A referred to as MTP-PE); and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2%

squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogen resulting from administration of the immunogen in vaccines which are also comprised of the various adjuvants. Such additional formulations and modes of administration as are known in the art may also be used.

[0099] *In vitro* acetylated lipooligosaccharide or oligosaccharides from *N. meningitidis* and advantageously containing cells of *N. meningitidis* may be formulated into immunogenic compositions as neutral or salt forms. Preferably, when whole cells are used, they are of attenuated or avirulent strains, or the cells are killed before use. Pharmaceutically acceptable salts include but are not limited to the acid addition salts (formed with free amino groups of the peptide) which are formed with inorganic acids, e.g., hydrochloric acid or phosphoric acids; and organic acids, e.g., acetic, oxalic, tartaric, or maleic acid. Salts formed with the free carboxyl groups may also be derived from inorganic bases, e.g., sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases, e.g., isopropylamine, trimethylamine, 2-ethylamino-ethanol, histidine, and procaine.

[00100] The immunogenic preparations provided herein are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of about 100 to 1,000 µg of *in vitro* acetylated oligosaccharide and/or lipooligosaccharide per dose, more generally in the range of about 1 to 500 µg per dose, depends on the subject to be treated, the capacity of the individual's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of the active ingredient required to be administered may depend on the judgment of the physician and may be peculiar to each individual, but such a determination is within the skill of such a practitioner.

[00101] The vaccine or other immunogenic composition may be given in a single dose or multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may include 1 to 10 or more separate doses, followed by other doses administered at subsequent time intervals as required to maintain and or reinforce the immune response, e.g., at 1 to 4 months for a second dose, and if needed, a subsequent dose(s) after several months.

[00102] As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the functional and novel characteristics of the claim. Any recitation herein of the term "comprising", particularly in a description of components of a composition or in a description of elements of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements. The invention may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein, provided that there would be no anticipation by or obviousness over prior art.

[00103] The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible without going beyond the scope of the invention claimed. Thus, it should be understood that although embodiments of the present invention have been specifically disclosed by certain embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[00104] In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art.

[00105] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art(s) to which the invention pertains, and all references cited herein are hereby incorporated by reference to the extent that there is no inconsistency with the present specification.

[00106] Monoclonal or polyclonal antibodies, preferably monoclonal, specifically reacting with a polypeptide or protein of interest may be made by methods known in the art. See, e.g., Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories; Goding (1986) *Monoclonal Antibodies: Principles and Practice*, 2d ed., Academic Press, New York; and Ausubel *et al.* (1995) *Current Protocols in Molecular Biology*, Wiley Interscience, New York, NY.

[00107] Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook *et al.* (1989) *Molecular Cloning*, Second Edition, Cold Spring Harbor Laboratory, Plainview, New York; Maniatis *et al.* (1982) *Molecular Cloning*, Cold Spring Harbor Laboratory, Plainview, New York; Wu (ed.) (1993) *Meth. Enzymol.* 218, Part I; Wu (ed.) (1979) *Meth. Enzymol.* 68; Wu *et al.* (eds.) (1983) *Meth. Enzymol.* 100 and 101; Grossman and Moldave (eds.) *Meth. Enzymol.* 65; Miller (ed.) (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Old and Primrose (1981) *Principles of Gene Manipulation*, University of California Press, Berkeley; Schleif and Wensink (1982) *Practical Methods in Molecular Biology*; Glover (ed.) (1985) *DNA Cloning* Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (eds.) (1985) *Nucleic Acid Hybridization*, IRL Press, Oxford, UK; Setlow and Hollaender (1979)

Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York; and Ausubel *et al.* (1995) *Current Protocols in Molecular Biology*, Greene/Wiley, New York, NY. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

[00108] The specifically exemplified sequences, compositions, cells, vectors, compounds and methods and accessory methods described herein are representative of embodiments of the present invention; they are not intended to limit the scope of the invention. Thus, additional embodiments are within the scope of the invention and within the following claims. The scope of the invention should be determined by the appended claims and their equivalents, rather than by the specific examples given.

EXAMPLES

Example 1. Bacterial strains, plasmids and growth conditions

[00109] *E. coli* strain JM109 (21) was used for the maintenance of plasmids. *E. coli* strains were grown in Luria-Bertani (LB) broth (Difco) at 37°C with agitation, or on LB agar plates supplemented with 1.5% agar, and where appropriate, with the following antibiotics: 100 µg/ml ampicillin, 25 µg/ml kanamycin, 150 µg/ml erythromycin or 10 µg/ml tetracycline. Meningococcal strains were grown on GC agar base (Oxoid) supplemented with 20 mM glucose, 0.43 µM thiamine pyrophosphate chloride, 6.8 mM glutamine and 12.4 µM Fe(NO₃)₃ in 5% carbon dioxide atmosphere. Liquid cultures were vigorously aerated at 37°C in GC broth with the same supplements and 0.51 M sodium bicarbonate (22). Where appropriate, media was supplemented with 7 µg/ml erythromycin, 1 µg/ml tetracycline or 40 µg/ml kanamycin.

Example 2. DNA manipulations.

[00110] All DNA manipulations were performed by standard methods as described previously (23). Plasmid DNA was purified using the Hi PURE Plasmid Isolation kit from Roche Diagnostics (Indianapolis, IN).

Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). DNA sequencing was performed with the BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and analyzed on an Applied Biosystems model 3730 DNA analyzer. Oligonucleotide primers were synthesized on an Applied Biosystems 394 oligonucleotide synthesizer. Competent *E. coli* JM109 was prepared by the method described by Chung et al. (24). Amino acid sequences were aligned either by CLUSTALW (on the internet, address ebi.ac.uk/clustalw/#) (25) or Blast 2 sequences (see the NCBI site on the internet: [website ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi](http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi)) (26).

Example 3. Inactivation of the lipooligosaccharide O-acetyltransferase (*lot3*).

[00111] The *lot3* gene in *N. meningitidis* strain NMB was inactivated by the incorporation of a *lot3::tetM* mutagenic cassette. To construct the *lot3::tetM* cassette, an internal fragment of *lot3* (formerly designated NMA2202 in *N. meningitidis* strain Z2491) was amplified using the primer pair DAP379 (5'-gtctcggtcgccgtaaagatagc-3', SEQ ID NO:1) and DAP377 (5'-gcagtagaggaacagtattacctcc-3', SEQ ID NO:2) (Figure 2). The resulting 1.5 kb PCR product was treated with T4 DNA polymerase and ligated into pHSG576 (27) digested with *Sma*I to create pJKD2709. The *tetM* cassette was released from pJKD2401 by *EcoRV* digestion and ligated to *Sma*I digested pJKD2709. Those transformants which were tetracycline and chloramphenicol resistant were assessed by colony PCR for the correct insertion of the *tetM* cassette into pJKD2709, and were designated pJKD2710.

[00112] To create NMBlot3, pJKD2710 was transformed in *N. meningitidis* strain NMB using the protocol of Janik et al. (28) and transformants were identified by resistance to tetracycline. To ensure these transformants contained the *lot3::tetM* cassette in the correct chromosomal location, the *lot3* locus was PCR amplified with DAP371 (5'-CGATTTGTCGCGGAAAGAAACCG-3', SEQ ID NO:3) and DAP372 (5'-gaagccaaagccaaattgcttgagc-3', SEQ ID NO:4), located outside the *lot3*

open reading frame and the correct mutants were designated NMB/*lot3* (JKD5172). To create a meningococcal double mutant containing inactivated *lot3* and *lgtG*, the *lgtG::kan* cassette was introduced into NMB/*lot3* by transformation with pCK49 (29), to create the strain designated NMB/*lot3/lgtG* (JKD5173).

Example 4. Isolation of oligosaccharides.

[00113] The LOS preparations, prepared as previously described (30), were washed three times with 9:1 ethanol/ water (v/v) mixture to remove contaminating phospholipids. The washed LOS preparations were suspended in water and lyophilized. Samples were then subjected to mild acid hydrolysis in 1% aqueous HOAc (v/v) for 2.5 h at 100°C with constant stirring. The lipid A precipitate that formed during hydrolysis was collected by centrifugation at 3000 x g for 15 min at 4°C, and supernatants containing the released oligosaccharides (OSs) were decanted and lyophilized. The lyophilized OSs were further purified by gel-filtration chromatography using Bio-Gel P-4 (Bio-Rad) column (120 x 1 cm) and water as eluent.

Example 5. Glycosyl composition and linkage analysis of the oligosaccharides.

[00114] Glycosyl compositional analysis was performed by gas chromatography -mass spectrometry (GLC-MS) of trimethylsilyl (TMS) methyl glycosides with myoinositol used as an internal standard (31). The samples were methanolized with methanolic 1 M HCl at 80°C for 18 h. The released monosaccharides were dried under a stream of dry air and acetylated with 3:1:1 methanol/ pyridine/ acetic anhydride (v/v/v) at 100°C for 1 h. After cooling, samples were dried-down and trimethylsilylated with Tri-sil reagent (Pierce) for 30 min at 80°C. The resulting TMS derivatives were analyzed by GLC-MS, on Hewlett-Packard HP5890/HP5970 MSD gas chromatograph/mass spectrometer equipped with Supelco DB-1 fused silica capillary column (30m x 0.25 mm I.D.) with helium as the carrier gas.

[00115] Linkage analyses were carried out by the slurry NaOH method modified from that of Ciucanu and Kerek (32). Samples were dissolved in 0.5 mL dimethyl sulfoxide (DMSO) by stirring overnight at room temperature under a N₂ atmosphere. After dissolution, a freshly prepared slurry of NaOH in DMSO was added (0.5 mL) and the reaction mixture was stirred for 2 h at room temperature. Methylation was performed by the sequential addition of iodomethane (250 µL followed by 100 µL) at 30 min intervals. The permethylated monosaccharide was extracted into the organic phase (dichloromethane) after partitioning the reaction mixture between water and dichloromethane. The organic phase was then removed by evaporation under a stream of N₂. The permethylated OS was further purified using a Sep-Pak C18 cartridge to remove any remaining DMSO. The permethylated OS was hydrolyzed with 4 M TFA (100°C, 6h), reduced with NaBH₄, acetylated and the resulting partially methylated alditol acetates (PMAAs) were analyzed by GLC/MS using a SPB capillary column (25 m × 0.25 mm, from Supelco) and DB-1 capillary column.

Example 6. Mass spectrometry.

[00116] Oligosaccharides were analyzed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) using a 4700 Proteomics Analyzer instrument (Applied Biosystems) in reflectron mode. The OS samples were dissolved in water (1 mg/mL) and mixed in a 1:1 (v/v) ratio with 0.5 M 2,5-dihydroxybenzoic acid (DHB) in methanol matrix solution. Spectra were acquired in both the positive and negative acquisition modes.

Example 7. NMR Spectroscopy.

[00117] NMR spectra were collected on Varian Inova500 and 600 spectrometers using standard software supplied by Varian. The samples were exchanged several times with D₂O (99.8% Aldrich), and final measurements were made in 0.5mL D₂O solutions (100% D; Cambridge Isotope Laboratories) at 25°C. Proton NMR spectra were measured at 600 MHz using spectral width of 8 kHz and the data were processed with HOD

signal referenced at 4.78 ppm on proton scale. The correlated spectroscopy (gCOSY) spectra were measured over a spectral width of 2.25 kHz using a dataset of ($t_1 \times t_2$) of 256×2048 points with 16 scans. The total correlated spectroscopy (TOCSY) spectra were collected using the same sized data set with 32 scans with a mixing time of 80 msec. For the heteronuclear single quantum coherence (HSQC) experiment, the spectral widths in the proton and carbon dimensions were 2.2 and 13.9 kHz, respectively, and 96 scans were acquired. $^1\text{D} \ ^{31}\text{P}$ NMR spectra were done at pH 7.0 using a Varian Inova-500 instrument with a broadband probe adjusted to 202.38MHz. Proton decoupled ^{31}P spectra were acquired with spectral width of 10 kHz calibrated with phosphoric acid (85%) as the external standard ($\delta p=0.0\text{ppm}$). The 2D proton detected $^1\text{H}-^{31}\text{P}$ heteronuclear multiple bond quantum coherence (HMQC) and HMQC-TOCSY experiments were performed using the standard pulse sequence supplied by Varian. The HMQC spectrum was collected using a data set of 128×2048 ($t_1 \times t_2$) points with total of 32 scans. The measurements of HMQC and HMQC-TOCSY spectra were done using $J_{\text{H,P}}$ coupling value of 12.0 Hz. The mixing time used for HMQC-TOCSY spectrum was 60msec and total of 64 scans were collected. The spectral width in $^{31}\text{P}-^1\text{H}$ HMQC and HMQC-TOCSY experiments were set to 5kHz in phosphorus dimension and 2.25 kHz in proton dimension respectively.

Example 8. PCR to determine presence or absence of the *lot* gene

[00118] Chromosomal DNA of representative immunotyped stains L1 - L12 were used as the template for PCR amplification of *lot*. Primers DAP 371 (5'- cgattgtcgcggaagaaaccg-3', SEQ ID NO:3) and DAP 372 (5'- gaagccaaagccaaattgcttgagc-3', SEQ ID NO:4) and an extended PCR cycle with a 50°C annealing temperature and 4 minute auto-extend were used to amplify the gene, which is expected to be 2 Kb. All of the 12 representative strains contained *lot*. Both strands for each PCR product were sequenced and a consensus was generated (See below).

[00119] Immunotyping strains are shown in Table 11. All strains were obtained from Dr Wendell Zollinger at the Walter Reed Army Institute of Research.

Table 1: Expression matrix for meningococcal LOS immunotypes.

Strain	Immunotype	LOS biosynthesis genes*					
		lgtA	**lgtC	§lgtG	†lpt-3	§lpt-6	‡lot3
126E	L1	P “-“	P”+”	P”-“ or A	P	A	P”+”
NMB	L2	P “+”	A	P”+”	P or A	P	P”+”
MC58	L3	P “+”	A	P”-“ or A	P	A	P”-”
89I	L4	P “+”	A	P”-“ or A	A	P	P”+”
M981	L5	P”+”	A	P”+”	A	A	P”+”
M922	L6	P”+”	A	P”-“ or A	A	P	P”+”

* “A” indicates absent, “P” indicates present, “-“ indicates phase off, “+” indicates phase on.

**The presence or absence and phase of expression for these genes in the representative isolates has been established by Jennings et al (15).

§ lgtG and lpt-6 are present on two separate islets that are carried separately or together (18).

† lpt-3 is carried by most isolates but may contain internal deletions which inactivate the locus (17).

‡ The presence of lot3 has been established in representative meningococcal strains expressing all twelve immunotypes (7). O-acetylation of the chemical structures for immunotypes L1, L2, L4, L5, and L6 has been

Residue	H1	H2	H3	H4	H5	H6a,b	H7a,b
α-L-D-HepII (A)	5.77	4.25	4.23	4.17	3.76	4.57	3.83, 3.90
α-D-Glc (B)	5.43	3.6	3.72	3.48	n.d.	n.d.	n.d.
α-D-GlcNAc (C)	5.28	4.19	5.17*	3.68	n.d.	n.d.	n.d.
α-L-D-HepI (D)	5.09	4.18	4.16	4.08	n.d.	n.d.	n.d.
β-D-GlcNAc (E)	4.72	3.81	3.74	3.59	n.d.	n.d.	n.d.
β-D-Glc (F)	4.56	3.44	3.64	3.56	n.d.	n.d.	n.d.
β-D-Gal (G)	4.48	3.55	3.67	3.93	n.d.	n.d.	n.d.
β-D-Gal (H)	4.43	3.59	3.75	4.16	n.d.	n.d.	n.d.

established (see Figure 1 for references).

Table 2: Proton chemical shift values for the OS from NMB LOS (partial assignment)

*Indicates the position of O-acetyl group; n.d. = not detected. The residues are labeled A-H in descending order of their chemical shifts. The arrangement of these glycosyl residues are as shown on the structures given in Figure 3.

Table 3: Proton chemical shift values of the OS from NMBlot3 OS (partial assignment)

*Indicates the position of O-acetyl group; n.d. = not detected.

Residue	H1	H2	H3	H4	H5	H6a,b	H7a,b
α -L-D-HepII (A)	5.71	4.18	4.21	4.16	3.73	4.56	3.74, 3.82
α -D-Glc (B)	5.41	3.58	3.72	n.d.	n.d.	n.d.	n.d.
α -D-GlcNAc (C)	5.18	3.89	3.85	3.48	3.86	3.9	n.d.
α -L-D-HepI (D)	5.09	4.17	4.11	n.d.	n.d.	n.d.	n.d.
β -D-GlcNAc (E)	4.72	3.82	3.76	3.59	n.d.	n.d.	n.d.
β -D-Glc (F)	4.56	3.44	3.65	3.56	n.d.	n.d.	n.d.
β -D-Gal (G)	4.48	3.55	3.68	3.94	3.56	3.44	n.d.
β -D-Gal (H)	4.46	3.59	3.75	4.16	3.92		n.d.

Table 4A. L1 lot (SEQ ID NO:5)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
 QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTATSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFGKISYSYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVPRFEAQSFLIPGF
 PARFRET VKRIA AVKP VYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNQAVFDLVKDIPNVHWVDAQKYL PKNTVEIHGRYLYGDQDHLTYFGSYY
 MGREFHKHERLLKSSRDGALQ

Table 4B L2 lot (SEQ ID NO:6)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
 QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTASSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFGKISYSYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVPRFEAQSFLIPGF
 QARFRET VKRIA AVKP VYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI

GKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSYY
MGREFHKHERLLKSSRDGALQ

Table 4C L3 lot (SEQ ID NO:7)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
RNISIILFLILTATSFLPSRFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
RRQTASGKRQLLSLLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
GTLPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
WVDTTLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGF
QARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
GKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGRYLYGDQDHLTYFGSYY
MGREFHKHERLLKSSRGGALQ.

Table 4D L4 lot (SEQ ID NO:8)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
RNISIILFLILTASSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
RRQTANGKRQLLSLLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
GTLPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGF
QARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
GKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSYY
MGREFHKHERLLKSSRDGALQ

Table 4E L5 lot (SEQ ID NO:9)

MQAVRYRPEIDGLRAVAVLSVMIFHLNDRWLPGGFLGVDIFFVISGFLITGIILSEI
IQNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
RNISIILFLILTATSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
RRQTANGKRQLLSLLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
GTLPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGF
PARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
GKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIHGRYLYGDQDHLTYFGSYM
GREFHKHERLLKSSRGGALQ

Table 4F L6 lot (SEQ ID NO:10)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
 QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTASSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFVGKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSF LIPGF
 QARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSYY
 MGREFHKHERLLKSSRDGALQ

Table 4G L7 lot (SEQ ID NO:11)

MQTVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
 QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 CHISIILFLILTASSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFVGKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSF LIPGF
 PARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSYYM
 GREFHKHESLLKHSHGNALQ

Table 4H L8 lot (SEQ ID NO:12)

MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEI
 QNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTGEFS AVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTATSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASSIVFVGKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSF LIPGF
 QARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSYYM
 GREFHKHERLLKSSRDGALQ

Table 4I L9 lot (SEQ ID NO:13)

MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSE
 IQNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTATSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGF
 PARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNAQAVFDLIK DIPNVHWVDAQKYL PKNTVEIYGRYLYGDQDHLTYFGSYYM
 GREFHKHERLLKSSRDGALQ

Table 4J L10 lot (SEQ ID NO:14)

MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSE
 IQNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
 LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
 RNISIILFLILTATSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNG
 RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQY
 GTLPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTA
 GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
 PGAPLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
 WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGF
 PARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDI
 GKSNAQAVFDLIK DIPNVHWVDAQKYL PKNTVEIYGRYLYGDQDHLTYFGSYYM
 GREFHKHERLLKSSRDGALQ

Table 4K L11 lot (SEQ ID NO:15)

MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSE
 IQ
 NGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLS
 NIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL RN
 ISIILFLILTATSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRR
 QTANGKRQLLSSLCFGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGT
 LPTRILSASPIVFGVKISYSLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGF
 SLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPLPG
 APLAAENHFPETVLT LGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLVWV
 DEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVP RFEAQSFLIPGFPA
 RFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYLRPIQAMGDIGK
 SNQAVFDLIK DIPNVHWVDAQKYL PKNTVEIYGRYLYGDQDHLTYFGSYYMGR
 EFHKHERLLKSSRDGALQ

Table 4L L12 lot (SEQ ID NO:16)

MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSE
IQNGSFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVF
LSNIYLGFGQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVL
RNISIILFLILTATSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNG
RRQTANGKRQLLSSLCFGALLACLFVIDKHNPFIPIGMTLLLPCLLTALLIRSMQY
GTLPTRILSASPIVFGVKISYSLYLHWIFIAFAHYITGDKQLGLPAVSABAALTA
GFSLLSYYLIEQPLRKRKMTFKKAFFCLYLAPSLILVGYNLYARGILKQEHLRPL
PGAPLAAENHFPETVLTGDSHAGHLRGFLDYVGSREGWKAKILSLDSECLV
WVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQPVPRFEAQSFILPGF
PARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYLRPIQAMGDI
GKSNQAVFDLIKDIPNVHWVDAQKYLKNTVEIYGRYLYGDQDHLTYFGSYYM
GREFHKHERLLKSSRDGALQ

Table 5: Results of CLUSTAL W (1.82) multiple sequence alignment for the twelve O-acetyl transferase proteins in Table 4A-4L (SEQ ID NOs:5-16, respectively).

L2lot	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L4	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L6lot	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L3lot	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L1lot	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L8lot	MQAVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L11lot	MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L12lot	MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L10lot	MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L9lot	MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L5lot	MQAVRYRPEIDGLRAVAVLSVMIFHLNDRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
L7lot	MQTVRYRPEIDGLRAVAVLSVIIIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
60	
	.:***.*.....*
L2lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L4	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L6lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L3lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L1lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L8lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTGEFSAVFLSNIYLGF
120	
L11lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L12lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L10lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L9lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	
L5lot	SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLGF
120	

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L71ot    SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLG
120
*****.*****

L21ot    QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L4        QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L61ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L31ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L11ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L81ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L111ot    QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L121ot    QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L101ot    QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L91ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L51ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA
180
L71ot     QQGYFDLSADENPVLHIWVSLAVEEQYYLLYPLLLIFCCKKTKSLRVLCHISIILFLILTA
180
*****:*****

L21ot     SSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L4         SSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L61ot      SSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L31ot      TSFLPSRFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTASGKRQLLSLLC
240
L11ot      TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L81ot      TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L111ot     TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L121ot     TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L101ot     TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L91ot      TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L51ot      TSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
L71ot      SSFLPSGFYTDILNQPNNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
240
:***** *****.***** **

L21ot     FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L4         FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L61ot      FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L31ot      FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L11ot      FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L81ot      FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASSIVFGKISY
300
L111ot     FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L121ot     FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L101ot     FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300
L91ot      FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
300

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L51ot    FGALLACLFVIDKHNPFI PGMTLLL PCLLTALLIRSMQYGTLPTRILSASP I V F V G K I S Y
300
L71ot    FGALLACLFVIDKHNPFI PGMTLLL PCLLTALLIRSMQYGTLPTRILSASP I V F V G K I S Y
300
*****

L21ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L4        SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L61ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L31ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L11ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L81ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L111ot   SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L121ot   SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L101ot   SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L91ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L51ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
L71ot    SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
360
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L21ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L4        FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L61ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L31ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L11ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L81ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L111ot   FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L121ot   FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L101ot   FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L91ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L51ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
L71ot    FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
420
*****

L21ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L4        DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L61ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L31ot    DYVGSREGWKAKILSLDSECLVWVDTTLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L11ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L81ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L111ot   DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L121ot   DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L101ot   DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480

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L91ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L51ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
L71ot    DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
480
*****
L21ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L4        PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L61ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L31ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L11ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L81ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L111ot   PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L121ot   PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L101ot   PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L91ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L51ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
L71ot    PVPRFEAQSFILIPGFQARFRETVKRIAABKPVYVFANNTSISRSPLEEKLRFAANQYL
540
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L21ot    RPIQAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSY
600
L4        RPIQAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSY
600
L61ot    RPIQAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGCYLYGDQDHLTYFGSY
600
L31ot    RPIQAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGRYLYGDQDHLTYFGSY
600
L11ot    RPIQAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYLPKNTVEIHGRYLYGDQDHLTYFGSY
600
L81ot    RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
L111ot   RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
L121ot   RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
L101ot   RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
L91ot    RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
L51ot    RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIHGRYLYGDQDHLTYFGSY
600
L71ot    RPIQAMGDIGKSNQAVFDLIKDIPNVHWVDAQKYLPKNTVEIYGRYLYGDQDHLTYFGSY
600
*****
L21ot    YMGREFHKHERLLKSSRDGALQ 622
L4        YMGREFHKHERLLKSSRDGALQ 622
L61ot    YMGREFHKHERLLKSSRDGALQ 622
L31ot    YMGREFHKHERLLKSSRGALQ 622
L11ot    YMGREFHKHERLLKSSRDGALQ 622
L81ot    YMGREFHKHERLLKSSRDGALQ 622
L111ot   YMGREFHKHERLLKSSRDGALQ 622
L121ot   YMGREFHKHERLLKSSRDGALQ 622
L101ot   YMGREFHKHERLLKSSRDGALQ 622
L91ot    YMGREFHKHERLLKSSRDGALQ 622
L51ot    YMGREFHKHERLLKSSRGALQ 622
L71ot    YMGREFHKHESLLKSHGNALQ 622
*****

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Table 6A. L1 lot sequence (ATG translation start and TAG translation termination codons underlined)(SEQ ID NO:17)

AAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGAACGCGCGTTCAGACGGCATT
TTTAAGATAACGGGACATACGGGGCGATATTTATGCAAGCTGTCCGATACAGGCCTGAA
ATTGACGGATTGCGGGCCGTTGCCGTGCTATCCGTCATTATTTTCCACCTGAATAACCG
CTGGCTGCCCCGAGGGTTTTTGGGGGTGGACATTTTCTTTGTCTCATCTCGGGATTCTCTCA
TTACCGGCATCATTCTTTCTGAAATACAGAACGGTTCTTTTTCTTTCCGGGATTTTTAT
ACCCGCAGGATTAAGCGGATTTACCCTGCCTTTATTGCGGGCCGTGTCGCTGGCTTCGGT
GATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACCAAATGCGGAAAACCGTGGAGC
TTTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTTCAGCAGGGGTATTTTCGATTTG
AGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTGGCAGTAGAGGAACAGTATTA
CCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAAAACAAAATCGCTACGGGTGC
TGCGTAACATCAGCATCATCCTATTTCTGATTTTGACTGCCACATCGTTTTTTGCCAAGC
GGGTTTTATACCGATATTCTCAACCAACCCAATACTTATTACCTTTCGACACTGAGGTT
TCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACGGGCAAACGCAAAACGGCAGAC
GGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGCTTCGGCGCATTTGCTT
GCCTGCCTGTTCGTGATTGACAAACACAATCCGTTTATCCCGGGAATGACCCTGCTCCT
TCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGGACACTTCCGACCC
GCATCCTGTCGGCAAGCCCCATCGTATTTGTTCGGCAAATCTCTTATTCCCTATACCTG
TACCATTGGATTTTTTATTGCTTTCGCCCATTACATTACAGGCGACAAACAGCTCGGACT
GCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATTTTCCCTGTTGAGTTATTATT
TGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCAAAAAGGCATTTTTCTGCCTC
TATCTCGCCCCGTCCCTGATACTTGTTCGGTTACAACCTGTACGCAAGGGGGATATTGAA
ACAGGAACACCTCCGCCCCGTGCCCCGGCGCGCCCCCTTGCTGCGGAAAATCATTTTCCGG
AAACCGTCCCTGACCCTCGGCGACTCGCACGCCGGACACCTGCGGGGGTTCCTGGATTAT
GTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCCCTCGATTCCGAGTGTTTGGT
TTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTTCGAAAATACCGGGATGAAGTTG
AAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATTTGAGGATGGGCGGCCAGCCC
GTGCCGAGATTTGAAGCGCAATCCTTCCTAATAACCCGGGTTCAGCCCGATTTCAGGGA
AACCGTCAAAAGGATAGCCGCCGTCAAACCCGTCTATGTTTTTTGCAAACAACACATCAA
TCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTGAAAAGATTTGCCGCAAACCAATATCTC
CGCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGGT
TAAAGATATCCCCAATGTGCATTGGGTGGACGCACAGAAATACCTGCCTAAAAACACGG
TCGAAATACACGGCCGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTTCGGTTCT
TATTATATGGGGCGGGAATTTACAAACACGAACGCCTGCTTAAATCTTCTCGCGACGG
CGCATTCAGTAGCCTGCCTTGCCGTCCGATATCGTTTGTGCCGCGGTTTGCCTTTTCGG
GGCGGCGGCTTTTATAGTGGATTAACAAAAATCAGGACAAGGCAACGAAGCCGCAGACA
GTACAAATAGTACGGAACCGATTCACTTGGTGCTTCAGCACCTTAGAGAATCGTTCTCT
TTGAGCTAAGGCGAGGCAACGCCGTACTGGTTTTTTGTAAATCCACTATATTTTGCCGTT
TTGAGGCCGGGGTCGGAATAACCGTTTTTTTGATGATTTTCCCTCCCTGGCTGTGTCATC
AAAACCCCAATTGCCTTTCCAAACTCTCCACCAGATTGTCATCCAGTTTCAAAGCCTGC
GACAGGCGGGCGAGGAAGACGGTTTCTTTCCGGGAACGGAATCGAA

Table 6B. L2 lot consensus coding sequence; ATG translation start codon and TAG translation termination codons underlined. (SEQ ID NO:18)

AACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGAACGCGCGTTCAGACGGCATT
TTAAGATAACGGGACATACGGGGCGATATTTATGCAAGCTGTCCGATACAGGCCTGAAA
TTGACGGATTGCGGGCCGTTGCCGTGCTATCCGTCATTATTTTCCACCTGAATAACCGC

TGGCTGCCCCGGAGGGTTTTTGGGGGTGGACATTTTCTTTGTCATCTCGGGATTCCTCAT
 TACCGGCATCATTTCTTTCTGAAATACAGAACGGTTCTTTTTCTTTCCGGGATTTTTATA
 CCCGCAGGATTAAGCGGATTTACCCTGCCTTTATTGCGGCCGTGTCGCTGGCTTCGGTG
 ATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACCAAATGCGGAAAACCGTGGAGCT
 TTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTTCAGCAGGGGTATTTTCGATTTGA
 GTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTGGCGGTAGAGGAACAGTATTAC
 CTCCTGTATCCCCTTTTTGCTGATATTTTGCTGCAAAAAAACCAAATCGCTACGGGTGCT
 GCGTAACATCAGCATCATCCTGTTTTTTGATTTTGACTGCCTCATCGTTTTTTGCCAAGCG
 GGTTTTATACCGACATCCTCAACCAACCCAATACTTATTACCTTTTCGACACTGAGGTTT
 CCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACGGGCAAACGCAAAACGGCAGACG
 GCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGCTTCGGCGCATTTGCTTG
 CCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTTATCCCGGGAATGACCCTGCTCCTT
 CCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGGACACTTCCGACCCG
 CATCCTGTTCGGCAAGCCCCATCGTATTTGTTCGGCAAATCTCTTATTCCCTATACCTGT
 ACCATTGGATTTTTTATTGCTTTCGCCCATTACATTACAGGCGACAAACAGCTCGGACTG
 CCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATTTTCCCTGTTGAGTTATTATTT
 GATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCAAAAAGGCATTTTTCTGCCTCT
 ATCTCGCCCCGTCCCTGATACTTGTTCGGTTACAACCTGTACGCAAGGGGGATATTGAAA
 CAGGAACACCTCCGCCCCGTTGCCCGGCGCGCCCCCTTGCTGCGGAAAATCATTTTCCGGA
 AACCGTCCCTGACCCTCGGCGACTCGCACGCCGGACACCTGCGGGGGTTTCTGGATTATG
 TCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCCCTCGATTCGGAGTGTTTGGTT
 TGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTGCAAAATACCGGGATGAAGTTGA
 AAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATTTGAGGATGGGCGGCCAGCCCCG
 TGCCGAGATTTGAAGCGCAATCCTTCCTAATACCCGGGTTCCAAGCCCGATTTCAGGGAA
 ACCGTCAAAGGATAGCCGCCGTCAAACCCGTCTATGTTTTTTGCAAACAACACATCAAT
 CAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTGAAAAGATTTGCCGCAAACCAATATCTCC
 GCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGGTT
 AAAGATATCCCCAATGTGCATTGGGTGGACGCACAGAAATACCTGCCTAAAAACACGGT
 CGAAATACACGGCTGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTTCGGTTCTT
 ATTATATGGGGCGGGAATTTACAAACACGAACGCCTGCTTAAATCTTCTCGCGACGGC
 GCATTGCAGTAGCCTGCCTTGCCGTCCGATATCGTTTTGTGCCGCCGTTTGCCTTTCGGG
 GCGGCGGCTTTTTATAGTGGATTAACAAAAATCAGGACAAGGCAACGAAGCCGCAGACAG
 TACAAATAGTACGGAACCGATTCACTTGGTGCTTCAGCACCTTAGAGAATCGTTCTCTT
 TGAGCTAAGGCGAGGCAACGCCGTACTGGTTTTTTGTTAATCCACTATATTTTGCCGTTT
 TGAGGCCGGGGTCGGAATAACCGTTTTTTTGATGATTTTCCCTCCCCGGCTGTGTCATCA
 AAACCCCAATTGCCTTTCCAAACTCTCCACCAGATTGTCATCCAGTTCCAAAGCCTGCG
 ACAGGCGGGCGAGGAAGACGGTTTCTTTCGG

Table 6C. L3 lot consensus sequence (SEQ ID NO:19); ATG translation start codon and TAG translation termination codons underlined.

CGCGAGTGGCAGCTGAGCGTTGGCGAACGGATTTGAGCGTTTACTGAAACCGATGCCGT
 CTGAACGCGCGTTCAGACGGCATTTTTTAAGATAACGGGACATACGGGGGCGATATTTAT
GCAAGCTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTTGCCGTGCTATCCG
TCATTATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGGTTTTTGGGGGTGGACATT
 TTCTTTGTCATCTCGGGATTCCTCATTACCGGCATCATTTCTTTCTGAAATACAGAACGG
 TTCTTTTTTCTTTCCGGGATTTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTTTA
 TCGCGGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTT
 AACCAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTTCTTGTCCAATATTTATCTGGG
 GTTTCAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGT

CTTTGGCGGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGC
 AAAAAAACCAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATTTT
 GACTGCCACATCGTTTTTTGCCAAGCAGGTTTTATACCGACATCCTCAACCAACCCAATA
 CTTATTACCTTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTT
 TACGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAGCGGAAAACGGCAGTTGCTTTC
 ATTACTCTGCTTCGGCGCATTTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGT
 TTATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGT
 ATGCAATACGGGACACTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTCGG
 CAAAATCTCTTATTCCCTATACCTGTACCATTGGATTTTTTATTGCCTTCGCCCATTACA
 TTACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCATTGACGGCC
 GGATTTTTCCCTGTTGAGCTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGAC
 CTTCAAAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTCTGGTTACA
 ACCTGTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCGTTGCCCGGCGCGCCC
 CTTGCTGCGGAAAATCATTTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCCGG
 ACACCTGCGGGGTTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCC
 TGTCCCTCGATTTCGGAGTGTTTGGTTTGGGTGGATACGACACTGGCAGACAACCCGTTA
 TGTGCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTA
 TGATTTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATAC
 CCGGGTTCCAAGCCCGATTTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCGTC
 TATGTTTTTTGCAAACAACACATCAATCAGCCGTTCGCCCCCTGAGGGAGGAAAAATTGAA
 AAGATTTGCCGCAAACCAATATCTCCGCCCCATTCAGGCTATGGGCGACATCGGCAAGA
 GCAATCAGGCGGTCTTTGATTGTTTAAAGATATCCCCAATGTGCATTGGGTGGACGCA
 CAGAAATACCTGCCTAAAAACACGGTCGAAATACACGGACGCTATCTTTACGGCGACCA
 AGACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGAAC
 GCCTGCTTAAATCTTCCCGCGGCGGCGCATTCAGTAGCCTGCCTTCTTGTCTGGATATT
 GCCTTTGGCAGCCTATGCCGCTGTTTGGCCCTTCGGGGCGGCGGCTTTTTATAGTGGATTA
 ACAAAAATCAGGACAAGGCGACGAAGCCGCAGACAGTACAAATAGTACGGAACCGATTTC
 ACTTGGTGCTTCAGCACCTTAGAGAATCGTTCTCTTTGAGCTAAGGCGAGGCAACGCCG
 TACTGGTTTTTTGTTAATCCACTATATTTTGCCGTTTTTGAGGCCGGGGTTCGGAATAACCG
 TTTTTTTGATGATTTTCCCTCCCTGGCTGTGTTCATCAAAACCCCAATTGCCTTTCCAAAC
 TCTCCACCAGATTGTCATCCAGTTTCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTT
 TCTTTCGG

Table 6D. L4 lot consensus sequence (SEQ ID NO:20); ATG translation start codon and TAG translation termination codons underlined.

AGCGTTGGCGAAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGAACGCGCGTTC
 AGACGGCATTTTTTAAGATAACGGGACATACGGGGCGATATTTATGCAAGCTGTCCGATA
 CAGGCCTGAAATTGACGGATTGCGGGCCGTTGCCGTGCTATCCGTCATTATTTTCCACC
 TGAATAACCGCTGGCTGCCCCGAGGGTTTTTGGGGGTGGACATTTTCTTTGTCATCTCG
 GGATTCCTCATTACCGGCATCATTTCTTTCTGAAATACAGAACGGTTCTTTTTCTTTCCG
 GGATTTTTTATACCCGCAGGATTAAGCGGATTTACCCTGCCTTTATTGCGGCCGTGTCGC
 TGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACCAAATGCGGAAA
 ACCGTGGAGCTTTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTTCAGCAGGGGTA
 TTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTGGCGGTAGAGG
 AACAGTATTACCTCCTGTATCCCCTTTTTGCTGATATTTTGCTGCAAAAAACCAAATCG
 CTACGGGTGCTGCGTAACATCAGCATCATCCTGTTTTTTGATTTTGACTGCCTCATCGTT
 TTTGCCAAGCGGGTTTTATACCGACATCCTCAACCAACCCAATACTTATTACCTTTTCA
 CACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACGGGCAAACGCAA
 AACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGCTTCGG
 CGCATTTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTATCCCGGGAATGA

CCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGGACA
 CTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTCGGCAAATCTCTTATTC
 CCTATACCTGTACCATTTGGATTTTTTATTGCTTTCGCCCATTACATTACAGGCGACAAAC
 AGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATTTTCCCTGTTG
 AGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCAAAAAGGCATT
 TTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTCTGGTTACAACCTGTACGCAAGGG
 GGATATTGAAACAGGAACACCTCCGCCCCGTGCCCCGGCGCGCCCCCTTGCTGCGGAAAAT
 CATTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCCGGACACCTGCGGGGGTT
 TCTGGATTATGTCTGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCCCTCGATTCTGG
 AGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTCTGAAAATACCGG
 GATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATTTGAGGATGGG
 CGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATAACCCGGGTCCAAGCCC
 GATTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCGTCTATGTTTTTTGCAAAC
 AACACATCAATCAGCCGTTCTGCCCCCTGAGGGAGGAAAAAATTGAAAAGATTTGCCGCAAA
 CCAATATCTCCGCCCCATTCTAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCT
 TTGATTTGGTTAAAGATATCCCCAATGTGCATTGGGTGGACGCACAGAAATACCTGCCT
 AAAACACGGTCGAAATACACGGCTGCTATCTTTACGGCGACCAAGACCACCTGACCTA
 TTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGAACGCCTGCTTAAATCTT
 CTCGCGACGGCGCATTCAGTAGCCTGCCTTGCCGTCCGATATCGTTTGTGCCGCCGT
 TGCCTTTCGGGGCGGCGGCTTTTATAGTGGATTAACAAAAATCAGGACAAGGCAACGAA
 GCCGCAGACAGTACAAATAGTACGGAACCGATTCACTTGGTGCTTCAGCACCTTAGAGA
 ATCGTTCTCTTTGAGCTAAGGCGAGGCAACGCCGTACTGGTTTTTTGTTAATCCACTATA
 TTTTGCCGTTTTTGAGGCCGGGGTCGGAATAACCGTTTTTTTGATGATTTTCCCTCCCCGG
 CTGTGTCATCAAACCCCAATTGCCTTTCCAACTCTCCACCAGATTGTCATCCAGTTC
 CAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTTTTCTTTCGGGGA

Table 6E. L5 lot consensus sequence (SEQ ID NO:21); ATG translation start codon and TAG translation termination codons underlined.

ACGCGAGTGGGCAGGCTGAGCGTTGGCGAACGGATTTGAGCGTTTACTGAAACCGATGC
 CGTCTGAACGCGCGTTTCAGACGGCATTTTTTAAGATAACGGGACATACGGGGGGCGATATT
TATGCAAGCTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTGCGCCGTGCTAT
CCGTCATGATTTTTCCACCTGAATGACCGCTGGCTGCCCGGAGGATTCCTGGGGGTGGAC
 ATTTTCTTTGTCATCTCAGGATTCCTCATTACCGGCATCATTTCTTCTGAAATACAGAA
 CGGTTCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTT
 TTATTGCGGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGAT
 TTCAACCAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGTCCAATATTTATCT
 GGGGTTTCAGCAGGGGTATTTGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCT
 GGTCTTTGGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGC
 TGCAAAAAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGAT
 TTTGACTGCCACATCGTTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCA
 ATACTTATTACCTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTGCTGCTGGCG
 GTTTACGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCT
 TTCATCACTCTGCTTCGGCGCATTGCTTGCCCTGCCTGTTCGTGATTGACAAACACAATC
 CGTTTATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGG
 AGTATGCAATACGGGACACTTCCGACCCGCATCCTGTCTGGCAAGCCCCATCGTATTTGT
 CGGCAAAATCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCCTTCGCCCATT
 ACATTACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACG
 GCCGGATTTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGAT
 GACCTTCAAAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTCTGGTT

ACAACCTGTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCG
 CCCCTTGCTGCAGAAAATCATTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGC
 CGGACACCTGCGGGGTTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAA
 TCCTGTCCCTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCG
 TTATGTCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATT
 CTATGATTTGAGGATGGGCGGCCAGCCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAA
 TACCCGGGTTCCCAGCCCGATTTCAGGGAAACCGTCAAAGGATAGCCGCCGTCAAACCC
 GTCTATGTTTTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATT
 GAAAAGATTTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCA
 AGAGCAATCAGGCGGTCTTTGATTTGATTAAAGATATTCCCAATGTGCATTGGGTGGAC
 GCACAAAAATACCTGCCTAAAAACACGGTCGAAATACACGGCCGCTATCTTTACGGCGA
 CCAAGACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACG
 AACGCCTGCTTAAATCTTCCCGCGGCGGCGCATTGCAGTAGCCTGCCTTCTTGTCGGAT
 ATTGCCTTTGGCAGCCTATGCCGCTGTTTGCCGTTTTGAGGCCGGGGTTCGGAATAACCG
 TTTTTTGATGATTTTCCCTCCCCGGCTGTGTCATCAAACCCCCAATTGCCTTTCCAAAC
 TCTCCACCAGATTGTCATCCAGTTTCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTT
 TCTTTCCGCGAACAAATCGA

Table 6F. L6 Lot consensus sequence (SEQ ID NO:22); ATG translation start codon and TAG translation termination codons underlined.

GGGCAGGCTGAGCGTTGGCGAAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGA
 ACGCGCGTTCAGACGGCATTTTTTAAGATAACGGGACATACGGGGCGATATTTATGCAAG
 CTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTTGCCGTGCTATCCGTCATT
 ATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGGTTTTTGGGGGTGGACATTTTCTT
 TGTTCATCTCGGGATTCTTCATTACCGGCATCATCTTTCTGAAATACAGAACGGTTCTT
 TTTCTTTCCGGGATTTTTTATACCCGCAGGATTAAGCGGATTTACCCTGCCTTTATTGCG
 GCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACCA
 AATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTTC
 AGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG
 GCGGTAGAGGAACAGTATTACCTCCTGTATCCCCTTTTTGCTGATATTTTGCTGCAAAAA
 AACCAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTGTTTTTTGATTTTGACTG
 CCTCATCGTTTTTTGCCAAGCGGGTTTTTATACCGACATCCTCAACCAACCCAATACTTAT
 TACCTTTTCGACACTGAGGTTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACGG
 GCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCAC
 TCTGCTTCGGCGCATTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTATC
 CCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCA
 ATACGGGACACTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTCGGCAAAA
 TCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCTTTTCGCCCATTTACATTACA
 GCGGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATT
 TTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCA
 AAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTTCGGTTACAACCTG
 TACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGCCCCCTTGC
 TCGGGAATAATCATTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCCGGACACC
 TCGGGGGGTTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCC
 CTCGATTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTCTG
 AAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATT
 TGAGGATGGGCGGCCAGCCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATAACCCGGG
 TTCCAAGCCCGATTTCAGGGAAACCGTCAAAGGATAGCCGCCGTCAAACCCGTCTATGT
 TTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTGAAAAGAT
 TTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGCAAT

CAGGCGGTCTTTGATTTGGTTAAAGATATCCCCAATGTGCATTGGGTGGACGCACAGAA
 ATACCTGCCTAAAAACACGGTCGAAATACACGGCTGCTATCTTTACGGCGACCAAGACC
 ACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGAACGCCTG
 CTAAATCTTCTCGCGACGGCGCATTGCAGTAGCCTGCCTTGCCGTCCGATATCGTTTG
 TGCCGCCGTTTGCCTTTCGGGGCGGCGGCTTTTATAGTGGATTAACAAAAATCAGGACA
 AGGCAACGAAGCCGCAGACAGTACAAATAGTACGGAACCGATTCACTTGGTGCTTCAGC
 ACCTTAGAGAATCGTTCTCTTTGAGCTAAGGCGAGGCAACGCCGTACTGGTTTTTTGTTA
 ATCCACTATATTTTGCCGTTTTGAGGCCGGGGTCGGAATAACCGTTTTTTTGATGATTTT
 CCCTCCCCGGCTGTGTCATCAAACCCCAATTGCCTTTCCAAACTCTCCACCAGATTGT
 CATCCAGTTCCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTTTCTTTCGGG

Table 6G. L7 lot consensus sequence (SEQ ID NO:23) ATG translation start codon and TAG translation termination codons underlined.

AGGGGCAGCTGAGCGTTGGCGAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGG
 ACGCGCGTTCAGACGGCATTTTTTAAAATACCGGATATACAGGGGCGATATTTATGCAAA
 CTGTCCGATACAGGCCTGAAATTGACGGATTACGGGCTGTCGCCGTCCTTTCCGTCATT
 ATTTTCCACCTGAATAACCGTTGGCTGCCCGGAGGATTCCTGGGGGTGGACATTTTCTT
 TGTCTCTCGGGATTCTCTATTACCGGCATCATTCCTTCTGAAATACAGAACGGTTCTT
 TTTCTTTCGGGATTTTTTATACCCGCAGGATTAAGCGGATTTACCCTGCCTTTATTGCG
 GCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACCA
 AATGCGGAAAACCGTGGAGCTTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTTC
 AGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG
 GCGGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAAA
 AACAAAATCGCTACGGGTGTTGTGCCACATCAGCATCATCCTGTTTTTTGATTTTGACTG
 CCTCATCGTTTTTTGCCAAGCGGGTTTTTATACCGACATCCTCAACCAACCCAATACTTAT
 TACCTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACGG
 GCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCAC
 TCTGCTTCGGCGCATTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTATC
 CCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCGCTGCTTATCCGGAGTATGCA
 ATACGGGACACTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTGCGGCAAAA
 TCTCTTATTCCCTATACCTGTACCATTGGATTTTTTATTGCCTTCGCCCATTACATTACA
 GCGGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATT
 TTCCTGTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCA
 AAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTGCGTTACAACCTG
 TACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTGCCCCGGCGCGCCCCCTTGC
 TGCGGAAAATCATTTTCCGGAAACCGTCCCTGACCCTCGGCGACTCGCACGCCGGACACC
 TGCGGGGGTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCC
 CTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTGCG
 AAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATT
 TGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATACCCGGG
 TTCCCAGCCCGATTTCAGGGAAACCGTCAAAGGATAGCCGCCGTCAAACCCGTCTATGT
 TTTTGCAAAACACATCAATCAGCCGTTCGCCCCCTGAGGGAGGAAAAATGAAAAGAT
 TTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGCAAT
 CAGGCGGTCTTTGATTTGATTAAAGATATTCCTCAATGTGCATTGGGTGGACGCACAAAA
 ATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGACCAAGACC
 ACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACATGAAAGCTTG
 CTCAAGCATTCACACGGCAACGCATTGCAGTAGCCTGCCTTCTTGTGCGGATATTGCCTT
 TGGCAGCCTATGCCGCTGTTTGCCCTTCGGGGCGGCGGCTTTTATAGTGGATTAACAAA
 AATCAGGACAAGGCGACGAAGCCGCAGACAGTACAAATAGTACGGAACCGATTCACTTG
 GTGCTTCAGCACCTTAGAGAATCGTTCTCTTTGAGCTAAGGCGAGGCAACGCCGTACTG

GTTTTTGTTAATCCACTATATTTTGCCGTTTTGAGGCCGGGGTCGGAATAACCGTTTTT
 TGATGATTTTCCCTCCCCGGCTGTGTCATCAAACCCCAATTGCCTTTCCAAACTCTCC
 ACCAGATTGTCATCCAGTTTCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTTTCTTT
 CGG

Table 6H. L8 lot consensus sequence (SEQ ID NO:24) ATG Translation start codon and TAG translation termination codons underlined.

ATGCAAGCTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTTGCCGTGCTATC
 CGTCATTATTTTCCACCTGAATAACCGCTGGCTGCCCCGAGGGTTTTTGGGGGTGGACA
 TTTTCTTTGTCATCTCGGGATTCCCTCATTACCGGCATCATTCTTTCTGAAATACAGAAC
 GGTTCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTACCCTGCCTT
 TATTGCGGCCGTGTGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATT
 TCAACCAAATGCGGAAAACCGGGGAGTTTTCTGCGGTTTTCTTGTTCCAATATTTATCTG
 GGGTTTCAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTG
 GTCTTTGGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCT
 GCAAAAAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATT
 TTGACTGCCACATCGTTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAA
 TACTTATTACCTTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTCGCTGCTGGCGG
 TTTACGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTT
 TCATCACTCTGCTTCGGCGCATTGCTTGCCCTGCCTGTTTCGTGATTGACAAACACAATCC
 GTTTATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCGCTGCTTATCCGGA
 GTATGCAATACGGGACACTTCCGACCCGCATCCTGTCGGCAAGCTCCATCGTATTTGTC
 GGCAAAATCTCTTATTCCCTATACCTGTACCATTGGATTTTTTATTGCTTTTCGCCCATTA
 CATTACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGG
 CCGGATTTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATG
 ACCTTCAAAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTCGGTTA
 CAACCTGTACGCAAGGGGGGATATTGAAACAGGAACACCTCCGCCCCGTGCCCCGGCGCGC
 CCCTTGCTGCGGAAAATCATTTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCC
 GGACACCTGCGGGGGTTTTCTGGATTATGTGCGGACCCGGGAAGGGTGGAAGCCAAAAT
 CCTGTCCCTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGT
 TATGTCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTC
 TATGATTTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAAT
 ACCCGGGTTCCAAGCCCGATTTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCG
 TCTATGTTTTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTG
 AAAAGATTTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCAA
 GAGCAATCAGGCGGTCTTTGATTTGATTAAAGATATTCCCAATGTGCATTGGGTGGACG
 CACAAAAATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGAC
 CAAGACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTCACAAACACGA
 ACGCCTGCTTAAATCTTCTCGCGACGGCGCATTGCAGTAG

Table 6I. L9 Lot consensus (SEQ ID NO:25)

ATGCAAGCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATC
 CGTCATGATTTTCCACCTGAATAACCGCTGGCTGCCCCGAGGATTCCTGGGGGTGGACA
 TTTTCTTTGTCATCTCAGGATTCCCTCATTACCGGCATCATTCTTTCTGAAATACAGAAC
 GGTTCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTT
 TATTGCGGCCGTGTGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATT
 TCAACCAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGTTCCAATATTTATCTG
 GGGTTTCAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTG
 GTCTTTGGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCT

GCAAAAAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATT
 TTGACTGCCACATCGTTTTTGGCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAA
 TACTTATTACCTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTGCTGCTGGCGG
 TTTACGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTT
 TCATCACTCTGCTTCGGCGCATTTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCC
 GTTTATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGA
 GTATGCAATACGGGACACTTCCGACCCGCATCCTGTGCGGCAAGCCCCATCGTATTTGTC
 GGCAAATCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCTTTCGCCCCATTA
 CATTACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGG
 CCGGATTTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATG
 ACCTTCAAAAAGGCATTTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTGCGGTTA
 CAACCTGTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGC
 CCCTTGCTGCGGAAAATCATTTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCC
 GGACACCTGCGGGGGTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAAT
 CCTGTCCCTCGATTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGT
 TATGTCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTC
 TATGATTTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAAT
 ACCCGGGTTCCCAGCCCGATTACAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCG
 TCTATGTTTTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTG
 AAAAGATTTGCCGCAAACCAATATCTCCGCCCCATTACAGGCTATGGGCGACATCGGCAA
 GAGCAATCAGGCGGTCTTTGATTTGATTAAAGATATTCCCAATGTGCATTGGGTGGACG
 CACAAAATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGAC
 CAAGACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGA
 ACGCCTGCTTAAATCTTCTCGCGACGGCGCATTTGCAGTAG

Table 6J. >L10 lot consensus sequence (SEQ ID NO:26)

GGGCAGGCTGAGCGTTGGCGAAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCTGA
 ACGCGCGTTTCAGACGGCATTTTTTAAGATAACGGGACATACAGGGGCGATATTTATGCAA
 GCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATCCGTCAT
 GATTTTCCACCTGAATAACCGCTGGCTGCCCCGAGGATTCCTGGGGGTGGACATTTTCT
 TTGTCATCTCAGGATTCCTCATTACCGGCATCATTTCTTTCTGAAATACAGAACGGTTCT
 TTTTCTTTCCGGGATTTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTTTATTGC
 GGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAACC
 AAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGTCCAATATTTATCTGGGGTTT
 CAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTT
 GGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAA
 AAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATTTTGACT
 GCCACATCGTTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAATACTTA
 TTACCTTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTACG
 GGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCA
 CTCTGCTTCGGCGCATTTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTAT
 CCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGC
 AATACGGGACACTTCCGACCCGCATCCTGTGCGCAAGCCCCATCGTATTTGTCGGCAA
 ATCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCTTTCGCCCATTACATTAC
 AGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGAT
 TTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTC
 AAAAAGGCATTTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTGCGTTACAACCT
 GTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGCCCCCTTG
 CTGCAGAAAATCATTTTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCCGGACAC
 CTGCGGGGGTTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTC

CCTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTC
 GAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTCATTGCCCAATTCTATGAT
 TTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATACCCGG
 GTTCCCAGCCCGATTTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCGTCTATG
 TTTTGTCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTGAAAAGA
 TTTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGCAA
 TCAGGCGGTCTTTGATTTGATTAAAGATATTCCCAATGTGCATTGGGTGGACGCACAAA
 AATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGACCAAGAC
 CACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGAACGCCT
 GCTTAAATCTTCTCGCGACGGCGCATTGCAGTAGCCTGCCTTGCCGTCCGATATCGTTT
 GTGCCGCCGTTTGCCTTTTCGGGGCGGCGGCGGTTTATTTTCTTCCCCTGCGGGAGG
 GAATTTTGAATCAAAACCCCAATTGCCTTTCCAAGTTTTCCACCAGATTGTCATCCAGT
 TCCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTTTCTTTCCCGAACAAATCGAA

Table 6K. L11 consensus sequence (SEQ ID NO:27); ATG translation start codon and TAG translation termination codons underlined.

GTGGGCAGGCTGAGCGTTGGCGAAACGGATTTGAGCGTTTACTGAAACCGATGCCGTCT
 GAACGCGCGTTCAGACGGCATTTTTTAAGATAACGGGACATACAGGGGCGATATTTATGC
 AAGCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTGCGCGTGCTATCCGTC
 ATGATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGATTCCTGGGGGTGGACATTTT
 CTTTGTCTATCTCAGGATTCCTCATTACCGGCATCATTTCTTCTGAAATACAGAACGGTT
 CTTTTTCTTTCCGGGATTTTTTATAACCCGCAGGATTAAGCGGATTTATCCTGCTTTTATT
 GCGGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAA
 CCAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTCTTGTCCAATATTTATCTGGGGT
 TTCAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCT
 TTGGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAA
 AAAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATTTTGA
 CTGCCACATCGTTTTTTGCCAAGCGGGTTTTTATAACCGATATTCTCAACCAACCCAATACT
 TATTACCTTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGGTTTA
 CGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCAT
 CACTCTGCTTCGGCGCATTTGCTTGCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTT
 ATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTAT
 GCAATACGGGACACTTCCGACCCGCATCCTGTTCGGCAAGCCCCATCGTATTTGTTCGGCA
 AAATCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCTTTCGCCCCATTACATT
 ACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGG
 ATTTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCT
 TCAAAAAGGCATTTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTTCGGTTACAAC
 CTGTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGCCCCCT
 TGCTGCGGAAAATCATTTTCCGGAACCGTCCTGACCCTCGGCGACTCGCACGCCGGAC
 ACCTGCGGGGGTTTCTGGATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTG
 TCCCTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATG
 TCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTCATTGCCCAATTCTATG
 ATTTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATACCC
 GGGTTCCCAGCCCGATTTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCGTCTA
 TGTTTTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTGAAAA
 GATTTGCCGCAAACCAATATCTCCGCCCCATTTCAGGCTATGGGCGACATCGGCAAGAGC
 AATCAGGCGGTCTTTGATTTGATTAAAGATATTCCCAATGTGCATTGGGTGGACGCACA
 AAAATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGACCAAG
 ACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTTACAAACACGAACGC
 CTGCTTAAATCTTCTCGCGACGGCGCATTGCAGTAGCCTGCCTTGCCGTCCGATATCGT

TTGTGCCGCCGTTTGCCTTTCGGGGCGGCGGCGGTTTTTATTTTCCTTCCCCTGCGGGA
GGGAATTTTGAATCAAAACCCCAATTGCCTTTCCTAAGTTTTCCACCAGATTGTCATCCA
GTTCCAAAGCCTGCGACAGGCGGGCGAGGAAGACGGTTTCTTTCCGCGAACAAATCGAA

Table 6L. L12 Lot consensus sequence (SEQ ID NO:28); ATG translation start codon and TAG translation termination codons underlined.

ATGCAAGCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATC
CGTCATGATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGATTCCTGGGGGTGGACA
TTTTCTTTGTCATCTCAGGATTCCTCATTACCGGCATCATTCTTTCTGAAATACAGAAC
GGTTCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTT
TATTGCGGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATT
TCAACCAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGCCAATATTTATCTG
GGGTTTCAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTG
GTCTTTGGCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCT
GCAAAAAACAAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATT
TTGACTGCCACATCGTTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAA
TACTTATTACCTTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTTCGCTGCTGGCGG
TTTACGGGCAAACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTT
TCATCACTCTGCTTCGGCGCATTTGCTTGCTGCCTGTTTCGTGATTGACAAACACAATCC
GTTTATCCCGGGAATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGA
GTATGCAATACGGGACACTTCCGACCCGCATCCTGTGCGGCAAGCCCCATCGTATTTGTC
GGCAAATCTCTTATTCCCTATACCTGTACCATTTGGATTTTTTATTGCTTTCGCCCCATTA
CATTACAGGCGACAAACAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGG
CCGGATTTTCCCTGTTGAGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATG
ACCTTCAAAAAGGCATTTTTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTGCGGTTA
CAACCTGTACGCAAGGGGGATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGC
CCCTTGCTGCGGAAAATCATTTTCCGGAAACCGTCTTGACCCTCGGCGACTCGCACGCC
GGACACCTGCGGGGGTCTTCTGGATTATGTGCGGAGCCGGGAAGGGTGGAAAGCCAAAAT
CCTGTCCCTCGATTTCGGAGTGTTTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGT
TATGTCGAAAATACCGGGATGAAGTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTC
TATGATTTGAGGATGGGCGGCCAGCCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAAT
ACCCGGGTTCAGCCCGATTTCAGGGAAACCGTCAAAAGGATAGCCGCCGTCAAACCCG
TCTATGTTTTTTGCAAACAACACATCAATCAGCCGTTTCGCCCCCTGAGGGAGGAAAAATTG
AAAAGATTTGCCGCAAACCAATATCTCCGCCCCATTCAGGCTATGGGCGACATCGGCAA
GAGCAATCAGGCGGTCTTTGATTTGATTAAAGATATTCCTCAATGTGCATTGGGTGGACG
CACAAAATACCTGCCCAAAAACACGGTCGAAATATACGGCCGCTATCTTTACGGCGAC
CAAGACCACCTGACCTATTTTCGGTTCTTATTATATGGGGCGGGAATTCACAAACACGA
ACGCCTGCTTAAATCTTCTCGCGACGGCGCATTTGCAGTAG

The lot genes are intact in *N. meningitidis* strain Z2491 and in *N. gonorrhoeae* strain FA1090. However, in *N. gonorrhoeae* the *lot* gene has been duplicated in two separate loci. There is a translational frameshift in *N. meningitidis* strain MC58.

See additional *lot* coding sequences below.

Table 7. *Neisseria meningitidis* Strain Z2491 (SEQ ID NO:29)

NMA2202 Coding sequence

ATGCAAGCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATCC
 GTCATGATTTTCCACCTGAATAACCGCTGGCTGCCCCGAGGATTCCTGGGGGTGGACATT
 TTCTTTGTCATCTCAGGATTCCTCATTACCGGCATCATTCTTTCTGAAATACAGAACGGT
 TCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTTTATT
 GCGGCCGTGTCGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAAC
 CAAATGCGGAAAACCGTGGAGCTTTCTGCGGTTTTCTTGCCAATATTTATCTGGGGTTT
 CAGCAGGGGTATTTGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG
 GCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAAAA
 ACAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATTTTGACTGCC
 ACATCGTTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAATACTTATTAC
 CTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTGCTGCTGGCGGTTTACGGGGCAA
 ACGCAAAACGGCAGACGGCAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGC
 TTCGGCGCATTGCTTGCCTGCCTGTTGCTGATTGACAAACACAATCCGTTTATCCCGGGA
 ATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGG
 ACACTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTCGGCAAAATCTCTTAT
 TCCCTATACCTGTACCATTGGATTTTTATTGCTTTCGCCCATTACATTACAGGCGACAAA
 CAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATTTTCCCTGTTG
 AGTTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCAAAAAGGCATTT
 TTCTGCCTCTATCTCGCCCCGTCCCTGATACTTGTCGGTTACAACCTGTACGCAAGGGGG
 ATATTGAAACAGGAACACCTCCGCCCCGTTGCCCGGCGCGCCCCCTTGCTGCGGAAAATCAT
 TTTCCGGAAACCGTCCTGACCCTCGGCGACTCGCACGCCGGACACCTGCGGGGGGTTTCTG
 GATTATGTCGGCAGCCGGGAAGGGTGGAAAGCCAAAATCCTGTCCCTCGATTCCGGAGTGT
 TTGGTTTGGGTAGATGAGAAGCTGGCAGACAACCCGTTATGTCGAAAATACCGGGATGAA
 GTTGAAAAAGCCGAAGCCGTTTTTCATTGCCCAATTCTATGATTTGAGGATGGGCGGCCAG
 CCCGTGCCGAGATTTGAAGCGCAATCCTTCCTAATACCCGGGTTCAGCCCGATTGAGG
 GAAACCGTCAAAGGATAGCCGCCGTCAAACCCGTCTATGTTTTTGCAAACAACACATCA
 ATCAGCCGTTGCCCCCTGAGGGAGGAAAAATTGAAAAGATTTGCCGCAAACCAATATCTC
 CGCCCCATTGAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGATT
 AAAGATATTCCCAATGTGCATTGGGTGGACGCACAAAAATACCTGCCCAAAAACACGGTC
 GAAATATACGGCCGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTGCGTTCTTAT
 TATATGGGGCGGGAATTTACAAACACGAACGCCTGCTTAAATCTTCTCGCGACGGCGCA
 TTGCAGTAG

NMA2202 Encoded amino acid sequence (SEQ ID NO:30)

MQAVRYRPEIDGLRAVAVLSVMIFHLNNRWLPGGFLGVDIFFVISGFLITGIILSEIQNG
 SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTVELSAVFLSNIYLG
 QQGYFDLSADENPVLHIWSLAVEEQYYLLYPLLLIFCCKKTKSLRVLRNISIILFLILTA

TSFLPSGFYTDILNQPNTYYLSTLRFPELLAGSLLAVYGQTQNGRRQTANGKRQLLSSLC
 FGALLACLFVIDKHNPFI PGMTLLLPCLLTALLIRSMQYGTLPTRILSASPIVFVGKISY
 SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
 FCLYLAPSLILVGYNLYARGILKQEHLRPLPGAPLAAENHFPETVLT LGDSHAGHLRGFL
 DYVGSREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
 PVPRFEAQSFLIPGF PARFRET VKRIA AVKPVYVFANNTSISR SPLREEKLKRFAANQYL
 RPIQAMGDIGKSNQAVFDLIK DIPNVHWVDAQKYL PKNTVEIYGRYLYGDQDHLTYFGSY
 YMGREFHKHERLLKSSRDGALQ

Table 8. *Neisseria gonorrhoeae* strain FA1090 (SEQ ID NO:31)

NGO1710 Coding sequence

ATGCAAGCTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATCC
 GTCATTATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGATTCCTGGGGGTGGACATT
 TTCTTTGTCATCTCGGGATTCCTCATTACCAACATCATTCTTTCTGAAATACAGAACGGT
 TCTTTTTCTTTCCGGGATTTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTTTATT
 GCGGCCGTGTCCCTGGCTTCGGTGATTGCTTCTCAAATCTTCCTTTACGAAGATTTCAAC
 CAAATGAGGAAAACCATAGAGCTTTCTACGGTTTTTTTTGTCCAATATTTATTTGGGGTTC
 CGATTGGGGTATTTGATTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG
 GCGGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTCTGTTACAAAAA
 ACCAAATCACTACGGGTGCTGCGTAATATCAGCATCATCCTGTTTCTGATTTTGACCGCA
 TCATCGTTTTTTGCCGGCCGGGTTTTATACCGACATCCTCAACCAACCCAATACTTATTAC
 CTTTCGACACTGAGGTTTCCCGAGCTGTTGGTGGGTTGCTGTTGGCGGTTTACGGGCAA
 ACGCAAAACGGCAGACGGCAAACAGAAAATGGAAAACGGCAGTTGCTTTCATTACTCTGT
 TTCGGCGCATTGCTTGTCTGCCTGTTGCTGATCGACAAACACGATCCGTTTATCCCGGGA
 ATAACCCTGCTCCTTCCCTGCCTGCTGACGGCGCTGCTTATCCGGAGTATGCAATACGGG
 ACACTTCCGACCCGCATCCTGTCGGCAAGCCCCATCGTATTTGTCGGCAAAATCTCTTAT
 TCCCTATACCTGTACCATTGGATTTTTATTGCCTTCGCCCATTACATTACAGGCGACAAA
 CAGCTCGGACTGCCTGCCGTATCGGCGGTTGCCGCGTTGACGGCCGGATTTTCCCTGTTG
 AGCTATTATTTGATTGAACAGCCGCTTAGAAAACGGAAGATGACCTTCAAAAAGGCATTT
 TTCTGCCTTTATCTCGCCCCGTCCCTGATGCTTGTGCGGTTACAACCTGTATTCAAGAGGG
 ATATTGAAACAGGAACACCTCCGCCCCGCTGCCCGGCACGCCCCGTTGCTGCGGAAAATAAT
 TTTCCGGAAACCGTCTTGACCCTCGGCGACTCGCACGCCGGACACCTGCGGGGGTTTCTG
 GATTATGTCGGCGGCAGGGAAGGGTGGAAAGCTAAAATCCTGTCCCTCGATTGCGGAGTGT
 TTGGTTTGGGTGGATGAGAAGCTGGCAGACAACCCGTTGTGCCGAAAATACCGGGATGAA
 GTTGAAAAAGCCGAAGCTGTTTTTATTGCCCAATTCTATGATTTGAGGATGGGCGGCCAG
 CCCGTGCCGAGATTTGAAGCGCAATCCTTCCTGATACCCGGGTTCAAAGCCCGATTCAGG
 GAAACCGTCAAGAGGATAGCCGCCGTCAAACCTGTATATGTTTTTGCAAACAATACATCA
 ATCAGCCGTTCTCCCTTGAGGGAGGAAAAATTGAAAAGATTTGCTATAAACCAATACCTC

CGGCCTATTCGGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGGTT
 AAAGATATTCCCAATGTGCATTGGGTGGACGCACAAAAATACCTGCCCAAAAACACGGTC
 GAAATACACGGACGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTCGGTTCTTAT
 TATATGGGGCGGGAATTTACAAACACGAACGCCTGCTCAAGCATTCCCGAGGCGGCGCA
 TTGCAGTAG

Table 9. NGO1710 Encoded amino acid sequence (SEQ ID NO:32)

MQAVRYRPEIDGLRAVAVLSVIFHLNNRWLPGGFLGVDIFFVISGFLITNIILSEIQNG
 SFSFRDFYTRRIKRIYPAFIAAVSLASVIASQIFLYEDFNQMRKTIELSTVFLSNIYLG
 RLG YFDLSADENPVLHIWSLAVEEQYYLLYPLLLIFCYKKTSLRVLRNISILFLILTA
 SSFLPAGFYTDILNQPNTYYLSTLRFPELLVGSLLAVYGQTQNGRRQTENGKRQLLSLLC
 FGALLVCLFVIDKHDPFIPGITLLLPCLLTALLIRSMQYGTLPTRILSASPIVFGKISY
 SLYLYHWIFIAFAHYITGDKQLGLPAVSAVAALTAGFSLLSYYLIEQPLRKRKMTFKKAF
 FCLYLAPSLMLVGYNLYSRGILKQEHLRPLPGTPVAAENNFETVLTGLGDSHAGHLRGFL
 DYVGGREGWKAKILSLDSECLVWVDEKLADNPLCRKYRDEVEKAEAVFIAQFYDLRMGGQ
 PVPRFEAQSFIPGFKARFRET VKRIA AVKPVYVFANNTSISRSP LREEKLKRFAINQYL
 RPIRAMGDIGKSNQAVFDLVKDIPNVHWVDAQKYL PKNTVEIHGRYLYGDQDHLTYFGSY
 YMGREFHKHERLLKHSRGGALQ

Table 10. NGO0065 Coding sequence (SEQ ID NO:33)

ATGAGCCAAGCCTTACCCTACCGCCCGGACATCGACACATTGCGCGCCGCGCCGTCTTG
 TCCGTCATCGTGTTCCATATCGAAAAGGATTGGCTGCCGGGCGGGTTTCTCGGTGTCGAT
 ATATTCTTTGTGATTT CAGGCTTTTTGATGACGGCGATCCTCCTTCGCGAAATGTCCGGG
 GGGCGTTTCTTCCTCAAGACATTTTATATCCGCCGCATCAAACGGATTTTGCCCGCATTT
 TTCGCCGTATTGGCGGCAACGCTGGCAGGCGGCTTCTTTTTATTACCAAAGATGATTTC
 TTTCTTTTGTGGAAATCCGCGCTGACCGCCTTGGGTTTCGCCTCCAACCTGTATTTTGCA
 AGGGGGAAGGATTATTTTCGATCCCGCGCAGGAAGAAAAGCCCCTGCTGCACATCTGGTCT
 TTGTCGGTCGAAGAACAATTTTACTTTGTCTTTCCGATATTGCTGTTGCTTGTCGCCCCG
 AAAAGCCTGCGCGTACAGTTCGGCTTCCTCGCCGCATTGTGCGCCTTAAGCCTTGCCGCT
 TCCTTTATGCCTTCGCGCTCGATAAATATTACCTGCCCCACCTGCGCGCCTGCGAAATG
 CTGGTCGGATCGCTGACCGCCGTGCGGATGCGGTACCGGCAACAGCGGAATCCCGCCGT
 C
 GGGAAACGGTATGCCGCCGTGCGCGCATTGTTTTCCGCGTGCATACTGTCCGCCTGCCTG
 TTTGCCTATTGGAACAAACCGCCTATTTCCCGGGCCCCGCGCTTTGATTCCCTGTCTG
 GCTGTTGCCGCGCTGATTTATTTCAACCATTACGAACACCCGCTTAAAAAATTTTCCAA
 TGGAATACACCGTTGCCGCCGGTTTGATTTCTATTGCTTTATCTGTGGCATTGGCCG
 ATATTGGCCTTTATGCGCTATATCGGCCCGGACAACCTGCCGCCTTATTCGCCGGCGGCA
 GCGATCGTCCTGACCCTGGCGTTTTCCCTGATTTCTTATCACTGCATCGAAAAGCCGTTT

AAAAAATGGAAAGGCTCGTTCGCACAATCCGTTTTATGGATTTATGCCTTGCCTATGCTC
 GTTTTGGGCGCGGGCTCGTTTTTCGCGATGAGGCTGCCGTTTATGGCGCAATACGACCGC
 TTGGGGCTGACGCGTTCCAACACCTCCTGCCACAACAATACCGGCAAACAATGCCTGTGG
 GGGGATACGGAAAAACAGCCGGAAGTCTGGTTTTGGGCGACTCCCACGCCGACCATTAC
 AAAACATTCTTCGATGCCGTGGGCAAAAAAGAAAAATGGTCCGCCACTATGGTTTCCGCC
 GACGCCTGCGCCTATGTGGAAGGCTACGCGTCCCGTGTGTTCCAAAAGTGGGCCGCCTGC
 CGCGCCGTTTACCGCTATGCCGAAGAACACCTGCCCCGGTATCCGAAAGTGGTTTTGGCG
 ATGCGCTGGGGCAGCCAGATGCCCGAAAACAGCCGCTCCCTTGCCTACGATGCCGGTTTT
 TTCCAAAATTTCGACCGTATGCTGCACAACTCTCATCCGAAAAACAAGCCGTTTACCTG
 ATGGCGGACAACCTTGGCTTCGTCTTACAACGTCCAGCGCGCCTATATCTTGTCTTCACGC
 ATACCGGGTTGCCGCCAAACACTGCGCCCGGACGACGAAAGCACCTGAAAGCCAATGCC
 CGCATCAGGGAATTGGCAGCCAAATACCCCAACGTCTATATTATTGATGCCGCCGCCTAT
 ATCCCCGCAGATTTCCAAATCGGCGGATTGCCGGTTTACTCGGACAAAGACCACATCAAC
 CCTTACGGCGGCACAGAATTGGCGAAGCGTTTTTCCGAAAAACAAGGTTTCTCGATACG
 CGCCATAACCATTGA

Table 11. NGO0065 Encoded amino acid sequence (SEQ ID NO:34)

MSQALPYRPDIDTLRAAAVLSVIVFHIEKDWLPGGFLGVDIFFVISGFLMTAILREMSG
 GRFFLKTFYIRRIKRILPAFFAVLAATLAGGFFLFTKDDFFLLWKSALTALGFASNLYFA
 RGKDYFDPAQEEKPLLHIWSLSVEEQFYFVPILLLLVARKSLRVQFGFLAALCALSLAA
 SFMPSALDKYYLPHLRACEMLVGSLTAVRMRYRQQRNPAVGKRYAAVGALFSACILSACL
 FAYSEQTAYFPGPAALIPCLAVAALIYFNHYEHPLKKFFQWKITVAAGLISYSLYLWHWP
 ILAFMRYIGPDNLPPYSPAAAIVLTLAFSLISYHCIEKPFKKWKGSFAQSVLWIYALPML
 VLGAGSFFAMRLPFMAQYDRLGLTRSNTSCHNNTGKQCLWGDTEKQPELLVLGDASHADHY
 KTFFDAVGKKEKWSATMVSADACAYVEGYASRVFQNWAACRAVYRYAEEHLPRYPKVVLA
 MRWGSQMPENSRLAYDAGFFQKFDRMLHKLSSEKQAVYLMADNLASSYNVQRAYILSSR
 IPGCRQTLRPDDESTLKANARIRELAAKYPNVYIIDAAAYIPADFAQIGGLPVYSDKDHIN
 PYGGTELAKRFESEKQRFLDTRHNH

This Sanger Center database contains *N. meningitidis* strain FAM18
 (serogroup C) and *N. lactamica* (commensal neisseria). Neither database is
 annotated. Nucleotide blast alignments are given below indicating co-ordinates
 in the respective genomes.

Table 12. *Neisseria meningitidis* serogroup C FAM18 (SEQ ID NO:37, Query;
SEQ ID NO:38, Subject)

Plus Strand HSPs:

Score = 9120 (1374.4 bits), Expect = 0., P = 0.

Identities = 1844/1869 (98%), Positives = 1844/1869 (98%), Strand = Plus / Plus

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Query:      1  ATGCAAGCTGTCCGATACAGACCGGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATCC  60
           |||
Sbjct: 292641 ATGCAAGCTGTCCGATACAGGCCTGAAATTGACGGATTGCGGGCCGTCGCCGTGCTATCC
292700

Query:      61  GTCATGATTTTCCACCTGAATAACCGCTGGCTGCCCGGAGGATTCCTGGGGGTGGACATT  120
           |||
Sbjct: 292701 GTCATGATTTTCCACCTGAATGACCGCTGGCTGCCCGGAGGGTTTTTGGGGGTGGACATT
292760

Query:     121  TTCTTTGTCATCTCAGGATTCCTCATTACCGGCATCATTCCTTTCTGAAATACAGAACGGT  180
           |||
Sbjct: 292761 TTCTTTGTCATCTCGGGATTCCTCATTACCGGCATCATTCCTTTCTGAAATACAGAACGGT
292820

Query:     181  TCTTTTCTTTCCGGGATTTTATACCCGCAGGATTAAGCGGATTTATCCTGCTTTTATT  240
           |||
Sbjct: 292821 TCTTTTCTTTCCGGGGTTTTTATACCCGCAGGATTAAGCGGATTTACCTGCTTTTATT
292880

Query:     241  GCGGCCGTGTGCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAAC  300
           |||
Sbjct: 292881 GCGGCTGTGTCCCTGGCTTCGGTGATTGCCTCTCAAATCTTCCTTTACGAAGATTTCAAC
292940

Query:     301  CAAATGCGGAAAACCGTGAGCTTTCTGCGGTTTTCTTGTTCCAATATTTATCTGGGGTTT  360
           |||
Sbjct: 292941 CAAATGCGGAAAACCGTGAGCTTTCTGCGGTTTTCTTGTTCCAATATTTATCTGGGGTTT
293000

Query:     361  CAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG  420
           |||
Sbjct: 293001 CAGCAGGGGTATTTTCGATTTGAGTGCCGACGAGAACCCCGTACTGCATATCTGGTCTTTG
293060

Query:     421  GCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAAAA  480
           |||
Sbjct: 293061 GCAGTAGAGGAACAGTATTACCTCCTGTATCCTCTTTTGCTGATATTTTGCTGCAAAAAA
293120

Query:     481  ACAAATCGCTACGGGTGCTGCGTAACATCAGCATCATCCTATTTCTGATTTTGACTGCC  540
           |||
Sbjct: 293121 ACCAAATCGCTACGGGTGTTGTGCCACATCAGCATCATCCTGTTTTTGATTTTGACTGCC
293180

Query:     541  ACATCGTTTTTGCCAAGCGGGTTTTATACCGATATTCTCAACCAACCCAATACTTATTAC  600
           |||
Sbjct: 293181 TCATCGTTTTTGCCAAGCGGGTTTTATACCGACATCTCAACCAACCCAATACTTATTAC
293240

Query:     601  CTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTGCTGCTGGCGGTTTACGGGCAA  660
           |||
Sbjct: 293241 CTTTCGACACTGAGGTTTCCCGAGCTGTTGGCAGGTTGCTGCTGGCGGTTTACGGGCAA
293300

Query:     661  ACGCAAAACGGCAGACGGCAAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGC  720
           |||
Sbjct: 293301 ACGCAAAACGGCAGACGGCAAAACAGCAAATGGAAAACGGCAGTTGCTTTCATCACTCTGC
293360

Query:     721  TTCGGCGCATTGCTTGCCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTATCCCGGGA  780
           |||
Sbjct: 293361 TTCGGCGCATTGCTTGCCCTGCCTGTTTCGTGATTGACAAACACAATCCGTTTATCCCGGGA
293420

Query:     781  ATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGG  840
           |||
Sbjct: 293421 ATGACCCTGCTCCTTCCCTGCCTGCTGACGGCACTGCTTATCCGGAGTATGCAATACGGG
293480

Query:     841  ACACTTCCGACCCGCATCCTGTCCGCAAGCCCCATCGTATTTCTCGGCAAAATCTCTTAT  900

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Query: 1621 CGCCCCATTCAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGATT
1680
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 294261 CGCCCCATTCAGGCTATGGGCGACATCGGCAAGAGCAATCAGGCGGTCTTTGATTTGATT
294320

Query: 1681 AAAGATATTCCCAATGTGCATTGGGTGGACGCACAAAAATACCTGCCCAAAAACACGGTC
1740
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 294321 AAAGATATTCCCAATGTGCATTGGGTGGACGCACAAAAATACCTGCCCAAAAACACGGTC
294380

Query: 1741 GAAATATACGGCCGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTTCGGTTCCTAT
1800
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 294381 GAAATATACGGCCGCTATCTTTACGGCGACCAAGACCACCTGACCTATTTTCGGTTCCTAT
294440

Query: 1801 TATATGGGGCGGGAATTTACAAAACACGAACGCCTGCTTAAATCTTCTCGCGACGGCGCA
1860
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 294441 TATATGGGGCGGGAATTTACAAAACACGAACGCCTGCTTAAATCTTCTCGCGACGGCGCA
294500

Query: 1861 TTGCAGTAG 1869
      |||||||
Sbjct: 294501 TTGCAGTAG 294509

```

N. lactamica has not been assembled into genome. The position indicators below are for an assembled read. In addition, note this is the reverse complement of the *lot* gene.

Table 10. >lact221a11.p2kA115 373210 bp, 7825 reads, 47.57 AT

Length = 373,210

Minus Strand HSPs:

```

Score = 8907 (1342.5 bits), Expect = 0., P = 0.
Identities = 1825/1869 (97%), Positives = 1825/1869 (97%), Strand = Minus /
Plus
Query: 1869 CTACTGCAATGCGCCGCTCGCGAGAAGATTTAAGCAGGCGTTTCGTGTTTGTGAAATTCCCG 1810
      ||||||||||||||||| || ||||||||||||||||||||||||||||||||||||
Sbjct: 14561 CTACTGCAATGCGCCGCTGGGAAGATTTAAGCAGGCGTTTCGTGTTTGTGAAATTCCCG
14620

Query: 1809 CCCCATATAATAAGAACCGAAATAGGTCAGGTGGTCTTGGTCGCCGTAAAGATAGCGGCC 1750
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 14621 CCCCATATAATAAGAACCGAAATAGGTCAGGTGGTCTTGGTCGCCGTAAAGATAGCGTCC
14680

Query: 1749 GTATATTTTCGACCGTGTTTTTTGGGCAGGTATTTTTGTGCGTCCACCCAATGCACATTGGG 1690
      || ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 14681 GTGTATTTTCGACCGTGTTTTTTGGGCAGGTATTTTTGTGCGTCCACCCAATGCACATTGGG
14740

Query: 1689 AATATCTTTAATCAAATCAAAGACCGCCTGATTGCTCTTGCCGATGTGCCCATAGCCTG 1630
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 14741 AATATCTTTAATCAAATCAAAGACCGCCTGATTGCTCTTGCCGATGTGCCCATAGCCTG
14800

Query: 1629 AATGGGGCGGAGATATTGGTTTGGCGCAAATCTTTTCAATTTTTCCTCCCTCAGGGGCGA 1570
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 14801 AATGGGGCGGAGATATTGGTTTGGCGCAAATCTTTTCAATTTTTCCTCCCTCAGGGGCGA
14860

Query: 1569 ACGGCTGATTGATGTGTTGTTTGCAAAAACATAGACGGGTTTGACGGCGGCTATCCTTTT 1510
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 14861 ACGGCTGATTGATGTGTTGTTTGCAAAAACATAGACGGGTTTGACGGCGGCTATCCTTTT
14920

Query: 1509 GACGGTTTCCCTCAATCGGGCTGGGAACCCGGGTATTAGGAAGGATTGCGCTTCAAATCT 1450

```

```

Sbjct: 14921 |||||GACGGTTTCCCTGAATCGGGCTTGGAACCCGGGTATTAGGAAGGATTGCGCTTCAAACTCT
14980

Query: 1449 CCGCACGGGCTGGCCGCCCATCCTCAAATCATAGAATTGGGCAATGAAAACGGCTTCGGC 1390
|||

Sbjct: 14981 CCGCACGGGCTGGCCGCCCTATCCTCAAATCATAGAATTGGGCAATGAAAACGGCTTCGGC
15040

Query: 1389 TTTTTCAACTTCATCCCGGTATTTTCGACATAACGGGTGTCTGCCAGCTTCTCATCTAC 1330
|||

Sbjct: 15041 TTTTTCAACTTCATCCCGGTATTTTCGACATAACGGGTGTCTGCCAGCTTCTCATCTAC
15100

Query: 1329 CCAAACCAAACACTCCGAATCGAGGGACAGGATTTTGGCTTTCCACCCTTCCCGGCTGCC 1270
|||

Sbjct: 15101 CCAAACCAAACACTCCGAATCGAGGGACAGGATTTTGGCTTTCCACCCTTCCCGGCTGCC
15160

Query: 1269 GACATAATCCAGAAACCCCGCAGGTGTCCGGCGTGCGAGTCGCCGAGGGTCAGGACGGT 1210
|||

Sbjct: 15161 GACATAATCCAGAAACCCCGCAGGTGTCCGGCGTGCGAGTCGCCGAGGGTCAGGACGGT
15220

Query: 1209 TTCCGGAAAATGATTTTCCGCAGCAAGGGGCGCGCCGGGCAACGGGCGGAGGTGTTCTCTG 1150
|||

Sbjct: 15221 TTCCGGAAAATGATTTTCCGCAGCAAGGGGCGCGCCGGGCAACGGGCGGAGGTGTTCTCTG
15280

Query: 1149 TTTCAATATCCCCCTTGCGTACAGGTGTGTAACCGACAAGTATCAGGGACGGGGCGAGATA 1090
|||

Sbjct: 15281 TTTCAATATCCCCCTTGCGTACAGGTGTGTAACCGACAAGTATCAGGGACGGGGCGAGATA
15340

Query: 1089 GAGGCAGAAAAATGCCTTTTGAAGGTCATCTTCCGTTTCTAAGCGGCTGTTCAATCAA 1030
|||

Sbjct: 15341 GAGGCAGAAAAATGCCTTTTGAAGGTCATCTTCCGTTTCTAAGCGGCTGTTCAACCAA
15400

Query: 1029 ATAATAACTCAACAGGGAAAAATCCGGCCGTCAACGCGGCAACCGCCGATACGGCAGGCAG 970
|||

Sbjct: 15401 ATAATAACTCAACAGGGAAAAATCCGGCCGTCAATGCGGCAACCGCCGATACGGCAGGCAG
15460

Query: 969 TCCGAGCTGTTTGTGCGCTGTAATGTAATGGGCGAAAGCAATAAAAAATCCAATGGTACAG 910
|||

Sbjct: 15461 TCCGAGCTGTTTGTGCGCTGTAATGTAATGGGCGAAAGCAATAAAAAATCCAATGGTACAG
15520

Query: 909 GTATAGGGAATAAGAGATTTTGCCGACAAATACGATGGGGCTTGCCGACAGGATGCGGGT 850
|||

Sbjct: 15521 GTATAGGGAATAAGAGATTTTGCCGACAAATACGATGGGGCTTGCCGACAGGATGCGTGT
15580

Query: 849 CGGAAGTGTCCCGTATTGCATACTCCGGATAAGCAGTGCCGTCAGCAGGCAGGGAAGGAG 790
|||

Sbjct: 15581 CGGAAGTGTCCCGTATTGCATACTCCGGATAAGCAGCGCCGTCAGCAGGCAGGGAAGGAG
15640

Query: 789 CAGGGTCATTCCCGGATAAACGGATTGTGTTTGTCAATCACGAACAGGCAGGCAAGCAA 730
|||

Sbjct: 15641 CAGGGTCATTCCCGGATAAACGGATTGTGTTTGTCAATCACGAACAGGCAGGCAAGCAA
15700

Query: 729 TGCGCCGAAGCAGAGTGATGAAAGCAACTGCCGTTTCCATTTGCTGTTTGCCGTCCTGCC 670
|||

Sbjct: 15701 TGCGCCGAAGCAGAGTGATGAAAGCAACTGCCGTTTCCATTTGCTGTTTACCGT-T ---
15756

Query: 669 GTTTTGCCTTTGCCCGTAAACCGCCAGCAGCGAACCTGCCAACAGCTCGGGAAACCTCAG 610
|||

Sbjct: 15757 --TTC-CGTTTGCCCATAAACCGCCAGCAGCGAACCTGCCAACAGCTCGGGAAACCTCAG
15813

Query: 609 TGTCGAAAGGTAATAAGTATTGGGTTGGTTGAGAATATCGGTATAAAACCCGCTTGGCAA 550
|||

```

Sbjct: 15814 TGTCGAAAGGTAATAAGTATTGGGTTGGTTGAGGATGTCGGTATAAAACCCGCTTGGCAA
15873

Query: 549 AAACGATGTGGCAGTCAAAATCAGAAATAGGATGATGCTGATGTTACGCAGCACCCGTAG 490
|||||
Sbjct: 15874 AAACGATGAGGCAGTCAAAATCAGAAACAGGATGATGCTGATGTTACGCAGCACCCGTAG
15933

Query: 489 CGATTTTGTFTTTTTCAGCAAAATATCAGCAAAAGAGGATACAGGAGGTAATACTGTTC 430
|||||
Sbjct: 15934 CGATTTGGTFTTTTTCAGCAGAATATCAGCAAAAGGGGATACAGGAGGTAATACTGTTC
15993

Query: 429 CTCTACTGCCAAAGACCAGATATGCAGTACGGGGTTCTCGTCGCCACTCAAATCGAAATA 370
|||||
Sbjct: 15994 CTCTACCGCCAAAGACCAAATATGCAGTACGGGGTTCTCGTTGGCACTCAAATCGAAATA
16053

Query: 369 CCCCTGCTGAAACCCAGATAAATATTGGACAAGAAAACCGCAGAAAGCTCCACGGTFTT 310
|||||
Sbjct: 16054 CCCCTGCTGAAACCCCAAATAAATATTGGACAAGAAAACCGCAGAAAGCTCCACGGTFTT
16113

Query: 309 CCGCATTTGGTTGAAATCTTCGTAAAGGAAGATTTGAGAGGCAATCACCGAAGCCAGCGA 250
|||||
Sbjct: 16114 CCGCATTTGGTTGAAATCTTCGTAAAGGAAGATTTGAGAGGCAATCACCGAAGCCAGCGA
16173

Query: 249 CACGGCCGCAATAAAAGCAGGATAAATCCGCTTAATCCTGCGGGTATAAAAATCCCGGAA 190
|||||
Sbjct: 16174 CACGGCCGCAATAAAAGGCAGGATAAATCCGCTTAATCCTGCGGGTATAAAAATCCCGGAA
16233

Query: 189 AGAAAAAGAACCGTTCTGTATTTTCAGAAAGAATGATGCCGGTAATGAGGAATCCTGAGAT 130
|||||
Sbjct: 16234 AGAAAAAGAACCGTCCCGTATTTTCGGCAAGAATGATGCCGGTAATGAGGAATCCCGAGAT
16293

Query: 129 GACAAAGAAAATGTCCACCCCCAGGAATCCTCCGGGCAGCCAGCGGTTATTCAGGTGGAA 70
|||||
Sbjct: 16294 GACAAAGAAAATGTCCACCCCCAGGAATCCTCCGGGCAGCCAACGGTTATTCAGGTGGAA
16353

Query: 69 AATCATGACGGATAGCACGGCGACGGCCCCGCAATCCGTCAATTTCCGGTCTGTATCGGAC 10
|||||
Sbjct: 16354 AATAATGACGGATAGCACGGCGACAGCCCCGCAATCCGTCAATTTCCGGTCTGTATCGGAC
16413

Query: 9 AGCTTGCAT 1
|||||
Sbjct: 16414 AGCTTGCAT 16422

Without wishing to be bound by any particular theory, it is believed that the *N. lactamica* sequence provided above encoded a functional O-acetyl transferase enzyme with that equivalent to that of the *N. meningitidis* Lot3 protein exemplified herein.

Table 11. Immunotyping strains used in this study.

Immunotype	Strain which has been chemically defined	Reference	Strains used in this study
L1	126E	(9)	126E
L2	Serogroup C 2241	(10)	35E
L3	Serogroup B MC58	(13)	6275

L4	Serogroup C strain 89I	(11)	89I
L5	Serogroup B 981	(12)	M981
L6	M992	(9)	M992
L7	Serogroup C strain M982B	(11)	6155
L8			M978
L9	Serogroup A		120M
L10	Serogroup A		7880
L11	Serogroup A		7889
L12	Serogroup A		7897

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DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

CLAIMS

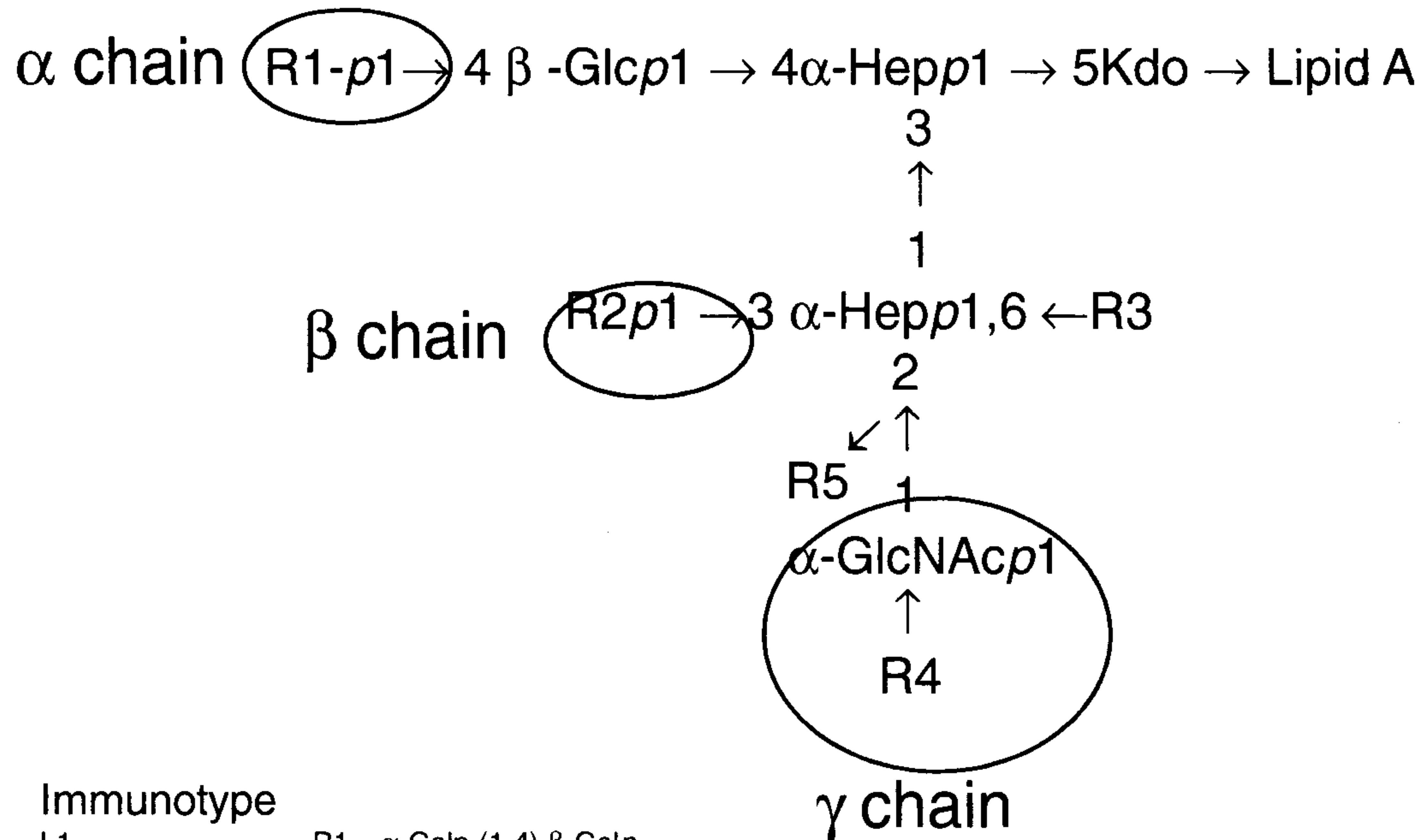
We claim:

1. A DNA molecule comprising a *Neisseria lot* 3-O-acetyltransferase protein coding sequence and a transcriptional control sequence with which said 3-O-acetyltransferase coding sequence is not associated in nature, said coding sequence and said transcriptional control sequence being operably linked.
2. The DNA molecule of claim 1, wherein said O-acetyltransferase protein comprises the amino acid sequence of any of SEQ ID NOs:5 to 16 or SEQ ID NO:30, or a sequence having at least 25%, at least 50%, at least 75%, at least 85%, at least 90% or at least 95% sequence identity to SEQ ID NO:5, and wherein said protein has enzymatic activity of a lipooligosaccharide 3-O-acetyltransferase.
3. The DNA molecule of claim 2, wherein said 3-O-acetyltransferase coding sequence comprises the amino acid sequence as given in any of SEQ ID NOs:32 or 34 or comprises an amino acid sequence with at least to 25%, at least 50%, at least 75%, at least 85%, at least 90% or at least 95% sequence identity to the amino acid sequence set forth in SEQ ID NO:32.
4. The DNA molecule of any of claim 1 to 3, wherein the 3-O-acetyltransferase coding sequence comprises the sequence set forth in any of SEQ ID NO:17-32 or a sequence functionally equivalent to one of the foregoing.
5. The DNA molecule of claim 1, further comprising a sequence encoding an affinity tag, said sequence being fused in frame, selected from the group consisting of a streptavidin tag, a flagellar antigen epitope tag, a polyhistidine tag, a glutathione-S-transferase tag or a calmodulin tag or a streptactin tag.
6. The DNA molecule of claim 1, further comprising a vector sequence functional in a bacterial cell.

7. The DNA molecule of claim 6, wherein the vector sequence is functional in *Escherichia coli*.
8. The DNA molecule of claim 6, wherein the vector sequence is functional in *Neisseria meningitidis* or *Neisseria gonorrhoeae*.
9. A bacterial host cell in which the DNA molecule of any of claims 1 to 8 is stably maintained.
10. A method for recombinantly producing an O-acetyltransferase comprising the step of culturing the bacterial host cell of claim 9 under conditions such that the Lot3 O-acetyltransferase is expressed.
11. The method of claim 9 further comprising recovering the Lot3 O-acetyltransferase.
12. A method for acetylating lipooligosaccharide prepared from *Neisseria meningitidis*, said method comprising the step of contacting an isolated lipooligosaccharide with the 3-O-acetyltransferase produced by the method of claim 11.
13. An improved immunogenic composition comprising an acetylated lipooligosaccharide of *Neisseria meningitidis*, wherein the improvement comprises acetylation of the lipooligosaccharide according to the method of claim 12.
14. A lipooligosaccharide composition prepared from a *lot3* mutant of *Neisseria meningitidis*.
15. A lipooligosaccharide composition prepared from a mutant of *Neisseria meningitidis* in which a *lot3* and a *lgt* gene product are not functional.

16. A mutant of *Neisseria meningitidis* in which a *lot3* gene product is not functional.
17. A mutant of *Neisseria meningitidis* in which a *lot3* gene product and a *lgt* gene product are not functional.

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Immunotype

L1	R1 = α -Galp-(1-4)- β -Galp R2 = 3-PEA R3 = H R4 = Ac
L2	R1 = α -Neu5Ac-(2 $\rightarrow$ 3)- β -Galp1 $\rightarrow$ 4 β -Glc pNAc1 $\rightarrow$ 3 β -Galp R2 = α -Glc p R3 = 6-PEA R4 = Ac (40%) R5 = Gly or O-7 PEA*
L3	R1 = α -Neu5Ac-(2 $\rightarrow$ 3)- β -Galp1 $\rightarrow$ 4 β -Glc pNAc1 $\rightarrow$ 3 β -Galp R2 = 3-PEA R3 = H R4 = H R5 = Gly
L4	R1 = α -Neu5Ac-(2 $\rightarrow$ 3)- β -Galp1 $\rightarrow$ 4 β -Glc pNAc1 $\rightarrow$ 3 β -Galp R2 = H R3 = 6-PEA R4 = Ac (50%) R5 = Gly
L5	R1 = α -Neu5Ac-(2 $\rightarrow$ 3)- β -Galp1 $\rightarrow$ 4 β -Glc pNAc1 $\rightarrow$ 3 β -Galp1 $\rightarrow$ 4 β -Glc p1 R2 = α -Glc p R3 = H R4 = Ac (60%)
L6	R1 = β -Glc pNAc1 $\rightarrow$ 3 β -Galp R2 = H R3 = H R4 = Ac R5 = 7-PEA
L8	R1 = β -Galp R2 = 3-PEA R3 = H R4 = H

Figure 1

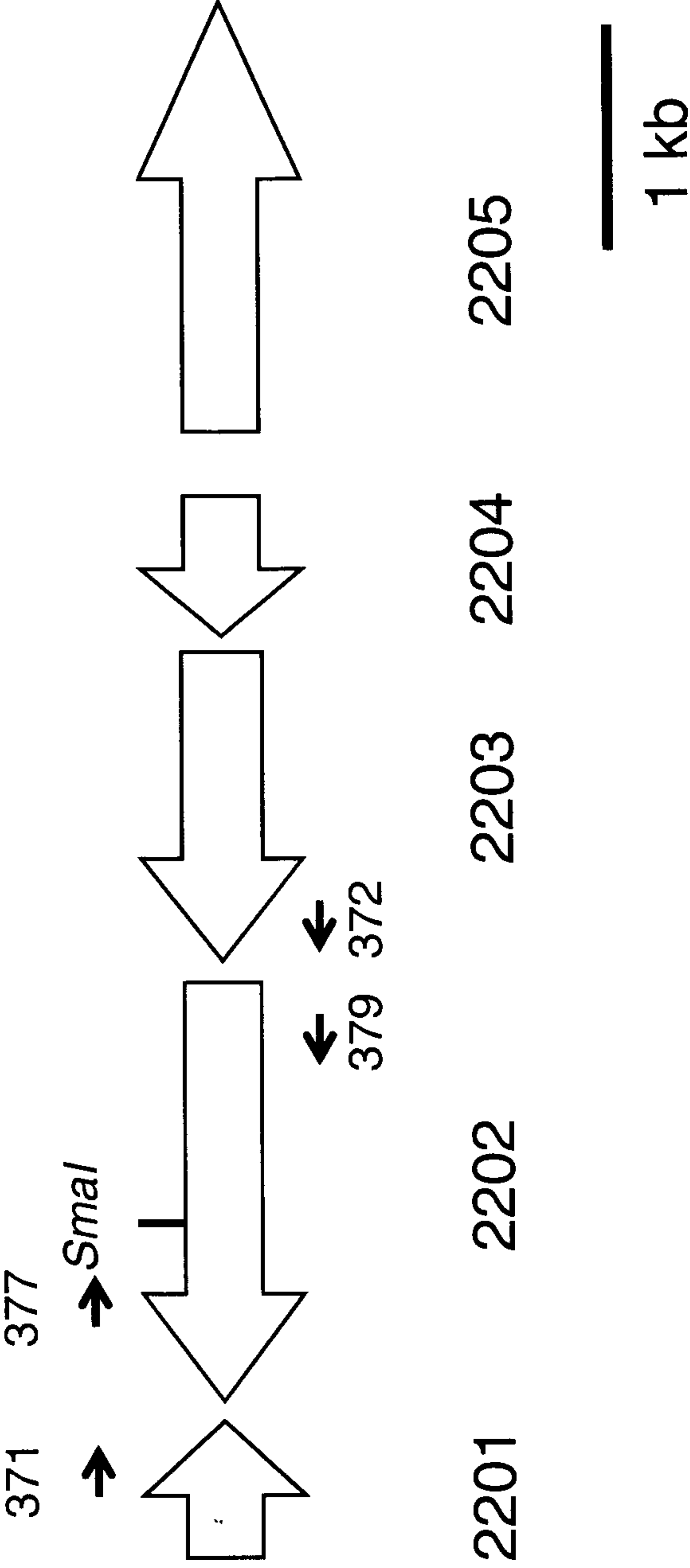


Figure 2

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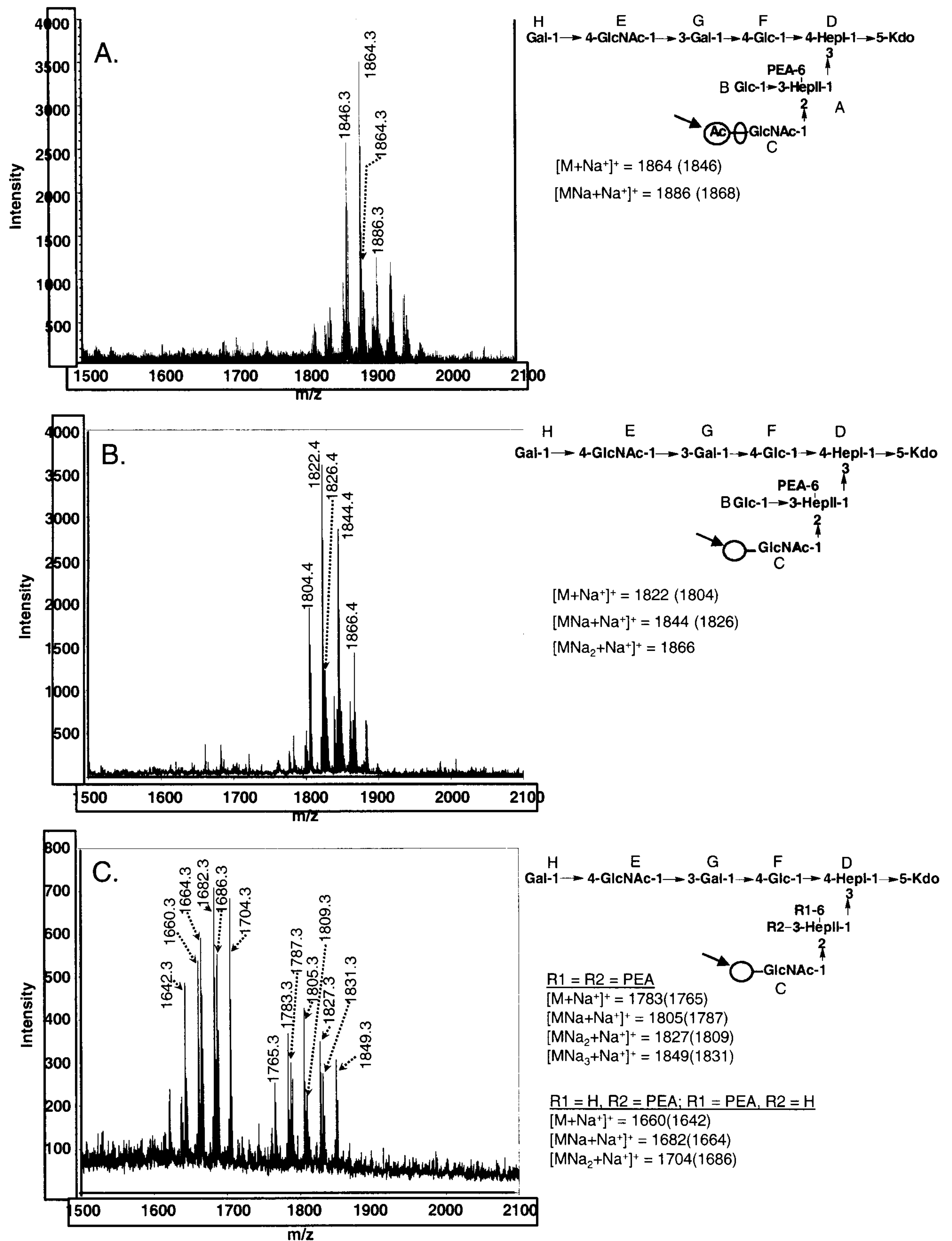


Figure 3

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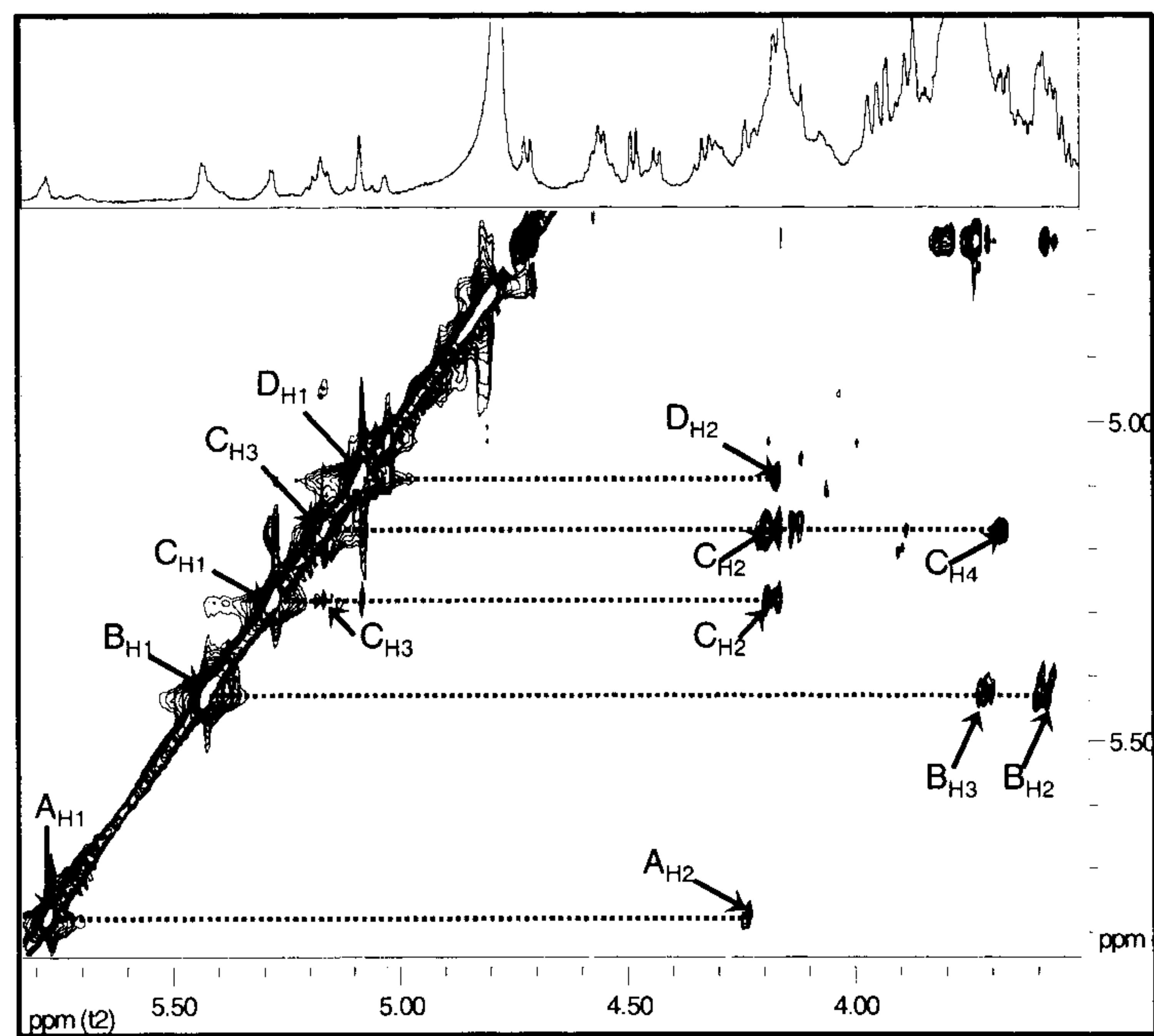


Figure 4

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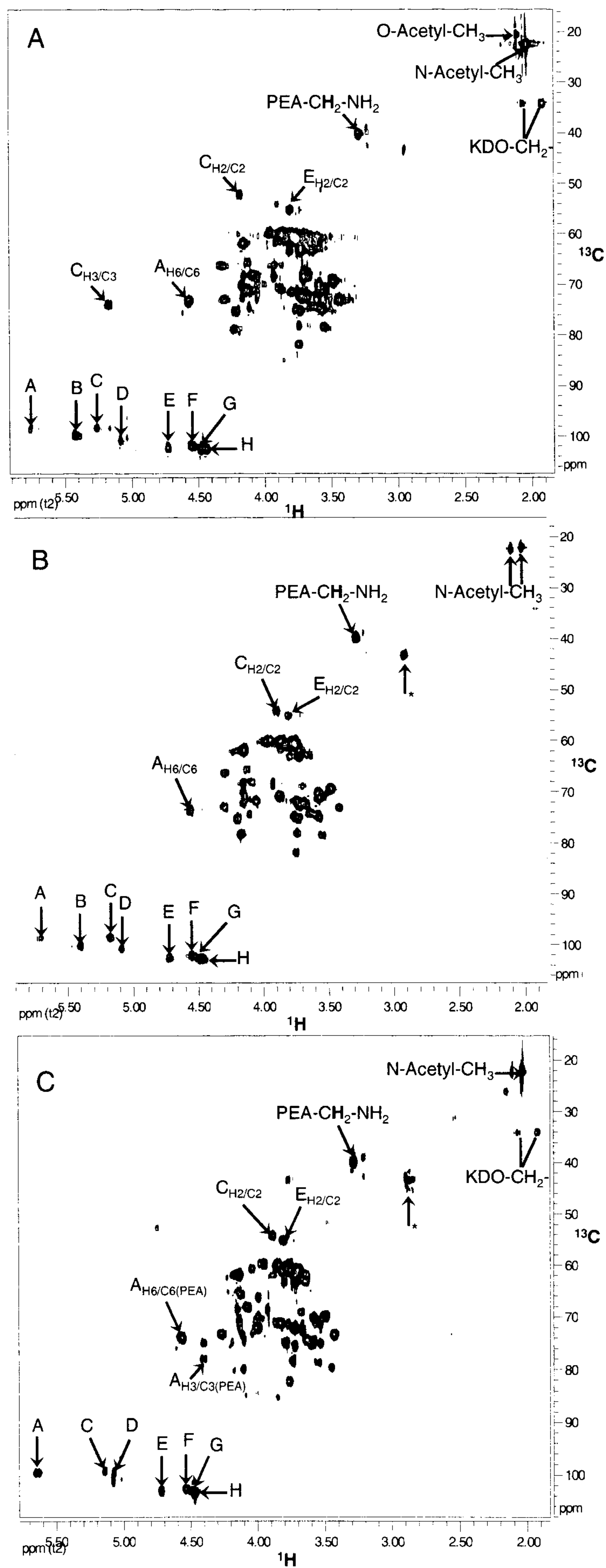


Figure 5

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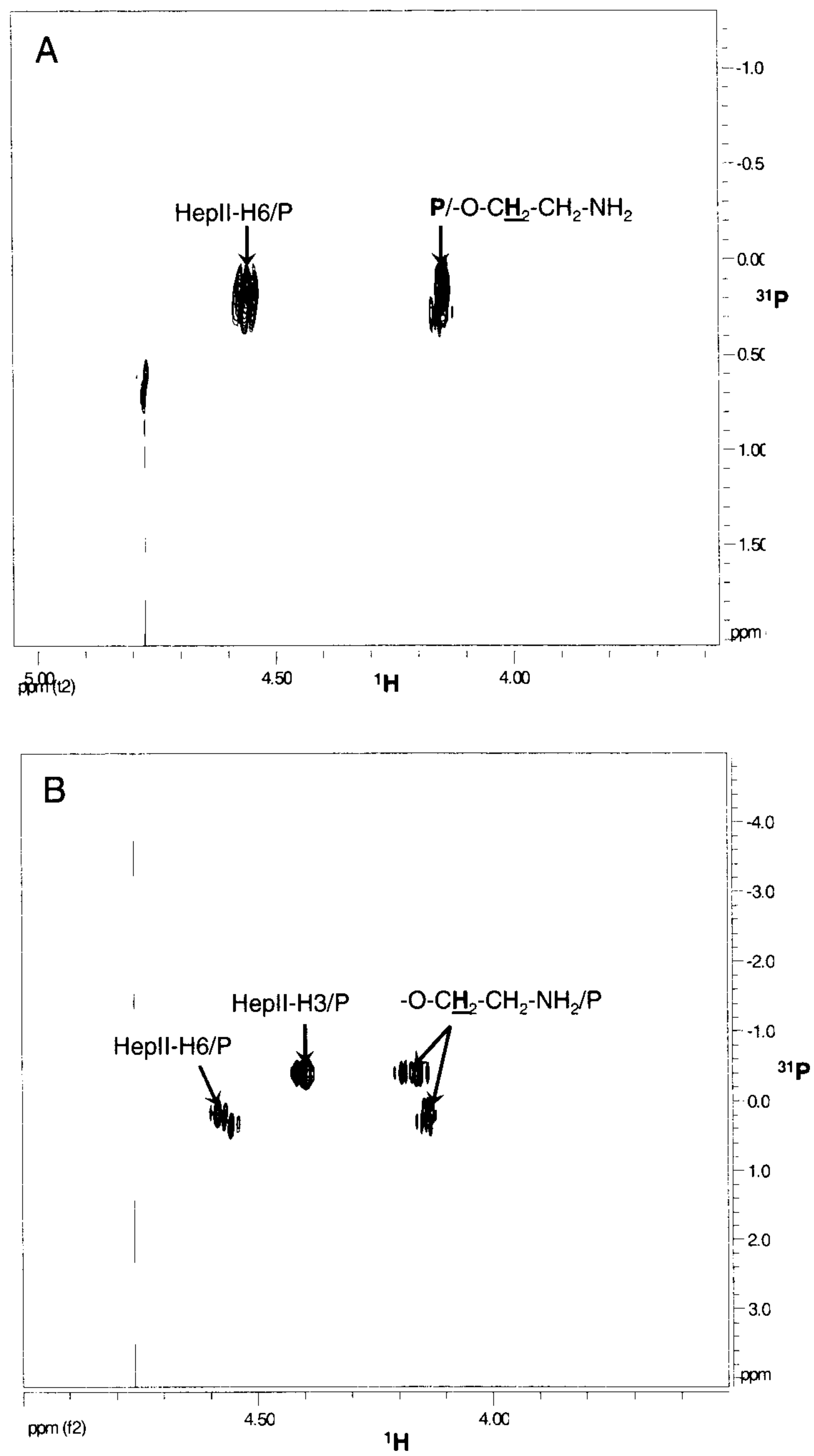


Figure 6

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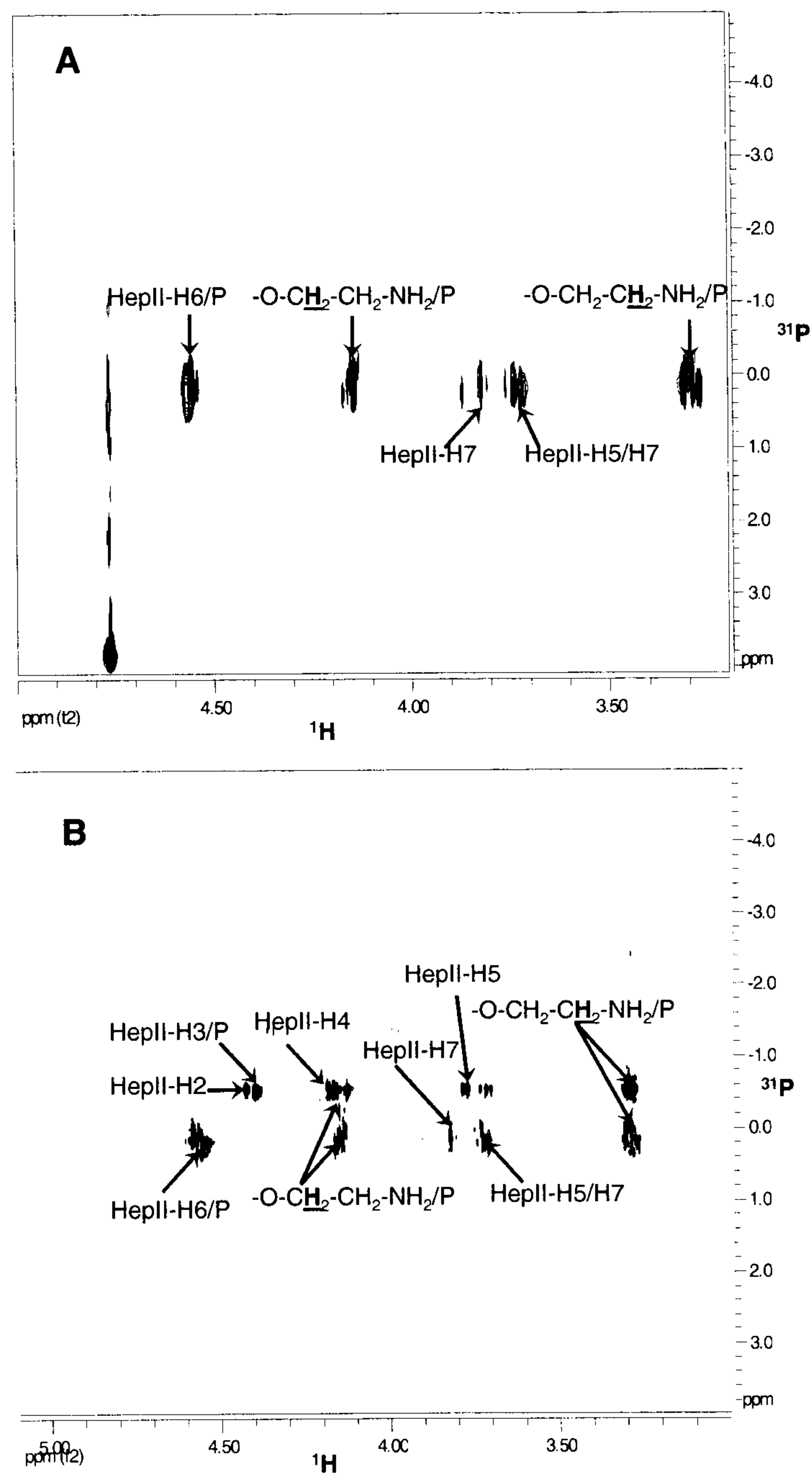
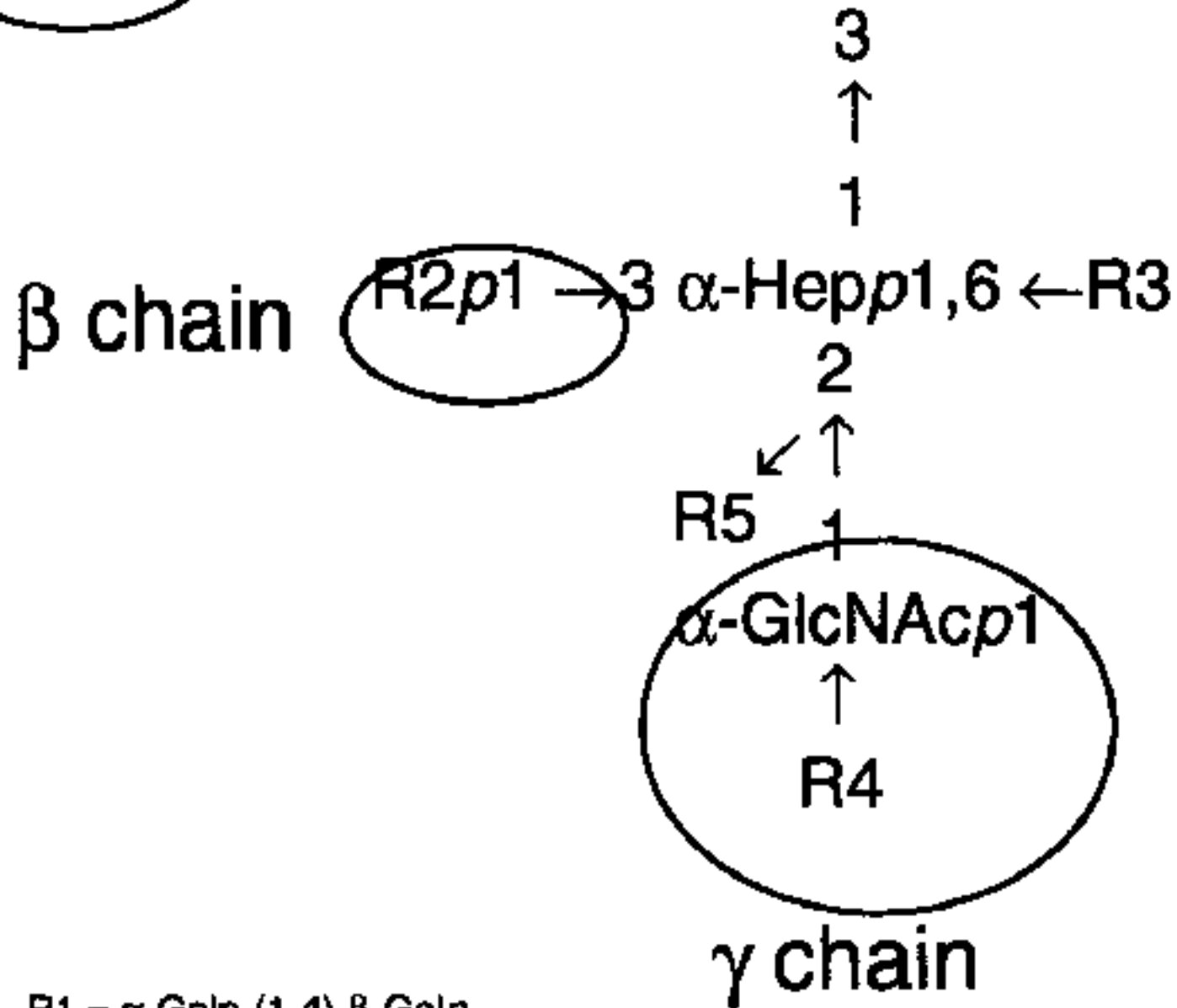


Figure 7

α chain $(R1-p1 \rightarrow) 4 \beta -Glc p1 \rightarrow 4\alpha -Hepp1 \rightarrow 5Kdo \rightarrow Lipid A$



Immunotype

L1	$R1 = \alpha -Galp-(1-4)-\beta -Galp$ $R2 = 3-PEA$ $R3 = H$ $R4 = Ac$
L2	$R1 = \alpha -Neu5Ac-(2 \rightarrow 3)-\beta -Galp1 \rightarrow 4 \beta -Glc pNAc1 \rightarrow 3 \beta -Galp$ $R2 = \alpha -Glc p$ $R3 = 6-PEA$ $R4 = Ac (40\%)$ $R5 = Gly \text{ or } O-7 PEA^*$
L3	$R1 = \alpha -Neu5Ac-(2 \rightarrow 3)-\beta -Galp1 \rightarrow 4 \beta -Glc pNAc1 \rightarrow 3 \beta -Galp$ $R2 = 3-PEA$ $R3 = H$ $R4 = H$ $R5 = Gly$
L4	$R1 = \alpha -Neu5Ac-(2 \rightarrow 3)-\beta -Galp1 \rightarrow 4 \beta -Glc pNAc1 \rightarrow 3 \beta -Galp$ $R2 = H$ $R3 = 6-PEA$ $R4 = Ac (50\%)$ $R5 = Gly$
L5	$R1 = \alpha -Neu5Ac-(2 \rightarrow 3)-\beta -Galp1 \rightarrow 4 \beta -Glc pNAc1 \rightarrow 3 \beta -Galp1 \rightarrow 4 \beta -Glc p1$ $R2 = \alpha -Glc p$ $R3 = H$ $R4 = Ac (60\%)$
L6	$R1 = \beta -Glc pNAc1 \rightarrow 3 \beta -Galp$ $R2 = H$ $R3 = H$ $R4 = Ac$ $R5 = 7-PEA$
L8	$R1 = \beta -Galp$ $R2 = 3-PEA$ $R3 = H$ $R4 = H$